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AMPEROMETRIC BIOSENSOR WITH CYCLIC REACTION IN DYNAMIC MODE

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ABSTRACT
In the present paper is investigated a mathematical model of hybrid biosensor with cyclic reaction in dynamic mode. Those models have been present in other issue of the journal in steady state mode. Biosensor system is amperometric co-substrate sensitive with substrate cyclic reaction. Bio-chemical amplification is due to combination of enzyme and reducing agent. Enzyme kinetics adopted to be ping-pong. Concentration profiles of substrate, co-substrate reducing agent and medial product have been received. Influence of reducing agent, enzymatic rate, membrane thickness; over output current have been investigated. For solving system of non-linear partial differential equations (PDE) is used Matlab solver *pdepe*. It use both finite difference and finite element methods. *pdepe solve* initial-boundary value problems for system of parabolic-elliptic PDEs in the one space variable x and time t.
KEY WORDS: hybrid biosensor, modeling cyclic reaction, catechol, dynamic mode.

1. INTRODUCTION

Tissue biosensors are very complex devices, because they use biological recognition element such plant or animal tissue in which flows very specific processes. Mathematical modeling is the way to understand their behavior. Generally biosensors work in steady state mode. Decay time of transient process is long (for tissie biosensors 3 to 5 min) and therefore dynamic measurements can be used. A comprehensive study of the mathematical modeling of amperometric biosensors is given in [1]. Recently Baronas et all are developed a mathematical model of amperometric product-sensitive biosensors [2]. The model is based on non-stationary diffusion equations containing a non-linear term related to Michaelis-Menten kinetics of the enzymatic reaction [3]. The same authors in another paper examine the dynamic response of amperometric biosensor in stirred and non-stirred solutions.

Hybrid biosensors are biosensors with more then one biosensitive material – enzyme, tissue microorganism [4] or other agents [5]. 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 [6,7] 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 [8]. For example hybrid biosensor [9] 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 applications of those biosensors. An amperometric system with a chemically amplified response for neurotransmitters and their metabolites is presented in [10]. 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 goal of this paper is to simulate the same model of biosensor with cyclic reaction that have been investigated previously in steady state mode in [11]. The hybrid biosensor is with chemically amplified response.

2. DESCRIPTION OF THE MATHEMATICAL MODEL

The active membrane is made from potato tissue in which preliminary are removed phenols. x –is the current coordinate. Biosensor is dip into solution with concentration of substrate So / catechol / and there is added the reducing agent Ro / L – ascorbic acid /, which will turn the cyclic reaction. In the active membrane whish is like enzyme reactor, the measuring substrate is converted into product. Oxygen which is necessary for the running of the enzyme catalyzing reaction ensured from the air. The rest of the oxygen is passing through the gas-permeable membrane and by depolarizing the cathode is resulted an output current I. That current is measured with oxygen-meater. At fig.1 is given the scheme of cyclic reaction which is flowing in the medium.

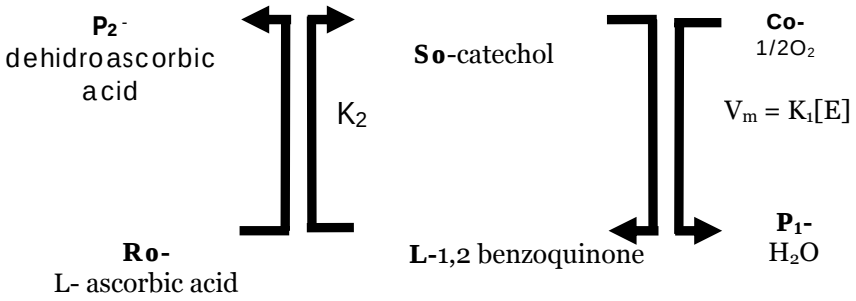

Figure 1. Cyclic reaction in the research medium

where K_1,K_2 – are reaction rate constants; $\boldsymbol{K_1}$ is connected with the velocity of enzyme reaction. $\boldsymbol{K_2}$ is connected with velocity of chemical reaction; $\boldsymbol{P_1}$ – first product; $\boldsymbol{P_2}$ – 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 because it reduce 1,2 benzoquinone to catechol and turn the reaction towards enzyme oxidizing of catechol in the presence of enzyme tirozinaze [12]. 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 while concentration of substrate is run out, after some cycles. This scheme shows that current is amplificated when L – ascorbic acid presences in the research medium. The reactions can be explain with following equations:

