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Optimization of Bi-Layer Biosensors: Trade-off Between Sensitivity and Enzyme Volume

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Abstract. The research is focused on investigation of impact of parameters of bi-layer mono-enzyme amperometric biosensor to its characteristics: the sensitivity and the enzyme volume. The bi-objective optimization problem of determination of the optimal thicknesses of the layers and maximal enzymatic rate of the biosensor with respect to maximization of the sensitivity and simultaneously minimization of the enzyme volume is formulated. The influence of the parameters of the biosensor to the objectives of the problem has been experimentally investigated. The simulated biosensor responses and the set of different parameters' values systematically chosen with respect to the real-life experiments have been used in the investigation.

Keywords: multi-objective optimization, modeling, simulation, biosensor

1 Introduction

Biosensors are analytical devices in which specific recognition of the chemical substances is performed by biological material, usually an enzyme, which serves as recognition element and is used in combination with a transducer (Scheller and Schubert, 1992, Turner et al., 1990). The transducer transforms concentration of substrate or product to measurable signal that is amplified and further processed. Amperometric biosensors measure changes in the cathodic or anodic current on the working electrode. The amperometric biosensors are reliable devices for clinical diagnostics, food analysis and environment monitoring (Cooper and Cass, 2004, Banica, 2012).

The action of catalytic biosensors is associated with the substrate diffusion from a bulk solution into a biocatalytic membrane and an enzyme-catalyzed substrate

conversion to a product (Bartlett and Whitaker, 1987, Schulmeister, 1990). The action can be mathematically modeled by partial differential equations of the reaction-diffusion type (Schulmeister, 1990, Baronas et al., 2010). The mathematical models of biosensors response are widely used to optimize their configurations. Usually, an optimization in biosensor engineering is concentrated on a unique objective (Shoorideh and Chui, 2012). The complex nature of practical biosensors involves consideration of the simultaneous optimization of several objectives (multi-objective optimization). These objectives are often conflicting, which means that if it is desired to improve one of them, it must allow others to get worse (Sadana and Sadana, 2010).

Multi-objective optimization of chemical and biochemical processes and systems has been shown beneficial in many applications. Sendín et al. (2006, 2009) investigated solution of multi-objective optimization problems arising from the domain of biochemical systems, namely metabolic pathways, with the aim to maximize the rate of production of ethanol and simultaneously minimize several internal metabolite concentrations. Vera et al. (2010) presented an optimization framework for the technological improvement of biochemical systems. Taras and Woinaroschy (2012) proposed an interactive multi-objective optimization framework for sustainable design of bioprocesses. Ardao and Zeng (2013) applied a multi-objective genetic algorithm to maximize productivity and yield of a multi-enzymatic system. The importance of multi-objective optimization in chemical and biochemical engineering permanently increases due to the development of new and improved methods sustained by increased computational resources (Rangaiah, 2009).

This paper focuses on the determination of the parameters of the bi-layer mono-enzyme biosensor utilizing the Michaelis-Menten kinetics with respect to the maximization of the biosensor sensitivity and simultaneously minimization of the enzyme amount. The amperometric biosensor is treated as a flat electrode covered with a relatively thin layer of an enzyme (biocatalytic membrane) applied onto the electrode surface by using a dialysis membrane.

The reminder of the paper is organized as follows: Section 2 describes the mathematical model of the relevant biosensor and its characteristics; Section 3 is devoted for the formulation of the multi-objective mathematical optimization problem and results of the computational experiments are presented and discussed in Section 4. Finally, conclusions of the investigation are formulated in Section 5.

2 Mathematical Model

We consider a mono-enzyme biosensor utilizing the Michaelis-Menten kinetics (Scheller and Schubert, 1992, Turner et al., 1990),

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_3} \mathrm{E} + \mathrm{P} \tag{1}$$

where E denotes the enzyme, S – the substrate to be determined, ES – the enzyme-substrate complex, P – the reaction product, and k_{+1}, k_{-1}, k_3 are the kinetic constants.

The model of the amperometric biosensor involves three regions: the enzyme layer, where the biochemical reaction (1) as well as the mass transport by diffusion take place,

the dialysis membrane (diffusion layer), where only the mass transport by diffusion of the substrate as well as product takes place, and a convective region, where the concentrations of the substrate and product remain constant. Fig. 1 shows the principal structure of the biosensor, where $x = 0$ represents the electrode surface, $x = d$ corresponds to the boundary between the enzyme layer and the dialysis membrane, and δ is the thickness of the dialysis membrane.

