JOURNAL OF ENGINEERING annals OF FACULTY OF ENGINEERING HUNEDOARA
Tome V (year 2007), Fascicole 1, (ISSN 1584 – 2665)

MODELING AMPEROMETRIC BIOSENSOR WITH CYCLIC REACTION

Vania RANGELOVA

TECHNICAL UNIVERSITY - SOFIA, BRANCH PLOVDIV, BULGARIA

ABSTRACT
Mathematical model of hybrid biosensor with cyclic reaction has been developed. Biosensor system was amperometric co-substrate sensitive with substrate cyclic reaction. Bio-chemical amplification was due to combination of enzyme and reducing agent. Measuring range was expanded to low values. Enzyme kinetics adopted to be ping-pong . A diffusion limitation and steady state condition were performed. Concentration profiles of substrate, co-substrate reducing agent and medial product were received. Influence of reducing agent and reaction rates over output current were investigated. To solve non-linear system of equations an numerical method is used. Digital simulations were done with Matlab product and finite difference technique for boundary value problems. Concentration profiles for all four reagents are pictured for different values of reducing agent. It was seen that with cyclic reaction current responses is amplificated about hundred times. Ping-pong kinetic fully described all processes and can be used for other biosensors with the same reaction mechanisms. Starting concentration of reducing agent has a significant meaning for receiving more sensitive output but for concentration upper 25mM current response stop changed the system is coming in saturation.
KEY WORDS:
hybrid biosensor, modeling cyclic reaction , catechol.

1.INTRODUCTION

The development of amperometric biosensors provokes a very big interest. This is due to their possibilities and namely – detection of concentration of different substances cheap, selective and highly sensitive. Small devices are needed for medical diagnostics, health care, environmental monitoring , quality control and biosensors can realized that. The areas of interest continually expanded.

Amperometric biosensors based on oxidase enzymes are very popular. During the enzymatic bioconversion oxygen is consumed (for co-substrate mode) or hydrogen peroxide is generated (for product mode). A small, but significant, proportion of the oxygen present in the bulk is consumed by this process and the oxygen electrode measuring the rate of a process. A typical application for this simple type of biosensor is the determination of glucose concentrations by the use of an immobilised glucose oxidase membrane. The reaction results in a reduction of the oxygen concentration as it diffuses through the biocatalytic membrane to the cathode, this being detected by a reduction in the current between the electrodes . Other oxidases may be used in a similar manner for the analysis of their substrates (e.g. alcohol oxidase, D - and L-amino acid oxidases, cholesterol oxidase, galactose oxidase, and urate oxidase) [1,2].

Hybrid biosensors are biosensors with more then one biosensitive material – enzyme, tissue microorganism [3] or other agents [4]. The running processes are more then one, in result is received a biosensor with new properties - improved selectivity and sensitivity for detection of substrates.

Enzyme electrode with a chemically amplified response [5,6] can be performed like a hybrid biosensor. Here has one reaction for enzyme and another for chemical reagent, because those two reactions are in cycle the amplified response is observed. The substrate regeneration cycle established by coupling of oxidase and reducing agents is useful to amplify the output signal of oxygen-detection type enzyme sensors. During this amplification reaction, the amount of consumed oxygen in oxidase reaction exceeded the initial amount of the substrates added. Significantly amplified current response of oxygen electrode modified with oxidase-membrane was obtained when the reducing agents coexist in the sample solution. The concept of this chemical amplification could be introduced to flow-injection analysis (FIA) system consisting with immobilized oxidase reactor, oxygen electrode detector and the carrier containing reducing agents. These chemically amplified biosensors were applied for highly sensitive detection of not only substrates but also enzymatic inhibitors and cell population [7]. For example hybrid biosensor [8] with cyclic reaction gave highly sensitive measurements of catechol. The detection limit was found to be 5.10^{-8}M. The determination of biogenic monoamines is one of the main application of those biosensors. An amperometric system with a chemically amplified response for neurotransmitters and their metabolites is presented in [9]. The principle is the rapid cyclic oxidation of the analyte on the amperometric detector in the presence of the redoxactive enzyme glucose oxidase in the capillary electrophoresis buffer. With this approach, detection limits in the range of 10^{-7}-10^{-8} M could be reached. Because of the good linearity between the current response and the concentration of catecholamines and their metabolites at concentrations up to 300microM, this method is attractive for the analytical detection at low concentration levels such as in biological fluids.

