APPLICATION OF TWO PHASE MULTI-OBJECTIVE OPTIMIZATION TO DESIGN OF BIOSENSORS UTILIZING CYCLIC SUBSTRATE CONVERSION

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ABSTRACT
A method for the optimal design of amperometric biosensors with cyclic substrate conversion is proposed. The design is multi-objective since biosensors must meet numerous, frequently conflicting, requirements of users and manufacturers. Moreover, they should be technologically and economically competitive. To apply a multi-optimization technique, a mathematical model should be developed where the most important characteristics of the biosensor are defined as objectives, and the other characteristics and requirements are defined as constraints. For the considered biosensors the following characteristics are taken as objectives: the output current, the enzyme amount, and the biosensor sensitivity. The proposed method consists of two phases. At the first phase an approximated Pareto front is constructed, and a preliminary solution is selected. The second phase is aimed at specification of the Pareto front around the preliminary solution, and at making the final decision. A numerical example is presented using a computational model of an industrially relevant biosensor.

INTRODUCTION
A biosensor is a device capable to measure the concentration of an analyte (Scheller and Schubert 1992; Turner et al. 1987). A catalytic biosensor is based on the enzymatic reaction where the analyte is turned to a measurable product (Banica 2012). Amperometric biosensors measure the changes in the output current on the working electrode due to the direct oxidation or reduction of the products of biochemical reactions (Grieshaber et al. 2008). In this work a biosensor utilizing the cyclic substrate conversion was considered. Biosensors with cyclic substrate conversion are of particular interest due to high sensitivity possible by utilizing a cyclic substrate conversion in a single enzyme membrane (Kulys and Vidziunaite 2003). The efforts to design biocatalytical systems can be mightily reduced by the aid of computer tools (Dagan and Bercovici 2014). The multi-objective optimization has been successfully applied to design biochemical systems (Vera et al. 2010; Taras and Woinaroschy 2012), to increase the productivity of multi-enzyme systems (Ardao and Zeng 2013) and for optimal design of synergistic amperometric biosensors (Baronas et al. 2016).

The multi-objective optimization is often used as a tool to get a representative set of trade-off solutions for a product to be designed (Žilinskas et al. 2006). The trade-off solutions, also known as Pareto optimal solutions, together with trade-off curves as their visualization are widely used for learning and making decisions when designing products (Maksimovic et al. 2012). Amperometric biosensors can be rather efficiently designed and optimized by combining the multi-objective optimization and multi-dimensional visualization with computational modelling (Baronas et al. 2016).

One and two compartment mathematical and corresponding numerical models for particular amperometric biosensors utilizing cyclic substrate conversion are already known (Sorochinskii and Kurganov 1996; Baronas et al. 2004a,b). In this work, a more complex biosensor involving a dialysis membrane is modelled and optimized. The modelling biosensor comprises three compartments, an enzyme layer, a dialysis membrane and an outer diffusion layer. The model is based on non-stationary reaction-diffusion equations containing a non-linear term related to Michaelis-Menten kinetic of the enzymatic reaction (Baronas et al. 2010). The numerical simulation of the biosensor action was carried out using the finite difference technique (Britz and Strutwolf 2016).

The objective functions in such applications are often non-linear and therefore the multi-objective optimization consumes much computational time, especially when the objectives are considered as expensive black-box functions, whose values can be obtained by solving nonlinear partial differential equations numerically. Because of this a two phase multi-objective optimization was applied. Firstly, a particular trade-off solution is obtained and a Pareto front is analyzed. Then the preliminary solution is adjusted in a specific part of the Pareto front, the decision variables are calculated, and finally pilot plant tests can be performed. For the amperometric biosensors with cyclic substrate conversion the output current, the enzyme amount and the gain of the current were consid-
erade in the optimization.

MATHEMATICAL MODELLING

Modelling biosensor

An amperometric biosensor may be considered as enzyme membrane attached to an electrode. In the case of the biosensor utilizing cyclic substrate conversion, a measured substrate (S) is electrochemically converted to a product (P) in which an enzyme (E) reaction in turn is converted to the substrate (S) (Baronas et al. 2004b),

\[ S \rightarrow P \stackrel{E}{\rightarrow} S. \] (1)

The modelling biosensor involves four regions: the enzyme layer where the enzymatic reaction as well as the mass transport by diffusion take place, a dialysis membrane and a diffusion limiting region where only the mass transport by diffusion takes place, and a convective region where the analyte concentration remains constant. The schematic view of the biosensor is presented in Fig. 1, where \( d_1, d_2 \) and \( d_3 \) are the thicknesses of the enzyme, dialysis and diffusion layers, respectively, \( x = a_0 = 0 \) corresponds to the electrode surface, and \( a_1, a_2, a_3 \) denote boundaries between the adjacent regions.

\[ \begin{array}{c}
\text{Solution} \\
\text{Diffusion layer} \\
\text{Dialysis membrane} \\
\text{Enzyme layer} \\
\text{Electrode}
\end{array} \]

\[ \begin{array}{c}
a_3 \\
a_2 \\
a_1 \\
a_0 = 0
\end{array} \]

Figure 1: The Schematic View of the Biosensor.

