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INFLUENCE OF PARAMETERS OVER BIOSENSORS RESPONSE IN DYNAMIC MODE AT DIFFERENT ENZYME KINETIC

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ABSTRACT
In the paper is investigated amperometric biosensors response for the three basic type of enzyme kinetic – first order, Michaelis – Menten, and ping - pong. The models are described in non stationary diffusion conditions. System of differential equations is solved numerically in MATLAB medium. For putting in action of solving machine is used parameters developed in other works. In the 3D vision are given substrate and co-substrate concentration changing in active membrane. The influence of diffusion parameters – diffusion coefficients and active membrane thickness and kinetic parameters – reaction rate and reaction constants of biosensors over output current are investigated. Form the received results is seen very interesting effects.
KEY WORDS:
biosensor, mathematical modeling, dynamic mode, enzyme kinetic.

1. INTRODUCTION

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). The efficient reduction of oxygen at the surface of the cathode causes the oxygen concentration there to be near zero. The rate of this electrochemical reduction depends on the rate of diffusion of the oxygen from the bulk solution, which is dependent on the concentration gradient and hence the bulk oxygen concentration. 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.

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.

The biosensors are very complex devices, because they use biological recognition element such us pure enzyme, 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 tissue 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 al 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.

The goal of this paper is to simulate a model in dynamic mode of three type amperometric co-substrate-sensitive biosensors and investigate the influence of the diffusion and kinetic parameters on the response of the biosensor. Enzyme kinetics are: **first orde**r, Michaelis – Menten (**M - M**), and ping – pong (**p - p**). Digital simulations are done with Matlab solver *pdepe*, system of non-linear partial differential equations are coded in advance.

2. PRESENTATION OF MATHEMATICAL MODELS

The investigated biosensors are amperometric biosensors based on oxygen electrode [5]. In the active membrane flows an enzyme catalyzed reaction which can be – first order

$$E + S \rightleftharpoons \{ES\} \longrightarrow E + P$$

We use that model when measured concentrations are very little and the velocity of reaction is proportional directly to substrate. For Michaelis – Menten

$$E + S \rightleftharpoons \{ES\} \longrightarrow E + P$$

We use that model for typical enzyme reaction with non-linear enzyme kinetic. When enzyme has a strong affinity to substrate, co-substrate taking part in enzyme reaction limiting the reaction and for that we use ping – pong kinetic

$$Eo + S \rightleftharpoons \{EoS\} \longrightarrow Er + P_1$$

$$Er + C \rightleftharpoons \{ErC\} \longrightarrow Eo + P_2$$

It is seen that here both substrate and co- substrate taka part in enzyme reaction. We will investigate biosensors systems only in active membrane, because it is known that concentration of substrate *S(x)* and co – substrate *C(x)* in other two membranes are changed linear. Biosensors are operated under diffusion control. We admit that electrode has symmetrical geometry. and enzyme is homogeneous distributed in active membrane. We assume that diffusion is one-dimensional in space and is described with second Fick's law. The equation described those amperometric systems in dynamic mode is

$$\frac{\partial Q}{\partial t} = D_Q \frac{\partial^2 Q}{\partial x^2} + R_Q \tag{1}$$

Where Q is the concentration of any species involved in enzyme or electrochemical reactions, D_Q is the corresponding diffusion constant for the active membrane and R_Q is the term related with enzyme kinetic.

For the firs order kinetic R_Q is

$$R_Q = \frac{Vm}{K_S} S \tag{2}$$

for Michaelis – Menten is

$$R_Q = \frac{VmS}{K_S + S} \tag{3}$$

and for the ping – pong is

$$R_Q = \frac{Vm}{1 + \frac{K_S}{S} + \frac{Kc}{C}}, \tag{4}$$

where: Ks - reaction rate constant for substrate; Kc - for co-substrate; Vm – maximal velocity of enzyme reaction - enzymatic rate.

The output current is proportional to gradient of co-substrate concentration at the electrode surface

$$I = nFADc\frac{\partial C}{\partial x}\Big|_{x=d} \quad , \text{ [A]} \tag{5}$$

where: n = 4, is the number of electrons taking part in electrochemical reaction, F = 96485 C/mol is the Faraday's number, A = 7,85.10^{-7} m^2, because the diameter of cathode is 1mm is the electrode surface [m^2].