The aim of this paper is to make a model of hybrid biosensor with chemically amplified response and to simulate so that to investigated kinetic and technical parameters over response of the biosensor. Very extensive material for mathematical modeling of amperometric biosensors for co-substrate mode was made by Shulmeister [10]. Some cyclic reactions were given too.

For digital simulations was used Matlab and finite difference technique for boundary value problems.

2. MATHEMATICAL MODEL

For the given example [8] cyclic reaction can be shown like

$$\begin{aligned} S + C &\xrightarrow{K_1} L + P_1 \\ L + R &\xrightarrow{K_2} S + P_2 \end{aligned} \qquad (1)$$

where K_1,K_2 – are reaction rate constants; K_1 is connected with the velocity of enzyme reaction. Let we denote standard kinetics parameters Vm and Km for enzyme tyrosinase. K_2 is connected with velocity of chemical reaction; P_1 – first product; P_2 – second product – dehidroascorbic acid; *S* – measured substrate – catechol; *C* – co-substrate – oxygen; *R* – reducing agent – L- ascorbic acid; *L* – medial product –1,2 benzoquinone.

As a reducing agent is used L- ascorbic acid (L-AA) because it reduce 1,2 benzoquinone to catechol and turn the reaction towards enzyme oxidizing of

catechol in the presence of enzyme tyrosinase. The oxygen consumed with enzyme reaction is not compensating from cyclic reduction of L- ascorbic acid. Therefore if L-ascorbic acid is not influence of enzyme there will be cyclic reaction on substrate. Consuming of dissolved oxygen will continue until its concentration becomes zero. This scheme shows that current is amplificated when L –ascorbic acid presences in the research medium.

The electrode (cathode) is electrochemical active. The active membrane was made from potato tissue in which preliminary have been removed phenols. Biosensor was dip into solution with measuring concentration of substrate So and reducing agent Ro.

We assume that diffusion of reactants in enzyme membrane, with thickness x is one-dimension and diffusion coefficients are constant. In those reactions predominate processes of diffusion and the second Fik's low is in action. Products P_1 and P_2 are not electrochemically. Enzyme kinetics for interaction between substrate and co-substrate adopted to be ping-pong [10]. System of differential equations for the steady – state regime then is

$$\begin{aligned} D_S \frac{d^2[S]}{d\delta^2} &= \frac{V_m}{1+\frac{K_S}{[S]}+\frac{K_C}{[C]}} - K_2[L][R] \\ D_L \frac{d^2[L]}{d\delta^2} &= K_3[L] - \frac{V_m}{1+\frac{K_S}{[S]}+\frac{K_C}{[C]}} \\ D_R \frac{d^2[R]}{d\delta^2} &= K_4[R] \\ D_C \frac{d^2[C]}{d\delta^2} &= \frac{V_m}{1+\frac{K_S}{[S]}+\frac{K_C}{[C]}} \end{aligned} \qquad (2)$$

where: D_S, D_L, D_R, D_C are diffusion coefficients in [m²/s] of substrate with concentration *[S]* in [mM], medial product with concentration *[L]* in [mM], reducing agent with concentration *[R]* in [mM],and co-substrate with concentration *[C]* in [mM], in active membrane with thickness *d*, δ – is the current coordinate, reaction rate for *S* we denote with maximal rate $V_m = K_1[E]$ in [mmol/(l.s)], *[E]* – total enzyme, reaction rate constant K_2 in [mmol/(l.s)], for *L* with K_3 in [mmol/(l.s)] and for *R* with K_4 in [mmol/(l.s)], K_S and K_C - reaction rate constants for substrate and co-substrate.

In the result of enzyme and chemical reaction in the research medium concentration decreasing of oxygen producing decreasing of output current. In steady state regime this diffusion limiting current is given with expression

$$I = nFAD_C \left.\frac{dC}{d\delta}\right|_{\delta=d} \qquad (3)$$

where: *n* is the number of electrons taking part in the electrochemical reaction on the cathode, *F* is Faraday's constant in [As/mmol], *A* is surface of cathode in [m²], I is the output current in [A].

