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The influence of external factors on the operational stability of the biosensor response

Olga Štikonienė [a,b,*], Feliksas Ivanauskas [b], Valdas Laurinavicius [c]

[a] Institute of Mathematics and Informatics, Akademijos 4, Vilnius, LT-08663, Lithuania
[b] Vilnius University, Naugarduko 24, Vilnius, LT-03225, Lithuania
[c] Institute of Biochemistry, Mokslininku 12, Vilnius, LT-08662, Lithuania

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ABSTRACT

The behaviour of the electrochemical glucose biosensor based on the glucose oxidase was examined in the diffusion and the kinetic modes of the action. The sensitivity and linearity of the biosensor can be monitored changing the permeability of the outer membrane of the biosensor. The mathematical model based on the enzymatic conversion of the substrate and the diffusion of the substrate was created. The influence of the fluctuations of the membrane thickness, the diffusion coefficients and pH were modelled and their impact was evaluated at different modes of an action of the biosensor. Taking into account that limited acceptable fluctuations of the biosensor response should not exceed 5%, we calculated how $K_{M(app.)}$ and V_{max} can move to satisfy this requirement. In a deep diffusive mode (thick highly acetylated membrane), the fluctuations of $K_{M(app.)}$ up to 400% do not influence significantly the biosensor response. In the diffusion mode of action of the biosensor, the limit of the V_{max} fluctuations is on the level of 34%. The increase of the thickness of the membrane 5 times, increases the limit of fluctuations only to 19%. The reduction of the permeability of the membrane 4 times increases the level of limited fluctuations about 10–16%. The novelty of this work is binding into one system the fluctuations of pH and diffusion parameters and demonstrating the interdependence of them as an integrated factor of the reliability of the biosensor response.

1. Introduction

Biosensors as an analytical instrument have found wide application in medicine, environment, and food-quality control [1,2]. However, the application of the biosensors is limited by a low reliability of the biosensor operation. This is due to a number of different parameters. The stability of the biosensor action depends both on the stability of the sensing element of the biosensor, i.e. usually an enzyme or an enzymatic complex, and the stability of the matrix. Enzymes are not stable for a long time and are sensitive to a number of factors. The activity of an enzyme is limited by the pH. A number of compounds can activate and even more often inhibit the activity of the enzyme. The complicated construction of the biosensor, usually consisting of several semi-permeable layers is very often sensitive to the fluctuations of the pressure in the bulk, especially in mixing or flow-through conditions. Fluctuations of the pH and the concentration of salts can change the diffusion parameters of the biosensor membranes, thereby, changing the response of the biosensor.

The main goal of this paper is mathematical modelling and evaluation of the influence of two main parameters of the biosensor–pH fluctuations and diffusion fluctuations. pH fluctuations can occur in the bulk during a number of processes outside the biosensor, such as the microbiological, the action of enzymes, and as a result of different chemical reactions taking place in the bulk. The shift of the pH can be the result of an enzyme reaction inside the biosensor. The shift of the pH can change the activity of the enzyme, because almost all enzymes are pH-dependent. The shift of the pH can also evoke the shrinking of the membranes, thus the diffusion parameters and the diffusion distance can be changed. All these shifts will influence the response of the biosensor. We evaluated the weight of these factors on the biosensor response.

2. Experimental

2.1. Biosensor

As a model biosensor, a well-known electrochemical glucose biosensor based on glucose oxidase was selected [3]. Glucose oxidase (*Asp. niger* sp., $K_M = 0.23$ mM) in albumin gel layer was

* Corresponding author at: Institute of Mathematics and Informatics, Numerical Analysis Department, Akademijos 4, Vilnius, LT-08663, Lithuania.
Tel.: +370 5 2109346; fax: +370 5 2729209.
E-mail address: olgast@ktl.mii.lt (O. Štikonienė).

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deposited on the surface of the flat Pt electrode and covered with diffusion membrane made from cellulose or acetylated cellulose. Electrochemical registration of the oxidation current of the produced (hydrogen peroxide) was performed at potential 0.6 V *vs.* Ag/AgCl reference electrode.