Taking in mind all that the dynamic mode for the thee type of biosensors will be described with the following system of partial differential equations – for the firs order kinetic

$$\frac{\partial S}{\partial t} = Ds\frac{\partial^2 S}{\partial x^2} - \frac{VmS}{K_S} \qquad \frac{\partial C}{\partial t} = Dc\frac{\partial^2 C}{\partial x^2} - \frac{VmS}{K_S} \tag{6}$$

for Michaelis – Menten

$$\frac{\partial S}{\partial t} = Ds\frac{\partial^2 S}{\partial x^2} - \frac{VmS}{K_S + S} \qquad \frac{\partial C}{\partial t} = Dc\frac{\partial^2 C}{\partial x^2} - \frac{VmS}{K_S + Vm} \tag{7}$$

and for the ping – pong

$$\frac{\partial S}{\partial t} = Ds\frac{\partial^2 S}{\partial x^2} - \frac{Vm}{1 + \frac{Ks}{S} + \frac{Kc}{C}} \qquad \frac{\partial C}{\partial t} = Dc\frac{\partial^2 C}{\partial x^2} - \frac{Vm}{1 + \frac{Ks}{S} + \frac{Kc}{C}} \tag{8}$$

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

$t = 0$ $\quad S(x,0) = So$
$\quad C(x,0) = Co$

Limiting conditions are:

$x = 0$ $\quad S(0,t) = So,$
$\quad C(0,t) = Co$

The substrate, didn't react with the electrode, medium is well stirred and it remain constant at the electrode surface, then the limiting conditions are

$x = d$

$$\frac{\partial S}{\partial x}\Big|_{x=d} = 0,$$

The Thiele module is known [] and it is

$$\Phi^2 = \frac{d^2}{D_S}.\frac{Vm}{K_S} \text{ ,} \tag{9}$$

where ***d*** is the thickness of active membrane. It is seen that Thiele module depends from **diffusion** parameters – diffusion coefficients – *Ds* and active membrane thickness - d and **kinetic** parameters – reaction rate *Vm* and reaction constants *Ks.* We will investigate those parameters of biosensors for the three type of enzyme kinetic over output current in dynamic mode.

3. RESULTS FROM DIGITAL SIMULATIONS

For solving systems (6,7,8) of non-linear partial differential equations (PDE) we use Matlab solver ***pdepe.*** It use both finite difference and finite element methods as

described in [6]. The ***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 taken from [7], where the real tissue biosensor have been investigated. At fig.1, 2 and 3 in three dimensional size are given concentration profiles of substrate S(x,t) and co-substrate C(x,t)] in active membrane.

The following values of parameters are used:

$So = 0{,}65mM$, $Co = 0{,}27mM$ $\qquad$ $Vm = 0.28mM/s$

$Kc = 0{,}5\ mM$, $Ks = 0{,}65mM$ $\qquad$ $Ds = 1.10^{-9}m^2/s$, $Dc = 2{,}5.10^{-9}m^2/s$.

$d = 70\mu m$

The maximal time is $t = 5s$. One can see that for the first order kinetic the dynamic range is bigest – 38%, for the p – p is lowest – 15%. We can tell that the participation of co-substrate in enzyme kinetic leads to limiting of dynamic of concentartion profiles of substrate.

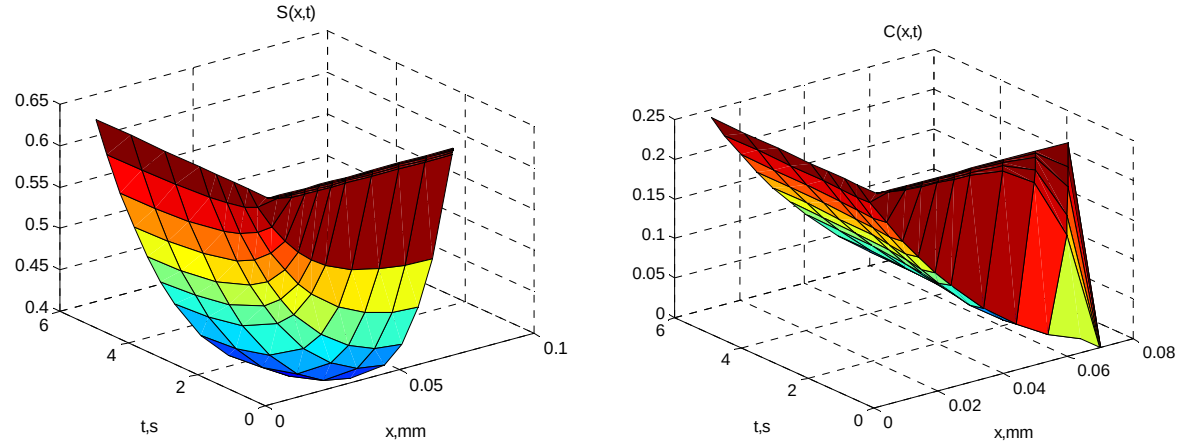

FIG.1. First order enzyme kinetic.