We denote with $\delta = 0$ dimension for the bulk/membrane interface, where the start concentration of substrate co-substrate and reducing agent are

$\delta = 0$

$S(0) = So,\ R(0) = Ro,\ C(0) = Co$ (4)

The oxygen concentration during the reaction continues to decrease nearly to zero because there is the cyclic reaction and at

$\delta = d$ co-substrate is run out and

$C(d) = 0$ (5)

The substrate, medial product and reducing agent didn't react with the electrode, medium is well stirred and they remain constant at the electrode surface then the limiting conditions for them are

$$\left.\frac{dS}{d\delta}\right|_{\delta=d} = 0, \qquad \left.\frac{dL}{d\delta}\right|_{\delta=d} = 0 \qquad \left.\frac{dR}{d\delta}\right|_{\delta=d} = 0 \tag{6}$$

Because in numerical simuling dimensionless coordinates have more advantages we use them. System will be

$$\begin{aligned}
\frac{d^2S}{dx^2} &= \phi^2 \frac{1}{1+\frac{1}{S}+\frac{1}{C}} - m_1\phi^2 LR \\
\frac{d^2L}{dx^2} &= m_2\phi^2\mu_2 K_m L - \phi^2\mu_2 \frac{1}{1+\frac{1}{S}+\frac{1}{C}} \\
\frac{d^2R}{dx^2} &= m_3\phi^2\mu_3 K_m R \\
\frac{d^2C}{dx^2} &= \phi^2\mu_1\rho \frac{1}{1+\frac{1}{S}+\frac{1}{C}}
\end{aligned} \tag{7}$$

where $\phi^2 = (d^2.Vm)/(Ds.Km)$, $m_1 = K_2/Vm$, $m_2 = K_3/Vm$, $m_3 = K_4/Vm$, $\mu_1 = D_S/D_C$, $\mu_2 = D_S/D_L$, $\mu_3 = D_S/D_R$, $\rho = K_m/K_C$, The activity of enzyme in potato tissue membrane is lower then the pure enzyme membrane and for the given example we chouse $\phi = 3$. Numerical solution was evaluated for different values of measuring substrate concentration So, reducing agent concentration *Ro,* reaction rates of enzyme Vm, and of chemicals K_2. For chousing the correct values of parameters were used results of own experiments with plant tissue biosensors [11] and paper sources [12, 13]. We take in mind too that reaction rate for the L-AA in the research medium that pH is *7* (it is optimum for enzyme reaction) is very low [14] and because of that m_1 is chosen 0.001 Diffusion coefficients are chousing nearly equal.

3. RESULTS AND DISCUSSION

Solving numerically system is received the concentration profiles for all 4 reactants - substrate S(x), medial product L(x), co-substrate C(x) and the reducing agent R(x) in enzyme membrane. Following values were used for $m_1 = 0,2$; $m_2 = 0,05$; $m_3 = 0,001$; $K_m = 0,6$mM; $K_C = 0,5$mM; $\mu_1 = 0.8$; $\mu_2 = 0,1$; $\mu_3 = 0,6$; $\rho = 1,2$. Different cases are pictured. Concentration of reagents were dimensionless, for the reducing agent scale is normalized to 1 because the difference in values are very big..

The profiles pictured on firg.1 and 2 for the measured concentration $[S_o]$ = *0.06mM* show that with increasing the Ro the gradient of co-substrate is increasing too and the output current *I* is increased, and we see that system work proper and no-limits seen. Increasing of Ro, increased sensitivity of biosensor and output current from105nA for fig.1 is become to *130nA* for fig.2.

Influence of concentration of reducing agent *Ro* for the interval of 0-25 mM over the output current, is shown to fig.3. It is seen that at some value of *Ro* saturation area is reached. It corresponding to saturation current which is proportional only to maximal rate of enzyme reaction *Vm.* And those value is constant for the biosensor with concentration of given enzyme [E].

Current response is received for the three values of concentartion of redusing agent *Ro* and is pictured to fig.4. One can seen that regime of p-p kinetics corresponding fully to real function given in [8]. The curve is s-shaped too. It is seen that all three curves incline to the same value of output current and than stopped increasing. Explanetion for that reason is given above. We see that the slope of the

curve is decreased with increase of concentration of substrate *S* This indicate that cycling rate of the substrate increasesq but at that time enzyme activity and concentration of redusing agent *R* are constant.

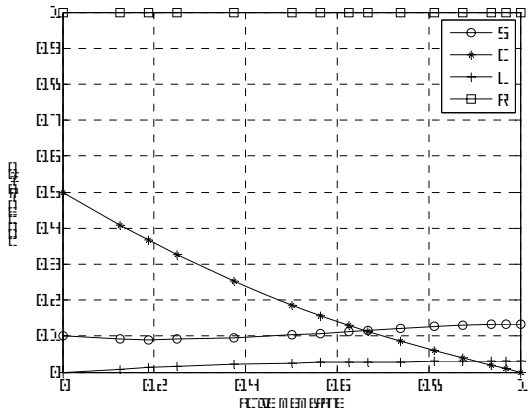

FIG.1. CONCENTRATION PROFILES OF 4 REAGENTS, [R_O] = 10mM, [S_O] = 0.06mM, ϕ = 3.

