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ORIGINAL PAPER

Mathematical modeling of the action of biosensor possessing variable parameters

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Abstract Electrochemical biosensor containing flat semi-permeable membrane covering enzyme-containing layer has been investigated. Mathematical modeling of the action modes of electrochemical biosensors with outer diffusion membrane was performed. Operation of the biosensor under the conditions when the permeability of the membrane and the activity of the biocatalytic layer depend on the parameters of the probe has been examined. The pH and temperature were selected as the main parameters which often affect the action of biosensors. A set of parameters was selected when the biosensor operates in kinetic and diffusion modes of action. The response time of the biosensor was shown to be sensitive to the mode of the biosensor action especially in the boundary region of the biosensor action. The linearity of the biosensor (the linear dependence of the biosensor response on the substrate concentration) in the deep diffusion mode can be increased by several magnitudes, whereas the response time increases only several times.

Keywords Biosensor · pH · Temperature · Membrane permeability

1 Introduction

Biosensor is a complicated device, which consists of a sensing element of a biological nature, and a signal converting system. Usually enzymes, enzyme complexes, antibodies, or even whole cells are used as sensing elements. Very often electrochem-

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ical devices are used as a signal converting systems. The action of the biosensor is determined by a number of the parameters attributed to the: (1) biological system, such as the catalytical capacity of the biosensor, the rate of the bounding of the substrate, rate of the conversion of the substrate; (2) the transducer, such as the rate of the conversion of the product of the enzymatic reaction, the rate of the regeneration of the electrochemical system; (3) diffusion parameters and the rates of the substrate diffusion into active center, the rate of diffusion of the product to the electrochemical system, the diffusion of the products of electrochemical conversion [1]. All these mentioned parameters are constant during the considerable time of biosensor action. The slowest process is identified as a rate limiting step and determines the parameters of the biosensor. The electrochemical reactions are usually very fast compared to the enzymatic process and diffusion parameters. Thereby, there are two groups of parameters, responsible for biosensor action: biocatalytic—determined by the parameters of enzymatic conversion, and diffusion—determined by the construction of the biosensor. On the basis of these two groups of parameters two limiting biosensor action modes were identified. The first one—the kinetic mode is realized when the activity of the enzymatic conversion of the substrate is very low in comparison with the diffusion parameters. In this case all parameters of the biosensor are determined by the parameters of the catalytic process. It means, that the pH dependence of the biosensor response will be the same as the pH dependence of the enzyme activity, and the temperature dependence of the response will be determined by activation energy of the catalytic process (usually about 10%/degree) etc. The linearity of the biosensor response (linear dependence of the biosensor response to the substrate concentration) is usually determined by the value of K_M and is very short. The sensitivity of the response (response to concentration ratio) is quite high.

Another limiting mode—the diffusion mode, is realized when the catalytic activity of the biosensor is very high and the slowest step is a substrate diffusion to the active center of enzyme. At this mode the parameters of the biosensor are determined by the diffusion parameters. If the pH change does not affect diffusion parameters, it means that biosensor response is insensitive to the pH fluctuations in the bulk. The influence of the temperature on the diffusion is much lower (about 2–3%/degree). The sensitivity of the biosensor is low. When the biosensor operates in the diffusion mode, a long linear calibration curve of the biosensor can be expected. It is a good feature, because biosensor can operate at high concentrations of the substrate. For example, linearity of glucose biosensors, designed on glucose oxidase [2] or PQQ glucose dehydrogenase [3] usually reach only few mM. Only in some cases it can be extended up to 15 mM [2,4]. This can be achieved by switching biosensor action into deep diffusion mode, or artificially lowering the concentration of the substrate on the outer surface of the outer membrane [5]. These modes of the biosensor action were described in a (large) number of papers [6–11].

The possibility of switching the modes of action opens up a nice opportunity to manage analytical parameters of the biosensor. Sometimes it is useful, especially, when the biosensor operates in a system where the substrate concentration varies on large scale.

The goal of this paper is the mathematical modeling of the biosensor action, where the catalytic capacity of the biosensor is affected by the pH, and when the diffusion

parameters of the membrane can be regulated. The response time and the linearity of the biosensor will also be analyzed.