2.2. Mathematical model

Suppose that the substrate (S) conversion to product (P) was catalysed by the enzyme:

$$S \xrightarrow{E} P \tag{1}$$

Consider a biosensor as a flat amperometric device with a layer of enzyme and outer membrane. It follows that the model has two regions. In the first region (outer membrane) only mass transport limited by diffusion takes place. In the second region (enzyme layer) enzymatic conversion of glucose to gluconic acid, oxygen to hydrogen peroxide and mass transport are limited by diffusion. A mathematical model of a biosensor is based on the system of the diffusion equations with a non-linear term corresponding to the Michaelis–Menten kinetics of the enzymatic reaction [4–8]. Let us suppose that the symmetry of a biosensor allows to describe changes in the concentration of the substrate and the product in the biosensor by the following system of reaction–diffusion equations. In the porous membrane region only the processes of diffusion take place:

$$\begin{aligned} \frac{\partial S_m}{\partial t} &= d_{S_m}\frac{\partial^2 S_m}{\partial x^2}, \quad a_e \le x \le a_e + a_m, \\ \frac{\partial P_m}{\partial t} &= d_{P_m}\frac{\partial^2 P_m}{\partial x^2}, \end{aligned} \tag{2}$$

where x and t are space and time respectively, $S_m(x,t)$ is the concentration of the first substrate (glucose) and $P_m(x,t)$ is the concentrations of the reaction product (hydrogen peroxide) in the membrane region. a_e is the thickness of the enzyme layer, a_m is the thickness of the diffusion layer (outer membrane). d_{S_m} and d_{P_m} are the diffusion coefficients of the substrate and the reaction product in the membrane. The thickness of the diffusion layer remains constant. The concentration of the substrate as well as the product over the outer membrane surface (bulk solution/membrane interface) remains constant while the biosensor keeps in touch with the substrate ($t > 0$). So, the following boundary conditions are satisfied:

$$S_m(t,\ a_e + a_m) = S_0, \quad P_m(t,\ a_e + a_m) = 0. \tag{3}$$

S_0 is the substrate concentration in the bulk solution.

In the second region the diffusion of both the substrate and the product, and the reaction take place:

$$\begin{aligned} \frac{\partial S_e}{\partial t} &= d_{S_e}\frac{\partial^2 S_e}{\partial x^2} - \frac{V_{\max} S_e}{K_M + S_e}, \quad 0 \le x \le a_e, \\ \frac{\partial P_e}{\partial t} &= d_{P_e}\frac{\partial^2 P_e}{\partial x^2} + \frac{V_{\max} S_e}{K_M + S_e}, \end{aligned} \tag{4}$$

where $S_e(x,t)$, $P_e(x,t)$ are the concentrations of the substrate and the reaction product in the enzyme layer, respectively. $V_{\max}$ is the maximal enzymatic rate. K_M is the Michaelis–Menten constant for glucose. d_{S_e} and d_{P_e} are the diffusion coefficients of the substrate and the reaction product in the enzyme layer, respectively. The concentration of the reaction product at the surface of electrode ($x = 0$) is equal to zero due to the fast electrochemical oxidation of the hydrogen peroxide. As the substrate is an electro-inactive substance, the following boundary conditions for $t > 0$ can be applied to the system:

$$\frac{\partial S_e}{\partial t}(t,\ 0) = 0, \quad P_e(t,\ 0) = 0. \tag{5}$$

Let $x = 0$ represent the surface of the electrode, while $x = a_e + a_m$ is the boundary between the diffusion layer and the buffer solution. The biosensor operation starts when some substrate appears in the bulk solution. This is used in the initial conditions ($t = 0$):

$$\begin{aligned} &S_e(0,\ x) = 0, \quad S_m(0,\ a_e + a_m) = S_0, \\ &P_e(0,\ x) = 0, \quad P_m(0,\ x) = 0. \end{aligned} \tag{6}$$

On the boundary between two regions with different diffusion coefficients we define the compatibility conditions ($t > 0$). These conditions mean that the concentration and the fluxes of the substrate and the product through the outer membrane are equal to the corresponding concentrations and fluxes entering the surface of the enzyme layer:

$$\begin{aligned} &S_e(t,\ a_e - 0) = S_m(t,\ a_e + 0), \\ &P_e(t,\ a_e - 0) = P_m(t,\ a_e + 0), \\ &d_{S_e}\frac{\partial S_e}{\partial x}(t,\ a_e - 0) = d_{S_m}\frac{\partial S_m}{\partial x}(t,\ a_e + 0), \\ &d_{P_e}\frac{\partial P_e}{\partial x}(t,\ a_e - 0) = d_{P_m}\frac{\partial P_m}{\partial x}(t,\ a_e + 0). \end{aligned} \tag{7}$$

We introduce the concentration S of the substrate and the concentration P of the reaction product in the entire domain as follows ($t > 0$):

$$\begin{aligned} S(t,\ x) &= \begin{cases} S_e(t,\ x), & 0 \le x \le a_e, \\ S_m(t,\ x), & a_e \le x \le a_e + a_m, \end{cases} \\ P(t,\ x) &= \begin{cases} P_e(t,\ x), & 0 \le x \le a_e, \\ P_m(t,\ x), & a_e \le x \le a_e + a_m. \end{cases} \end{aligned} \tag{8}$$

Both concentration functions S and P are continuous in the entire domain.