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

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. System of differential equations for dynamic regime then is

$$\frac{\partial S}{\partial t} = D_S \frac{\partial^2 S}{\partial x^2} - \frac{V_m}{1 + \frac{Ks}{S} + \frac{Kc}{C}} + K_2 .L.R \; ; \; \frac{\partial L}{\partial t} = D_L \frac{\partial^2 L}{\partial x^2} - K_3 .L + \frac{V_m}{1 + \frac{Ks}{S} + \frac{Kc}{C}} \qquad (2)$$

$$\frac{\partial R}{\partial t} = D_R \frac{\partial^2 R}{\partial x^2} - K_4 R \; ; \; \frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} - \frac{V_m}{1 + \frac{Ks}{S} + \frac{Kc}{C}}$$

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, active membrane is with thickness **d,** x – is the current coordinate, reaction rate for S we denote with maximal rate Vm = K_1[E] in mM/s E – total enzyme, reaction rate constant K_2 in mM, for L with K_3 in mM and for R with K_4 in mM, K_S and K_C - reaction rate constants for substrate and co-substrate in mM. The output current is proportional to gradient of co-substrate concentration at the electrode surface

$$I = nFAD_C \frac{\partial C}{\partial x}\Big|_{x=d} \quad , \text{[A]} \qquad (3)$$

where: n is the number of electrons taking part in electrochemical reaction, F = 96485 C/mol is the Faraday's number, A is the electrode surface [m²].

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

$$t = 0 \qquad S(x,0) = So \qquad L(x,0) = 0 \qquad R(x,0) = Ro \qquad C(x,0) = Co \tag{4}$$

Limiting conditions are

$$x = 0 \qquad S(0,t) = So \qquad L(0,t) = 0 \qquad R(0,t) = Ro \qquad C(0,t) = Co \tag{5}$$

The substrate, and co-sumstrate didn't react with the electrode. Consuming of dissolved oxygen will continue while concentration of substrate is run out, after some cycles. Oxygen is fully exhausted and medium is well stirred and it remain constant at the electrode surface, then the limiting conditions are

$$x = d \qquad S(d,t) = 0 \qquad C(d,t) = 0 \tag{6}$$

$$\left.\frac{\partial S}{\partial x}\right|_{x=d} = 0, \qquad \left.\frac{\partial L}{\partial x}\right|_{x=d} = 0, \qquad \left.\frac{\partial R}{\partial x}\right|_{x=d} = 0 \tag{7}$$

3. RESULTS AND DISCUSSION

For solving system (2) of non-linear partial differential equations (PDE) we use Matlab solver *pdepe*. It use both finite difference and finite element methods as described in [13]. *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.

Because oxygen is consumed during enzymatic conversion output current of biosensor is descending function and we normalized and centered it that to see it at the pictures increasing functions of substrate concentration. Parameters used for simulations are

n = 4 , So =0,5mM, Lo = 0, Ro = 10mM,
Co = 0,27mM
F = 96,5A.s / mmol - Faraday's number
A = 7,85.10-7 m2 - diameter of cathode is 1mm
K_s = 0,65mM - reaction constant for substrate
K_c = 0,5 mM - reaction constant for oxygen

K_2 = 0,15 mM, K_3 = 0,05 mM, K_4 = 0,005 mM
D_S =1,0. 10^{-9} m²/s, D_C =1,0. 10^{-10} m²/s, D_L =1,0. 10^{-9} m²/s, D_R =1,0.10^{-9} m²/s,
d = 70 μm
Vm = 0,28 mM/s

4. CONCENTRATION PROFILES OF THE FOUR REAGENTS – S, L, R, C

At fig.2,3,4 and 5 in three dimensional size are given concentration profiles of the four reagents – substrate S(x,t), medial product L(x,t), reducing agent R(x,t) and co-substrate C(x,t) in active membrane with thickness d = 70μm for the time t = 10s. The system is running for the case when cycle of substrate is go on. It is seen that concentration of S is increase, which is due to the presence of reducing agent R in the research medium. The consuming of oxygen increases too.