2 Biosensor structure

The flat biosensor with the enzyme layer deposited on the flat electrode and covered with the flat membrane has been investigated. A number of electrochemical biosensors have such construction. Even if the outer membrane is omitted, the thickness of enzymatic layer on the surface of the electrode and non- mixing solvent layers act like the outer membrane. We assume that the thickness of the enzyme layer is c and stable during all procedure. Flat porous membrane of thickness $\delta = d - c$ possess flexible thickness and permeability characterized by diffusion coefficients for substrate and reaction product $D_{\mathrm{SM}} = D_{\mathrm{PM}} = D_{membr}$. The enzyme activity and the membrane permeability are dependent on the pH. Currently modeled system is schematically presented in Fig. 1.

3 Mathematical model

We assume the classical scheme, where enzyme (E) converts substrate (S) into reaction product (P):

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

Such a biosensor mathematical model could be described by using two dimensional reaction–diffusion equations containing a nonlinear term related to the Michaelis-Menten kinetics with the reaction product inhibition. In our case, it is additionally assumed that the diffusion coefficients and the enzyme activity are dependent on the pH and the temperature. Equations governing the processes occurring in area 2 (Fig. 1) are [12]:

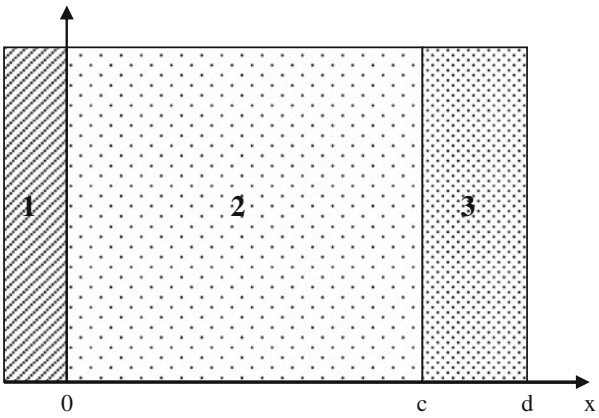

Fig. 1 Cross-section scheme of the model used in the present study: 1 electrode, 2 enzyme layer, 3 membrane

$$\begin{cases} \dfrac{\partial S}{\partial t} = D_{\mathrm{SE}}(T)\dfrac{\partial^2 S}{\partial x^2} - \dfrac{V_{\max}(pH,T)\cdot S}{K_{\mathrm{M}}(1 + P/K_{\mathrm{P}}) + S} \\ \dfrac{\partial P}{\partial t} = D_{\mathrm{PE}}(T)\dfrac{\partial^2 P}{\partial x^2} + \dfrac{V_{\max}(pH,T)\cdot S}{K_{\mathrm{M}}(1 + P/K_{\mathrm{P}}) + S} \end{cases}, \quad x \in (0,c), \tag{2}$$

Equations describing the processes occurring in area 3 are:

$$\begin{cases} \dfrac{\partial S}{\partial t} = D_{\mathrm{SM}}(pH,T)\dfrac{\partial^2 S}{\partial x^2} \\ \dfrac{\partial P}{\partial t} = D_{\mathrm{PM}}(pH,T)\dfrac{\partial^2 P}{\partial x^2} \end{cases}, \quad x \in (c,d), \tag{3}$$

where symbols in italics are S, substrate concentration, D_{SE}; D_{PE}, substrate and reaction product diffusion coefficients inside area 2; D_{SM}, D_{PM}, substrate and reaction product diffusion coefficients inside membrane (area 3); $V_{\max}$, maximum enzymatic rate; K_{M}, Michaelis constant; K_{P}, reaction product inhibition constant; T, temperature; pH, pH inside membrane.

