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Introduction

Biosensor is a device for detection and measurement of substrate concentration in analysed solution. It is a cheap and reliable device used in environment monitoring, food industry and medicine [1, 2]. This dissertation analyses application of neural networks to determine multiple substrate concentrations from biosensor response. Furthermore this dissertation analyses biosensor optimisation as a design measure.

Amperometric biosensor has some disadvantages: usually it can measure concentration of only one substrate and has relatively small measure range, enzyme have to be selective, expensive production of the enzyme, signal is noise sensitive [3, 4]. By using chemometrical methods the concentrations of several substrates can be determined [5, 6, 7, 8, 9]. Optimisation was used to solve the inverse problem, i.e., to find multiple concentrations of substrates from biosensor response [8, 9]. Moreover several substrates was determined using artificial neural networks [5, 6, 7].

Commercially successful biosensor usually needs to have the following characteristics: a measure range sould be as long as possible, amount of enzyme should be small, a signal should not be sensitive to noise and etc. Mathematical models are widely applied in order to get biosensor of required characteristics [3]. Multicriteria optimisation with mathematical models may be utilised during design of the biosensor [10]. The multi-objective optimization of biochemical processes and systems has been successfully performed in different applications, particularly, for the technological improvement of biochemical systems [11, 12], for increasing the productivity and yield of a multi-enzymatic system [13], for the optimal design of a pressure swing adsorption system [14], for finding trade-off between sensitivity and enzyme volume of biosensors [15] and for the optimal design of a metal ion biosensor [16].

Tasks and Goal of Dissertation

The goal of this dissertation is to investigate the influence of biosensor parameters towards accuracy of multiple substrates concentration determination with artificial neural networks, furthermore an application of optimisation methods to find appropriate parameters of biosensors is investigated.

To reach the goal of dissertation, the following tasks were solved:

1. Application of neural networks to determine multiple substrates concentration:
    - Define mathematical biosensor model, including the external (Nernst) diffusion layer and substrate interaction. Approximate the model of biosensor by a numerical model and implement it by a computer model. Apply computer simulation to get pseudo-experimental data.
    - Apply artificial neural networks to determine multiple substrates concentration from biosensor response and steady state currents.
    - Investigate and find best biosensor parameters for multiple substrates concentration determination.
2. Biosensor optimisation:
    - Define multi-objective optimisation problem for biosensor: determine optimisation variables and objective functions.
    - Implement objective functions.
    - Investigate objective functions ant select optimisation method.
    - Optimise biosensor and analyse optimisation results. Give recommendation based on optimisation result analysis.

Means and Methods of Investigation

Biosensors analysed in this work were modeled using reaction-diffusion equations [17]. A mathematical model was solved using a numerical finite difference method

[3]. Computer models were implemented using the C programming language [18]. Multiple substrate concentrations were determined using artificial neural networks [19]. The Matlab Neural Network Toolbox was used [20]. To reduce imput data dimension, the principal component analysis was used [21]. Parallel calculations were done using OpenMPI protocol [22]. Optimisation was done using the Hooke-Jeeves optimisation algorithm [23] and the Chebyshev scalarisation [24]. Multi-dimensional scaling was done using the SMACOF algorithm [25].

Novelty of Dissertation Results

1. In this dissertation a mathematical model of biosensor was used: it includes the external Nernst diffusion layer and substrate interaction. Artificial neural networks were used to find concentrations of substrates from biosensor response.

2. Artificial neural networks were used to determine concentrations of substrates using multiple steady state currents generated by biosensors.

3. A dimensionless model was used to investigate the influence of biosensor parameters on errors when concentrations were determined by neural networks.

4. Multi-objective optimisation problem for biosensor was defined.

5. The proposed method of biosensor design that integrates the multi-objective optimisation with visualisation facilitates exploration of a relation between the Pareto optimal decision and solution spaces aiming at search for an appropriate trade-off between conflicting objectives.

Practical Value of Dissertation Results

Biosensors capable to measure several substrates would enable primary analysis in pollution detection. As shown by our investigation, a biosensor response or steady state currents can be used to determine concentrations of multiple substrates.

Mathematical model of biosensor including the external (Nernst) diffusion layer and substrate interaction was used.

The proposed method of biosensor design that integrates the multi-objective optimisation with visualisation facilitates exploration of a relation between the Pareto optimal decision and solution spaces aiming at search for an appropriate trade-off between conflicting objectives. It was applied for glucose and phenol biosensors and recommendations was given.

Dissertation results were used for an EU project "Developing computational techniques, algorithms and tools for efficient simulation and optimisation of biosensors of complex geometry", under the European Social Fund measure No. VP1-3.1-ŠMM-07-K (2014–2015).

Propositions to be Defended

1. Using mathematical model of biosensor (including the external Nernst diffusion layer and substrate interaction) and artificial neural networks it is possible to determine concentration of several substrates.
2. Using multiple steady state currents generated by biosensors (differing only in parameters) and artificial neural networks it is possible to determine concentrations of several substrates.
3. Applying multi-objective optimisation and multi-dimensional data visualisation in the phase of biosensor design allows to get most suited Pareto optimal trade-off solutions.

Approval of Dissertation Results

Two publications related to multi-objective biosensor optimisation were published. The first one published in a journal indexed in Clarivate Analytics Web of Knowledge [A1]. The second one was published in a scientific conference proceedings indexed in SCOPUS [A5].

Material related to the application of artificial neural networks to determine multiple substrate concentrations was published in Lithuanian journal of computer science [A4] and in a proceedings of the conferences [A3, A2].

Dissertation results were presented in four international and three Lithuanian conferences:

1. ECMS 2017 (Budapest, Hungary): *31th European Conference on Modelling and Simulation*. 23–26th May 2017.
2. FTMTT 2017 (Vilnius, Lithuania): *Fizinių ir technologijos mokslų tarpdalykiniai tyrimai 2017*. 9th February 2017.
3. MMA 2016 (Tartu, Estonia): *Mathematical Modelling and Analysis 2016*. 1–4th June 2016.
4. OR 2016 (Vilnius, Lithuania): *Open Readings 2016*. 15–18th March 2016.
5. DAMSS 2015 (Druskininkai, Lithuania): *Data Analysis Methods for Software Systems 2015*. 3–5th December 2015.
6. KODI 2015 (Panevėžys, Lithuania): *Computer Days 2015*. 17–19th September 2015.
7. IVUS 2015 (Kaunas, Lithuania): *International Conference on Information Technology 2015*. 24th April 2015.

Chapter 1

Modeling and Analysis of Biosensors

1.1 Mathematical Modeling of Biosensors

Biosensors are based on the enzyme reaction of substrates [26]. In case of amperometric biosensor the generated current is based on oxidation-reduction reaction of enzyme reaction products. The abundance of ferment reaction allows creating many schemes of bioelectrocatalysis [3, 27]. Biosensor can be created using various bioelectrocatalysis schemes and semi-permeable membranes. The selection of the geometry and enzyme parameters is essential in project phase of biosensors.

Usually biosensor is composed of multiple membranes [3]. Figure 1.1 shows the principal scheme of multi-layer biosensor. Thickness of all layers varies: $d_1, d_2, ..., d_n$. a_0 is the surface of an electrode, a_n is the solution boundary and $a_1, ..., a_{n-1}$ is layer boundaries.

Biosensors are modeled using diffusion-reaction equations. In the l layer the diffusion movement of particles and the kinetics of reaction can be expressed by a system of diffusion-reaction equations [3],

$$\partial_t \boldsymbol{c}^{(l)} = \boldsymbol{D}^{(l)} \Delta \boldsymbol{c}^{(l)} + \boldsymbol{R}^{(l)}(\boldsymbol{c}^{(l)}), \tag{1.1}$$

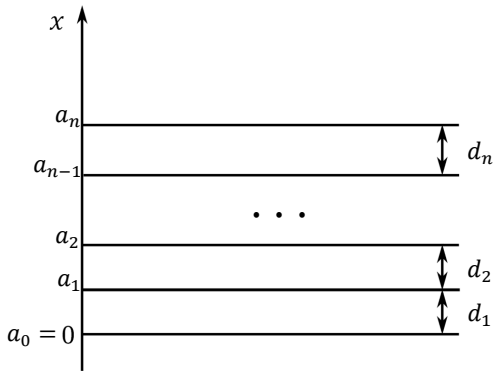

Figure 1.1: Principal scheme of multi-layer biosensor. Diffusion is present in all layers and the reaction is present in some layers. Ferment reaction is present in the first layer $x \in [a_0, a_1]$.

where $\boldsymbol{c}^{(l)}(\boldsymbol{x}, t) = (c_1^{(l)}(\boldsymbol{x}, t), c_2^{(l)}(\boldsymbol{x}, t), ..., c_k^{(l)}(\boldsymbol{x}, t))^T$ is a vector of regents concentrations in the l layer, $\boldsymbol{x} = (x, y, z)$ is a space coordinate, t is time, $\boldsymbol{D}^{(l)}$ is the diagonal diffusion coefficient matrix, $\boldsymbol{R}^{(l)}(\boldsymbol{c}^{(l)}) = (R_1^{(l)}(\boldsymbol{c}^{(l)}), R_2^{(l)}(\boldsymbol{c}^{(l)}), ..., R_k^{(l)}(\boldsymbol{c}^{(l)}))^T$ is a function that describes the kinetics of a reaction. When no reaction occurs we get a diffusion equitation, i.e., $\boldsymbol{R}^{(l)}(\boldsymbol{c}^{(l)}) = (0, 0, ..., 0)^T$, index l indicates a specific layer $1 \leq l \leq n$.

Biosensor can be modeled in one dimentional space without losing of accuracy if some assumptions are adopted [3]. The system of one dimensional diffusion-reaction equations is used,

$$\partial_t \boldsymbol{c}^{(l)} = \boldsymbol{D}^{(l)} \partial_{xx} \boldsymbol{c}^{(l)} + \boldsymbol{R}^{(l)}(\boldsymbol{c}^{(l)}), \tag{1.2}$$

where x is a one dimensional space coordinate.