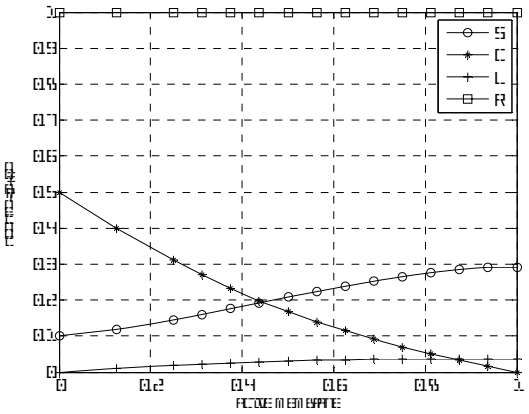

FIG.2. CONCENTRATION PROFILES OF 4 REAGENTS, [R_O] = 15mM, [S_O] = 0.06mM, ϕ = 3.

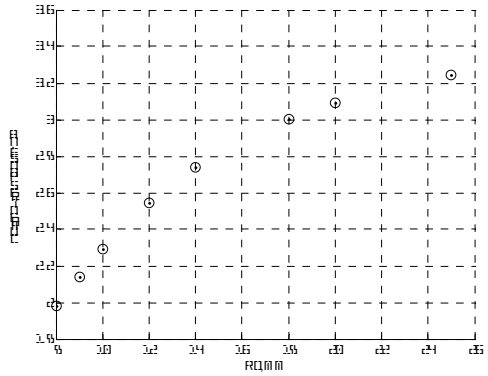

FIG.3. DEPENDENCE OF CURRENT RESPONSE FROM Ro.

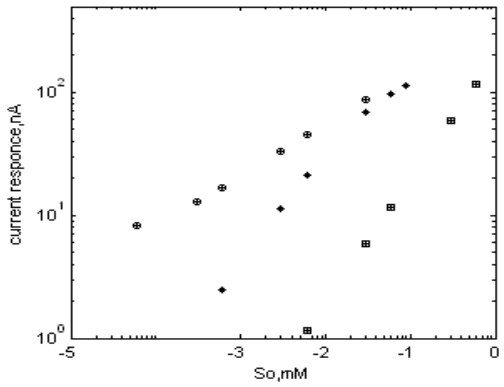

FIG.4. CURRENT RESPONSE FOR [Ro] = 0,10,15mM.

At the next pictures fig.5 is given the influence over the output current of biosensor for the given values of proportion of reaction rates of chemical reaction K_2 and maximal enzyme reaction *Vm.*

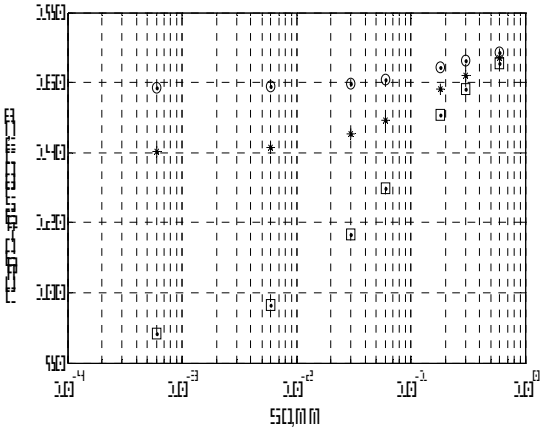

FIG.5. RESPONSE FOR m_1: SQUARE = 0,3; *= 0,5;

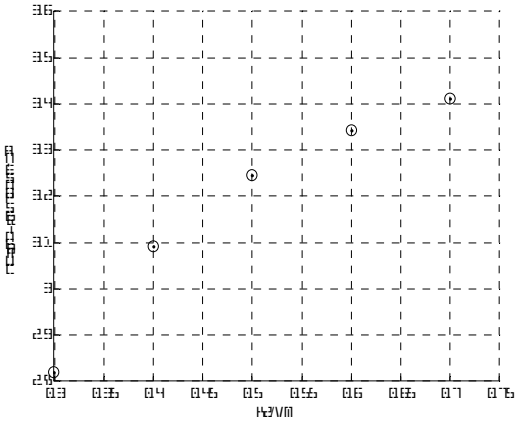

FIG.6. CURRENT RESPONSE FOR K_2/Vm. o = 0.9,

The influence is significant with the decreasing the value of measured substrate and that is in the real state. For *[Ro] = 10mM* the amplification of *I* is *125*. (in [8] it was 120) and for *[Ro] =15mM* amplification of *I* is *525.*

