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Modeling Trienzyme Biosensor at Internal Diffusion Limitation*

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Abstract. A model of biosensor containing three immobilized enzymes utilizing consecutive substrate conversion in the chain was developed. The modeling was performed at an internal diffusion limitation and a steady-state condition. The calculations showed that significant response of biosensors was produced if diffusion modules were larger than 1 for all enzyme reactions. Due to diffusion limitation the apparent stability of biosensor response increased many times in comparison to stability of the most labile enzyme of the chain.

Keywords: biosensor, modeling, polyenzymes.

1 Introduction

Amperometric biosensors may utilize one, two, three or multi enzymes [1]. The classical example of mono enzyme biosensor might be the biosensor that contains membrane with immobilized glucose oxidase. The glucose oxidase specifically oxidizes glucose to hydrogen peroxide that is determined amperometrically on platinum electrode [1]. The example of successful application of three enzymes might be sensitive to creatinine biosensor [2]. In membrane of this biosensor creatininase (E_1) hydrolyzes creatinine (S) to creatine (P_1). The

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creatine is further hydrolyzed with creatinase (E_2) to sarcosine (P_2). The oxidation of sarcosine with sarcosine oxidase (E_3) produces hydrogen peroxide (P_3) that is determined amperometrically:

$$S \overset{E_1}{\rightarrow} P_1 \overset{E_2}{\rightarrow} P_2 \overset{E_3}{\rightarrow} P_3. \tag{1}$$

The rate of each reaction (V_i) can be characterized by standard enzyme parameters $V_{max(i)}$ and $K_{m(i)}$ where $i = 1, 2$ and 3 for $E_1,\ E_2$ and E_3 catalyzed process respectively. At concentration of $S,\ P_1$ and P_2 less than Michaelis-Menten constants ($K_{m(i)}$), $V_1 = V_{max(1)}s/K_{m(1)}$, $V_2 = V_{max(2)}p_1/K_{m(2)}$, $V_3 = V_{max(3)}p_2/K_{m(3)}$ where s, p_1 and p_2 is a concentration of S, P_1 and P_2.

Two principal regimes, i.e. external and internal limitation, are considering during biosensors modeling [1]. The action of polyenzymes chain under *external diffusion* limitation has been analyzed in [3]. The analysis of the action of the biosensors containing one and two enzymes was performed under *internal diffusion* limitation in [4, 5].

The task of this investigation is to model biosensors containing three immobilized enzymes utilizing consecutive substrate conversion like as a biosensor designed to creatinine determination [2]. The modeling of biosensor action is performed at an internal diffusion limitation and a steady-state condition.

2 Mathematical model

If we restrict ourselves to cases in which the diffusion of compounds in enzyme membrane of biosensor is one-dimension, the diffusion equation for a constant diffusion coefficient and enzymatic conversion takes the form :

$$1/D \cdot \partial s/\partial t = \partial^2 s/\partial x^2 - \alpha_1^2 s, \tag{2}$$

$$1/D \cdot \partial p_1/\partial t = \partial^2 p_1/\partial x^2 + \alpha_1^2 s - \alpha_2^2 p_1, \tag{3}$$

$$1/D \cdot \partial p_2/\partial t = \partial^2 p_2/\partial x^2 + \alpha_2^2 p_1 - \alpha_3^2 p_2, \tag{4}$$

$$1/D \cdot \partial p_3/\partial t = \partial^2 p_3/\partial x^2 + \alpha_3^2 p_2, \tag{5}$$

where t – time, x – dimension, D – diffusion coefficient of all compounds ($3 \cdot 10^{-6}$ cm^2/s), $\alpha_i = (V_{max(i)}/K_{m(i)}D)^{1/2}$.

The solution of (2)–(5) was performed at steady state ($\partial s/\partial t = \partial p_1/\partial t = \partial p_2/\partial t = \partial p_3/\partial t = 0$) with boundary conditions: $s = s_0$, $p_1 = 0$, $p_2 = 0$, $p_3 = 0$ at $x = d$, $\partial s/\partial x = 0$, $\partial p_1/\partial x = 0$, $\partial p_2/\partial x = 0$, $p_3 = 0$ at $x = 0$ where d – membrane thickness (0.01 cm). The biosensor response was calculated:

$$i = 2FD(\partial p_3/\partial x)_{x=0}, \tag{6}$$

where F – Faraday number.

3 Results and discussion

The solving (2)–(5) gives compounds steady-state distribution in enzyme membrane. Calculations show that significant concentration of products in membrane is produced if all diffusion modules ($\alpha_i d$) are larger than 1 (Fig. 1).