Consuming of dissolved oxygen will continue while concentration of substrate is run out, after some cycles /not given to the figures that case/. The reducing agent R is act in medium with pH = 7, this is optimum for enzyme reaction and because of that its rate is very low. From the picture is seen that its concentration change very little / diffusion coefficients for R is chosen to be K_4 = 0.005/.

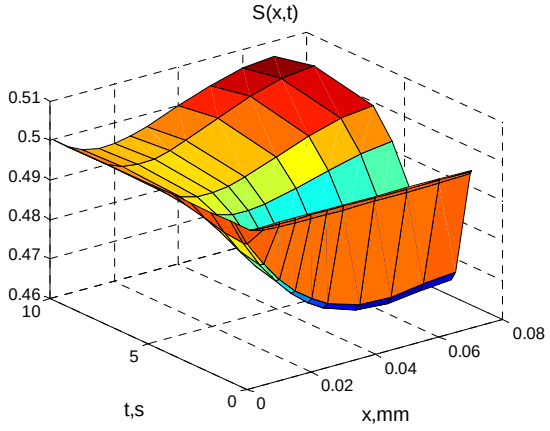

Figure 2

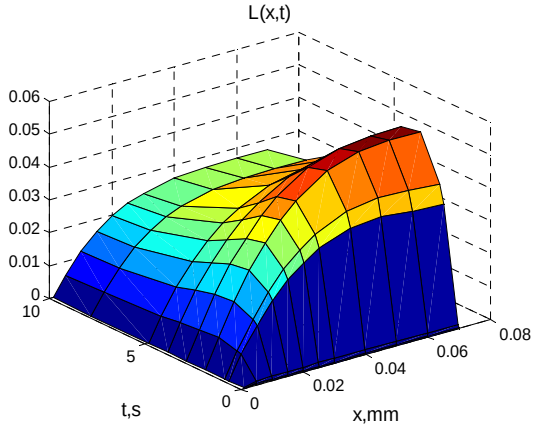

Figure 3

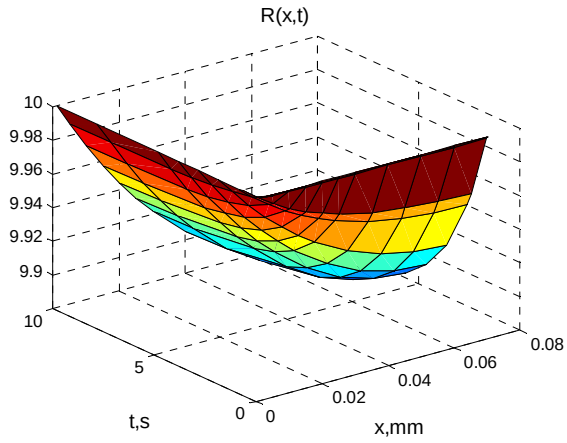

Figure 4

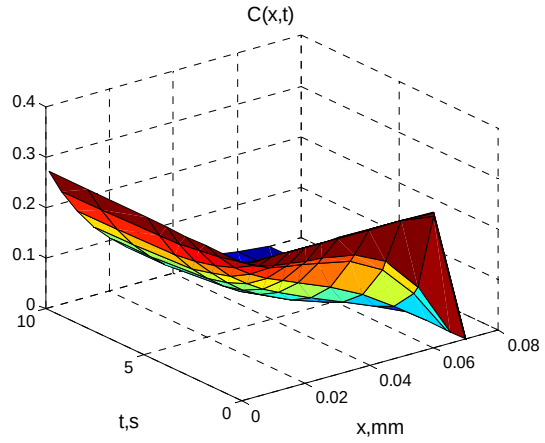

Figure 5

Concentration profiles of substrate S(x,t), medial product L(x,t), reducing agent R(x,t) and co-substrate C(x,t)