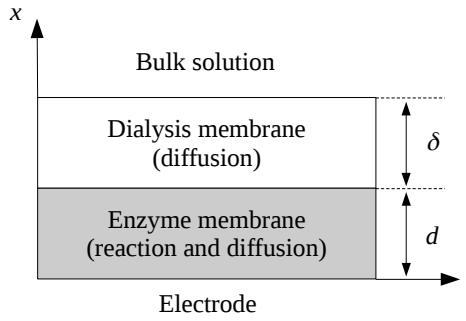

Fig. 1. Principal structure of the biosensor to be optimized.

Usually, the steady-state current is used as a response of commercial amperometric biosensors. Further, for the quantitative analysis, the measurement of the steady-state response is one of the easiest electrochemical methods (Scheller and Schubert, 1992, Banica, 2012). A steady-state electrochemical signal is reached when the rate of the reaction product formation equals the rate at which the product diffuses out of enzyme membrane.

Assuming a symmetrical geometry of the electrode and a homogeneous distribution of the immobilized enzyme in the enzyme layer of a uniform thickness, the mathematical model of the biosensor response can be defined in a one-dimensional-in-space domain (Schulmeister, 1990, Baronas et al., 2010).

2.1 Governing equations

The mass transport and the kinetics of the enzyme-catalyzed reaction (1) in the enzyme layer under steady-state conditions can be described by the following system of stationary reaction-diffusion equations:

$$\begin{aligned} D_{S,e}\frac{\mathrm{d}^2S}{\mathrm{d}x^2} &= \frac{VS}{K_M+S}, \\ D_{P,e}\frac{\mathrm{d}^2P}{\mathrm{d}x^2} &= -\frac{VS}{K_M+S}, \quad 0<x<d, \end{aligned} \tag{2}$$

where x stands for space; $S(x)$ and $P(x)$ are the molar concentrations of the substrate S and the product P in the enzyme layer, respectively; V is the maximal enzymatic

rate; K_M is the Michaelis constant; d is the thickness of enzyme layer; $D_{S,e}$ and $D_{P,e}$ are the diffusion coefficients; $V = k_3 E_0$, $K_M = (k_{-1} + k_3)/k_{+1}$; and E_0 is the total concentration of the enzyme.

In the dialysis membrane, only the mass transport by diffusion of the substrate and product takes place during the biosensor operation. Away from the biosensor the solution is assumed to be in motion and uniform in the concentration,

$$S(d+\delta) = S_0, \quad P(d+\delta) = P_0, \tag{3}$$

where S_0 and P_0 are the concentrations of the substrate and product in the buffer solution. Usually, the zero concentration of the reaction product in the bulk is assumed, $P_0 = 0$, while the concentration S_0 is to be quantitatively determined from the biosensor response.

In the case of the amperometric biosensors, due to the electrode polarization the concentration of the reaction product at the electrode surface ($x = 0$) is being permanently reduced to zero. The substrate does not react at the electrode surface, and therefore the non-leakage (zero flux) boundary condition is applied to the substrate,

$$D_{S,e} \frac{\mathrm{d}S}{\mathrm{d}x}\bigg|_{x=0} = 0, \quad P(0) = 0. \tag{4}$$

At the steady-state, fluxes of the substrate and product through the boundary of the dialysis membrane/bulk solution ($x = d + \delta$) are equal to the corresponding fluxes through the boundary of the biocatalytic/dialysis membranes ($x = d$) (Schulmeister, 1990, Baronas et al., 2010),

$$\begin{aligned} D_{S,m} \frac{S_0 - S(d)}{\delta} &= D_{S,e} \frac{\mathrm{d}S}{\mathrm{d}x}\bigg|_{x=d}, \\ D_{P,m} \frac{P_0 - P(d)}{\delta} &= D_{P,e} \frac{\mathrm{d}P}{\mathrm{d}x}\bigg|_{x=d}, \end{aligned} \tag{5}$$

where δ is the thickness of the external diffusion layer; $D_{S,m}$ and $D_{P,m}$ are the diffusion coefficients of the species in the dialysis membrane.

2.2 Biosensor characteristics

The measured current is usually assumed as the response of an amperometric biosensor. The density I of the biosensor current is directly proportional to the flux of the reaction product at the electrode surface and can be expressed explicitly from the Faraday and the Fick laws (Gutfreund, 1995),

$$I = n_e F D_{P,e} \frac{\mathrm{d}P}{\mathrm{d}x}\bigg|_{x=0}, \tag{6}$$

where n_e is the number of electrons involved in a charge transfer at the electrode surface, and $F = 96,486\ \mathrm{C/mol}$ is the Faraday constant.