Biosensor development and modelling from both chemical and mathematical point of view are presented in a monograph [9].

3. Results and discussion

3.1. Digital simulation

Serious difficulties can arise when one tries to solve analytically a multidimensional non-linear system of partial differential equations with complex boundary conditions. Therefore, the problem was solved numerically [10,11]. All simulations were carried out using MATLAB. We solved the initial-boundary value problems for systems of parabolic partial differential equations. The finite difference technique was applied for discretization of the mathematical model. A uniform discrete grid was introduced in x direction. The second order approximation to the solution is made on the mesh in x direction. The time integration was achieved with dynamically selected time step. Having a numerical solution of the system of partial differential equations, the density of the biosensor current was calculated.

The steady-state biosensor current I (the biosensor response) is one of the most important characteristics of biosensors. The recorded current is a response of a biosensor to the glucose concentration in the bulk. The current depends upon the flux of the reaction product at the electrode surface. The current density $i(t)$ at time t can be obtained explicitly from Faraday's law and Fick's law using the flux of the product concentration at the surface of the electrode:

$$i(t) = n_e F d_{Se}\frac{\partial P_e}{\partial x}(t,\ 0), \tag{9}$$

where n_e is a number of electrons, involved in the charge transfer at the electrode surface, and F is the Faraday constant, $F = 96{,}485$ C/mol. The steady-state current I is defined as:

$$I = \lim_{t\to\infty} i(t). \tag{10}$$

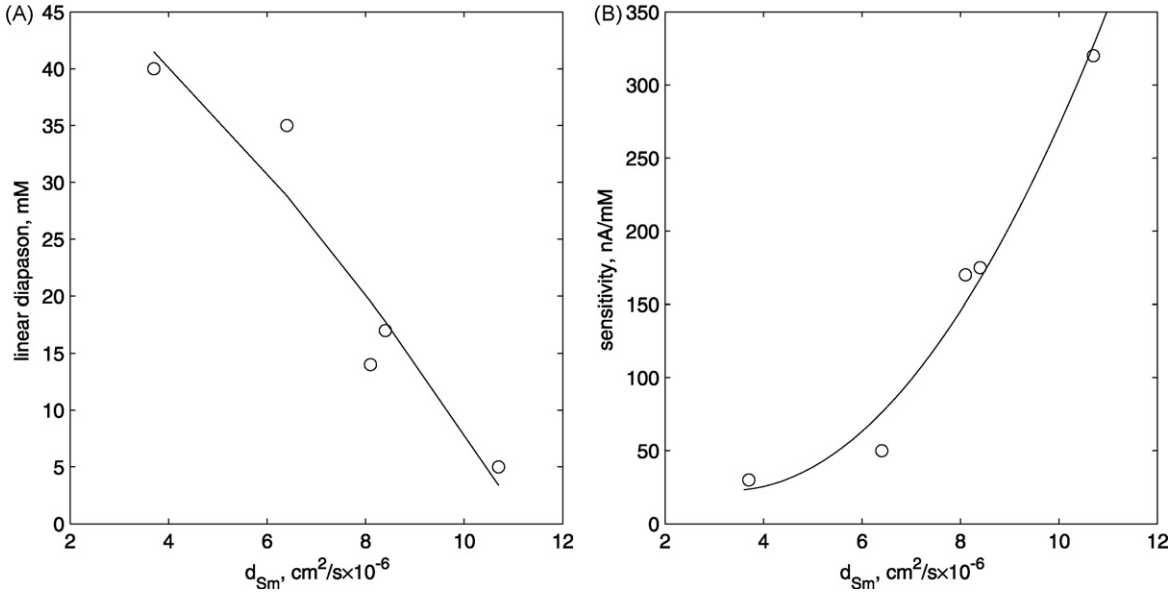

Fig. 1. Dependence of the linear diapason of action of the biosensor (A) and sensitivity of the biosensor (B) on diffusion coefficient (degree of acetylating) of the outer cellulose membrane.