5. INFLUENCE OF MEMBRANE THICKNESS – d

We study the dynamic effect of membrane thickness - d [μm] over output current. At fig.6 is given output current of biosensors with different thickness of active membrane d: 10, 30, 70, 150 and 300μm. For the thinner membranes (10μm, 30μm) biosensor response increases with increasing the thickness, but for the thicker is opposite biosensor response decreases with increasing the thickness (70μm, 150μm, 300 μm). In order to see variation of current for the small values of time we use logarithmic scale – fig.7 and one can see that thick membrane has delay and it increases with increasing the thickness of active membrane. Response time is less than 0.5 s for thinner membranes and about 5 s for medial membranes (70-100 μm) and bigger for the very thick.

For the very thick membranes / d = 300μm /, it is start to appear processes of limiting of substrate in the face layer of membrane and cosubstrate which reach to the indicatory electrode did't change his concentration significantly and because of that and output current didn't change very much, which is seen from the fig.6.

It is known that membrane thickness is one of the main technical parameters and most impotant for the detremination of $\Phi^2 = (Vm / Ks).(d^2 / Ds)$ - so called the Thile's module. When the biosensor system is constructed with very thick active membrane then the measured substrate is consumed in the face layer of membrane. Thile's module Φ will be very big, because it is proportional to the thickness d. Values of S(x,t) and C(x,t) in the area of electrode will be near zero. But in our case the situation is different because there has cyclic reaction. And concentrations profiles depends significantly of membrane thickness. For that we made some simulations and show results for the two types of membrans.

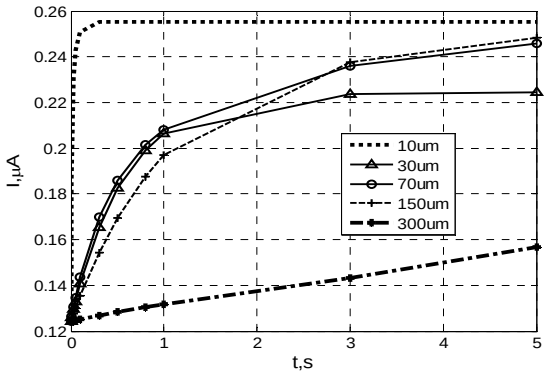

Figure 6. *Influence of membrane thickness - d.*

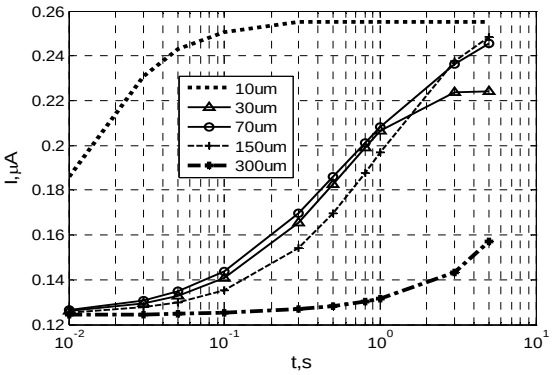

Figure 7. *Influence of membrane thickness - d.*

If membrane thickness is bigger and equal to 150 μm the concentration profiles of substrate S(x,t), medial product L(x,t), reducing agent R(x,t) and co-substrate C(x,t), are like those shown to fig 8,9,10 and 11. S(x,t) is increase, L(x,t) has maximum, R and C decreases.

If membrane thickness is vey small and equal to 10 μm the concentration profiles of substrate S(x,t), medial product L(x,t), reducing agent R(x,t) and co-substrate C(x,t), are like those shown to fig 12,13,14 and15. It is seen that concentrations of S and R decreases very litlle, the medial product and C significantly.