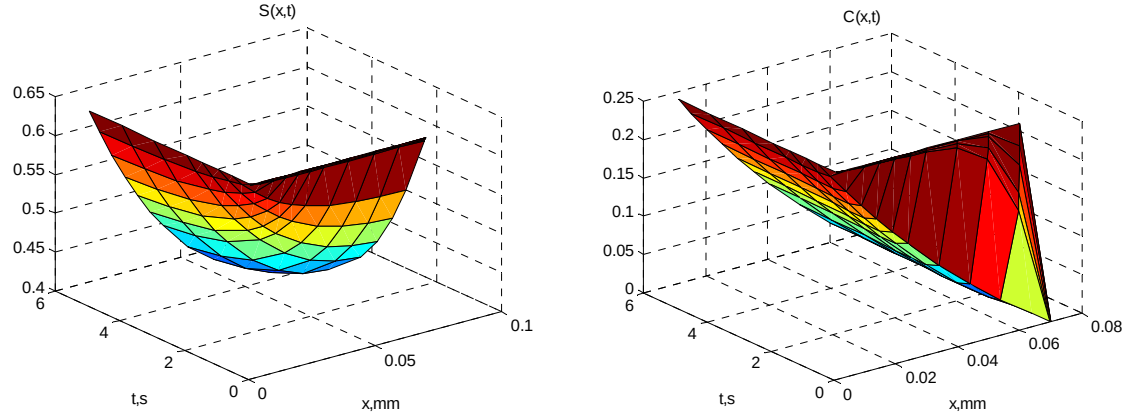

FIG.2. MICHAELIS – MENTEN ENZYME KINETIC.

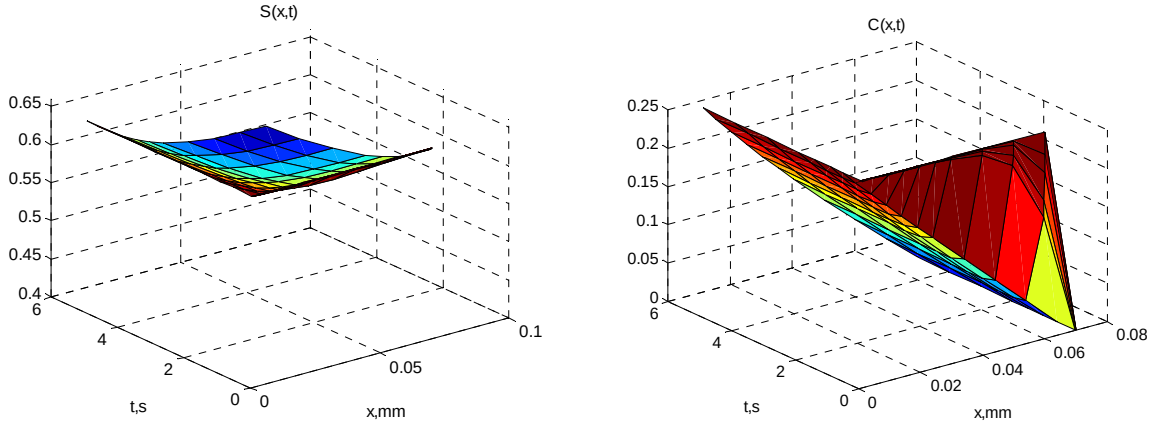

FIG.3. PING – PONG ENZYME KINETIC.

4. INFLUENCE OF KINETIC PARAMETERS

4.1. INFLUENCE OF ENZYMATIC RATE - Vm

Fig. 4, 5 and 6 shows the response of biosensors for different values of enzymatic rate – Vm [mM/s].

