REVERSIBLE INHIBITOR BIOSENSOR SYSTEMS IN DYNAMIC MODE

Vania Rangelova[1], Pavlina Kasarova[2],Vasil Spasov[3],

Techical University– Sofia, Branch Plovdiv[1,2,3], BULGARIA

Abstract

The biosensor amperometric transducers can work in the case of three basic types of reversible inhibitor enzyme systems – with competitive inhibition, with non-competitive inhibition and mixed inhibition. Tipicaly they work in static mode. Now they are investigated in dynamic mode. The kinetic in those type biosensors is generally discussed in terms of a simple extension to the Michaelis-Menten reaction scheme. The investigated biosensors are amperometric product sensitive. The parameters for simulations are chosen from some real experiments with biosensors. The models are described in non stationary diffusion conditions. Solving system of non-linear partial differential equations is reseived in three dimensional size and the concentration profiles in active membrane of substrate $S(x,t)$, inhibitor $I(x,t)$ and product $P(x,t)$ are reseived. The systems of non-linear differential partial equations have been solved numerically in MATLAB medium. The influence of starting concentration of substrate , inhibitor and kinetic parameters - reaction rate and reaction constants of biosensors over output current have been investigated.

Key words: amperometric biosensor, inhibition, dynamic mode, simulations.

1.Introduction

The biosensors now days are very popular devices and have a very big area of uses from health-care industry through food quality appraisal to environmental monitoring. It is due to their main advantages – to detect concentration in very low range of different substances cheap, selective and highly sensitive. Measurements in enzyme based biosensor systems with enzyme layers are either made through stationary mode or flow-through mode [1]. Our investigation is done over stationary mode biosensors. Now days are used different sensing principles for determining the concentration of substrate - electrochemical, calorimetric, optical and piezoelectric. Biosensor systems with enzyme inhibition are very popular in environmental monitoring [1] used with a proper intelligent terminals [2] they could do their job – to serve humanity for better life. Recently biosensors are used for the detection of pollutants, such as pesticides, heavy metal ions and other toxic compounds. Botre et all [3] use inhibition – based biosensor for the detection of environmental contaminants. A new bienzymatic inhibition biosensor based on the combined catalytic activity of the enzymes alkaline phosphatase and glucose oxidase is proposed and discussed for the direct determination of 2,4-dichlorophenoxyacetic acid (2,4-D), one of the most powerful and diffused defoliants, which also is endowed with estrogenic properties. Lee S. et all [4] use urease-immobilized biosensor for the determination of heavy-metal ions. Multinzime biosensors systems are investigated also [5], from SP. Ganesan, et all , but again is investigated only the same steady-state mode. Generally those type of biosensors - stationary mode, work in steady state mode and the majority research papers are in this mode and dynamic mode is still poorly examined. But it is known that the 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 [6]. Recently Baronas et al are developed a mathematical models of amperometric product-sensitive biosensors [7,8,9] in dynamic mode. Baronas and Kulys have a lots of papers dedicated expecialy on modelling biosensors, so they wrote a book called – "Mathematical modeling od biosensors". Where are done very comrehensive marhematical modeling and simulations on biosensors [10]. In the chapter 8 very shortly they investigate the enzyme inhibitions, they describe and gave solutions for substrate and product inhibition using Michaele – Menteh kinetic but their equations don't include the inhibitor equation. They use only substrate inhibition constants – Ks which is not enough to descibe the full

model of the enzymatic reaction . In our paper are investigated three type of inhibition.Yupeng Liu [11] had done some interesting modelling and simulation and synamic mode also for free enzyme model electorchemical biosensor, but it is not inhibitor type biosensor.

The goal of this paper is to investigate the all three different type of enzyme inhibitor kinetics models of biosensors in dynamic mode, and they are – with competitive inhibition, with non-competitive inhibition and mixed inhibition. In the enzyme reactions, enzyme E and substrate S react and ES complex is formed. After that ES is transformed to an transition complex ES^*. The latter convert S to product P and the result is free enzyme and product

$$E + S \longleftrightarrow ES \longleftrightarrow ES^* \longleftrightarrow EP \longleftrightarrow E + P$$

The kinetic of those reaction is $v = \dfrac{V_S S}{K_S + S}$ and connect enzyme reaction rate v with concentration of substrate S and two constants, **maximal reaction velocity** V_S and Michaelis-Menten constant for given substrate K_S. Those two are called **kinetic** parameters.

2.Presentation of mathematical models.