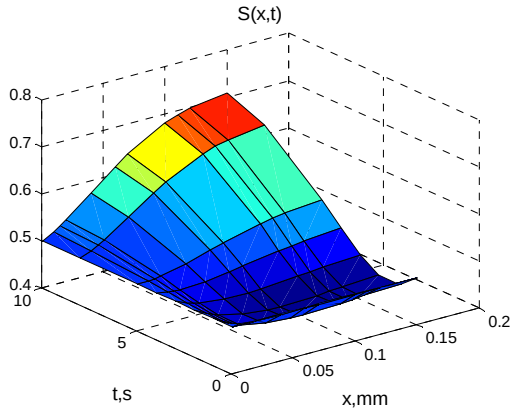

Figure 8

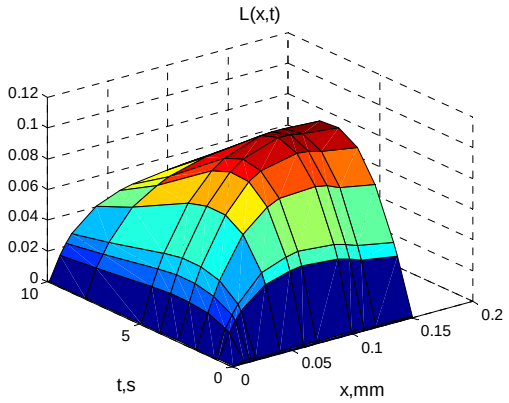

Figure 9

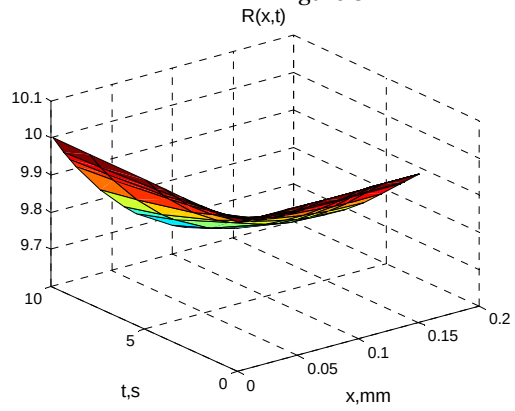

Figure 10

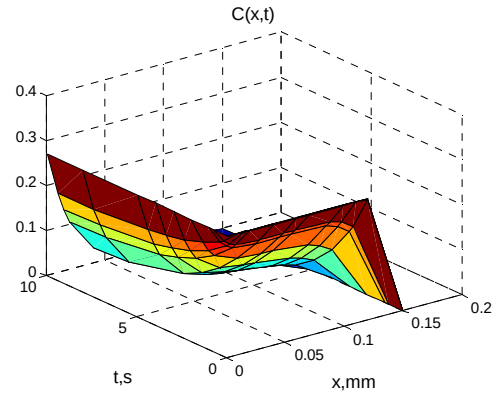

Figure 11

Concentration profiles of substrate S(x,t), medial product L(x,t), reducing agent R(x,t) and co-substrate C(x,t)