Mathematical model of biosensor

Assuming symmetric geometry of the enzyme electrode, homogeneous distribution of the enzyme in the enzyme membrane and the uniform thickness of the dialysis membrane, the dynamics of the biosensor action can be described by the following reaction-diffusion system \((t > 0)\):

\[ \begin{align*}
\frac{\partial S_i}{\partial t} &= D_{S_i} \frac{\partial^2 S_i}{\partial x^2} + \frac{V_{\text{max}} P_i}{K_M + P_i}, \quad i = 1, 2, 3, \\
\frac{\partial P_i}{\partial t} &= D_{P_i} \frac{\partial^2 P_i}{\partial x^2} - \frac{V_{\text{max}} P_i}{K_M + P_i}, \quad x \in (0, a_1), \\
\frac{\partial S_i}{\partial t} &= D_{S_i} \frac{\partial^2 S_i}{\partial x^2}, \quad x \in (a_i-1, a_i), i = 2, 3, \\
\frac{\partial P_i}{\partial t} &= D_{P_i} \frac{\partial^2 P_i}{\partial x^2}, \quad x \in (a_i-1, a_i), i = 1, 2.
\end{align*} \] (2a-b-d)

where \( x \) and \( t \) stand for space and time, respectively, \( S_i(x, t), P_i(x, t), i = 1, ..., 3 \) are the substrate (S) and reaction product (P) concentrations in the enzyme layer \((i = 1)\), dialysis membrane \((i = 2)\) and diffusion layer \((i = 3)\), \( D_{S_i}, D_{P_i}, i = 1, ..., 3 \) are the diffusion coefficients, \( V_{\text{max}} \) is the maximal enzymatic rate, and \( K_M \) is the Michaelis constant (Baronas et al. 2004a,b).

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

\[ \begin{align*}
P_i(x, 0) &= 0, x \in [a_{i-1}, a_i], i = 1, ..., 3, \quad (3a) \\
S_i(x, 0) &= 0, x \in [a_{i-1}, a_i], i = 1, 2, \quad (3b) \\
S_3(x, 0) &= 0, x \in [a_2, a_3], S_3(a_3, 0) = S_0, \quad (3c)
\end{align*} \]

where \( S_0 \) is the concentration of the substrate to be analyzed.

The substrate is assumed to be an electro-active substance. Due to the electrode polarization the concentration of the substrate at the electrode surface is permanently reduced to zero. The substrate at the electrode surface is electrochemically converted to the product. The product is generated at the same rate as the substrate is reduced. On the boundary between the adjacent regions having different diffusivities the matching conditions are defined \((t > 0)\):

\[ \begin{align*}
S_i(0, t) &= 0, S_i(a_3, t) = S_0, P_3(a_3, t) = 0, \quad (4a) \\
D_{P_i} \frac{\partial P_i}{\partial x} \bigg|_{x=0} &= -D_{S_i} \frac{\partial S_i}{\partial x} \bigg|_{x=0}, \quad (4b) \\
D_{S_i} \frac{\partial S_i}{\partial x} \bigg|_{x=a_{i+1}} &= D_{S_{i+1}} \frac{\partial S_{i+1}}{\partial x} \bigg|_{x=a_i}, \quad (4c) \\
S_i(a_i, t) &= S_{i+1}(a_i, t), \quad (4d) \\
D_{P_i} \frac{\partial P_i}{\partial x} \bigg|_{x=a_i} &= D_{P_{i+1}} \frac{\partial P_{i+1}}{\partial x} \bigg|_{x=a_i}, \quad (4e) \\
P_i(a_i, t) &= P_{i+1}(a_i, t), \quad i = 1, 2. \quad (4f)
\end{align*} \]

For simplicity, the functions \( S_i \) and \( P_i \) applicable to particular intervals \([a_{i-1}, a_i] (i = 1, ..., 3)\) are replaced with \( S(x, t) \) and \( P(x, t) \) applicable to whole domain, \( x \in [0, a_3], t \geq 0 \). Both concentration functions are continuous in the entire domain.