Enzyme inhibitors are molecules that bind to enzymes and decrease their activity [12]. Loss of activity may be either reversible, where activity may be restored by the removal of the inhibitor, or irreversible, where the loss of activity is time dependent and cannot be recovered during the timescale of interest. The main is that kinetic parameters K_S and V_S is changed. More important for most enzyme-catalysed processes is the effect of reversible inhibitors. These are generally discussed in terms of a simple extension to the Michaelis-Menten reaction scheme.

There are known three type **reversible inhibition** - Competitive inhibitions, Non-competitive inhibitions (sub type - Uncompetitive inhibitions, *rarely encountered and we don't investigate it*) and Mixed-type inhibitions.

- **Competitive inhibitors** can bind to E, but not to ES. Competitive inhibition increases K_S (i.e., the inhibitor interferes with substrate binding), but does not affect V_S (the inhibitor does not hamper catalysis in ES because it cannot bind to ES).

- **Non-competitive inhibitors** have identical affinities for E and ES ($K_I = K_I'$). Non-competitive inhibition does not change K_S (i.e., it does not affect substrate binding) but decreases V_S (i.e., inhibitor binding hampers catalysis).

  - **Uncompetitive inhibitors** bind only to ES complexes at locations other than the catalytic site. Substrate binding modifies enzyme structure, making inhibitor- binding site available. Inhibition cannot be reversed by substrate. Apparent V_S decreased; K_S is decreased.

- **Mixed-type inhibitors** bind to both E and ES, but their affinities for these two forms of the enzyme are different ($K_I \neq K_I'$). Thus, mixed-type inhibitors interfere with substrate binding (increase K_S) and hamper catalysis in the ES complex (decrease V_S).

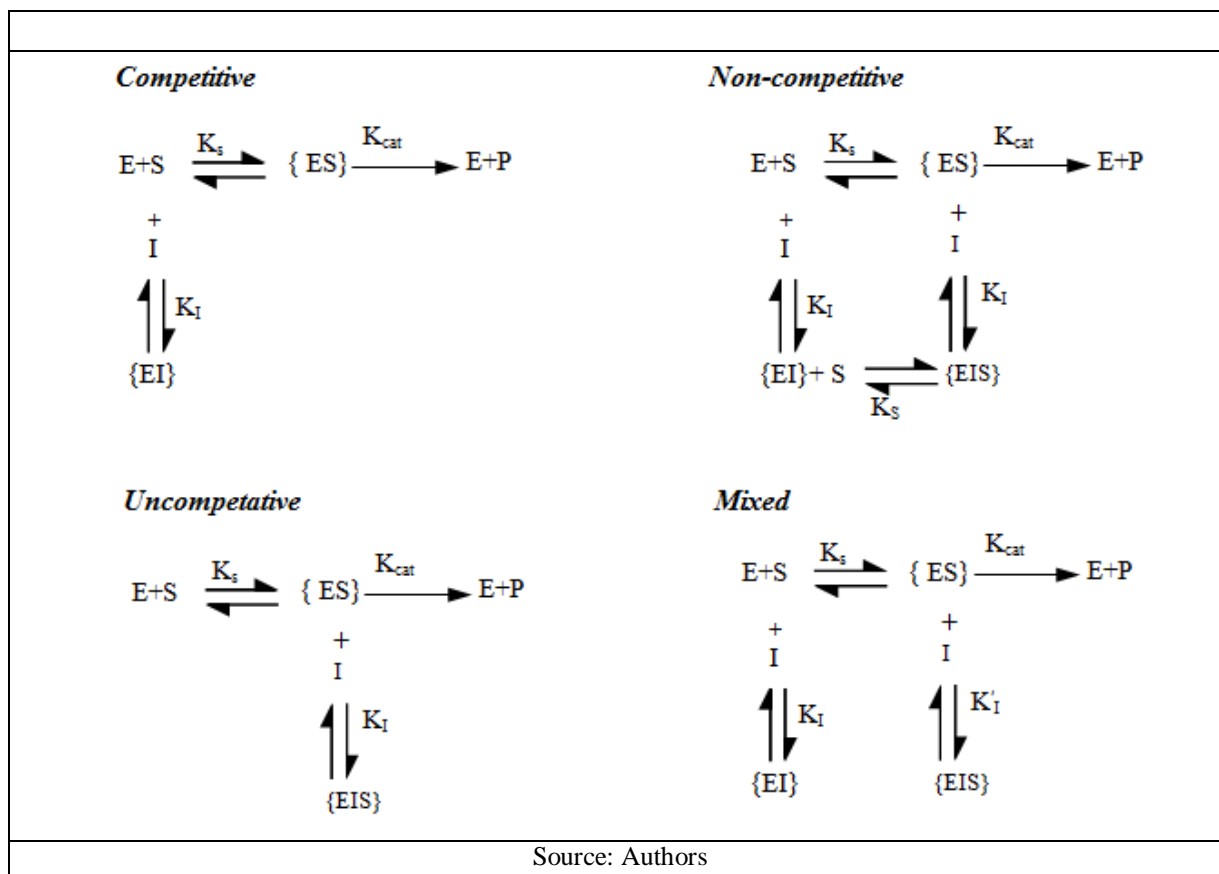The reaction follows the schemes, given in the fig.1

Source: Authors

Fig. 1: The reaction schemes of three type inhibitions.

where I represents the reversible inhibitor and the inhibitory (dissociation) constants K_I and K_I', K_{cat} is the rate constant of product formation.