In case when the i-th regent can diffuse by a limit ($x = a_l$) of layers, the flux by a limit of layers l and $l + 1$ (i.e. through surface $x = a_l$) is equal to the flux that is equal to the corresponding flux of the same compound entering the surface of a layer l [3],

$$D_i^{(l)} \partial_x c_i^{(l)}\big|_{x=a_l} = D_i^{(l+1)} \partial_x c_i^{(l+1)}\big|_{x=a_l}, \tag{1.3a}$$

$$c_i^{(l)}\big|_{x=a_l} = c_i^{(l+1)}\big|_{x=a_l}, \tag{1.3b}$$

where $D_i^{(l)}$ and $D_i^{(l+1)}$ is the i-th regent diffusion coefficient in the l-th and the $l+1$-th layers.

If the $l+1$ layer has a constant concentration of the i-th substrate, the Dirichlet boundary condition is applied to the l layer on a boundary ($x = a_l$) [3],

$$c_i^{(l)}\big|_{x=a_l} = c_0, \tag{1.4}$$

where c_0 is a concentration value.

If i-th regent doesn't diffuses by the limit ($x = a_l$) of layers l and $l+1$ because of the non-permeability, the Neumann boundary condition is applied [3],

$$D_i^{(l)} \partial_x c_i^{(l)}\big|_{x=a_l} = 0. \tag{1.5}$$

In case of amperometric biosensor electrochemically active regents (lets say: $c_{k_1}^{(1)}$, $c_{k_2}^{(1)}$,....,$c_{k_m}^{(1)}$) transfer charge on the electrode surface ($x = a_0$) and generate an electric current. It can be calculated using Fick and Faraday laws [3],

$$I(t) = \sum_{i=1}^{m} n_i F D_{k_i}^{(1)} \partial_x c_{k_i}^{(1)}\Big|_{x=a_0}, \quad I_\infty = \lim_{t\to\infty} I(t), \tag{1.6}$$

where n_i is a number of electrons involved in the charge transfer on the electrode surface ($x = a_0$), I_∞ is a steady state current, F is the Faradays constant.

Diffusion-reaction problem is specified by specifying a function that describes the kinetics of the reaction $\boldsymbol{R}^{(l)}$, $1 \leq l \leq n$ also boundary and initial conditions. Specified problem can be solved using analytical or numerical methods [3].

1.2 Multi-objective Optimisation of Biosensor

Multi-objective optimisation allows to get Pareto optimal trade-off solutions (any single objective of the Pareto optimal solution cannot be improved by not worsen other objectives) [24]. Design of biosensor can be reduced to multi-objective op-

timisation with an optimised function [28],

$$\mathbf{F_P} = \min_X F(X) = \begin{pmatrix} f_1(X) \\ f_1(X) \\ ... \\ f_k(X) \end{pmatrix}, \tag{1.7}$$

where $F(X)$ is a vector of minimised objective functions that describe the characteristics of biosensors ($k \geq 2$), $X \in A$ is a decisions vector (optimisation variables) from the decision area A. The decision area is defined by the following constraints: $A = \{X \in \mathbb{R}^n : g_1(X) = 0, ..., g_m(X) \geq 0\}$. In the case of maximisation, a negative value of an optimised function is analysed.

Multi-objective optimisation result is the Pareto front aproximation $\mathbf{F_P}$, i.e., a set of Pareto optimal solutions. Besides computing an approximation of $\mathbf{F_P}$ we are also interested in the representation of the set of Pareto optimal decisions,

$$\mathbf{X_P} = \{X : F(X) \in \mathbf{F_P}\}. \tag{1.8}$$

Optimised objectives (characteristics) depend on particular biosensor. It may be maximisation of a steady state current I_∞, minimisation of a biosensor response time T, minimisation of a ferment amount $E \times d_1$ etc. Decisions variables also depend on particular biosensor which may be the thickness of biosensor layers $d_1, d_2, ..., d_n$, a ferment concentration E, regent concentrations etc.

It is important to select the method of optimisation to get a representative Pareto front. The initial objective function analysis is used to select a proper method. Optimisation may be complicated if the optimsed function is an expensive (needs a lot computing resources) black box function. Also convexity of optimised functions should be analysed. Classical methods [24] and their adaptations [29] are well suited for continuous and convex functions, they do not suit for non-convex and non-continuous functions. Functions may be non-continuous due to numerical errors. The application of metaeuristic methods is irrational if optimised functions are expensive [30]. The most suitable option for expensive black box function optimisation is an algorithm based on a statistical model of the objectives [31]. However, at present the corresponding software is only available for bi-objective

problems [31]. Among other available alternatives the most promising method for the considered problem is the Chebyshev scalarisation method [24]. Using the latter, the minimisation problem (1.7) is reduced to a single objective problem.

Applied optimisation algorithm gives the Pareto front representation. Pareto optimal solutions are analysed by visualisation. Results of visualisation allows selecting the proper trade-off solution determined by a human expert [32].

1.3 Application of Artificial Neural Networks to Determine Substrates Concentrations

A linear analysis was applied in analysis of biological systems [33]. Complex signals are analysed using multi-variate analysis methods [34, 35]. Artificial neural networks can be used to increase the selectivity and sensitivity of sensors [36]. Artificial neural networks were applied to the classification of biosensor response [5, 6]. In case of measured substrate changing evenly and artificial neural networks can be applied to the determination of substrates concentrations from biosensor response [7].

In comparison to previous papers, this dissertation uses a mathematical model of biosensor including the external diffusion layer and substrate interaction. Artificial neural networks were applied to determine substrates concentrations from biosensor response [A3]. Artificial neural networks were also applied to determine substrates concentrations from multiple steady state currents [A2]. Furthermore, was found biosensor parameter values for most accurate concentrations determination [A4].

1.4 Optimisation of Biochemical Systems and Biosensors

Modeling was used to investigate biosensor action principles and give some recommendations for design, for example, modeling glucose dehydrogenase [27] and cyclic reaction based biosensors [37, 38]. Optimal parameters of biosystems can be

obtained using multi-objective optimisation, for example, technological improvement of biochemical systems [11, 12], increasing the productivity and yield of a multi-enzymatic system [13], the optimal design of a pressure swing adsorption system [14], finding trade-off between sensitivity and enzyme volume of biosensors [15] and the optimal design of a metal ion biosensor [16]. The importance of the multi-objective optimisation in biochemical engineering constantly increases due to development of new methods sustained by increased computational resources [12]. Computer based design of industrial analytical systems is still a challenging task due the fact that there are not only multiple often conflicting objectives, but also a combination of factors with complex non-linear mathematical models [12, 14, 39].

Optimisation was used to solve the inverse problem, i.e., to determine concentrations of substrates from biosensor response [8, 9]. On the other hand it important to find optimal often conflicting characteristics of biosensor as in the case of finding trade-off between sensitivity and enzyme volume of biosensors [15] and the optimal design of a metal ion biosensor [16]. In this dissertation multi-objective optimisation and visualisation was applied to find optimal trade-off characteristics of glucose [A1] and phenol biosensors [A5].

Chapter 2

Analysis of Biosensor Response

In this chapter an artificial neural networks is applied to the biosensor to determine multiple substrate concentrations. In subsection 2.1 the case of the k substrate is analysed. The biosensor model involves substrate interaction and the external (Nernst) diffusion layer. The response of the biosensor is used to determine multiple substrates concentrations. Moreover, the effect of the external diffusion layer is investigated [A3]. In subsection 2.2 the determination of concentration of two substrates using two biosensor static currents is investigated [A2, A4]. Furthermore, the effect of a diffusion module is analysed.

2.1 Multiple Substrate Concentration Determination from Biosensor Response

Biosensor response was used to determine multiple substrates concentrations. Besides that, the effect of the external diffusion layer was investigated. The principal component analysis was used to reduce the dimension of artificial neural networks input vector. The results show the effect of using external diffusion layer.

2.1.1 Model of the Biosensor

Using quasy-steady state assumption, enzyme reaction can be expressed [26],

$$\mathrm{S}_1 + \mathrm{S}_2 + ... + \mathrm{S}_k \xrightarrow{\mathrm{E}} \mathrm{P}_1 + \mathrm{P}_2 + ... + \mathrm{P}_k, \tag{2.1}$$

substrates $\mathrm{S}_i,\ i = 1, ..., k$, do not react with each other, but are competing in the enzyme reaction.

The analysed model of the biosensor involves three parts: an enzyme layer, where enzyme reaction and diffusion proceed, the external diffusion layer, where only diffusion proceed, and the analysed solution, where substrate concentrations remain constant [8]. Lets denote d as the thickness of the enzyme layer and δ as the thickness of the external diffusion layer. Model of the biosensor was specified by a reaction-diffusion system [A3]. The specified problem was solved using the finite difference method [3].

2.1.2 Numerical Experiments

In this case four substrates were analysed ($k = 4$). Neural networks determined normalised substrates (S_i, $i = 1...k$) concentrations c_i,

$$c_i = S_{i,0}/K_i, i = 1...k, \tag{2.2}$$

where S_i is the concentration of the ith substrate and K_i is the Michaelis constant [26]. Selected concentration $c = (c_1, ..., c_k)$ change range $C = [3.2; 12.8]^k$ [8]. Biosensor numerical simulation gives response $Z(c) = (z(1, c), ..., z(n, c))$, i.e., biosensor generated currents at time moments $t_i = i$ s.

Biosensor involves a diffusion layer. In this case the Biot number is important. It defines the ratio between the internal and the external mass transport resistance [40],

$$\beta_i = \frac{d/D_{S_{i,e}}}{\delta/D_{S_{i,b}}} = \frac{dD_{S_{i,b}}}{\delta D_{S_{i,e}}}, \quad i = 1, ..., k, \tag{2.3}$$

where d is the thickness of an enzyme membrane, δ is the thickness of the diffusion layer, $D_{S_{i,e}}$ is S_i substrate diffusion coefficient in an enzyme membrane, $D_{S_{i,b}}$ is

S_i substrate diffusion coefficient in the diffusion layer.