During a physical experiment the anodic or cathodic current is measured as the biosensor response. The current depends on the flux of electro-active substrate (S) at electrode surface \((x = 0)\). The current density \( I(t) \) at time \( t \) can be explicitly calculated from Faraday’s and Fick’s laws,

\[ I(t) = n_e F D_{S_1} \frac{\partial S}{\partial x} \bigg|_{x=0} = -n_e F D_{P_1} \frac{\partial P}{\partial x} \bigg|_{x=0}, \quad (5) \]

where \( n_e \) is the number electrons involved in charge transfer, \( F \) is Faraday’s constant, \( F \approx 9.65 \times 10^4 \) C/mol.

During the biosensor operation the system \((2)-(4)\) as well as the biosensor current \( I(t) \) approaches the steady state,

\[ I_\infty = \lim_{t \to \infty} I(t). \quad (6) \]
Computational simulation

The initial boundary value problem (2)-(4) is a nonlinear. Because of this the problem was solved numerically by applying the finite difference technique (Baronas et al. 2010). An explicit finite difference scheme was build as a result of the model discretization (Baronas et al. 2004a,b).

Some parameters of the model (2)-(4) are application-specific and cannot be changed or optimized by a biosensor designer (Banica 2012; Grieshaber et al. 2008). Meanwhile, values of some other parameters, e.g., the concentration of the enzyme as well as the biosensor geometry, can be selected by the designer quite freely. The following values specific to phenol sensitive biosensors and commercially available dialysis membranes were assumed to be constant (Kulys and Vidziumaite 2003; Banica 2012):

\[ D_{S_0} = D_{P_1} = 3 \times 10^{-6} \text{cm}^2/\text{s}, \quad (7a) \]
\[ D_{S_2} = D_{S_1}/10, \quad D_{P_2} = D_{P_1}/10, \quad (7b) \]
\[ D_{S_3} = 2D_{S_1}, \quad D_{P_3} = 2D_{P_1}, \quad (7c) \]
\[ K_M = 10^{-7}\text{mol/cm}^3, \quad n_e = 2. \quad (7d) \]

The chemical signal amplification is one the main features of amperometric biosensors utilizing cyclic substrate conversion (Kulys and Vidziumaite 2003). The rate of the steady state current of enzyme active electrode \( V_{\text{max}} > 0 \) to the steady state current of the corresponding enzyme inactive electrode \( V_{\text{max}} = 0 \) is considered as the gain \( G \) of the biosensor sensitivity (Baronas et al. 2004b),

\[ G(V_{\text{max}}) = \frac{I_\infty(V_{\text{max}})}{I_\infty(0)}. \quad (8) \]

Due to the substrate cyclic conversion, the gain \( G \) of the sensitivity significantly depends on the geometry as well as catalytic activity of the biosensor and can be increased in some tens of times (Baronas et al. 2004a,b).

OPTIMAL DESIGN OF THE BIOSENSOR

The design of a biosensor can be mathematically reduced to a multi-objective optimization problem (Baronas et al. 2016). The complexity of biosensors involves consideration and simultaneous optimization of several often conflicting objectives, which means that if one of them is improved, the others get worse (Sadana and Sadana 2011). The solutions of multi-objective optimization is called Pareto optimal front (Deb 2009).

The goal of the optimal design is to find Pareto optimal solutions and by use of expert evaluation to find a particular trade-off solution which would satisfy needs of user and manufacturer (Žilinskas et al. 2006; Maksimovic et al. 2012; Žilinskas 2013; Žilinskas et al. 2015).

Most enzymes are expensive products and some of them are produced in very limited quantity (Sadana and Sadana 2011; Banica 2012). In such cases the optimization of the enzyme amount is important though the greater amount of the enzyme in some cases increases the range of calibration curve (Baronas et al. 2010).

The biosensor response is often perturbed by noise, e.g. white noise, sinusoidal power electrical noise, or if the biosensor response is biased, e.g. by temperature change (Hassibi et al. 2007). Miniaturized biosensors with small sensing area has low signal-to-noise ratio and it may result problems in measurement (Sadana and Sadana 2011). To reduce the negative influence of the signal noise to the biosensor sensitivity the biosensor current should be as high as possible.

The current of the biosensor utilizing cyclic substrate conversion monotonously increases with increasing the substrate concentration (Baronas et al. 2004b). Because of this, the biosensor current \( I_M \) calculated at a moderate concentration \( S_0 = K_M \) of the substrate was assumed as the characteristics of the magnitude of the current of a particular biosensor,

\[ I_M = I_\infty(K_M), \quad (9) \]

where \( I_\infty(K_M) \) is density of the steady state current calculated assuming \( S_0 = K_M \).