To obtain the concentration profiles in the steady-state, the calculations were continued while the concentration profiles stopped changing. In digital simulation, the biosensor response time, namely the time moment of occurrence of the steady-state current, was calculated using the following formula:

$$T = \min_{i(t) > 0} \left(t : \frac{1}{i(t)} \left| \frac{di(t)}{dt} \right| < \varepsilon \right), \tag{11}$$

where ε is the given dimensionless decay rate. $\varepsilon = 10^{-3}$ was used for the calculations. Simulation time 5000 s was chosen so that it guarantees an accurate simulation of the steady-state current. The steady-state currents were calculated for the modelled biosensor response when the substrate concentration S_0 changes up to 35 mM.

The activity of the enzyme depends on pH of solution. It is described by the following equation:

$$V(\mathrm{pH}) = \frac{V_{\max}}{10^{-\mathrm{pH}+pKa_1} + 10^{\mathrm{pH}-pKa_2} + 1}, \tag{12}$$

pKa_1 and pKa_2 are the values of the ionogenic groups of the enzyme active centre responsible for the activity.

Using computer simulation the influence of the variations of pH as well as the variations of the diffusion coefficient and the thickness of the outer membrane on the biosensor response was investigated. The following values of the parameters were constant in the numerical simulation of all the experiments: the square of the flat electrode $S = 2\ \mathrm{mm}^2$; the thickness of the enzyme layer $a_e = 4\ \mu\mathrm{m}$; the diffusion coefficients of the substrate and the reaction product in the enzyme layer $d_{P_e} = d_{S_e} = 70\ \mu\mathrm{m}^2/\mathrm{s}$; $K_M = 0.23$ mM; $V_{\max} = 1.1$ mM/s, $pKa_1 = 3.5$, $pKa_2 = 7.3$. The numerical solution of the model was evaluated for different values of input parameters. In all numerical experiments the diffusion coefficients of the substrate d_{S_m} and the reaction product d_{P_m} in the membrane were changed from 3 to 12 $\mu\mathrm{m}^2/\mathrm{s}$. The thickness of the diffusion layer (outer membrane) a_m was changed from 5 to 25 μm. In this simulation pH value was calculated at the point of maximal activity of the enzyme (the maximal enzymatic rate). In the case of the given values of ionogenic groups pK_a pH = 5.4.

3.2. Biosensor response

The response of the electrochemical glucose oxidase based biosensors was monitored by the outer membrane possessing different permeability. The sensitivity and the linear response of the biosensor depend on acetylating degree of the cellulose membrane. When the enzyme layer on the electrode surface is covered with a cellophane possessing high permeability for small molecules, like glucose, the linearity of the biosensor (the concentration substrate when the response of the biosensor differs from the linear mode more than 5%) is short (Fig. 1A), but the biosensor possesses a high sensitivity (Fig. 1B). It is a typical feature of the biosensor acting in a kinetic mode. Increasing the acetylating degree of the cellulose membrane (decreasing the permeability), the linearity of the biosensor is increasing, and the sensitivity is decreasing. The biosensor is switching to the diffusion mode of action. It is obvious that a set of parameters affecting the response of the biosensor depends on the biosensor action mode, and the impact of the same parameters is different.

3.3. $K_{M(app.)}$ fluctuations

As a sensitive element of the mode of the biosensor action $K_{M(\mathrm{app.})}$ and $V_{\max(\mathrm{app.})}$ were selected. Parameter K_M was selected, because it is easy to determine the value of $K_{M(\mathrm{app.})}$ (from the concentration of substrate/response curve). Calculated from experimental results $K_{M(\mathrm{app.})}$ covers all parameters responsible for the enzyme action (denaturation of the enzyme, the shape of the active centre, the inhibition of an enzyme, and other parameters affecting the capability of the enzyme to recognize its substrate). The fluctuations of $K_{M(\mathrm{app.})}$ directly reflect the integrated number of impacts of a number of factors affecting the biosensor action. Taking into account that limited acceptable fluctuations of the biosensor response should not exceed 5%, we calculated how $K_{M(\mathrm{app.})}$ can be changed to satisfy this requirement. Data are presented in Fig. 2.