Biosensor response asymptotically goes to a steady state, so experiment should be stopped when the current increase is negligible. The normalised current increase was used and an acceptable value ϵ was selected [3],

$$\epsilon \leq \frac{t}{i(t)} \frac{di(t)}{dt}, \tag{2.4}$$

where $i(t)$ is biosensor currents, t is time. Biosensor simulation continues while (2.4) the condition is met.

2.1.3 Application of Artificial Neural Networks

Biosensor response can have large dimension. To reduce data dimension the principal component analysis was applied [21, 41]. First ten ($J = 10$) principal components were used as input for artificial neural networks, because remaining ones have a small dispersion (sum less than 10%).

Artificial neural networks that use superposition of a sigmoidal function were used. It can approximate any continuous function in a selected precision [42]. To find weights of neural networks the Levenberg-Marquardt optimisation algorithm was used [5, 43]. Artificial neural networks gives output vector of determined values of substrate concentrations $(\tilde{c}_1, \tilde{c}_2, ..., \tilde{c}_k)$.

Artificial neural networks were trained on records evenly covering concentration change range,

$$c \in \{q_i : q_i = c_{min} + i \times (c_{max} - c_{min})/M, i = 0, ..., M\}^k \in$$

$$C = [c_{min}; c_{max}]^k = [3.2; 12.8]^k, k = 4, M = 10.$$

To validate the accuracy of neural networks a validation set was used. It consisted of 1000 records with random concentrations. During biosensor modeling the stop condition $\epsilon = 0.01$ (see (2.4)) was used and t_{max} shows how long the experiment lasted. In experiments various external diffusion layer thickness $\delta \in \{0;\ 0.004;\ 0.016;\ 0.04;\ 0.1\}$ and, respectively, Biot numbers: $\beta = 0.04/\delta$

Table 2.1: Average values of substrate concentration determination relative errors.

δ	β	t_{max}	$\bar{\varepsilon}_1$	$\bar{\varepsilon}_2$	$\bar{\varepsilon}_3$	$\bar{\varepsilon}_4$
0	∞	120	0.0427	0.0465	0.0177	0.0122
0.004	10	146	0.05	0.0577	0.0222	0.0148
0.016	2.5	309	0.0376	0.0407	0.0162	0.0145
0.04	1	910	0.0051	0.0061	0.0032	0.0026
0.1	0.4	3468	0.002	0.0021	0.0013	0.001

were used. Expression of β_i (2.3) is simplified to β because constant values were applied.

2.1.4 Results

To estimate substrate concentration determination errors the relative errors value was used: $\varepsilon_i = \frac{|c_i - \tilde{c}_i|}{c_i}$, $i = 1, ..., k$, where $\tilde{c}_i$ is a value determined by neural networks, c_i is the real value of the ith substrate concentration. Experiments were carried out ten times and the average values are indicated in table 2.1.

The table shows that the external diffusion layer improves accuracy of results, i.e., comparing rows with $\delta = 0$ and $\delta = 0.1$ we see that accuracy is improved $\min_{i \in \{1,...,k\}} \frac{\bar{\varepsilon}_i(\delta=0)}{\bar{\varepsilon}_i(\delta=0,1)} = \frac{0.0177}{0.0013} \approx 13.6$ times. One the other hand, the experiment time increases $\frac{t_{max}(\delta=0.1)}{t_{max}(\delta=0)} = \frac{3408}{120} = 28.4$ times. Results confirm that external diffusion layer improves the sensitiveness of biosensors [40, 44, 45, 46, 47].

2.2 Determination of Several Substrates by Using Steady State Currents

Two biosensors that have the same enzyme (differing only in enzyme concentration) were used to determine two substrate concentrations. Steady state currents generated by two biosensors were used as an input of artificial neural networks to determine concentrations of two substrates. Furthermore, the influence of biosensor parameters (diffusion module) was investigated.

2.2.1 Model of the Biosensor

The analysed model of the biosensor involves three parts: an enzyme layer, where enzyme reaction and diffusion proceed, the external diffusion layer, where only diffusion proceed, and the analysed solution, where substrate concentrations remain constant. The model of the biosensor was specified by a steady state reaction-diffusion system [A2]. The anlaysed model was simplified by deriving a dimensionless model [A4]. The specified problem was solved using the finite difference method [3].

One of most important parameters that define biosensor is a diffusion module: $\alpha_i^2 = (d^2 V_i)/(D_{S_{i,e}} K_i)$ [3]. It describes the ratio between an enzyme reaction speed (V_i/K_i) and a diffusion speed in enzyme layer $(D_{S_{i,e}}/d^2)$. There V_i is the enzyme reaction speed, K_i is the Michael constant, $D_{S_{i,e}}$ is the diffusion coefficient in enzyme layer, d is the thickness of diffusion layer. The influence of the diffusion module was investigated.

2.2.2 Modeling Steady State Current of Biosensor

Two biosensors that have a different enzyme concentration were analysed. Biosensors generate two different steady state currents: I_1 and I_2. The diffusion module differs because different enzyme concentrations were used. Steady state currents can be expressed as functions of diffusion modules: $I_1(\alpha_{1,1}^2, \alpha_{2,1}^2)$ and $I_2(\alpha_{1,2}^2, \alpha_{2,2}^2)$. Diffusion modules of both biosensors $(\alpha_{1,1}^2, \alpha_{2,1}^2)$ and $(\alpha_{1,2}^2, \alpha_{2,2}^2)$ can be expressed by parameters p, q, α^2,

$$\alpha_{1,1}^2 = \alpha^2, \quad \alpha_{2,1}^2 = p\alpha^2 \tag{2.5a}$$

$$\alpha_{1,2}^2 = q\alpha^2, \quad \alpha_{2,2}^2 = qp\alpha^2. \tag{2.5b}$$

where α^2 is a constant that defines the enzyme reaction speed, p is the enzyme reaction speeds ratio of substrates, q is the enzyme concentrations ratio of biosensors.

Having steady state currents I_1 and I_2 an artificial neural networks determined normalised substrate S_i concentrations $\hat{S}_{i,0} = S_{i,0}/K_i, i = 1, 2$. Substrate concentration $s = (\hat{S}_{1,0}, \hat{S}_{2,0})$ change range is $s \in S = [3.2; 12.8]^2$ [8]. Parameters p, q,

α^2 influence on the substrate determination using artificial neural networks was investigated.

2.2.3 Application of Artificial Neural Networks

An artificial neural networks that uses superposition of a sigmoidal function was used. It can approximate any continuous function in the selected precision [42]. Therefore, it was used to find concentrations $(\hat{S}_{1,0}, \hat{S}_{2,0})$ from steady state currents (I_1, I_2). Neural networks weights were found using the Levenberg-Marquardt optimisation algorithm [43].

An artificial neural networks was trained on records evenly covering concentration change range,

$$s \in \{q_i : q_i = \hat{S}_{0,min} + i \times (\hat{S}_{0,max} - \hat{S}_{0,min})/M, i = 0, ..., M\}^2 \tag{2.6a}$$

$$S = [\hat{S}_{0,min}; \hat{S}_{0,max}]^2 = [3.2; 12.8]^2, M = 20. \tag{2.6b}$$

To validate the accuracy of neural networks a validation set was used. It consists of 100 records with random concentrations.

2.2.4 Results

To estimate substrate concentration determination errors a relative error value was used: $\varepsilon_i = |\hat{S}_{i,0} - \tilde{c}_i|/\hat{S}_{i,0},\ i = 1, 2$, where $\tilde{c}_i$ is the value determined by neural networks, $\hat{S}_{i,0}$ is the real value of the ith substrate concentration. Experiments were carried out ten times and the average values were calculated $\bar{\varepsilon}_i, i = 1, 2$.

Experiments were carried out with different values of p and q so we get a relative error function $\bar{\varepsilon}_i(p, q), i = 1, 2$. Results were normalised using the maximum error value $\bar{\varepsilon}_{max} \approx 0,45$ to get percent error function: $e_i(p, q) = (\bar{\varepsilon}_i(p, q)/\bar{\varepsilon}_{max}) \times 100\%, i = 1, 2$. The set $A = \{1, 2, ..., 10\}$ was used as a change range of parameters $p \in A$ and $q \in A$ so function values $e_i(p, q), i = 1, 2$ calculated in the area $(p, q) \in A^2$. Results were calculated using $\alpha^2 \in \{0.1; 1; 10\}$ and are presented in Fig. 2.1.