The maximum enzymatic rate dependence on the pH is modeled using the Gauss function with the center $pH_V = 6$ and the dependency on temperature is modeled by the linear function with 10%/degree rate and the center at 20 °C:

$$V_{\max}(pH,T) = V_{\max 0}\cdot\frac{T-10}{10}\cdot \mathrm{e}^{-(pH-6)^2}, \tag{4}$$

where $V_{\max 0}$, a typical maximum enzymatic rate at 20 °C and the $pH = 6$. The diffusion coefficients inside the enzymatic layer (area 2) are modeled using the linear function with 3%/degree rate and the center at 20 °C:

$$\begin{cases} D_{\mathrm{SE}}(pH) = D_{\mathrm{SE}0}\cdot\dfrac{T+13}{33} \\ D_{\mathrm{PE}}(pH) = D_{\mathrm{PE}0}\cdot\dfrac{T+13}{33} \end{cases}, \tag{5}$$

where $D_{\mathrm{SE}0}$ and $D_{\mathrm{PE}0}$, the substrate and the reaction product diffusion coefficient inside the enzymatic layer (area 2) at 20 °C temperature. The diffusion coefficients dependency on time inside membranes modeled using several different models—aussian, linear and constant:

$$\begin{cases} D_{\mathrm{SM}}(pH,T) = D_{\mathrm{SE}}(T)\cdot \mathrm{e}^{-(pH-pH_M)^2} \\ D_{\mathrm{PM}}(pH,T) = D_{\mathrm{PE}}(T)\cdot \mathrm{e}^{-(pH-pH_M)^2} \end{cases}, \tag{6}$$

or

$$\begin{cases} D_{\mathrm{SM}}(pH,T) = D_{\mathrm{SE}}(T)\cdot(0.2\cdot pH - 0.7) \\ D_{\mathrm{PM}}(pH,T) = D_{\mathrm{PE}}(T)\cdot(0.2\cdot pH - 0.7) \end{cases}, \tag{7}$$

or

$$\begin{cases} D_{\mathrm{SM}}(pH,T) = D_{\mathrm{SE}}(T)\cdot 0.2 \\ D_{\mathrm{PM}}(pH,T) = D_{\mathrm{PE}}(T)\cdot 0.2 \end{cases}. \tag{8}$$

Initial conditions:

$$S(0,x) = 0, \quad \text{when} \quad 0 \le x < d;\ \ S(0,d) = S_0;\ \ P(0,x) = 0. \tag{9}$$

Boundary conditions:

$$\begin{aligned} &\frac{\partial S}{\partial x}(t,0) = 0,\ S(t,d) = S_0,\ \ P(t,0) = 0,\ \ P(t,d) = 0, \\ &D_{\mathrm{SE}}(T)\frac{\partial S}{\partial x}(t,c-0) = D_{\mathrm{SM}}(pH,T)\frac{\partial S}{\partial x}(t,c+0), \\ &D_{\mathrm{PE}}(T)\frac{\partial P}{\partial x}(t,c-0) = D_{\mathrm{PM}}(pH,T)\frac{\partial P}{\partial x}(t,c+0), \quad 0 \le t. \end{aligned} \tag{10}$$

The observed sensor characteristics:

Reaction product gradient (proportional to sensor amperometric response)

$$i\colon i(pH,T,S_0,t) = \left.\frac{\partial P}{\partial x}\right|_{x=0}. \tag{11}$$

Sensor steady state response:

$$i_{\mathrm{fin}}(pH,T,S_0). \tag{12}$$

Sensor steady state achievement time t_{fin}:

$$t_{\mathrm{fin}}(pH,T,S_0) = \max\{t : i(pH,T,S_0,t) < 0.95\cdot i_{\mathrm{fin}}(pH,T,S_0)\}. \tag{13}$$

Sensor linear range $S_{\mathrm{linear\_range}}$:

$$S_{\mathrm{linear\_range}} = \max\left\{ S_0 : 0.95 < \frac{i_{\mathrm{fin}}(pH,T,S_0)S_{0\_\mathrm{initial}}}{i_{\mathrm{fin}}\left(pH,T,S_{0\_\mathrm{initial}}\right)S_0} < 1.05 \right\}. \tag{14}$$