As can be seen in the case of a thin membrane and a high diffusion coefficient the biosensor operates in a kinetic mode and the response of the biosensor is very sensitive to any fluctuations of the value of the $K_{M(\mathrm{app.})}$. If the membrane is not tightly fixed on the electrode (this can be observed in flow-throw systems when the pressure of the liquid fluctuates, especially in the case when the

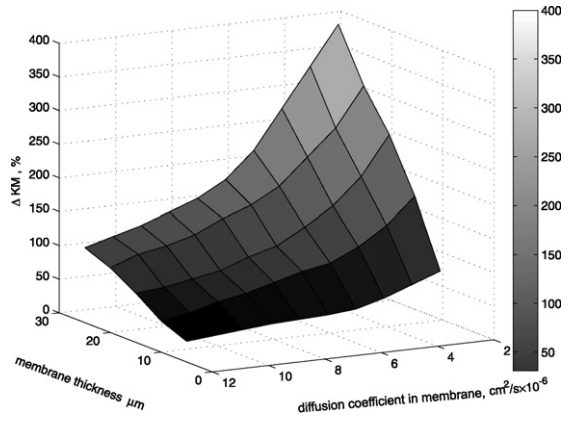

Fig. 2. Dependence of the limited increase of $K_{M(app.)}$ on membrane thickness and diffusion coefficient. Limited decrease of $K_{M(app.)}$ will be symmetric (not shown).

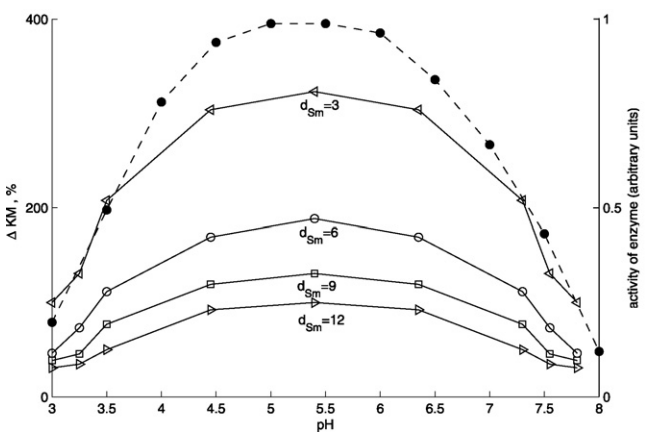

Fig. 4. Dependence of the limited increase of $K_{M(app.)}$ (left axis) on pH and diffusion coefficient in membrane (cm^2/s). Dash line (right axis) is pH dependence of activity of the native glucose oxidase.

peristaltic pumps are used), it can wave. The membrane can swell. In both cases the thickness of the membrane (thereby, the diffusion way) can be changed. Calculations show, that the increase of the membrane thickness 5 times increases the allowed fluctuations of the $K_{M(app.)}$ from 30.8 to 111.5%. It means that the biosensor is switching to the diffusion mode of action and the impact of fluctuations of the enzyme structure and the capacity to bind the substrate reduces 3.6 times. Thereby, the impact of the enzyme selectivity on the biosensor selectivity also decreases. The obtained data can be useful in modeling the action of the artificial catalytic activities operating in the heterogeneous catalytic systems, both analytical and reactor.

The biosensor is sensitive to the fluctuations of the diffusion coefficient. The outer membrane can be glued by adhesive components from the bulk. This can be observed very often when the biosensor operates in the blood. The decrease of the permeability of the membrane 4 times switches the mode of action of the biosensor to the diffusive mode. This allowed the fluctuations of $K_{M(app.)}$ to increase up to 3 times. In a deep diffusive mode (thick highly acetylated membrane), the fluctuations of $K_{M(app.)}$ up to 400% do not influence significantly the biosensor response.

3.4. V_{max} fluctuations

V_{max} of the immobilized enzyme can be affected by a number of factors of the matrix. How important is it to the biosensor response? The activity of the enzyme directly impacts the response of the biosensor, thereby, the allowed fluctuations of the $V_{max.}$ can be applied in a much shorter diapason, than $K_{M(app.)}$. The fluctuations of the enzyme activity directly affect the ratio of the kinetic/diffusion modes of action, thereby, increasing or decreasing of the enzyme activity. The permeability of the membrane also regulates the ratio of the kinetic and the diffusion regimes of the biosensor. It is obvious that decreasing permeability of the membrane causes the impact of the fluctuations of the membrane thickness on the biosensor response to decrease slightly. Therefore their impacts will not be symmetric as can be seen in Fig. 3. By increasing the thickness of the membrane from 5 to 25 μm, we increase the diffusion mode of action, the response time, and thereby the time of the relaxation of any fluctuations inside the membrane. Obviously, the limited fluctuations of the enzyme activity also increase. In the case of highly permeable thin membrane the limit of the V_{max} fluctuations is on the level of 34%, that indicates the diffusion mode of action of the biosensor. In this case the increase of the thickness of the membrane 5 times, increases the limit of fluctuations only to 19%. The reduction of the permeability of the membrane 4 times increases the level of limited fluctuations about 10–16%. The decreased permeability of the membrane reduces the influence of the fluctuations of membrane thickness to 12%, as can be expected.