The following values of parameters are used:

So =0.65mM, Co = 0,27mM *Vm = 0,02; 02; 2,0; 10,0; and 20,0, mM/s*

Kc = 0,5 mM , Ks = 0.65mM *Ds = 1.10^{-9}m^2/s, Dc = 2,5.10^{-9}m^2/s .*

d = 70μm

Enzymatic rate depends on type of the enzyme and enzyme concentration. For the given thickness of active membrane and given enzyme Vm is constant. When we use different tissues (banana, potato, mushroom) with the same active membrane thickness the rate is different and when we use the same tissue but with different thickness of membrane again the rate is diffrent.

The calculated values of Thiele modules to coresponding values of Vm are - 0,14; 1,49; 15,05; 75,34; 150,53. For $\square^2 < 1$ the biosensors act in regime of limiting kinetic, for $\square^2 > 1$ biosensors act in diffusion regime. From figures we can see that in kinetic regime for the first order and for the M – M kinetic output current of the biosensors has pseudo- oscilations, but for the p – p kinetic transient proces is just asymptotic.

Form fig.6 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 in active membrane. The transient process is faster for the bigger values of Vm, because the bigger amount of enzyme has in membrane.

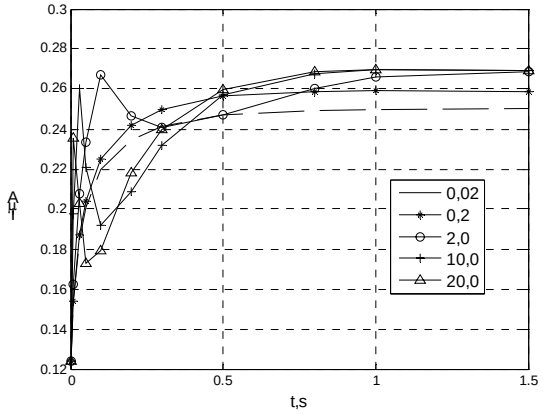

FIG.4. FOR THE1 - ORDER KINETIC.

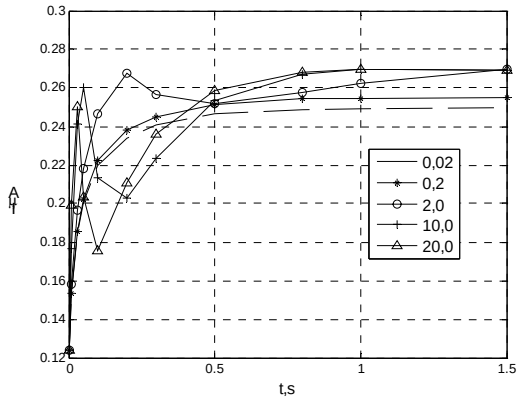

FIG.5. FOR THE M – M KINETIC.

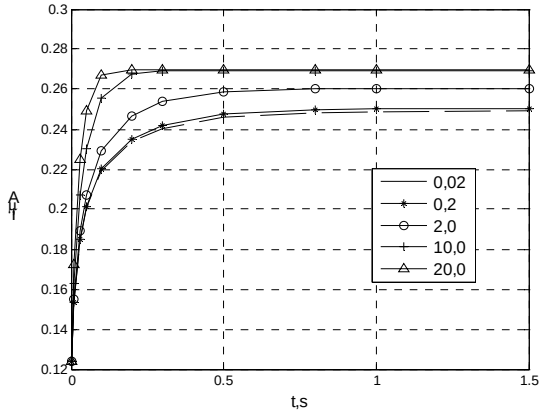

FIG.6. FOR THE P – P KINETIC.

4.2. INFLUENCE OF REACTION RATE CONSTANT – Ks

Reaction rate constant for substrate Ks [mM] is undependable from enzyme concentration and characterized enzyme. Different tissues have different enzymes and reaction rate constant for the given tissue is permanent. Low value of K mean strong affinity between enzyme and substrate High value mean poor affinity.

We investigate biosensor response for a wide range forom 0,05mM to 5,0 mM.