Sensor sensitivity B:

$$\begin{aligned} B &= \frac{i(pH,T,S_0,t_2) - i(pH,T,S_0,t_1)}{S_0\cdot(t_2-t_1)}, \\ t_2 &= \max\{t : i(pH,T,S_0,t) < 0.5\cdot i_{\mathrm{fin}}(pH,T,S_0)\}, \\ t_1 &= \max\{t : i(pH,T,S_0,t) < 0.3\cdot i_{\mathrm{fin}}(pH,T,S_0)\}. \end{aligned} \tag{15}$$

4 Solution method and experiment setup

The linear part of (2), (3) equation system was approximated and solved using Crank-Nicolson finite differences scheme [13]. The non-linear part of the system was handled using a simple iteration method.

A series of computational simulations were performed to investigate how electrode readings would differ if this amperometric biosensor worked under the presented model when: (1) the pH, the temperature and the membrane diffusion coefficients are constant, but the maximum enzymatic rate $V_{\max}$ is monotonously increased, the simulation targets are sensitivity B Eq. (15) and the linear range $S_{\text{linear\_range}}$ Eq. (14); (2) the pH, the temperature and the maximum enzymatic rate $V_{\max}$ are constant, but the membrane diffusion coefficient is monotonously increased up to the diffusion coefficients equal to the ones in area 2, the simulation targets are the same as for exp. (1); (3) the pH and temperature are increased linearly through the range, $V_{\max}$ is calculated according to Eq. (4), the 2nd area diffusion coefficients are calculated according to Eq. (5), the membrane diffusion coefficients are calculated according to Eq. (6), $pH_M > 6$, the simulation targets are the linear range $S_{\text{linear\_range}}$ Eq. (14) and the sensor steady state achievement time t_{fin} Eq. (13); (4) the same as (3), but $pH_M < 6$; (5) the same as (3), but $pH_M = 6$; (6) the same as (3), but the membrane diffusion coefficients are constant with regard to pH, Eq. (8); (7) the same as (3), but the membrane diffusion coefficients are calculated according to linear equation system (7).

The following values were used in simulation for experiment (1): $S_0 = 1.46 \times 10^{-6}\,\text{mol}\,\text{m}^{-3}$ (for sensitivity measurement and for linear range test substrate concentration by 10% with each iteration, while condition (14) is matched), $S_{0\_\text{initial}} = 1 \times 10^{-6}\,\text{mol}\,\text{m}^{-3}$, $D_{\text{SE}} = D_{\text{PE}} = D_{\text{SM}} = D_{\text{PM}} = 0.9 \times 10^{-10}\,\text{mol}\,\text{m}^{-3}$, $V_{\max} = (10^{-9}\text{–}10)\text{mol}\,\text{m}^{-3}\text{s}^{-1}$, $K_{\text{M}} = 10^{-1}\text{mol}\,\text{m}^{-3}$, $K_{\text{P}} = 10^{19}\,\text{mol}\,\text{m}^{-3}$ (value big enough to suppress the effect of inhibition for this experiment), $T = 20\,^{\circ}\text{C}$, $pH = 6$, sensor thickness (including membrane) $d = 2 \times 10^{-4}$ m, sensor enzymatic layer thickness $c = 1.6 \times 10^{-4}$ m, integration period $t_{\text{int}} = 15,000$ s. The values used for experiment (2) were the same except: $V_{\max} = 1 \times 10^{-6}\,\text{mol}\,\text{m}^{-3}\,\text{s}^{-1}$, $D_{\text{SM}} = D_{\text{PM}} = 8.79 \times 10^{-9}\text{–}0.9 \times 10^{-10}\,\text{mol}\,\text{m}^{-3}$. The values used in experiment (3) were the same as for (1) except, $S_0 = 2.62 \times 10^{-1}\,\text{mol}\,\text{m}^{-3}$ (for steady state time measurement), D_{SM}, D_{PM} calculated according to Eq. (6), $pH_M = 7$, $V_{\max} = 1 \times 10^{-4}\,\text{mol}\,\text{m}^{-3}\text{s}^{-1}$, $T = 15\text{–}25\,^{\circ}\text{C}$, $pH = 4\text{–}8$, $K_{\text{P}} = 1 \times 10^{-4}\,\text{mol}\,\text{m}^{-3}$. The values used in experiment (4) were the same as for experiment (3) except $pH_M = 5$. The values used in experiment V) were the same as for experiment (3) except $pH_M = 6$. The values used in experiment (6) were the same as for experiment (3) except that the membrane diffusion coefficients substrate and the reaction product were calculated according to Eq. (8). The values used in experiment (7) were the same as for experiment (3) except that the membrane diffusion coefficients substrate and the reaction product were calculated according to linear equation system (7).

