INHIBITOR MULTIENZYME BIOSENSOR SYSTEM
INDYNAMICMODE – PHOSPHATE MEASUREMENT

ABSTRACT:
In this paper a multienzyme inhibitor system is investigated. A hybrid inhibitor biosensor for measuring concentration of phosphate is used. Enzyme kinetic of Michaelis-Menten and ping-pong kinetics is accepted. Partial differential equations of that complex system are solved numerically and are received concentration profiles of five reagents. The influence of starting concentration of inhibitor is investigated and influence of reaction rate constant of inhibitor.

KEYWORDS:
mathematical modeling, inhibitor biosensor, simulations, phosphate

INTRODUCTION
Biosensors are analytical devices which tightly combine biorecognition elements and physical transducer for detection of the target compounds. Biosensors useful serve ecological purposes by enabling precision pollutant control [1, 2, 3]. In practice the most important are biosensors that identify water conditions [4, 5, 6, 7, 8] and to a lesser extent air [9, 10] and soil condition [11]. Two main water pollutant are phosphates and fluorides. For determination of phosphate and fluoride ions enzyme, microbial and multienzyme biosensors can be used. Multienzyme biosensors however are very complex devices.

DESCRIPTION OF THE MATHEMATICAL MODEL
The starting concentrations of substrate, co-substrate and inhibitor in the research medium are denoted with S0,C0, I0. The concentration profiles for substrate S(x), co-substrate C(x) and inhibitor I(x) are formed in the active membrane. In this paper a hybrid biosensor with two enzymes acid phosphatase (AP) and glucose oxidase (GOD) is used for the investigation. Operation principle of the hybrid biosensor is based on the given biochemical reaction:

\[
\text{glucose-6-phosphate} + H_2O \rightarrow \text{glucose} + HPO_4^{2-} \quad (1)
\]

under the activity of the enzyme acid phosphatase the glucose-6-phosphate is hydrolyzed to glucose and inorganic phosphate. In the second reaction the oxygen present oxidizes the obtained glucose. The amount of hydrogen peroxide being produced is measured electrochemically. In the presence of phosphate the hydrogen peroxide is produced at a slower rate. This happens because of the inhibitory effect of those element have on the catalytic activity of the acid phosphatase. As a result the glucose production is decreased which leads to more production of H2O2. As the AP is inhibited from the phosphate the substance can be identified with a biosensor according to its ability to support the formation.

The reactions above can be present with following successive enzyme reactions with competitive inhibition:

\[
\begin{align*}
E_1 & \quad S \qquad P_1 + P_2 \\
E_1 & \quad I \qquad \text{EI} \\
E_2 & \quad \text{P1 + C} \quad P_3 + P_4
\end{align*}
\]

AP is the first enzyme, let denote its reaction velocity with \( V_1 \), GOD is the second enzyme let denote its reaction velocity with \( V_2 \); \( P_1 \) - glucose, first product; \( P_2 \) - second product, not informative; \( S \) - glucose-6-phosphate, substrate; \( I \) - (KH2PO4) measured inhibitor, \( C \) - oxygen, co-substrate; \( P_3 \) - product H2O2 and \( P_4 \) - galactonic acid.

We admit that indicatory electrode has symmetrical geometry and assume that diffusion is one-dimensional in space and is described with second Fick’s law than we can write the system of equations for those bi-substrate sensitive amperometric system.
\[
\frac{\partial S}{\partial t} = D_s \frac{\partial^2 S}{\partial x^2} - \frac{V_1 S}{K_S \left[ 1 + \frac{I}{k_{i1}} \right] + S}
\]
\[
\frac{\partial I}{\partial t} = D_s \frac{\partial^2 I}{\partial x^2} - \frac{V_1 S}{K_S \left[ 1 + \frac{I}{k_{i1}} \right] + S}
\]
\[
\frac{\partial C}{\partial t} = D_c \frac{\partial^2 C}{\partial x^2} - \frac{V_2}{K_c + \frac{P_1}{P_1} C}
\]
\[
\frac{\partial P_1}{\partial t} = D_{p1} \frac{\partial^2 P_1}{\partial x^2} + \frac{V_1 S}{K_{p1} \left[ 1 + \frac{I}{k_{i1}} \right] + S} + \frac{K_c}{P_1} C
\]
\[
\frac{\partial P_2}{\partial t} = D_{p2} \frac{\partial^2 P_2}{\partial x^2} + \frac{V_2}{K_{p2} + \frac{K_c C}{P_1}}
\]