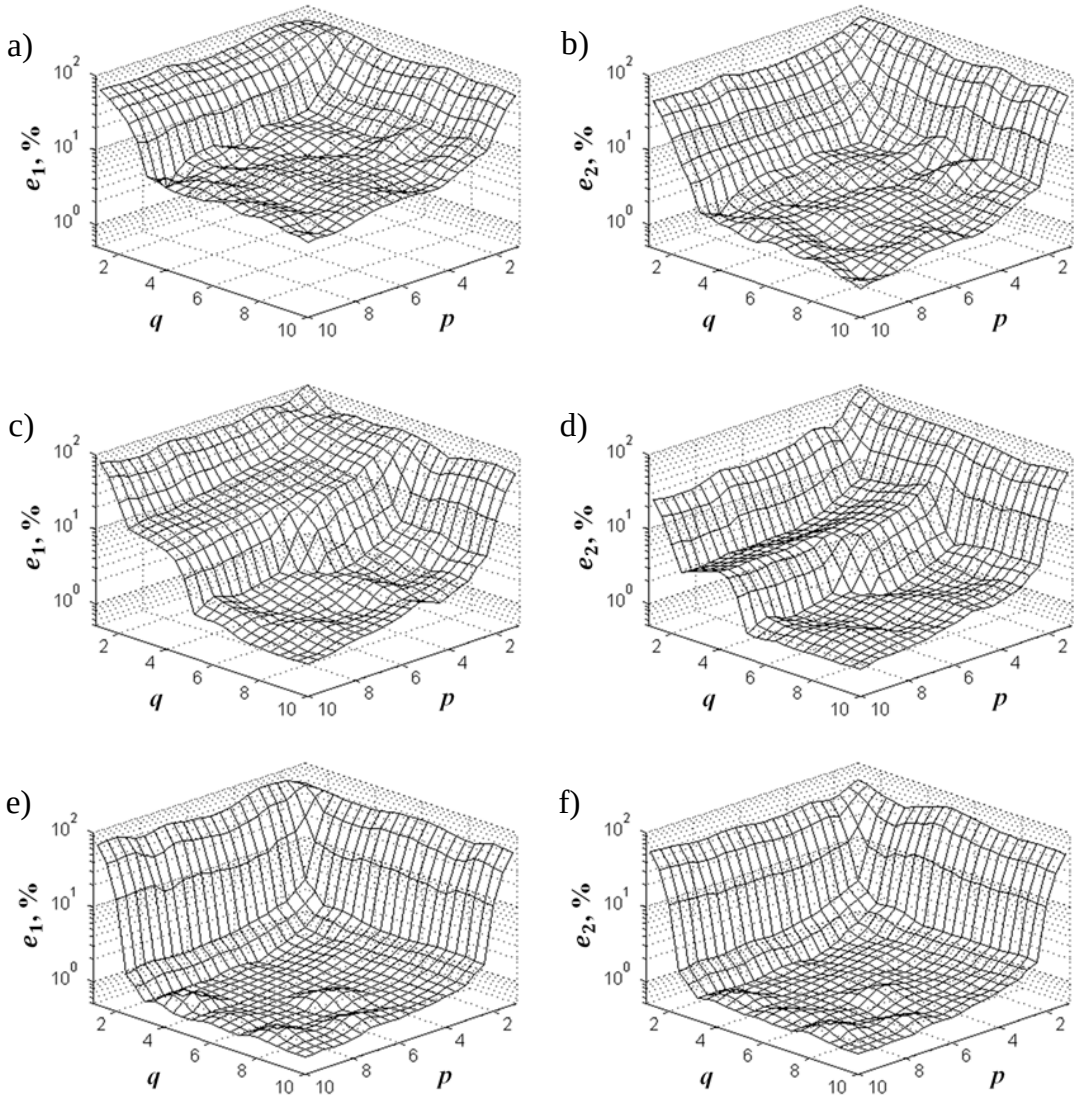

Figure 2.1: Relative error values $e_i(p,q), i = 1,2$. Used α^2 values: $\alpha^2 = 0.1$ (**a**)–(**b**), $\alpha^2 = 1$ (**c**)–(**d**), $\alpha^2 = 10$ (**e**)–(**f**).

The figure shows that the greatest relative error values occurred when $p = q = 1$, i.e., in this case both biosensors were identical and its impossible to find two substrate concentrations using one biosensor. Similarly when $p = 1$ or $q = 1$, i.e., it is impossible to find substrate concentration values. The minimal error $e_i(p,q) \approx 1\%, i = 1,2$, occurs when $p = q = 10$. When parameters p, q were reduced, error became greater.

In all cases $\alpha^2 \in \{0.1, 1, 10\}$ error values approach the minimum when $p > 4$

and $q > 4$. In all cases when $p > 1$ and $q > 1$ the error value of the second substrate is lower, because $\alpha_{1,1}^2 < \alpha_{2,1}^2$, $\alpha_{1,2}^2 < \alpha_{2,2}^2$, i.e., the seconds substrate influences biosensor current more. The lowest error values were obtained when $\alpha_i^2 \gg 1, i = 1, 2$, (Fig. 2.1e, 2.1f), i.e., when biosensor responses are mostly influenced by diffusion.

2.3 Conclusions and Results

Biosensors responding to multiple substrates are analysed. The model of the biosensor involves substrate interaction and external (Nernst) diffusion layer. Response of the biosensor was used to determine multiple substrate concentrations. The external diffusion layer improves the accuracy of results. One the other hand, the experiment time increases, so the trade-off should be selected.

Biosensors differing only in enzyme concentration were used to determine two substrate concentrations. The steady state current generated by two biosensors was used as an input for artificial neural networks to determine the two substrates concentrations. Moreover, the influence of biosensor parameters (diffusion module) was investigated. Error values approach the minimum when $p > 4$ and $q > 4$, i.e., the ratio of enzyme reaction speeds of substrates p and the ratio of enzyme concentrations of biosensors q is more than 4. Lowest error values were obtained when $\alpha_i^2 \gg 1, i = 1, 2,$, i.e., when biosensor response mostly influenced by diffusion.

Chapter 3

Optimisation of Biosensors

In order to make good biosensor, the device should meet many contradictory requirements [3]. This chapter presents a method that combines mathematical modeling, multi-objective optimisation and multi-dimensional visualisation that is intended for the design and optimisation of amperometric biosensors. The approach for optimising parameters of biosensors are based on the availability of a mathematical model of a catalytic biosensor. A multi-objective visualisation of trade-off solutions and Pareto optimal decisions is applied to select of the most favorable decision by a human expert when designing biosensors. The multi-objective optimisation was applied to glucose (subsection 3.1) [A1] and phenol (subsection 3.2) biosensors [A5].

3.1 Multi-objective Optimisation and Decision Visualisation of Biosensors with Synergistic Substrates Conversion

Biosensor that utilises the synergistic substrates conversion was optimised to get the optimal design. The following three objectives were optimised: the apparent Michaelis constant was maximised, the output current was maximised and the enzyme amount was minimised. The synergistic schemes of substrates conversion are of particular interest due to their application in order to produce highly

sensitive bioelectrodes and powerful biofuel cells [48].

3.1.1 Modeling of Biosensor with Synergistic Substrates Conversion

The glucose dehydrogenase (GDH)-based amperometric biosensor is a particular case of biosensors utilising the synergistic substrates conversion used to measure the glucose level in blood [27, 48]. The modeled GDH biosensor is assumed to be composed of a graphite electrode covered with an enzyme (GDH) layer [48]. The enzyme layer is separated from the bulk solution by means of the inert dialysis membrane.

Assuming the symmetrical geometry of the biosensor, the homogeneous distribution of the immobilised enzyme and coupling reactions in the enzyme layer with a one-dimensional-in-space diffusion, described by the Fick's second law, lead to reaction-diffusion type equations [3, 27]. The governing equations together with appropriate initial, boundary and matching conditions form the non-linear initial value and boundary value problem, which was numerically solved by applying the finite difference technique [3].

Values of some parameters are usually application-specific to a target biosensor: reaction rate constants, substrates diffusion coefficients, mediators, products and others [40]. Meanwhile, values of some other parameters, e.g., the concentration of the enzyme and mediators, as well as, the geometry, can be selected by the designer quite freely.

3.1.2 Optimal Design of Biosensor as a Problem of Multi-objective Optimisation

3.1.2.1 Patameters of the Biosensor to be Optimised

Designing biosensor, like designing in general, may be reducible to a multi-objective optimisation where the minimum or the maximum values of numerous parameters are desirable, e.g., the sensitivity of biosensors, the response time, material costs

and etc. In this work three objectives were considered: the apparent Michaelis constant K_M^{app}, the maximum current $I_{\max}$, and the enzyme amount Ad_1E_0, where E_0 is the total concentration of the enzyme.

An upper limit of the linear concentration range is an important parameter for electrochemical biosensors [40]. The greater value of K_M^{app} corresponds to a wider range of the linear part of the calibration curve [3].

In some cases of biosensors, enzymes are archival and only available in a limited quantity or are products of combinatorial synthesis procedures and thus are only produced in micrograms or milligrams [40]. Therefore, the amount of enzyme used in biosensors should be minimised. The total quantity of the enzyme (GDH) is expressed as a product of initial (total) concentration E_0 of GDH and the volume Ad_1 of the enzyme layer, i.e. the total quantity of GDH equals Ad_1E_0.

The limit of detection of sensors is also determined by a signal-to-noise ratio [49]. The signal amplification enhances the signal-to-noise ratio of biosensors. Therefore, it is reasonable to maximise the biosensor current $I_{\max}$.

3.1.2.2 Multi-objective Optimisation Problem

The considered optimal design problem mathematically is stated as a three-objective optimisation problem with an objective function $\Phi(x) = (\varphi_1(x), \varphi_2(x), \varphi_3(x))^T$, where $\varphi_1(x)$ is K_M^{app}, $\varphi_2(x)$ is $I_{\max}$ and $\varphi_3(x)$ is Ad_1E_0. The decision variables for the optimal biosensor design problem are provided in Table 3.1.

The minimum, as well as the maximum, values of decision parameters should be expertly estimated. Values of some of them depend on the technological possibilities, e.g. on the thicknesses of commercially available dialysis membranes or

Table 3.1: Decision variables $x = (d_1, d_2, E_0, S_{1,0}, S_{2,0})^T$ for the problem of biosensor design.

Variable		Description	Range	Units
1.	d_1	Enzyme layer thickness	$[2 \times 10^{-6}, 10^{-4}]$	m
2.	d_2	Dialysis membrane thickness	$[10^{-6}, 2 \times 10^{-5}]$	m
3.	E_0	Enzyme concentration	$[5 \times 10^{-8}, 5 \times 10^{-5}]$	mol dm^{-3}
4.	$S_{1,0}$	Ferricyanide concentration	$[10^{-3}, 10^{-2}]$	mol dm^{-3}
5.	$S_{2,0}$	Oxidised mediator concentration	$[0, 10^{-5}]$	mol dm^{-3}

on the of nylon nets thread thicknesses that is used to prepare of the enzyme layer [26, 40, 48].