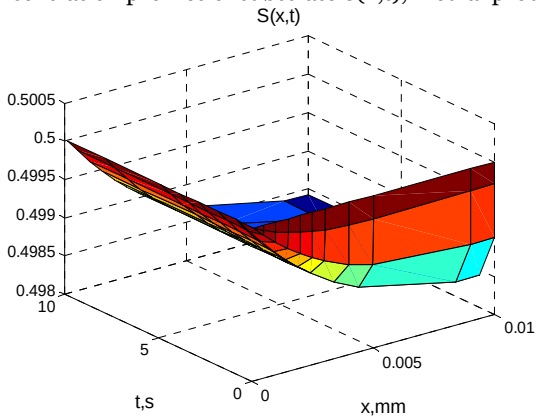

Figure 12

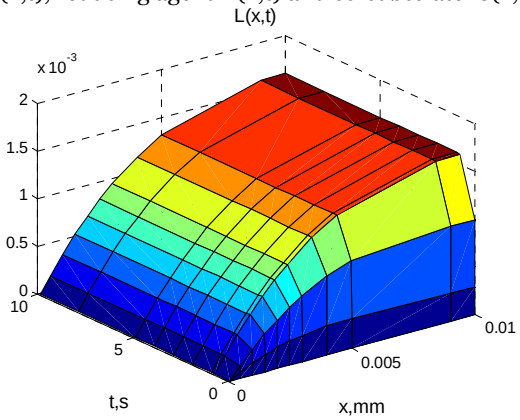

Figure 13

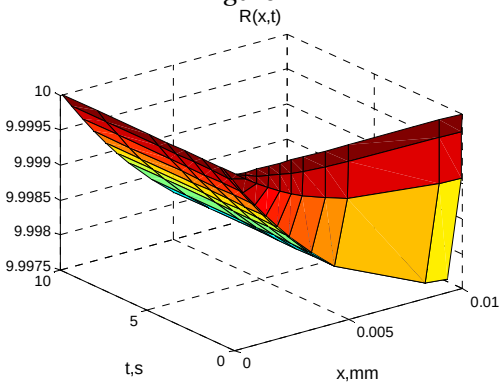

Figure 14

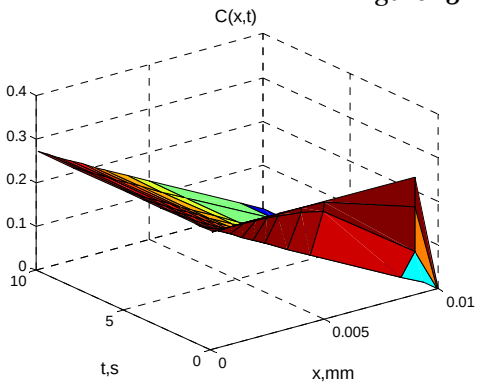

Figure 15

Concentration profiles of substrate S(x,t), medial product L(x,t), reducing agent R(x,t) and co-substrate C(x,t)

6. INFLUENCE OF CONCENTRATION OF REDUCING AGENT *Ro*

Influence of concentration of reducing agent *Ro* for the interval of 0 - 30 mM over the output current, is shown to fig.16. It is seen that at some value of *Ro* saturation area is reached. It is corresponding to saturation current which is proportional only to maximal rate of enzyme reaction *Vm*. And that value is constant for the biosensor with concentration of given enzyme [E].

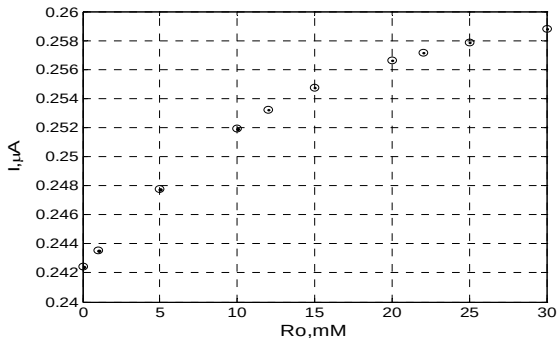

Figure 16. Influence of reducing agent Ro

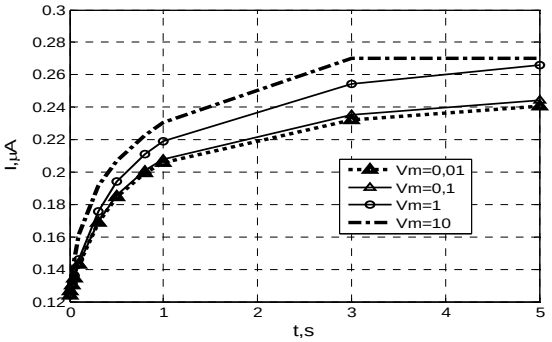

Figure17. Influence of enzymatic rate - Vm

7. INFLUENCE OF ENZYMATIC RATE – Vm

Fig. 17 show the response of biosensor for a different values of enzymatic rate – Vm[mM/s]. When we use different tissues (banana, potato, mushroom) with the same thickness of active membrane the rate is different. The rate is different and when we use the same tissue but with different thickness of membrane. It is seen that the maximal output current which corresponds to steady state current increase with increasing of Vm, because the enzymatic rate is directly related with enzyme concentration inactive membrane. The transient process is faster for the bigger values of Vm, because the bigger amount of enzyme has in membrane.

8. CONCLUSION

Numerical simulation of that tissue co-substrate sensitive amperometric biosensor system in dynamic mode shows that processes in the membrane are very complex. Response time like in other biosensors decreases for the thinner membranes and for the thick is much more. Cyclic reaction lead to amplification and a bigger output current is observed. The amplification depends from the starting concentration of reducing agent R.

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