5 Results and discussion

Using a combination of biosensor parameters and numerical simulation, biosensor action was extended into kinetic and diffusion modes. As a measure of the biosensor

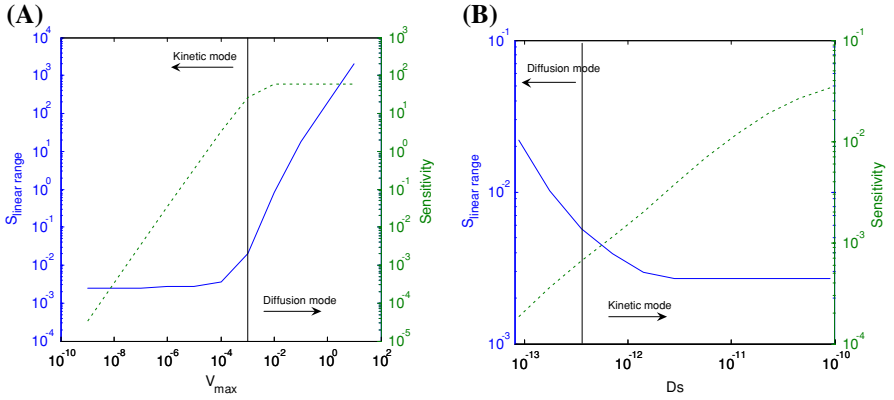

Fig. 2 **a** Dependence of the sensor response linear range (*left axis*), and sensitivity (*right axis*) on maximum enzymatic rate. Diffusion coefficient of the outer membrane $D_{SM} = D_{PM} = 0.9 \times 10^{-10}\,\mathrm{mol\,m^{-3}}$. Substrate concentration $S_0 = 1.46 \times 10^{-6}\,\mathrm{mol\,m^{-3}}$ (for sensitivity measurement). **b** Dependence of the sensor response linear range (*left axis*) and sensitivity (*right axis*) on outer membrane diffusion coefficient. $V_{max} = 1 \times 10^{-6}\,\mathrm{mol\,m^{-3}\,s^{-1}}$. Legend: *solid* – linear response range; *dashed* – sensitivity

action, the linearity of the biosensor response was calculated. As a criterion of the linearity the limit concentration of the substrate when the biosensor response curve differs more than 5% from the hypothetic linear dependence was considered. The biosensor response time was also considered as one of the important parameters. The response time was calculated as a time when 95% of the steady state signal is achieved.

In the Fig. 2 the typical parameters of the biosensor, operating in the kinetic and diffusion modes are presented. At the stable diffusion parameters (Fig. 2a) and low activity of the biocatalytic layer, the limiting process is an enzymatic conversion of the substrate, thereby, the concentration of the substrate inside enzymatic layer and in the bulk will be the same. If to take into account, that K_M of the enzyme is stable, then the linearity of the biosensor is stable and it is estimated about the third part from K_M value. The sensitivity of the biosensor (taking into account only linear part of the curve) increases with the increase of the activity of the enzyme.

At the high activity of the enzyme, the limiting step becomes the diffusion of the substrate through the enzymatic membrane. In this case the sensitivity of the biosensor depends only on the substrate supply rate. At the stabile substrate concentration, the sensitivity of the biosensor is also stable. In the diffusion mode of action the actual concentration of the substrate close to the active center of the enzyme will be much lower compared with the substrate concentration in the bulk. This difference of the substrate concentrations will increase increasing of the enzyme activity; thereby, the sensitivity of the biosensor will increase with increasing the activity of the biocatalytic layer.