First two objectives should be maximised, and the last one should be minimised. However, to facilitate the analysis it is convenient to reformulate the optimisation problem into a problem with all objectives aimed at minimisation. To equalise the range of objectives, their minimum and maximum φ_i^-, φ_i^+, $i = 1, 2, 3$, were computed (using the multi-start with the Hooke-Jeeves algorithm) and the ranges were normalised to [0, 1],

$$f_i(x) = \frac{\varphi_i^+ - \varphi_i(x)}{\varphi_i^+ - \varphi_i^-}, \qquad i = 1, 2, \tag{3.1a}$$

$$f_3(x) = \frac{\varphi_3(x) - \varphi_3^-}{\varphi_3^+ - \varphi_3^-}, \tag{3.1b}$$

$$\varphi_i^+ = \max_{x \in \mathbf{A}} \varphi_i(x), \qquad \varphi_i^- = \min_{x \in \mathbf{A}} \varphi_i(x), \quad i = 1, ..., 3, \tag{3.1c}$$

$$x = (x_1, \ldots, x_5)^T, \qquad \mathbf{A} = \{x : 0 \le x_j \le 1,\ j = 1, ..., 5\}, \tag{3.1d}$$

where x_j, $j = 1, 2, \ldots, 5$, denotes optimisation variables (decision parameters) rescaled to the unit interval; thus the considered optimal design problem is reduced to the following,

$$\mathbf{F_P} = \min_{x \in \mathbf{A}} F(x), \qquad F(x) = (f_1(x), f_2(x), f_3(x))^T. \tag{3.2}$$

The optimisation result $\mathbf{F_P}$ is the Pareto front of the formulated three-objective optimisation problem. The vector of variables that correspond to the Pareto optimal solution is named the Pareto optimal decision. Besides computing an approximation of $\mathbf{F_P}$, we are interested in the representation of a set of Pareto optimal decisions,

$$\mathbf{X_P} = \{x :\ F(x) \in \mathbf{F_P}\}. \tag{3.3}$$

3.1.2.3 Solution of the Stated Multi-objective Optimisation Problem

In selecting an appropriate algorithm to represent $\mathbf{F_P}$ and $\mathbf{X_P}$ the crucial difficulty is the characterisation of the problem as an expensive black-box problem. Moreover, numerical experiments showed the non-convexity of at least one objective function. Classical methods [24], which are efficient for smooth convex problems,

as well as their adaptive versions, e.g. [29], are not suitable here because of the non-convexity, and because of possible non-smoothness of the objective functions which is implied by numerical errors. The application of various metaheuristic methods [30] is limited because of expensiveness of the objectives; a vector value of objective function on average takes almost 6 minutes using a personal computer with Intel Core i7-4770 3.5 GHz processor. The most suitable algorythm for problems with characteristic of interest would be an algorithm based on a statistical model of the objectives [31], however, at present, the corresponding software is available only for biobjective problems. Among other available alternatives the most promising method for the considered problem is the so called Chebyshev scalarisation method [24]. Using the latter, the minimisation problem (3.2) is reduced to the following parametric single objective problem,

$$f(x) = \max_{1\le i\le 3} w_i f_i(x), \qquad x(w) = \arg\min_{x\in\mathbf{A}} f(x), \tag{3.4}$$

$$w = (w_1, w_2, w_3)^T,\ 0 \le w_i \le 1, \qquad \sum_{i=1}^{3} w_i = 1, \tag{3.5}$$

where the minimiser $x(w)$ is the Pareto optimal decision of the original multiobjective problem. All Pareto optimal decisions can be found by the solution of (3.4) with an appropriate vector of weights w. To represent whole set $\mathbf{F_P}$ and $\mathbf{X_P}$ the minimisation problem (3.4) should be solved repeatedly with different vectors of weights. The scalarised function $f(x)$ can be minimised by using a combination of a randomised selection of starting point with the Hooke-Jeeves optimisation algorithm [23].

The choice of weights aiming at the uniform distribution of solutions in $\mathbf{F_P}$ is complicated. We plan to compute the desirably distributed solutions using a two-step procedure. At the first step, the optimisation problem (3.4) was solved with weights shown in Fig. 3.1a. The determined Pareto optimal solutions are depicted in Fig. 3.1b, where triangles correspond to solutions with $f_2 > 0.3$ and squares to solutions with $f_2 \le 0.3$; this notation is held throughout the subsection. Different notation is used to highlight the change in structure of the representation of $\mathbf{F_P}$: for $f_2 \le 0.3$ $\mathbf{F_P}$ it looks like a curve, and for $f_2 > 0.3$ it seems to be extending to a surface. To verify the supposition that $\mathbf{F_P}$ transforms to a surface (not to separate curves), a more detailed representation of the Pareto front is desirable next to of points indicated by triangles. At the second step, the problem (3.4)

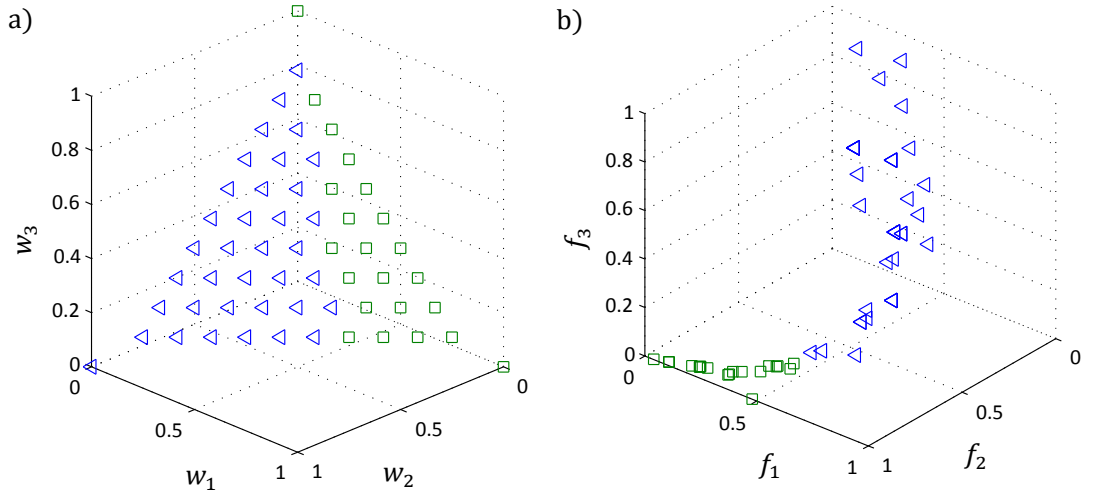

Figure 3.1: The weights (**a**) used and the Pareto optimal solutions (**b**) found at the first step of the optimisation procedure.

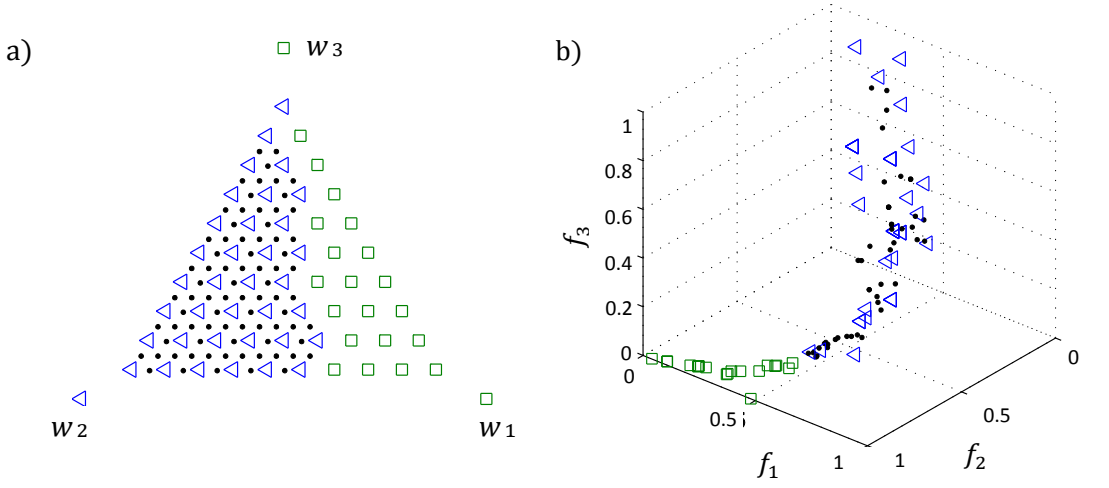

Figure 3.2: The supplementary weights indicated by black points in the triangle of weights (**a**) and the augmented representation of the Pareto front (**b**).

was solved using a set of weights depicted by solid points in Fig. 3.2a where the whole set of the used for approximation weights $\mathbf{W}$ is presented. The augmented representation of the Pareto front presented in Fig. 3.2b validates the supposition.

Although it requires a lot of computing time, the described implementation of the method for the representation of $\mathbf{F_P}(\mathbf{W})$, is still appropriate to be run on a personal computer. On our experiment, the computing of the representation of

the set of Pareto optimal solutions by the described implementation of the Chebyshev method (using weights shown in Fig. 3.1a) took about 168 hours. The total number of the objective function computations was equal to 1.3×10^4. The algorithm was run on a personal computer with Intel Core i7-4770 3.5 GHz processor, and 8 parallel threads were used to perform minimisation of different aggregated objectives $f(x)$ defined using different vectors of weights w. The optimisation was distributed by a master-slave approach using the Open MPI library. Open MPI provides the ability to parallelize task over a nonhomogenous distributed system (e.g., a supercomputer) [22].

3.1.3 Visualisation of Optimisation Results

By the analysis of a visual representation of the Pareto front $\mathbf{F_P}(\mathbf{W})$, such as presented in Fig. 3.2b, an appropriate Pareto solution can be selected as well as the decision $x(w)$ which corresponds to the selected Pareto solution. However, such a choice is not always satisfactory since it does not consider such properties of the corresponding decision as e.g. the location of the selected decision vector in the feasible region $\mathbf{A}$. The analysis of the location of the set of efficient points in $\mathbf{A}$ can be especially valuable in cases of structural properties of the considered set is important for the decision making [14]. To visualise a set of Pareto optimal decisions, which is a subset of a feasible region in a multi-dimensional space, special methods of visualisation of multi-dimensional data are required. A suitable method here is the multi-dimensional scaling [41].