where: \(D_s, D_c, D_{p1}, D_{p2}, D_{p3}\) are diffusion coefficients for substrate, co-substrate, product 1 and product 3, \(K_s\) - reaction constant for substrate, \(k_i\) - reaction constant for inhibitor, \(K_c\) - reaction constant for co-substrate, \(K_{p1}\) - reaction constant for product 1, \(K_{p2}\) - reaction constant for product 3. The output current is proportional to gradient of \(H_2O_2\) concentration at the electrode surface

\[
I = nFAD_{p3} \frac{\partial P_3}{\partial x} \big|_{x=d} \text{ [A]}
\]

where: \(n\) is the number of electrons taking part in electrochemical reaction, \(F\) is the Faraday’s number, \(A\) is the electrode surface \([m^2]\).

Let we denote \(x=0\) for the bulk/membrane interface and \(x=d\) for the electrode surface. The action in biosensor starts when some quality of substrate is appears into biological recognition element - active membrane. The initial conditions are:

\[
t=0 \quad S(x,0) = S_0 \quad I(x,0) = I_0 \quad C(x,0) = C_0
\]
\[
P_1(x,0) = 0 \quad P_3(x,0) = 0
\]

Limiting conditions are:

\[
x=0 \quad S(0,t) = S_0 \quad I(0,t) = I_0
\]
\[
C(0,t) = C_0 \quad P_1(0,t) = 0 \quad P_3(0,t) = 0
\]

The substrate, and co-substrate didn’t react with the electrode, oxygen and glucose fully exhausted and medium is well stirred and it remain constant at the electrode surface, then the limiting conditions are:

\[
x=d \quad \frac{\partial S}{\partial x} \big|_{x=d} = 0, \quad C(d,t) = 0 \quad P_1(d,t) = 0
\]
\[
\frac{\partial P_1}{\partial x} \big|_{x=d} = 0, \quad P_3(d,t) = 0
\]

\[\text{(5)}\]

\[\text{(6)}\]

\[\text{(7)}\]

RESULTS AND DISCUSSIONS

For solving system (4) of non-linear partial differential equations (PDE) we use Matlab solver pdepe. It use both finite difference and finite element methods as described [12]. pdepe solve initial-boundary value problems for system of parabolic-elliptic PDEs in the one space variable \(x\) and time \(t\). The ordinary differential equations resulting from discretization in space are integrated to obtain approximate solutions at times specified in a time vector. Time vector specifying the points at which a solution is requested for every value in distance vector. The pdepe function returns values of the solution on a mesh provided in a distance vector. Distance vector specifying the points at which a numerical solution is requested for every value in time vector.

Concentration profiles of substrate, co-substrate, inhibitor, product 1 and product 3

Because oxygen is consumed during enzymatic conversion output current of biosensor is descending function. Parameters used for simulations are \(n = 2\), \(S_0 = 100 \text{mM}, C_0 = 0.25 \text{mM}, I_0 = \text{changed}, P_{o1} = 0.0 \text{mM}, P_{o3} = 0.0 \text{mM}\)

\(A = 7.85.10^{-7} \text{m}^2\) - diameter of cathode is 1mm

\(K_i = 80 \text{mM}\) - reaction rate constant for substrate

\(K_i = 0.5 \text{mM}\) - reaction rate constant for oxygen

\(K_i = 0.1 \text{mM}, K_{p1} = 100 \text{mM}\) - reaction rate constant for inhibitor and products