Analogous data can be observed when the sensor operates with a variable permeability of the outer membrane (Fig. 2b). At high value of the D_S, the permeability of the membrane is high; thereby the limiting step is the activity of the enzyme. At stable V_{max} and K_M, both, linear response range of the biosensor and the sensitivity are stable. At lower diffusion coefficient the biosensor switches to the diffusion mode

of action. It leads to the decrease of the biosensor sensitivity, because the substrate concentration in the enzymatic layer will be lower than in the bulk. This difference in the concentrations will increase with decrease of the diffusion coefficient and it will reflect limited substrate capability to reach the active center of the enzyme. Thereby, the sensitivity of the biosensor will decrease, and linearity (linear response range) will increase, as we can see in Fig. 2b.

These data indicate that the selected parameters of the biosensor action are correct and we can apply them to our further calculations. The curves obtained on the basis of the mathematical simulations are close to the experimental data obtained in our previous experiments [2] where the behavior of the electrochemical biosensors with outer membranes possessing different permeability has been investigated. A number of membranes with different permeability have been obtained by acetylating of the cellulose membrane.

The activity of the enzyme is decreasing in time, and the outer membrane can be glued with outside proteins, lipids, cells, etc. This often occurs during a long time of exploitation of the biosensor. Let us consider the case when the permeability of the membrane or the activity of the enzyme, or both could be managed in the already prepared biosensor. This feature can be very useful when there is a need to control the activity of the biosensor, or to use the biosensor as a switcher. A very suitable instrument for this purpose can be the pH factor. A number of artificial membranes possess different permeability to the substrate with the pH. There can be several reasons of such behavior. Some membranes (especially of the protein nature) have ionogenic groups, which can be responsible for the charge of the membrane. The charge can influence the shrinking of the membrane, thereby, the permeability and thickness can be regulated by the pH. On the other hand, the charged substrate diffusion capability through the charged membrane can also be regulated by the pH. Several cases, including the different enzyme activity and membrane permeability, and sensitivity to the temperature changes, have been modeled. The results of the biosensor action are depicted in Fig. 3. The parameters of the biosensors have been selected so that in the case when the permeability of the membrane does not depend on the pH, the biosensor will operate in the kinetic mode. However, at high and low pH due to low activity of the enzyme, the biosensor action will be switched into the diffusion mode. The response time of the biosensor is mostly sensitive to this switch, and curve 5 of Fig. 3a clearly indicates the boundary regions of both modes of action. The linear range of the biosensor action expressed in the logarithm scale (Fig. 3b) is not the best way to visualize boundary regions; however it is a good method for the analysis of the deep diffusion mode.

Usually a membrane does not possess strongly expressed pH optima (like enzyme), and change its permeability in the wide region of the pH. Suppose, the permeability of the membrane depends linearly on the pH, is in the interval pH 4–8 and increases 9 times. It is a typical case for membranes that can shrink on the pH (Fig. 3a, curve 4). At high pH (pH 8) the permeability of the membrane is high, however the activity of the enzyme is very low (pH optima at 6) and the rate of the substrate supply is almost at the same value as the substrate consumption that leads to the kinetic or boundary mode of action. At pH 6 the activity of the enzyme is top high and the biosensor operates in the