During the exploitation of the biosensor we can expect inactivation of the enzyme rather than unexpected activation. The increase of the enzyme activity can switch the mode of the biosensor action

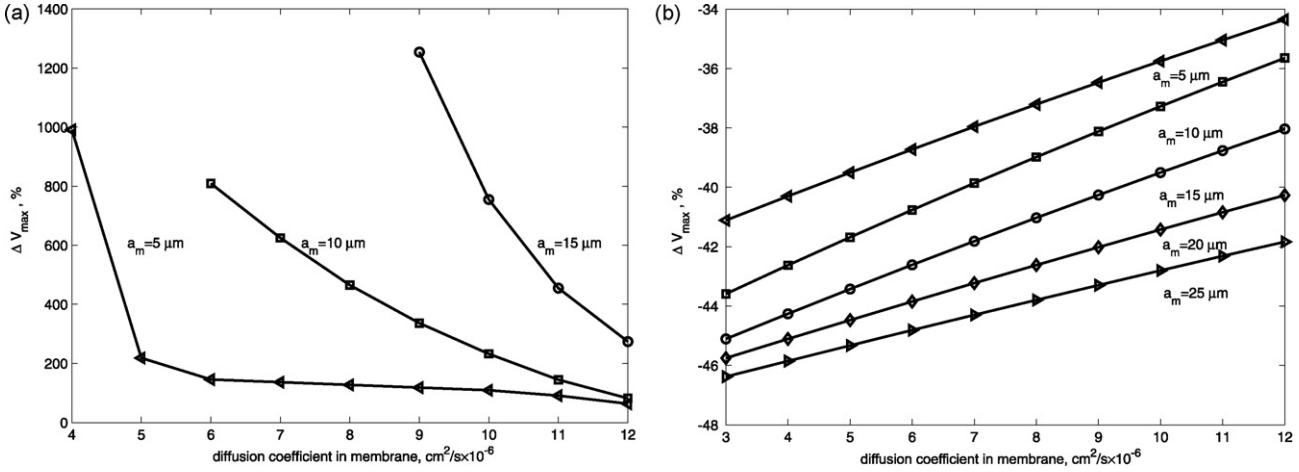

Fig. 3. Dependence of the limited increase and decrease of $V_{max.}$ on diffusion coefficient at different membrane thickness.

into the diffusion mode, thereby further increase of the enzyme activity does not influence the response of the biosensor, and the model does not describe the process.

3.5. pH fluctuations

Another parameter affecting the action of the biosensor is pH. Its dependence of glucose oxidase is determined by two ionogenic groups with *pKa* equal to 3.5 and 7.3 (calculated from the data presented in [12]), see Eq. (12). pH activity of the native glucose oxidase in the solution is depicted in Fig. 4 (dashed line, right axis).

As can be expected, pH affects the ratio of the diffusion/kinetic modes, thereby, it also affects the impact of the enzyme capacity to bound the substrate. The higher the activity of the enzyme, the deeper the diffusion mode of action is, the lower is the impact of the pH dependence.

4. Conclusion

In this study we have demonstrated a mathematical model, allowing to predict the operational stability of the biosensor and evaluate the influence of the membrane thickness, the diffusion coefficient and pH on the metrological parameters of the biosensor. In a deep diffusive mode (thick highly acetylated membrane), the fluctuations of $K_{M(\text{app.})}$ up to 400% do not influence significantly the biosensor response. The activity of the enzyme directly impacts the response of the biosensor. In the case of highly permeable thin membrane the limit of the $V_{\max}$ fluctuations is on the level of 34%, that indicates the diffusion mode of action of the biosensor. The increase of the thickness of the membrane 5 times, increases the limit of fluctuations only to 19%. The reduction of the permeability of the membrane 4 times increases the level of limited fluctuations about 10–16%. The model shows, that in the case of diffusion mode of action the fluctuations of $K_{M(\text{app.})}$ affects the response of the biosensor much weaker than the fluctuations of the enzyme activity. It means that the substrate specificity of the biosensor, operating in the diffusion mode of action will be smothered. The importance of the sensitivity of the enzyme activity on pH has been evaluated in different modes of the action of the biosensor.

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