The following values of parameters are used:

So =0,65mM, Co = 0,27mM Vm = 2 mM/s

Kc = 0,5 , Ks = changed Ds = $1.10^{-9}m^2/s$, Dc = $2,5.10^{-9}m^2/s$.

d = 70μm

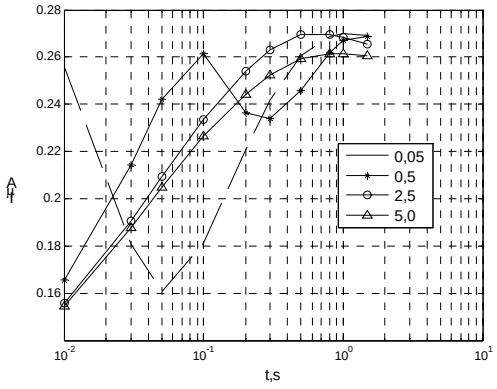

FIG.7 FOR THE 1 - ORDER KINETIC.

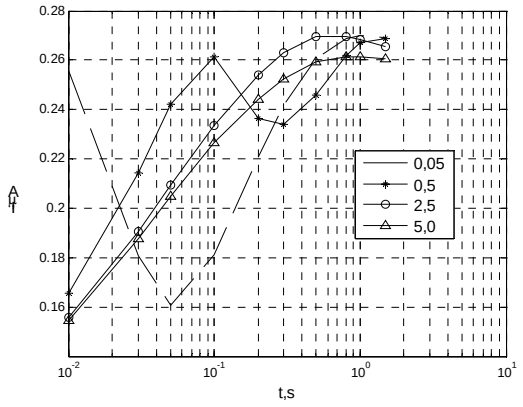

FIG. 8.FOR THER M – M KINETIC.

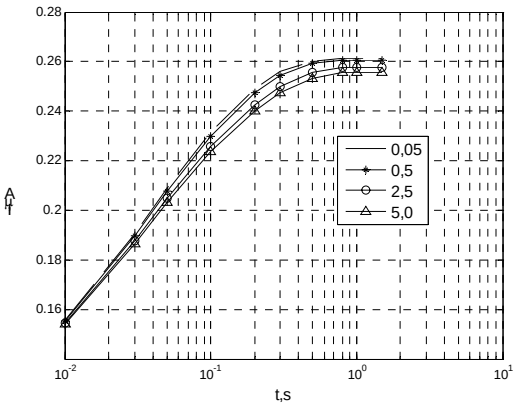

FIG.9. FOR THE P – P KINETIC.

At the fig. 7, 8 and 9 are given the results from simulations. In order to see the variation of current for the small values of time we use logarithmic scale. Again it is seen that for the first order and for the M–M kinetic output current has pseudo-oscillations, and for the p – p kinetic transient process is asymptotic.

Taking into account the participation of co-substrate in the strong enzyme kinetics leads to those effects – transient processes stand pure asymptotic.

5. INFLUENCE OF DIFFUSION PARAMETERS

5.1. INFLUENCE OF MEMBRANE THICKNESS - D

We study the dynamic effect of membrane thickness - d [μm]over output current. The following values of parameters are used:

So =0,65mM, Co = 0,27mM *Vm = 2 mM/s*

d = 10, 30, 70, 210μm , Ks = 0,65mM *Ds = 1.10^{-9}m^2/s, Dc = 2,5.10^{-9}m^2/s .*

At fig.10,11 and 12 is given the output current of biosensors for the different thickness of active membrane d: 10, 30, 70, 210μm. For the thinner one (10μm,30μm) biosensor response increases with increasing the thickness, but for the thicker is opposite biosensor response decreases with increasing the thickness (70μm, 210μm). again is seen the efect of pseudo- oscillations only for the firs two type of enzyme kinetic. In order to see variation of current for the small values of time we use logarithmic scale for the p – p kinetic – fig.13 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 2 s for the thicker membranes (70-210 μm).

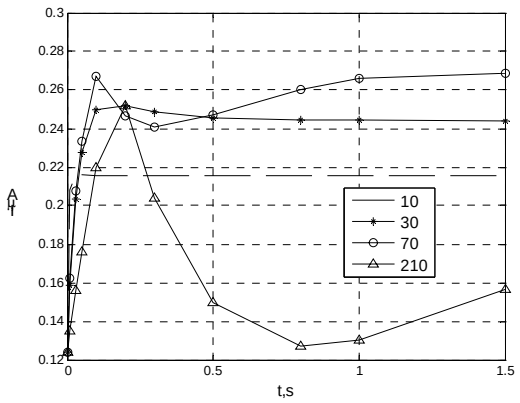

FIG.10 FOR THE 1 - ORDER KINETIC.