The approximation $\mathbf{F_P}(\mathbf{W})$ of the Pareto front $\mathbf{F_P}$ computed by the Chebyshev algorithm consists of $N = 136$ three-dimensional vectors. The corresponding set $\mathbf{X_P}(\mathbf{W})$ of five dimensional points $x(w^i) \in \mathbf{A}$, $i = 1, \ldots, N$, is an approximation of $\mathbf{X_P}$. To get an idea of the location of $\mathbf{X_P}(\mathbf{W})$ in a five dimensional unit cube, a multi-dimensional scaling based algorithm was applied to the two-dimensional visualisation of a set of five dimensional points consisting of $x(w^i)$, $i = 1, \ldots, N$, and the cube vertices. We decided to apply a multi-dimensional scaling procedure that is widely available on the internet [25]. The selected procedure is based on the well known SMACOF algorithm [50].

Before visualising the data for the considered problem, a two-dimensional image of the set of vertices of the five dimensional cube is presented in Fig. 3.3a. It is worth

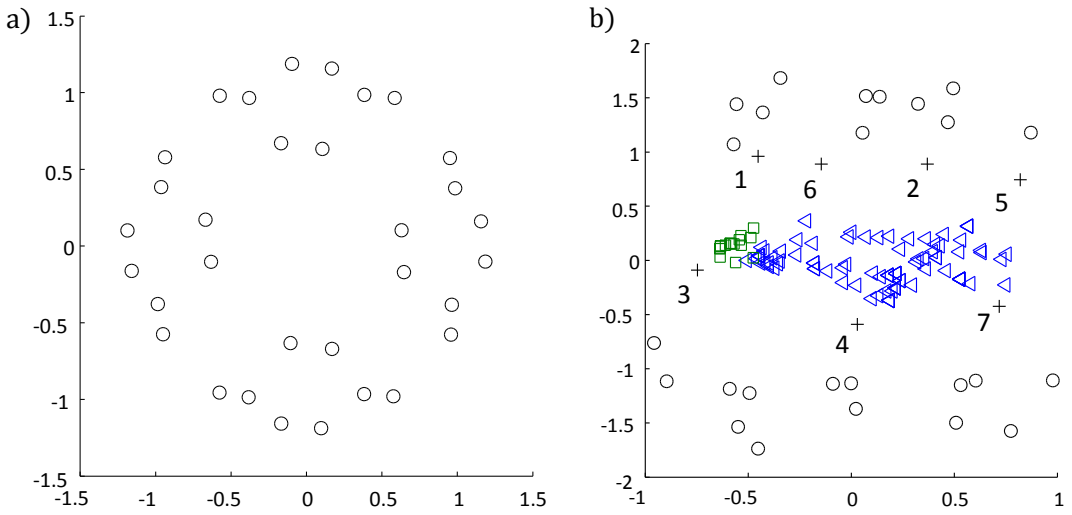

Figure 3.3: Two-dimensional images of the vertices of a five dimensional hypercube (**a**) and the vertices together with the points approximating the set of Pareto optimal decisions (**b**). Points marked by plus sign stand for closest to the Pareto front hypercube vertices.

Table 3.2: Hypercube vertices closest to Pareto front hypercube vertices.

Vertice	x_1	x_2	x_3	x_4	x_5
v^1	0	0	0	1	1
v^2	0	0	1	1	1
v^3	0	1	0	1	1
v^4	0	1	1	1	1
v^5	1	0	1	1	1
v^6	1	1	0	1	1
v^7	1	1	1	1	1

mentioning that a two-dimensional image is presented in the abstract coordinates, and the mutual distance between two-dimensional image points approximates the distance in a five dimensional space. In this way the structure of the set of two-dimensional points visualise the structure of the original set of multi-dimensional points. Images of points $x(w^i)$ are presented together with images of vertices in Fig. 3.3b. The seven hypercube vertices closest to the set of Pareto optimal decisions are depicted in Fig. 3.3b as numbers corresponding to vertice indexes $v^1, \ldots, v^7$. Their five coordinates are presented in Table 3.2.

The vertices closest to the set of Pareto optimal decisions have $x_4 = 1,\ x_5 = 1,$

meaning that values closest to the maximum of these decision variables should be chosen for the optimal biosensor design. If five dimensional Pareto optimal decisions $x(w)$ are projected onto a plane (x_4, x_5), the points are located near point $(1, 1)$ corresponding to the maximum values these decision variables.

The optimisation procedure as well as physical experiments showed that increasing concentrations of x_4 and x_5 (corresponding to $S_{1,0}$ and $S_{2,0}$) increases the biosensor sensitivity and thus increases the apparent Michaelis constant K_M^{app} [48].

3.2 Multi-objective Optimisation of Biosensor with Cyclic Substrate Conversion

Biosensor utilising cyclic substrate conversion was optimised. The following three objectives were optimised: the output current was maximised, the enzyme amount was minimised and sensitivity was maximised.

Biosensors with cyclic substrate conversion are of particular interest due to their high sensitivity made possible by utilising cyclic substrate conversion in a single enzyme membrane. Mathematical and corresponding numerical models for particular amperometric biosensors utilising cyclic substrate conversion are already known [37, 38]. In this work, a more complex biosensor involving a dialysis membrane was modelled and optimised. The modeled biosensor comprises of three compartments, an enzyme layer, a dialysis membrane and an outer diffusion layer.

3.2.1 Modeling of Biosensor with Cyclic Substrate Conversion

3.2.1.1 Model of the biosensor

In the case of a biosensor utilising cyclic substrate conversion, a measured substrate (S) is electrochemically converted into a product (P) which in an enzyme (E) reaction is then converted into a substrate (S) [37],

$$\mathrm{S} \longrightarrow \mathrm{P} \xrightarrow{\mathrm{E}} \mathrm{S}. \tag{3.6}$$

The modeled biosensor has four regions: the enzyme layer where the enzymatic reaction and the mass transport by diffusion takes place, a dialysis membrane and a diffusion limiting region where only mass transport by diffusion takes place, and a convective region where the analyte concentration remains constant. Where d_1, d_2 and d_3 is the thicknesses of an enzyme, dialysis and diffusion layers respectively.

Assuming symmetric geometry of the enzyme electrode, homogeneous distribution of enzyme in the enzyme membrane, and the uniform thickness of the dialysis membrane, the dynamics of the biosensor action can be described by a reaction-diffusion mathematical model [A5].

3.2.1.2 Computational simulation

The reaction-diffusion problem is a non-linear. Because of this, the problem was solved numerically by applying a finite difference technique. An explicit finite difference scheme was build as a result of the model discretisation [3].

Some model parameters are application-specific and cannot be changed or optimised by a biosensor designer [40]. Meanwhile, values of some other parameters, e.g., the concentration of the enzyme as well as geometry of biosensor, can be selected by the designer quite freely.

The chemical signal amplification is one the main features of amperometric biosensors that utilise a cyclic substrate conversion [37, 38]. The rate of the steady state current of enzyme active electrode ($V_{max} > 0$) to the steady state current of the corresponding enzyme inactive electrode ($V_{max} = 0$) is considered as the gain G of the biosensor sensitivity [37],

$$G(V_{max}) = \frac{I_\infty(V_{max})}{I_\infty(0)}. \tag{3.7}$$

3.2.2 Optimisation of Biosensor

Most enzymes are expensive products and some of them are produced in very limited quantity [40, 49]. In such cases the optimisation of the enzyme amount is important though a larger amount of enzymes in some cases increases the range of the calibration curve [3].

The response of biosensor is often perturbed by noise, e.g. white noise, sinusoidal power electrical noise etc [4]. Miniaturised biosensors with a small sensitive area has a low signal-to-noise ratio and this may result measurement problems [49]. To reduce the negative influence of signal noise on biosensor sensitivity, biosensor current I_M should be as high as possible.

The gain of biosensor sensitivity G shows an increase of the steady state current due to the catalized enzyme reaction. The high G indicates that biosensor with a particular configuration effectively uses enzymes to amplify the current.

The maximum enzymatic rate V_{max} is proportional to the enzyme amount ($V_{max} = kE$, k is reaction rate constant, E is enzyme concentration). This mean that, the maximum enzymatic rate can be changed by changing the enzyme concentration. The relative enzyme amount can be calculated as a product $V_{max}d_1$ of a maximum enzymatic rate and the thickness of enzyme layer.

The enzyme amount d_1V_{max}, the density I_M of a steady state current and the gain G of sensitivity were optimised for biosensor utilising cyclic substrate conversion.

3.2.2.1 Multi-objective Optimisation Problem

Design of the biosensor with the cyclic substrate conversion can be stated as a three-objective optimisation problem with an objective function $\Phi(x) = (\varphi_1(x), \varphi_2(x), \varphi_3(x))^T$, where $\varphi_1(x)$ is G, $\varphi_2(x)$ is I_M and $\varphi_3(x)$ is d_1V_{max}. Decision variables of the optimal design are given in Table 3.3. Range values of the decision parameters should be expertly evaluated. This depends on technological possibilities, e.g. the thicknesses of commercially available dialysis membranes or the thicknesses of nylon nets used for enzyme layer [26].

The stated multi-objective optimisation problem is similar to one solved in subsection 3.1.2.2. Therefore, the same method based on the Chebyshev scalarisation is applied [24]. By using scalarisation weight vectors: $w = (w_1, w_2, w_3)^T$, $0 \leq w_i \leq 1$, $\sum_{i=1}^{3} w_i = 1$ and optimising the scalarised function (3.4), the Pareto optimal solutions is found.

Table 3.3: Decision variables $x = (d_1, d_2, d_3, V_{max})^T$ for the cyclic biosensor design problem.