The influence over the output current of proportion of reaction rates of chemicall reaction K_2 and maximal enzyme reaction Vm is given at fig.6 we see that p-p kinetics is proper for the big values of reducing agent and for the low values have significant influence.

4. CONCLUSIONS

A hybrid biosensor model with cyclic reaction and bio-chemical amplification for the steady state regime was investigated. The system of 4 differential equations for pin-pong enzyme kinetics was solved numerically in Matlab medium.

Concentration profiles for all four reagents are pictured for different values of reducing agent. It was seen that with cyclic reaction current responses is amplificated about hundred times. Ping-pong kinetic fully described all processes and can be used for other biosensors with the same reaction mechanisms. Starting concentration of reducing agent has a significant meaning for receiving more sensitive output but for concentration upper 25mM current response stop changed the system is coming in saturation. The same is refer to proportion of reaction rates of two reactions-enzyme and chemical. There also is observe saturation area.

The mathematical investigation of biosensor confirmed the experimental research. Taking in mind the necessity of analytical detection at low concentration levels such as in biological fluids those biosensors will increase their production and developments.

REFERENCES

[1.] Turner John London South Bank University, http://www.lsbu.ac.uk/esbe

[2.] Кацарова П. Методи и алгоритми за диагностика на електрохимични биосензори. Автореферат за получаване на обр. и научна степен "доктор"-2006

[3.] Dubey, R S, Upadhyay, S N: Microorganizm based biosensor for monitoring of microbiologically influenced corrosion caused by fungal species. Indian J. Chem Technol 2003, 10(6), 607-10

[4.] Yao T, Handa S. Electroanalytical properties of aldehyde biosensors with a hybrid-membrane composed of an enzyme film and a redox Os-polymer film. Anal Sci. 19(5):767-70, 2003

[5.] Mizutani F., S. Yabuki, Y.Sato, S. Iijima. Amperometric measurement of ds-DNA content using a peroxidase-modified electrode. *Bioelectrochemistry*; 63:257-9, 2004

[6.] Mizutanui F., S. Yabuki, Y. Sato, S. Iijima Enzyme Electrode response in a solutioncontaining enzyme substrate, Proceedings of the 37th Chemical Sensor Symposium, September 11-12, 2003 Vol. 19, Supplement B, 2003

[7.] Hasebe Yasushi Highly Sensitive Biosensors Using the Oxidase-Amplified Reaction Induced by Reducing Agents *Chemical Sensors* Vol. 14, No. 3, 1998

[8.] Shunichi Uch. et all. Enzyme-based catechol sensor, Anal. Chim. Acta, 276, p.p. 341-345,1993

[9.] Schwarz Maria *Enzyme-catalyzed amperometric oxidation of neurotransmitters in chip-capillary electrophoresis *ELECTROPHORESIS* Volume 25, Issue 12 , Pages 1916 – 1922,2004

[10.] Schulmeister T. Mathematical modeling of the dynamic behaviour of amperometric enzyme electrodes, Selective Electrode Rev. Vol.12, pp 203-260,1990

[11.] Rangelova V., St. Gospodinov, A. Pandelova, Hybrid biosensor with bio-chemical amplification, "Ecological engineering and environment protection". vol.3 p.p.44-48,2003

[12.] Rodriguez-Lopez J., J. Ros, R. VARON, F. Garsia-Canova. Oxygen Michaelis constants for tyrosinase. *Biocliem. J.* 293, 859-866,1993.

[13.] Bru, R.; Sanchez-Ferrer, A.; Garcia-Carmona, F. Characteristics of tyrosinase in AOT-isooctane reverse micelles; *Biotechnol. Bioeng.* 34, 304-308, 1989

[14.] Moya, Horacio D. and Coichev, Nina Kinetic studies of the oxidation of L-ascorbic acid by tris(oxalate)cobaltate in the presence of CDTA metal ion complexes. *J. Braz. Chem. Soc.*, vol.17, no.2, p.364-368.,2006