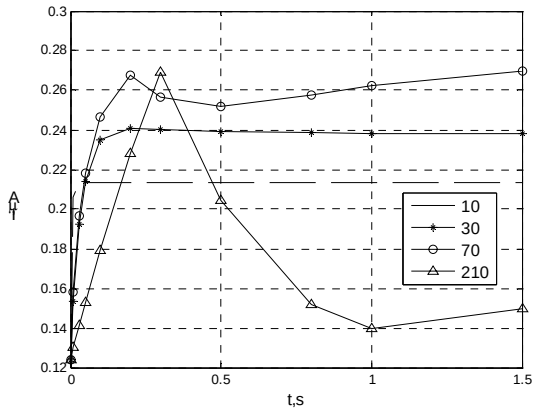

FIG. 11.FOR THE M –M KINETIC.

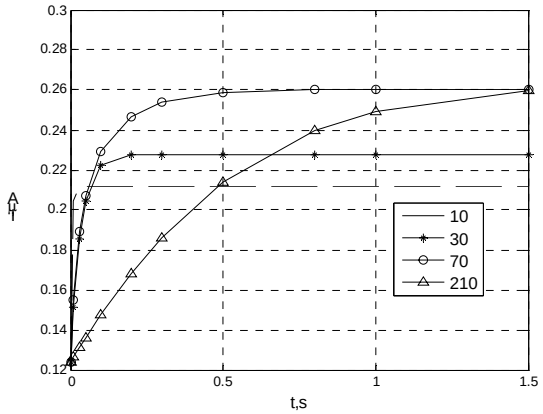

FIG.12. FOR THE P – P KINETIC.

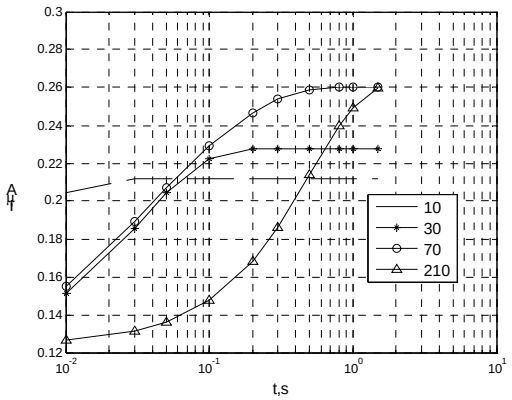

FIG.13 FOR THE, P – P KINETIC.

5.2. INFLUENCE OF DIFFUSION CONSTANTS – Ds,Dc

Those constants depend from the material of active membrane.

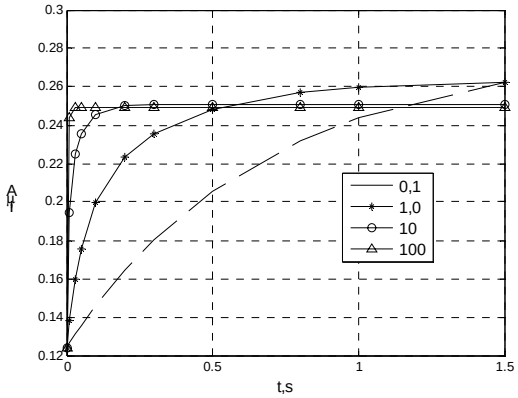

FIG.14. INFLUENCE OF Dc FOR THE P – P KINETIC.

Because the output current depends from the co-substrate gradient at the electrode surface Ds has not influence over biosensor response, but Dc has. The results are given only fot the p-p kinetic at the fig.14. Increasing the value of the diffusion constant Dc leads to small values of response time.

6. CONCLUSION

Numerical simulation of the three type of enzyme kinetic shows very interesting results. First we see that for the first order and M-M kinetic output current has pseudo – oscillations. Taking into account the participation of co-substrate in the strong enzyme kinetics leads to the effects – transient processes stand pure asymptotic. The second is about response time. If we measure with real biosensor response time is about 5-6min, simulations shows that in active membrane transient process complete about 5-10s. That mean that other two membranes take very big part of all time for measurement of substrate. Advantages of that is that we will receive a very cheap and rapid tissue biosensor (for enzyme biosensors procedure for purifying of enzyme is very expensive and continuous) and can use biosensors in very important area like medicine and environmental. The third result is that membrane thickness has specific influence over transient processes of biosensor, the fourth result is that enzyme loading didn't have very big influence over response time, the same we can say and for the reaction rate constants and diffusion constants.

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