\(D_s = 2.50.10^{-2} \text{m}^2/\text{s}, D_c = 2.5.10^{-11} \text{m}^2/\text{s}, D_{p1} = 2.50.10^{-9} \text{m}^2/\text{s}, D_{p2} = 2.5.10^{-11} \text{m}^2/\text{s}, D_{p3} = 2.5.10^{-10} \text{m}^2/\text{s},\)

\(d = 60 \mu\text{m}, V_{m1} = 1 \text{mM/s}, V_{m2} = 20 \text{mM/s}\)

At fig. 1, 2, 3, 4, and 5 in three dimensional size are given concentration profiles of substrate \(S(x,t)\), inhibitor \(I(x,t)\), co-substrate \(C(x,t)\), product 1 \(P_1(x,t)\), product 3 \(P_3(x,t)\) in active membrane with thickness \(d = 60 \mu\text{m}\) for the time \(t = 8\text{s}\), for values of reaction velocities \(V_1 = 1 \text{mM/s} \) and \(V_2 = 20 \text{mM/s}\). The value of inhibitor is \(I_0 = 0.0 \text{mM}\) and the value of substrate is \(S_0 = 100 \text{mM}\).

Figure 7 shows the output current \(I\) which is proportional to the concentration of the oxygen. It is seen that oxygen is consumed very rapidly for the case starting concentration \(I_0 = 0\), because there is no inhibitor in the research medium. Hydrogen peroxide (product P3) has value about 0.25 because the oxygen is almost exhausted. The velocity of changing of concentration of co - substrate depends of presence of the inhibitor (eq.3), because now there is no inhibitor oxygen is consumed very rapidly- fig.4.

Fig. 2. Concentration profile of substrate. \(I_0 = 0 \text{ mM}\)
The investigated biosensor is co-substrate sensitive and because of that it is important to analyze of changing of co-substrate C and inhibitor I. At the next pictures are given the dependence of the output current of the biosensor and concentration profiles of substrate, co-substrate, inhibitor and products for the values of I = 1.0 mM.

At fig. 8, 9, 10, 11, and 12 are given concentration profiles of substrate S(x,t), inhibitor I(x,t), co-substrate C(x,t), product 1 P1(x,t), product 3 P3(x,t) for the starting value of inhibitor Io = 5.0 mM.

It is seen clearly how the inhibitor effects over all the reagents. Substrate decreasing very little - from 100 mM to 98 mM, for the difference at figure 2 where...
the decreasing is from 100 mM to 20 mM when there is missing inhibitor in the medium. Consuming of the oxygen is less, product 3 formation is increase (fig. 12) with the time for the difference at fig. 6 where is poorly.

At fig. 7 is given the transient process of the output current for the four values of starting concentration of inhibitor \( \text{Io} = 0, 1, 5 \) and 10 mM. For the bigger starting concentration of \( \text{Io} \) the value of steady state of the current is increasing (this is the value for the time bigger then 7 s), but it is seen that the dependency is non linear. At fig. 13 it is seen more precise, value of \( \text{Io} \) are -0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 mM.

At fig. 14 is invstigated the influence of reaction rate constante for inhibitor \( \text{Ki} = 0.05, 0.1, 0.5, 1, 2, 5 \) mM at the constante starting concentration of \( \text{Io} = 5 \) mM over substrate concentration profile \( S(x,t) \) for \( x=d \). With increasing the \( \text{Ki} \) substrate consentration in active membrane is decreasing.

At fig. 15 is invstigated the influence of reaction rate constante for inhibitor \( \text{Ki} = 0.05, 0.1, 0.5, 1, 2, 5 \) mM for the constante starting concentration of \( \text{Io} = 5 \) mM over the output current. It is seen that transient proceses for the output current strongly depend from \( \text{Ki} \). With increasing the reaction rate constante for inhibitor transient process of the current loosing it first order system form.
CONCLUSION

In the paper is investigated the influence of inhibitor starting concentration over biosensor output current for the hybrid biosensor with two enzymes - acid phosphatase and glucose oxidase in the dynamic mode. Partial differential equations of that complex system are solved numerical and received concentration profiles of five reagents. In the future it will be investigated the influence of enzymes rate over biosensor response and some technical parameters.

REFERENCES


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