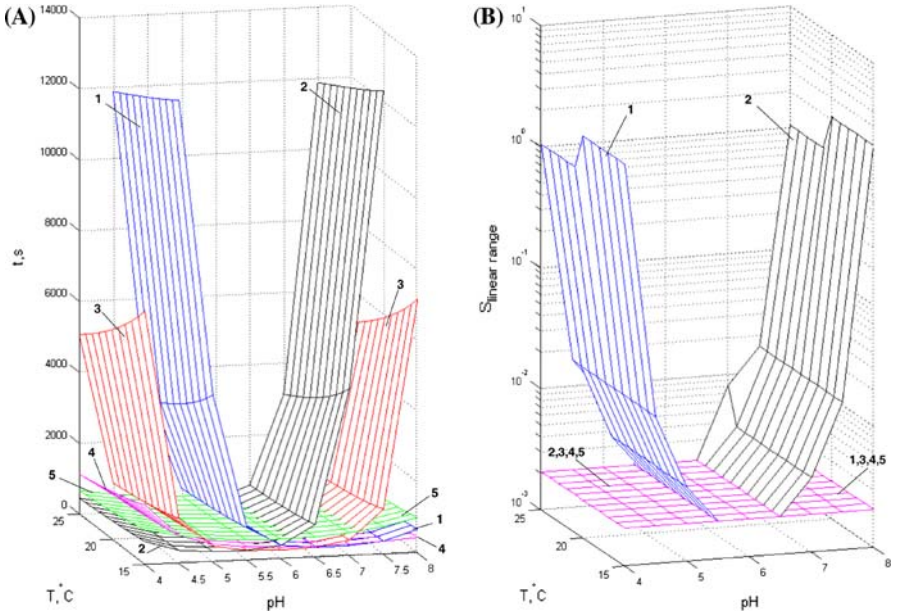

Fig. 3 **a** Dependence of sensor response time on temperature (T) and pH. **b** Dependence of sensor linear range length (logarithmic scale) on temperature and pH. pH optima of the enzyme $pH_V = 6$. 1 – membrane permeability depends on pH with pH optima at 7 ($pH_M = 7$, Eq. 6); 2 – membrane permeability depends on pH with pH optima at 5 ($pH_M = 5$, Eq. 6); 3 – membrane permeability depends on pH with pH optima at 6 ($pH_M = 6$, Eq. 6); 4 – membrane permeability linearly depends on pH (D_{SM}, D_{PM} increases nine times in the region from pH 4 to pH 8, Eq. 7); 5 – membrane permeability does not depend on pH (Eq. 8)

deep kinetic mode possessing relatively fast signal and a short linear range (Fig. 3b) At a lower pH both the enzyme activity and the membrane permeability are low and the biosensor operates again in the boundary or diffusion mode of the action.

Suppose the permeability of the membrane depends on the pH in the same manner as the activity of the enzyme. Such a situation can be observed when both the charge of the membrane and the charge of the substrate (or product) depend on the pH. Let both enzyme activity and membrane permeability dependency on pH be of the same Gaussian manner. Let us consider the situation when the pH optima of the membrane and the enzyme are the same, i.e., equal to pH 6. It means that the isoelectric point of the substrate and the membrane are the same—pH 6. In this case a well expressed diffusion regime of the action is observed (that indicates the increased time of the response (Fig. 3a, curve 3) at low and high pH, and quite a wide region (around pH 6), where the biosensor operates in the kinetic mode of action.

However, the diffusion regime is not very deep, and this could be seen from the negligible increase of the linearity of the biosensor action (Fig. 3b, curves 3–5).

If the pH optima of the enzyme and the membrane are different, a tremendous difference is observed on the biosensor action. When the pH optima of the permeability of the membrane is higher than the enzyme activity optima (Fig. 3, curve 1), the maximal permeability of the membrane is shifted to the region with a higher pH. The kinetic mode of the action is also shifted. In Fig. 3 only the left wing of the

curve is visible. If the pH optimum of the permeability of the membrane is lower than the enzyme (Fig. 3, curve 2), the same picture is observed, but it is shifted into the region with lower pH, and only the right wings of the curves are visible in Fig. 3. Getting deeper into the diffusion mode of the action of the biosensor, the response time is increasing several times, however the linearity of the biosensor is increasing by several magnitudes.

It is to be hoped that these observed dependencies will be useful for the creation of the biosensor monitoring systems.

6 Conclusions

The method of the mathematical modeling of the action modes of the electrochemical biosensors with an outer diffusion membrane was proposed. A set of parameters of the biosensor was selected indicating the boundary mode of the biosensor action. Special attention was paid to the case when the activity of the biocatalytic layer and the permeability of the outer membrane are dependent on the pH. The response time of the biosensor was shown to be sensitive to the mode of the biosensor action especially in the boundary region of the biosensor action. The linearity of the biosensor in the deep diffusion mode can be extended by several magnitudes.

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