The sensitivity is one of the most important characteristics of the biosensors (Banica, 2012). The biosensor sensitivity can be expressed as the gradient of the

steady-state current with respect to the substrate concentration. Since the biosensor current as well as the substrate concentration varies even in orders of magnitude, another useful parameter to consider is a dimensionless sensitivity. The dimensionless sensitivity B_S for the substrate concentration S_0 is given by (Baronas et al., 2010)

$$B_S(S_0) = \frac{\mathrm{d}I(S_0)}{\mathrm{d}S_0} \times \frac{S_0}{I(S_0)} \approx \frac{I(S_0 + \Delta S_0) - I(S_0)}{\Delta S_0} \times \frac{S_0}{I(S_0)}, \tag{7}$$

where $I(S_0)$ is the steady-state current obtained at the concentration S_0 of the substrate.

Despite the concentration S_0 the steady-state current $I(S_0)$ also depends on the maximal enzymatic rate V and thicknesses d and δ of the enzyme and diffusion layers, respectively. Aiming to increase the biosensor sensitivity, the biosensor parameters can be optimized at a certain concentration of the substrate. Since the Michaelis K_M constant is the concentration of the substrate at which half of the maximum velocity of an enzyme-catalyzed reaction is achieved, the concentration K_M of the substrate is widely used to evaluate the general sensitivity of biosensors. K_M is also used as a measure of the enzyme affinity for substrate, the higher the value of K_M, the lower is the affinity (Banica, 2012). Thus, the dimensionless biosensor sensitivity to be optimized at a specific substrate concentration $S_0 = K_M$ can be considered as a three-variable function,

$$f_1(V, d, \delta) = B_S(K_M). \tag{8}$$

In some applications of biosensors, enzymes are archival and only available in every limited quantity or are the products of combinatorial synthesis procedures and thus they are only produced in microgram to milligram quantities (Schuhmann and Habermüller, 2002). In such applications, the minimization of the enzyme volume is of crucial importance. In the case of enzyme mono-layer two-compartment model of biosensors (Fig. 1), the enzyme volume equals the product of the enzyme concentration E_0 ($E_0 = V/k_3$) and the thickness d of the enzyme layer. Without loss of generality, the mathematical model (2)–(5) involves the concentration E_0 implicitly as a parameter of V ($V = E_0 k_3$); for a discussion on the advantages of dimensionless modelling we refer to (Gutfreund, 1995, Baronas et al., 2010). Since the enzyme concentration E_0 can be freely selected and the maximal enzymatic rate V is directly proportional to the enzyme concentration, the rate V can be considered as a free variable. Therefore a relative enzyme volume, as the objective to be minimized, can be expressed as follows:

$$f_2(V, d) = V \times d. \tag{9}$$

2.3 Numerical simulation

Due to the nonlinearity of the governing equations (2) the boundary value problem (2)–(5) was solved numerically by applying the finite difference technique (Britz, 2005, Baronas et al., 2010).

The mathematical as well as the corresponding computational models of the biosensor were validated using known analytical and numerical solutions for

two-compartment model of mono-enzyme single substrate amperometric biosensors (Schulmeister, 1990, Baronas et al., 2010). Those analytical solutions were derived only for relatively low as well as high concentrations of the substrate. The relative difference between the numerical solution of (2)–(5) and known analytical and numerical solutions was less than 1% at different values of the model parameters (Britz et al., 2008, Baronas et al., 2010).

3 Bi-Objective Optimization Problem

In this research we are interested in determination of the maximal enzymatic rate V, and thicknesses d and δ of the enzyme and dialysis (diffusion) layers, respectively, on the aim to maximize the sensitivity of the biosensor (B_S) and simultaneously minimize the enzyme volume, considering the fixed substrate concentration S_0. This leads to solution of the multi-objective optimization problem by maximizing the function f_1 of the biosensor sensitivity,

$$\max_{V,d,\delta} f_1(V, d, \delta), \tag{10}$$

while minimizing the function f_2 of the enzyme volume,

$$\min_{V,d} f_2(V, d), \tag{11}$$

assuming that the maximal enzymatic rate V varies from the lower bound V_* to the upper bound V^*, and the thicknesses of the enzyme layer (d) and the dialysis membrane (δ) are defined as $d \in [d_*, d^*]$ and $\delta \in [\delta_*, \delta^*]$, respectively.