Variable	Description	Range
d_1	Enzyme layer thickness, cm	$[2 \times 10^{-4}, 5 \times 10^{-2}]$
d_2	Dialysis membrane thickness, cm	$[10^{-4}, 10^{-2}]$
d_3	Diffusion layer thickness, cm	$[10^{-4}, 10^{-1}]$
V_{max}	Maximal enzymatic rate, mol/(cm^{3}s)	$[0, 10^{-6}]$

3.2.2.2 Results of Optimisation

The selection of weights to get an uniform distribution of Pareto front is rather complicated task. Search of Pareto front solutions was performed by a two-step procedure. In the first step, to solve the task (3.4) uniformly distributed weights were used as shown in Fig. 3.4a. The determined Pareto optimal solutions are shown in Fig. 3.4b. In Fig. 3.4b a gap in the Pareto front near square points can be observed. The corresponding weight vectors are shown as squares in Fig. 3.4a. To eliminate the gap a more detailed representation of the Pareto front is needed nearby the square points. In the second step, additional weight vectors (black points) are added to find solutions nearby the square points in Fig. 3.5a. A supplemented representation of the Pareto front is presented in Fig. 3.5b. The gap is now filled with new solutions (black points). In figures, the Pareto front solutions were given in original dimensions $\Phi(x) = (\varphi_1(x), \varphi_2(x), \varphi_3(x))^T$ in order to evaluate solutions in further analysis.

The analysis of the Pareto front was performed to find an acceptable trade-off solution. A solution with the lowest enzyme amount $d_1 V_{max} = 8.4$ pmol/(cm^{2}s) corresponds to the lowest steady state current $I_M = 1.7$ μA/(cm^2) and the lowest gain of the sensitivity $G = 1.8$. A solution with the highest enzyme amount $d_1 V_{max} = 2.5$ nmol/(cm^{2}s) has the highest steady state current $I_M = 79.1$ μA/(cm^2) and the highest gain of sensitivity $G = 80.1$. So, the steady state current and the gain of the sensitivity are proportional to the amount of enzyme.

The steady state current and the sensitivity gain are not conflicting parameters, i.e. when one parameter increases, so does the other increases. An analysis of the

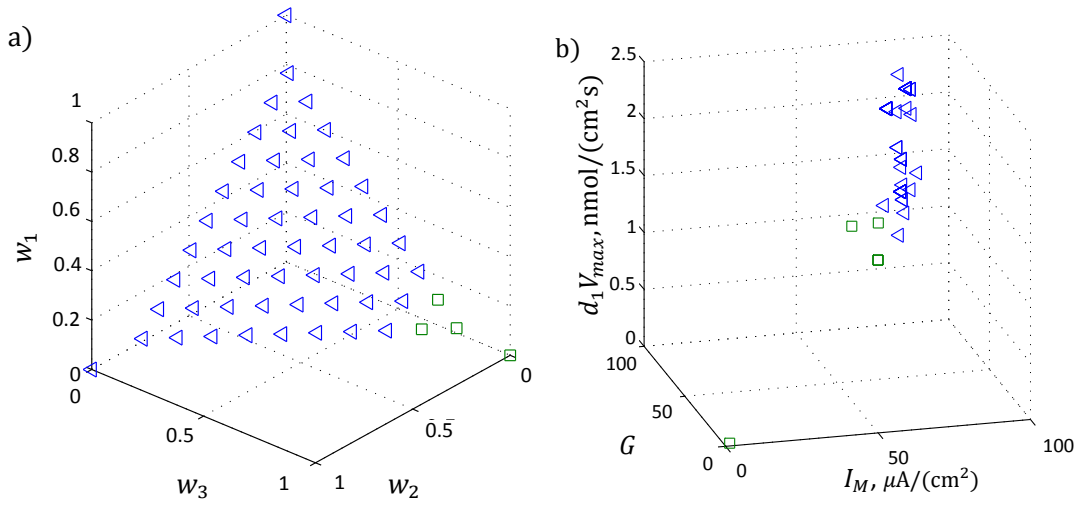

Figure 3.4: Weights (**a**) used in the first step of the optimisation procedure and the Pareto optimal solutions (**b**).

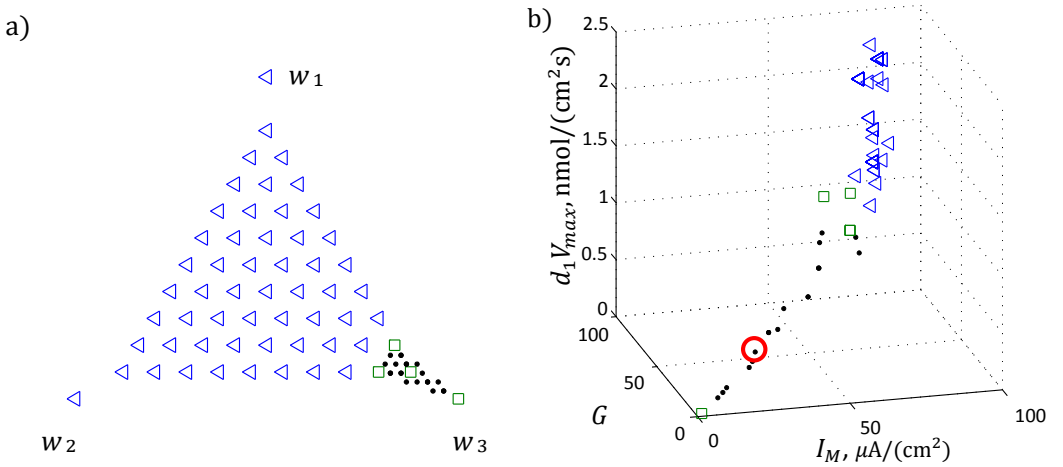

Figure 3.5: Additional weights indicated by black points in the triangle of weights (**a**) and the complementary representation of the Pareto front (**b**). Solution marked with red circle is one of most prommising trade-off solutions.

Pareto front revealed that a solution marked with a red circle (G, I_M, d_1V_{max}) = (25.3, 25.2 μA/(cm^2), 0.3 nmol/(cm^{2}s)) is most promissing trade-off solution (d_1, d_2, d_3, V_{max}) = (1.45×10^{-3} cm, 5.56×10^{-3} cm, 1.14×10^{-3} cm, 2.03×10^{-7} mol/(cm^{3}s)), as it uses a relatively small amount of enzyme and produces an acceptably high steady state current, as well as sensitivity gain.

The analysis of Pareto front decision variables revealed that a very thin en-

zyme layer is used in Pareto optimal solutions, i.e. the range of enzyme layer thickness is near the lowest limit of selected d_1 range (see Table 3.3): $d_1 \in (2.84 \times 10^{-4}\ \text{cm}, 2.52 \times 10^{-3}\ \text{cm})$. So, a thin enzyme layer should be used in the biosensor with the cyclic substrate conversion.

When comparing obtained most promissing trade-off solution (marked with red circle) with known configurations of the biosensor, particularly used for continuous flow-through measurements of phenol compounds in a alarm systems [37, 38, 51], one can see that optimised biosensor provides signal gain around tenfold stronger than others at approximately the same enzyme amount.

3.3 Conclusions and Results

The design of biosensor may use a multi-objective optimisation where optimal values of numerous parameters are desirable. The complex nature of biosensors involves consideration of the simultaneous optimisation of several often conflicting objectives.

The stated multi-objective optimisation problem is difficult to solve, since objectives are numerical solutions of the non-linear mathematical model. The Chebyshev scalarisation based method can be efficiently applied to find trade-off solutions (Pareto optimal decisions). Multi-dimensional scaling is a suitable method for visualisation of Pareto optimal decisions which are a subset of a feasible region in a multi-dimensional space.

The proposed method of biosensor design which integrates the multi-objective optimisation with visualsation helps exploring of the relation between the Pareto optimal decision and solution spaces that are aimed to look for an appropriate trade-off between conflicting objectives. An application of the proposed method to the optimisation of glucose biosensor that utilises the synergistic substrates conversion has showed that the advantage of the method is attained by combining advantages of mathematical methods to generate a set of admissible decisions with human heuristics to analyse the visual information. Multi-objective optimisation was also applied to phenol biosensors utilising the cyclic substrate conversion. Analysis of Pareto front give a solution having strong saturation current gain.

Conclusions

1. Using a mathematical model of biosensor (which includes an external (Nernst) diffusion layer and substrate interaction) and artificial neural networks it is possible to determine the concentration of several substrates. External diffusion layer improves accuracy of results. When large diffusion layer is used the relative error is about 0.2%, on the other hand, the experiment time increases, so a trade-off should be selected.

2. Using multiple steady state currents of biosensors (differing only in parameters) and artificial neural networks it is possible to determine the concentration of several substrates. The best accuracy was approached when was used a large diffusion module (more than 1) also the ratio of enzyme reaction speed of substrates and the ratio of enzyme concentration of biosensors are more than 4.

3. Applying multi-objective optimisation and multi-dimensional data visualisation in the phase of biosensor design allows to get the most suited Pareto optimal trade-off solutions. Hooke-Jeeves optimisation algorithm with Chebyshev scalarisation is effective enought for biosensor parameters optimisation with personal computer. The most suited Pareto optimal trade-off solutions can be sellected using multi-dimensional scaling (SMACOF algorithm) and projections of optimal solutions. Configurations of the glucose biosensor used in experiments are rather similar to optimal decisions, however the number of potentially good configurations can be reduced and configurations can be intentionally improved. Comparing the trade-off solution with the known phenol biosensor configurations show that the optimised biosensor produces a signal gain that is tenfold stronger compared to others at approximately the same enzyme amount.

Summary in Lithuanian (Santrauka)

Biojutikliai yra prietaisai, skirti aptikti ir matuoti medžiagų koncentracijas tirpaluose. Tai pakankamai pigūs ir patikimi prietaisai, plačiai taikomi aplinkosaugoje, maisto pramonėje ir medicinoje [1, 2]. Disertacijoje nagrinėjamas neuroninių tinklų pritaikymas kelių substratų koncentracijoms rasti iš biojutiklių atsako. Taip pat nagrinėjamas biojutiklio daugiakriterinis optimizavimas projektuojant.