We will investigate biosensors systems only in active membrane, because it is known that concentration of substrate $S(x)$ and inhibitor $I(x)$ 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

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

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 **competitive** reversible inhibition systems R_Q is

for **non - competiive** reversible inhibition system R_Q is

$$R_{Q\,1} = \frac{V_S}{K_S\left(1+\dfrac{I}{K_I}\right)+S}\,S \qquad (2)$$

$$R_{Q\,2} = \frac{V_S}{\left(1+\dfrac{I}{K_I}\right)}\frac{S}{(K_S+S)} \qquad (3)$$

and for **mixed** reversible inhibition system R_Q is

$$R_{Q\ 3} = \frac{V_S.S}{K_S\left(1+\dfrac{I}{K_I}\right) + S\left(1+\dfrac{I}{K_I'}\right)}, \tag{4}$$

where: Ks - reaction rate constant for substrate; K_I and K_I` - for inhibitor; V_S – maximal velocity of enzyme reaction - **enzymatic rate**.

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 **competative** inhibition

$$\frac{\partial S}{\partial t} = Ds\frac{\partial^2 S}{\partial x^2} - R_{Q1}$$

$$\frac{\partial I}{\partial t} = D_I\frac{\partial^2 I}{\partial x^2} - R_{Q1} \tag{5}$$

$$\frac{\partial P}{\partial t} = Dp\frac{\partial^2 P}{\partial x^2} + R_{Q1}$$

for **non - competiive** reversible inhibition

$$\frac{\partial S}{\partial t} = Ds\frac{\partial^2 S}{\partial x^2} - R_{Q2}$$

$$\frac{\partial I}{\partial t} = D_I\frac{\partial^2 I}{\partial x^2} - R_{Q2} \tag{6}$$

$$\frac{\partial P}{\partial t} = Dp\frac{\partial^2 P}{\partial x^2} + R_{Q2}$$

for **mixed** reversible inhibition

$$\frac{\partial S}{\partial t} = Ds\frac{\partial^2 S}{\partial x^2} - R_{Q3}$$

$$\frac{\partial I}{\partial t} = D_I\frac{\partial^2 I}{\partial x^2} - R_{Q3} \tag{7}$$

$$\frac{\partial P}{\partial t} = Dp\frac{\partial^2 P}{\partial x^2} + R_{Q3}$$

The output current is proportional to gradient of the product concentration at the electrode surface

$$I = nFAD_P\frac{\partial P}{\partial x}\Big|_{x=d} , \ [A] \tag{8}$$

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

$t = 0$ $S(x,0) = So$ $I(x,0) = Io$ $P(x,0) = 0$ (9)

Limiting conditions are

$x = 0$ $S(0,t) = So$ $I(0,t) = Io$ $P(0,t) = 0$ (10)

The substrate, and inhibitor didn't react with the electrode, but product fully exhausted. The medium is well stirred, then the limiting conditions are

$x = d$

$$\left.\frac{\partial S}{\partial x}\right|_{x=d} = 0, \qquad \left.\frac{\partial I}{\partial x}\right|_{x=d} = 0 \qquad P(d,t) = 0 \ . \tag{11}$$

### 3. Results from digital simulations and discussion

For solving systems (5,6,7) 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* *so*lve 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. Three programs defining the systems are developed, some appropriate substitutions were made, and also canonization of the equations, as well as fixation of the physical parameters.

And so will be investigated amperometric productsensitive inhibitor biosensor system. The parameters are chosen from some real experiments with biosensors from the authors [14, 15,16, 17] and for the simulations they are

$n = 2$, $So = 5$ mM, $Io = 0,5$ mM, $V_S = 0,5$ mM/s – or changed
$F = 96,5$A.s / mmol   - Faraday's number, $A = 7,85.10^{-7}$ m^2 - diameter of cathode is 1mm
$Ks = 0,6$ mM   - reaction constant for substrate,
$K_I = 0,6$ mM - reaction constant for inhibitor,
$K_{I'} = 2$mM   - reaction constant for inhibitor,
$Kp = 2$ mM - reaction constant for product,   - or changed, $d = 70$ μm,
$Ds = 1,510^{-9}$ m^2/s, $D_I = 5,5.10^{-9}$m^2/s, $Dp = 2,5.10^{-9}$ m^2/s.