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

The maximal enzymatic rate \( V_{\text{max}} \) is proportional to enzyme amount \( V_{\text{max}} = kE \), \( k \) - reaction rate constant, \( E \) - enzyme concentration and is attainable with that amount of enzyme, when the enzyme is fully saturated with the substrate. So, the maximal enzymatic rate can be changed by changing the enzyme concentration. The relative enzyme amount can be calculated as the product \( V_{\text{max}}d_1 \) of the maximal enzymatic rate and the thickness of enzyme layer.

The enzyme amount \( d_1V_{\text{max}} \), the density \( I_M \) of the steady state current and the gain \( G \) of the sensitivity were optimized for the optimization of the biosensor utilizing cyclic substrate conversion.

Multi-objective optimization problem

Design of the biosensor with the cyclic substrate conversion can be stated as a three-objective optimization problem with the 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 \( d_1V_{\text{max}} \). Decision variables of the optimal design are given in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>( d_1 )</td>
<td>Enzyme layer thickness, cm</td>
<td>( [2 \times 10^{-6}, 5 \times 10^{-6}] )</td>
</tr>
<tr>
<td>( d_2 )</td>
<td>Dialysis membrane thickness, cm</td>
<td>( [10^{-4}, 10^{-2}] )</td>
</tr>
<tr>
<td>( d_3 )</td>
<td>Diffusion layer thickness, cm</td>
<td>( [10^{-4}, 10^{-2}] )</td>
</tr>
<tr>
<td>( V_{\text{max}} )</td>
<td>Maximal enzymatic rate, mol/(cm²s)</td>
<td>( [0, 10^{-4}] )</td>
</tr>
</tbody>
</table>
Range values of the decision parameters should be expertly evaluated. It depends on technological possibilities, e.g. the thicknesses of the commercially available dialysis membranes or the thicknesses of nylon nets used for the enzyme layer (Scheller and Schubert 1992).

The defined multi-objective minimization problem is difficult since the objective function $\Phi$ was defined by expensive black-box functions calculated from the numerical solution of the system (2)-(4) of non-linear partial differential equations. This feature of the objective function is a crucial factor for selecting an appropriate algorithm to find a Pareto front representation. The classical methods (Miettinen 1999) are efficient for smooth convex problems and not suitable here because of the of non-smoothness of the objective functions implied by the numerical errors of the solution of equations (2)-(4).

The application of metaheuristic methods is limited due to expensive of the function to be optimized, i.e. calculation of the objective function $\Phi(x)$ takes about 5 minutes using Intel Core i7-4770 3.5 GHz based personal computer. For optimization problems with given characteristics the most suitable is statistical model based algorithm (Zilinskas 2014), however software currently available only for bi-objective problems. Among other alternatives Chebyshev scalarization based methods seem most promising (Miettinen 1999).

First two objectives functions $(\varphi_1(x), \varphi_2(x))$ are maximized while the last one $(\varphi_3(x))$ is minimized. The optimization task to minimize objectives normalised to unit interval $[0, 1]$ can be formulated as follows:

$$F(x) = (f_1(x), f_2(x), f_3(x))^T,$$

$$f_1(x) = \frac{\varphi_2^+ - \varphi_2(x)}{\varphi_2^+ - \varphi_1}, \quad i = 1, 2,$$

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

$$\varphi_1^+ = \max_{x \in A} \varphi_1(x), \quad i = 1, 2, 3,$$

$$\varphi_2^+ = \max_{x \in A} \varphi_2(x), \quad i = 1, 2, 3,$$

$$\varphi_3^+ = \max_{x \in A} \varphi_3(x),$$

$$x = (x_1, ..., x_4)^T,$$

$$A = \{x : 0 \leq x_j \leq 1, \quad j = 1, ..., 4\},$$

where $x_1, ..., x_4$ are the decision variables $d_1, d_2, d_3$ and $V_{\text{max}}$ re-scaled to the unit interval.

The ranges for the objective functions $(\varphi_1^-, \varphi_2^+, \varphi_3^-)$ were found by using the single criteria optimization of the corresponding function $\varphi_i(x), \quad i = 1, 2, 3$. The multi-start of Hooke-Jeeves algorithm was used for this (Kelly 1999).