In terms of multi-objective optimization, $p = (V, d, \delta)$ is called the *decision vector*, which is taken from the *search space*,

$$D = [V_*, V^*] \times [d_*, d^*] \times [\delta_*, \delta^*]. \tag{12}$$

The corresponding vector $f(p) = (f_1(p), f_2(p))$ representing values of the *objective functions* $f_1(p)$ and $f_2(p)$ obtained using decision vector p is called *objective vector*.

The joint optimization of $f_1(\cdot)$ and $f_2(\cdot)$ is a contradictory task. Depending on specific circumstances a certain trade-off between these objectives is accepted. To aid a rational decision the set of compromising decision vectors all of which are optimal in some sense will be constructed. But theretofore we will recall relevant definitions used in multi-objective optimization theory.

In general a decision vector is called *Pareto optimal* if value of any of objective functions cannot be improved without deterioration of value of any other objective; i.e. the values of parameters V', d', and δ' can be considered as Pareto optimal if there are no other values in the search space D which would increase the sensitivity of the biosensor without increment of enzyme value or would reduce the enzyme value without reduction of the sensitivity.

The set of Pareto optimal decision vectors is called *Pareto set* and the corresponding set of objective vectors is called *Pareto front*.

To derive an analytical expression for the Pareto front is a hard task even in the cases where the objective functions are defined by the analytical formulas. Therefore,

a discrete representation usually is computed to aid a decision maker in the selection of a proper trade-off between contradictive objectives. For the discussion on the arguments important in the constructing a discrete representation of Pareto fronts we refer to a recent paper (Faulkenberg and Wiecek, 2010). To substantiate a choice of a suitable algorithm for computation of a discrete representation of the Pareto front analytical properties of the objective functions would be very helpful. However, in the problem considered, objective functions are available only as computer algorithms, and their analytical investigation is difficult. In such circumstances, it seems reasonable to choose the algorithm which simply selects Pareto optimal decision vectors from the set of those computed at vertices of a quadratic lattice (in logarithmic scale). Let us mention that such an algorithm is approximately optimal in worst case setting as shown in (Žilinskas, 2013).

4 Computational Experiments

A number of numerical simulations of the biosensor response have been performed in order to determine a discrete approximation of the Pareto front of the multi-objective optimization problem (10)–(11). The numerical simulator of the biosensor response has been implemented by C++ programming language (Press et al., 2007).

The following constant values of the parameters of the mathematical model (2)–(5) have been used:

$$\begin{aligned} &D_{S,e} = D_{P,e} = 3 \times 10^{-6}\,\mathrm{cm}^2/\mathrm{s};\\ &D_{S,m} = D_{P,m} = 5 \times 10^{-7}\,\mathrm{cm}^2/\mathrm{s};\\ &K_M = 10^{-4}\,\mathrm{M}. \end{aligned} \tag{13}$$

Since the biosensor sensitivity increases with reduction of the concentration S_0, when values of other parameters (V, d, δ) are fixed, the constant value $S_0 = K_M$ of the substrate concentration was used. The remaining decision variables (V, d, δ) were varied in a wide range within their lower and upper bounds, as typical for practical biosensors (Banica, 2012, Grieshaber et al., 2008, Gough and Leypoldt, 1979):

$$V \in [10^{-13}, 10^{-2}]\,(\mathrm{M/s}); \quad d, \delta \in [0.001, 0.1]\,(\mathrm{cm}), \tag{14}$$

assuming that the difference of thicknesses of the layers would be no larger than an order of magnitude.

The boundary values of the decision variables has been chosen with respect to the real-life experiments. Polyvinyl alcohol, polyurethane, cellulose, latex or other membranes are often used to cover the enzyme layer in order to prevent it from dissolution and make biosensors more stable. The thickness most of them varies from several micrometers up to a millimeter (Grieshaber et al., 2008, Banica, 2012). The thickness of enzyme membranes in practical biosensors varies similarly (Cooper and Cass, 2004, Banica, 2012). The maximal enzymatic rate can vary in orders of magnitude (Gutfreund, 1995, Banica, 2012).