Amperometrinis biojutiklis turi trūkumų: be papildomų priemonių gali matuoti tik vieno substrato koncentraciją, sunku pagaminti selektyvų fermentą, turi santykinai trumpą išmatuojamų koncentracijų intervalą, gamybai naudojami brangūs fermentai, matuojamas signalas jautrus triukšmams [3, 4]. Chemometriniai metodai taikomi siekiant rasti kelių substratų koncentraciją naudojant biojutiklio atsaką [5, 6, 7, 8, 9]. Optimizavimo metodai taikyti spręsti atvirkštiniam uždaviniui, t. y. iš turimo biojutiklio atsako rasti kelių medžiagų (substratų) koncentracijas [8, 9]. Kelių tirpalo substratų koncentracijoms rasti naudotas dirbtinis neuroninis tinklas [5, 6, 7].

Biojutiklio projektavimas yra biojutiklio kintamųjų parametrų reikšmių, kurios duotų tinkamas charakteristikas gamintojams ir vartotojams, radimas. Toks parametrų parinkimas – sudėtingas uždavinys net kompetetingiems specialistams. Gaminant rinkoje konkurencingą biojutiklį, jis turi pasižymėti ypatingomis savybėmis: turėti kiek įmanoma ilgesnį matavimo intervalą, naudoti mažai brangaus fermento, būti mažai jautriu triukšmams ir kt. Norint gauti tinkamų charakteristikų biojutiklį, plačiai naudojami matematiniai modeliai [3]. Kaip ir kitų prietaisų projektavimui, biojutiklio projektavimui gali būti panaudoti daugiakriterinio optimizavimo metodai bei matematiniu modeliu išreikšta optimizavimo funkcija [10].

Optimalios biocheminių sistemų kintamųjų parametrų reikšmės gautos pritaikius daugiakriterinį optimizavimą daugelyje darbų: biocheminėms sistemoms gerinti [11, 12], multifermentinės sistemos produktyvumui ir efektyvumui didinti [13], nuo slėgio kitimo apsaugančiai sistemai optimaliai projektuoti [14], biojutikliui, pasižyminčiam aukštu jautrumu ir mažomis fermento sąnaudomis, rasti [15].

Disertacijos tikslas ir uždaviniai

Šio darbo tikslas yra ištirti biojutiklio fizinių ir cheminių savybių įtaką substratų koncentracijų radimo tikslumui, kai joms rasti naudojami dirbtiniai neuroniniai tinklai, taip pat pritaikyti optimizavimo metodus biojutiklių parametrams parinkti.

Disertacijos tikslui pasiekti buvo sprendžiami uždaviniai:

1. Dirbtinio neuroninio tinklo taikymas kelių substratų koncentracijoms nustatyti:
    - Apibrėžti biojutiklio matematinį modelį, kuriame substratai sąveikauja su vienu fermentu bei nagrinėjamas Nernsto išorinis difuzijos sluoksnis. Aproksimuoti biojutiklio matematinį modelį skaitiniu ir jį realizuoti kompiuteriniu modeliu. Atlikti kompiuterinį modeliavimą pseudoeksperimentiniams duomenims gauti.
    - Pritaikyti dirbtinius neuroninius tinklus kelių substratų koncentracijoms rasti naudojant biojutiklio atsaką bei įsisotinimo sroves.
    - Ištirti koncentracijų radimo tikslumą randant geriausias biojutiklio kintamųjų parametrų reikšmes.
2. Biojutiklio optimizavimas:
    - Apibrėžti biojutiklio daugiakriterinio optimizavimo uždavinį: parametrus, kurie gali būti keičiami, ir charakteristikas (kriterijus), kurias racionalu optimizuoti.
    - Realizuoti praktinių biojutiklių modelius ir funkcijas, skaičiuojančias optimizuojamas charakteristikas.

- Atlikti optimizuojamų funkcijų charakteristikų analizę ir parinkti optimizavimo metodą.
- Atlikti skaitinį biojutiklio optimizavimą, optimizavimo rezultatų analizę ir pateikti rekomendacijas biojutiklių parametrams parinkti.

Tyrimo metodai ir priemonės

Darbe nagrinėti biojutikliai modeliuojami reakcijos-difuzijos diferencialinėmis lygtimis [17]. Modeliai aproksimuoti skirtuminėmis schemomis, naudojant baigtinių skirtumų metodą [3]. Programos, įgyvendinančios biojutiklio modelius, rašytos C programavimo kalba [18]. Biojutiklių analizei taikomi dirbtiniai neuroniniai tinklai [19]. Dirbtiniams neuroniniams tinklams naudotas Matlab Neural Network Toolbox paketas [20]. Duomenų dimensijai mažinti naudota pagrindinių komponenčių analizė [21]. Lygiagretūs skaičiavimai ir optimizavimo algoritmai realizuoti C OpenMPI paketu [22]. Naudotas Huko-Dživso optimizavimo algoritmas [23] ir Čebyševo skaliarizacija [24]. Daugiamačių skalių metodui naudota SMACOF realizacija [25].

Mokslinis rezultatų naujumas

1. Kelių substratų koncentracijai rasti iš biojutiklio atsako naudoti dirbtiniai neuroniniai tinklai ir biojutiklio modelis, atsižvelgiantis į substratų tarpusavio sąveiką ir į Nernsto išorinį difuzijos sluoksnį. Toks modelis įvertina difuzijos sluoksnio poveikį.

2. Taikant dirbtinius neuroninius tinklus, kelių substratų koncentracijos randamos vien tik iš stacionariųjų (įsotinimo) srovių, kurias generuoja keli to paties tipo, bet skirtingų parametrų biojutikliai.

3. Ištirta biojutiklio parametrų įtaka, koncentracijoms rasti naudojant dirbtinius neuroninius tinklus.

4. Suformuoluotas biojutiklio daugiakriterinio optimizavimo uždavinys.

5. Pasiūlytas biojutiklio projektavimo metodas, apimantis daugiakriterinį optimizavimą ir daugiamatę vizualizaciją, naudojamas tiriant sąryšius tarp Pareto optimalių sprendinių ir jų kintamųjų vektorių bei siekiant rasti tinkamą kompromisinį sprendinį.

Praktinė rezultatų reikšmė

Biojutikliai, gebantys matuoti kelias medžiagas, leistų efektyviau atlikti pirminę analizę ir aptikti teršalus skysčiuose. Kaip parodė tyrimas, neuroniniais tinklais analizuojant biojutiklio atsaką ar biojutiklių įsisotinimo sroves, vienu matavimu galima rasti kelių substratų koncentracijas. Tyrimams naudotas biojutiklio matematinis modelis, atsižvelgiantis į substratų tarpusavio sąveiką ir išorinį Nernsto difuzijos sluoksnį.

Sudaryta metodika, naudojanti daugiakriterinį optimizavimą ir duomenų analizę, kurią taikant galima rasti optimalius projektuojamo biojutiklio parametrus. Darbe atlikta gliukozės ir fenolio biojutiklių optimizacija ir pateiktos rekomendacijos.

Disertacijos rezultatai panaudoti įgyvendinant projektą „Kompiuterinių metodų, algoritmų ir įrankių efektyviam sudėtingos geometrijos biojutiklių modeliavimui ir optimizavimui sukūrimas“, finansuojamą Europos socialinio fondo lėšomis pagal visuotinės dotacijos priemonę VP1-3.1-ŠMM-07-K „Parama mokslininkų ir kitų tyrėjų mokslinei veiklai (visuotinė dotacija)“(2014–2015).

Ginami disertacijos teiginiai

1. Naudojant dirbtinį neuroninį tinklą ir biojutiklio matematinį modelį, atsižvelgiantį į substratų tarpusavio sąveiką bei išorinį Nernsto difuzijos sluoksnį, iš biojutiklio atsako galima pakankamai tiksliai rasti kelių substratų koncentracijas.

2. Taikant dirbtinius neuroninius tinklus, kelių substratų koncentracijas galima rasti vien tik iš stacionariųjų (įsotinimo) srovių, kurias generuoja keli to paties tipo, bet skirtingų parametrų biojutikliai.

3. Biojutikliui pritaikant optimizavimo metodus, gaunamos Pareto optimalių biojutiklių kriterijų reikšmės, iš kurių naudojant vizualizacijos ir duomenų analizės metodus (daugiamačių skalių metodas, Pareto fronto grafinis vaizdavimas), galima išrinkti geriausias biojutiklių kriterijų reikšmes.

Rezultatų patvirtinimas

Doktorantūros metu paskelbtas straipsnis, nagrinėjantis biojutiklio optimizavimą, žurnale indeksuojamame *Clarivate Analytics Web of Knowledge* duomenų bazėje [A1]. Taip pat paskelbtas straipsnis tarptautinės konferencijos darbų rinkinyje indeksuojamame SCOPUS sistemoje [A5]. Dirbtinio neuroninio tinklo taikymo rezultatai aprašyti ir paskelbti Lietuvoje leidžiamame periodiniame recenzuojamame žurnale [A4], taip pat konferencijos darbų rinkiniuose [A2, A3].

Rezultatai pristatyti keturiose tarptautinėse ir trijose Lietuvos konferencijose:

1. ECMS 2017 (Budapeštas, Vengrija): *31th European Conference on Modelling and Simulation*. 2017 m. gegužės 23–26 d.
2. FTMTT 2017 (Vilnius, Lietuva): *Fizinių ir technologijos mokslų tarpdalykiniai tyrimai 2017*. 2017 m. vasario 9 d.
3. MMA 2016 (Tartu, Estija): *Mathematical Modelling and Analysis 2016*. 2016 m. birželio 1–4 d.
4. OR 2016 (Vilnius, Lietuva): *Open Readings 2016*. 2016 m. kovo 15–18 d.
5. DAMSS 2015 (Druskininkai, Lietuva): *Duomenų analizės metodai programų sistemoms 2015*. 2015 m. gruodžio 3–5 d.
6. KODI 2015 (Panevėžys, Lietuva): *Kompiuterininkų dienos 2015*. 2015 m. rugsėjo 17–19 d.
7. IVUS 2015 (Kaunas, Lietuva): *Informacinės technologijos 2015*. 2015 m. balandžio 24 d.

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