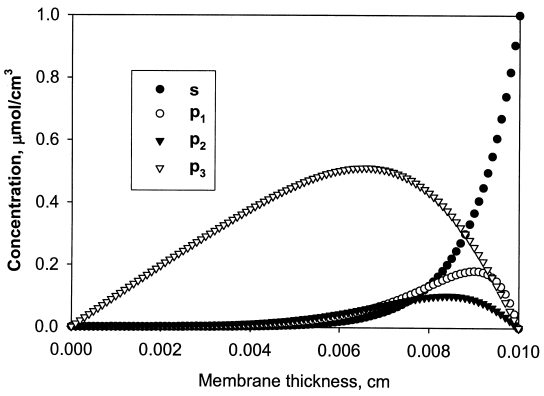

Fig. 1. The profile of compounds concentration in trienzyme membrane. For calculations $s_0 = 1$ μmol/cm^3, $\alpha_1 d = 10.0$, $\alpha_2 d = 10.1$, $\alpha_3 d = 10.3$ and $d = 0.01$ cm was used.

To proof correctness of the calculations the distribution of compounds in membrane was also performed at the boundary condition $\partial p_3/\partial x = 0$ ($x = 0$). In this case the sum of all compounds was equal to s_0 at all x values.

At $\alpha_1 \neq \alpha_2 \neq \alpha_3$ the expression of three enzyme biosensors response is

$$\begin{aligned} i =& 2FD\Big(\Big(\alpha_2^2\alpha_3^2/\big((\alpha_2^2-\alpha_1^2)(\alpha_3^2-\alpha_1^2)\big)\Big)\big(1-\cosh(\alpha_1 d)\big) \\ &- \Big(\alpha_1^2\alpha_3^2/\big((\alpha_2^2-\alpha_1^2)(\alpha_3^2-\alpha_2^2)\big)\Big)\big(1-\cosh(\alpha_2 d)\big) \\ &+ \Big(\alpha_1^2\alpha_2^2/\big((\alpha_3^2-\alpha_1^2)(\alpha_3^2-\alpha_2^2)\big)\Big)\big(1-\cosh(\alpha_3 d)\big)\Big) s_0/d. \end{aligned} \tag{7}$$

It is impossible in practice to achieve equal values of the diffusion modules for all enzymes. Therefore the biosensor response has not been derived at $\alpha_1 = \alpha_2 = \alpha_3$.

The dependence of response of biosensor on diffusion module of the least active enzymes E_1 and E_2 is shown in Fig. 2. It is easy to notice that response is very small still diffusion modules are less than 1. The maximal biosensor response 60 μA/cm^2 is achieved when the diffusion modules are more than 10.

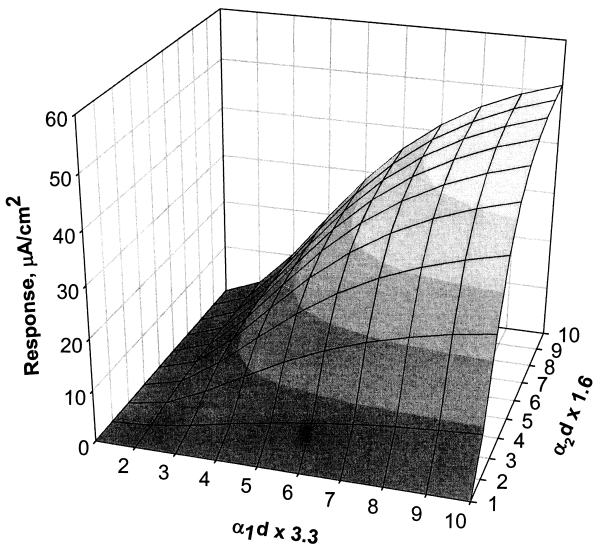

Fig. 2. The dependence of biosensor response on diffusion modules $\alpha_1 d$ and $\alpha_2 d$. For calculations $s_0 = 1$ μmol/cm^3 and $\alpha_3 d = 10.3$ was used.

Low stability of enzymes is a characteristic feature of enzymes. The activity of enzymes decreases during storage or biosensor action. Among three

immobilized enzymes the lowest stability shows creatininase (E_1). The model permits predict sensitivity change of biosensor during the enzyme inactivation. If inactivation follows exponential decay, for example with half-life 2 days, and at beginning biosensor contains large catalytic activity ($\alpha_i d \approx 10$), the response decreases just 34.7% during 10 days (Fig. 3). The apparent half-time of biosensor inactivation increase up to 11.6 days. In fact, this biosensor can be used even longer, i.e. during 15 days with permanent it calibration.

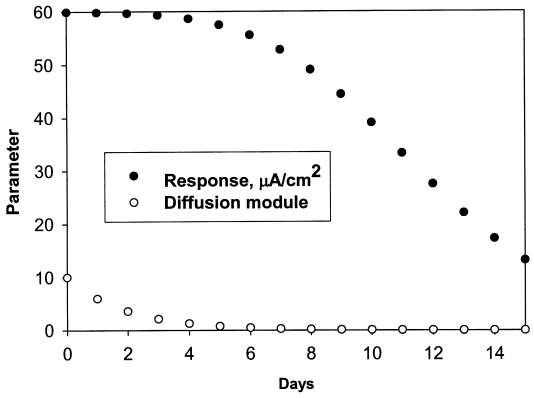

Fig. 3. The change of response and diffusion module of trienzyme biosensor during enzyme inactivation. Life-time of E_1 was 2 days, other parameters as in Fig. 1.

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