4.1 Dependence of the enzyme volume on the maximal enzymatic rate

The dependence of the biosensor dimensionless sensitivity $f_1 = B_S(K_M)$ on the maximal enzymatic rate V using different thicknesses of the enzyme layer (d) and the dialysis membrane (δ) is illustrated in Fig. 2, where the horizontal axis corresponds to the maximal enzymatic rate V, the vertical axis – to the biosensor sensitivity f_1, and different curves – to the different combinations (d, δ) of thicknesses of both layers. Computations have been performed for $V = 10^{-k}$, $k = 2, \ldots, 13$ except the cases $(d, \delta) = (0.1, 0.1)$ and $(d, \delta) = (0.01, 0.1)$ which have been investigated using intermediate values of V as these combinations of parameters appeared to be the most promising in the sense of Pareto optimality.

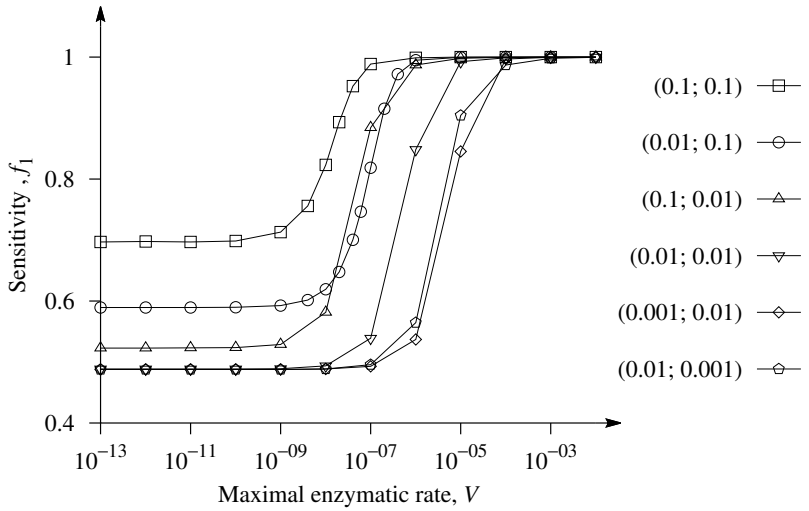

Fig. 2. The biosensor sensitivity f_1 versus the maximal enzymatic rate V at different thicknesses d and δ of the enzyme and dialysis layers.

One can see in Fig. 2, a higher value of the maximal enzymatic rate leads to the higher biosensor sensitivity. However the refractive point from which the increment of the maximal enzymatic rate is not useful in the sense of the sensitivity can be indicated as well as its dependence on the thicknesses of the catalytic and dialysis membranes.

It is also clear from the figure, that the best sensitivity is achieved with the thickest layers (curve denoted by rectangles), and thinning of the enzyme or dialysis layers leads to the lower sensitivity of the biosensor (see curves denoted by circles and triangles, respectively). The same tendency can be also envisaged for the layers, thinner by an order of magnitude.

Similarly, Fig. 3 shows the dependence of the biosensor sensitivity f_1 on the relative enzyme volume f_2, considering different thicknesses of the enzyme and dialysis layers. Since the enzyme volume f_2 was expressed as the product of the maximal enzymatic

rate V and the thickness d ($f_2 = Vd$), the latter dependency corresponds to the same curves as presented in Fig. 2, but shifted on the vertical axis.

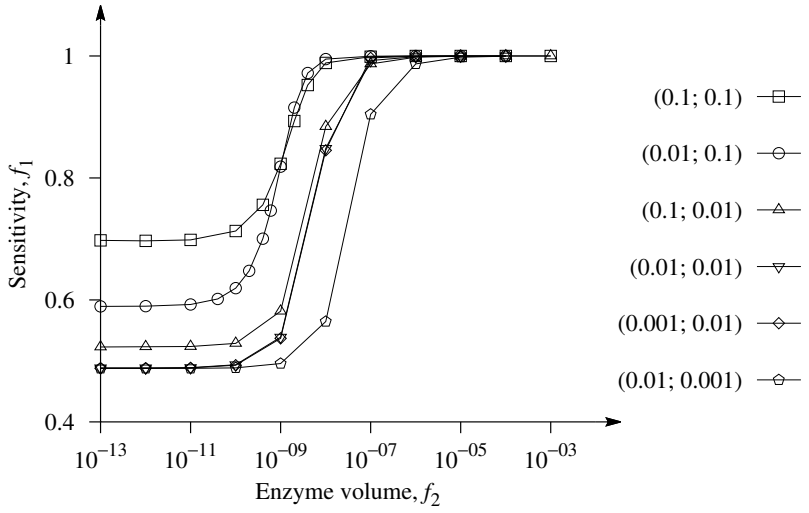

Fig. 3. The biosensor sensitivity f_1 versus the enzyme volume f_2 at different thicknesses of the enzyme and dialysis layers.