The multi-criteria optimization besides finding Pareto front approximation $F_P$ also finds optimal decision variables,

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

**Results of optimization**

The Chebyshev scalarization was used to the transform multi-objective problem (10a) to a single objective problem,

$$f(x) = \max_{1 \leq i \leq 3} w_i f_i(x), \quad x(w) = \arg \min_{x \in A} f(x),$$

$$w = (w_1, w_2, w_3)^T, \quad 0 \leq w_i \leq 1, \quad \sum_{i=1}^{3} w_i = 1,$$

where the minimizer $x(w)$ is the Pareto optimal solution of the original problem (10a).

All the Pareto optimal solutions can be found by solving (12a) with an appropriate weight vector $w$. To find an approximation of the Pareto front $F_P$ and the decision vectors $X_P$ the solution of (10a) should be found with a set of different weight vectors $w$. The optimization of the scalarized function $f(x)$ was performed by using the multi-start Hooke Jeeves algorithm (Kelly 1999), since it was successfully applied to similar problems (Zilinskas et al. 2006; Baronas et al. 2016). Some solutions of (10a) are weak Pareto optimal but they may be easily filtered.

The selection of weights to get an uniform distribution of $F_P$ is rather complicated task. Search of Pareto front solutions was performed by a two step procedure. In the first step, the uniformly distributed weights were used as shown in Fig. 2a to solve the task (12a). Found Pareto optimal solutions are shown in Fig. 2b. One can see in Fig. 2b a gap in the Pareto front near square points. The corresponding weight vectors are shown as squares in Fig. 2a. To abolish the gap a more detailed representation of the Pareto front is needed in the neighborhood of square points. In the second step, additional weight vectors (black points) are added to find solutions in neighborhood of the square points in Fig. 3a. The supplemented representation of the Pareto front is presented in Fig. 3b. The gap is now completed by new solutions (black points). In figures the Pareto front solutions were given in the original dimensions $\Phi(x) = (\varphi_1(x), \varphi_2(x), \varphi_3(x))^T$ to be expertly evaluate solutions in further analysis.

The described implementation of the two phase optimization procedure is still appropriate to run on a personal computer, even the multi-objective optimization was done with expensive black-box function. The computation of whole Pareto front took about a week (154 hours). The algorithm was parallelized by a master-slave approach using the Open MPI protocol (Gabriel et al. 2004). Eight parallel threads were used since the personal computer is based on Intel Core i7-4770 3.5 GHz processor. Each thread optimized function (12a) with different weight vectors $w$.

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

The steady state current and the sensitivity gain are not conflicting parameters, i.e. while one parameter increases also the other increases. An expert analysis of the Pareto front revealed that the solution marked with red circle \((G, I_M, d_1 V_{max}) = (25.3, 25.2 \mu\text{A/(cm}^2\text{)}, 0.3 \text{ nmol/(cm}^2\text{s})\) is best trade-off solution for the practical use \((d_1, d_2, d_3, V_{max}) = (1.45 \times 10^{-3} \text{ cm}, 5.56 \times 10^{-3} \text{ cm}, 1.14 \times 10^{-3} \text{ cm}, 2.03 \times 10^{-7} \text{ mol/(cm}^3\text{s})\). It uses a relatively small amount of enzyme and gives an acceptably high the steady state current as well as the sensitivity gain.

The analysis of Pareto front decision variables \(X_P\) revealed that a very thin enzyme layer is used in Pareto optimal solutions, i.e. the range of enzyme layer thickness is near the low boundary of selected \(d_1\) range (see Table 1); \(d_1 \in (2.84 \times 10^{-4} \text{ cm}, 2.52 \times 10^{-4} \text{ cm})\). So, a thin enzyme layer should be used in the biosensor with the cyclic substrate conversion.

When comparing obtained the best trade-off solution with known configurations of the modelling biosensor particularly used for continuous flow-through measurements of phenol compounds in a alarm systems (Kulys and Vidziunaite 2003; Baronas et al. 2004a,b), one can see that the optimized biosensor provides about ten times greater signal gain \(G = 25.3\) than the others at approximately the same enzyme amount \((d_1 V_{max} = 0.3 \text{ nmol/(cm}^2\text{s})\).

**CONCLUSIONS**

Optimal design of a biosensor is reducible to a problem of black-box multi-objective optimization. The objectives are computationally intensive since they are defined numerically via solution of a system of non linear partial differential equations. The Chebyshev scalarization based two phases method is appropriate to construct the approximation of the Pareto front the visualization of which greatly aids the design of the biosensor in question. The performed testing has shown that the computing resources of a personal computer are sufficient to de-
sign an industrially relevant biosensor by means of the proposed method.

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