One can see from Fig. 3 that usage of the thickest dialysis layer (δ) leads to the largest sensitivity. If we are interested in the lower enzyme volume rather than biosensor sensitivity then it is useful to use the thickest enzyme layer ($d = 0.1$ cm) – the sensitivity is higher for relatively small enzyme volumes. If we are more interested in the greater sensitivity rather that the saving the enzyme, then it is useful to use a thinner enzyme layer ($d = 0.01$ cm) as it produces slightly grater sensitivity when when the enzyme volume is larger.

4.2 The discrete approximation of Pareto front

The discrete approximation of the Pareto front with the context of all other decision vectors is illustrated in Fig. 4, where the horizontal axis stands for the sensitivity of the biosensor, the vertical axis – for the enzyme volume, and different marks correspond to different thicknesses $(d; \delta)$ of the enzyme and dialysis layers, correspondingly. Pareto optimal decision vectors are denoted by filled marks.

One can see from the figure that the Pareto front mainly consists of the decision vectors referring to the thickest dialysis layer, $\delta = 0.1$ cm. As it was shown in Fig. 2, the reduction of the value of δ always leads to the lower sensitivity of the biosensor without any impact to the enzyme volume.

More interesting is the thickness d of the enzyme layer as it has direct impact to the enzyme volume. One can see from Fig. 4 it is better to use $d = 0.01$ cm if the larger

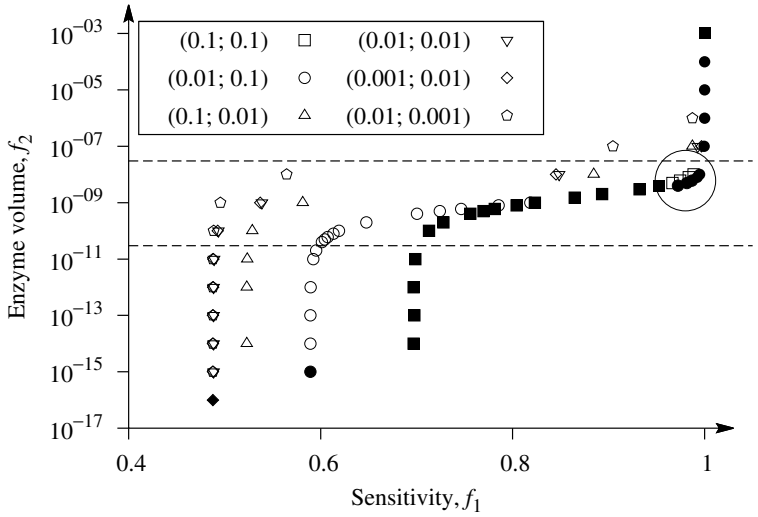

Fig. 4. The interdependence of the biosensor sensitivity f_1 and the enzyme volume f_2 with the distinguished Pareto front (filled circles).

sensitivity has priority against the saving of the enzyme, and $d = 0.1$ cm – if we have a limit for the enzyme volume.

We can also distinguish the Pareto optimal decision vector $(10^{-13}, 0.001, 0.01)$, referring to the lowest enzyme volume. However, this decision vector is not reasonable as it leads to the lowest sensitivity of the biosensor.

In general, the most interesting Pareto optimal decision vectors are illustrated between the dashed lines as they provide a reasonable trade-off between parameters – the dimensionless sensitivity of the biosensor can be significantly increased (from 0.7 to almost 1) without significant (relative) increment of the enzyme volume. Therefore the decision vectors indicated by the circle should be considered as the most relevant.

5 Conclusions

The multi-objective optimization problem for the determination of optimal maximal enzymatic rate and thicknesses of the layers of bi-layer mono-enzyme biosensor utilizing Michaelis-Menten kinetics with respect to maximize the biosensor sensitivity and minimize the enzyme volume has been formulated.

A number of the relevant values of biosensor parameters have been experimentally investigated and the discrete approximation of the set of Pareto-optimal solutions has been determined.

It was shown that it is always optimal to choose the thickest dialysis membrane. The thickness of the enzyme layer depends on whether the sensitivity or the enzyme volume is more relevant. If the saving of the enzyme has priority against the biosensor sensitivity, then it is reasonable to use the thickest enzyme layer – the sensitivity is notably

greater using relatively small enzyme volumes. On the other hand, if the sensitivity has priority against the enzyme volume, then it is reasonable to choose thinner enzyme layer – the sensitivity of the biosensor is slightly greater when the enzyme volume is larger.

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