3.1. Concentration profiles of substrate *S(x,t)*, inhibitor *I(x,t)* and product *P(x,t)*, for the biosensor with competitive inhibitions

In fig.2   in three dimensional coordinates   are given solutions for the concentration profiles of substrate *S(x,t)*, inhibitor *I(x,t)* and product *P(x,t)*, measurement unit is in mM, in the active membrane of the biosenosr with selected thickness equal to *d = 70*μm. Time is taken to be *t = 5*s and reaction rate *V_S = 0,5* mM/s. The value of starting concentration of substrate is chosen to be 5 mM which range is normal for the electrochemical biosensors. Reaction constans for substrate and inhibitor are chosen equal. In the up part of the figure 2 are resuts for the case without inhibitor, so they are for concentration of *I = 0mM*. The biosensor react like ordinary biosensor, the output product reaches maximal consentration of *0,12mM*, after that is fully exhausted at the electrode. Product is equal to zero at the beginning of the membraneand time, because still is not formed. Because the system is product sensitive the product concentration is increasing with the consuming of the substrate. The lower part of the figure 1 shows the profiles when there has presence of inhibitor, for *I = 5mM*. . It is seen that inhibitor is consumed very rapidly. The reaction constans for substrate and inhibitor are chosen equal, so due to the competition the output product reaches very small consentration of *0,08mM*, it is *1,5* times lower then when has no inhibitor in the system.
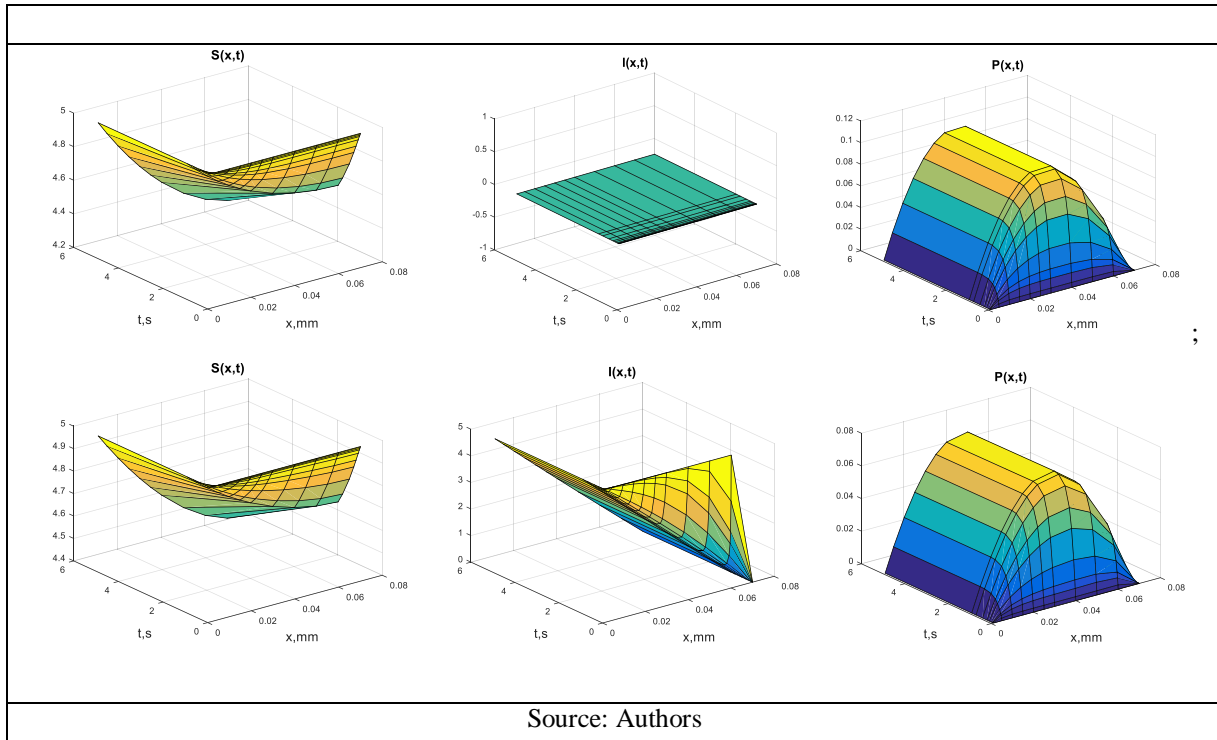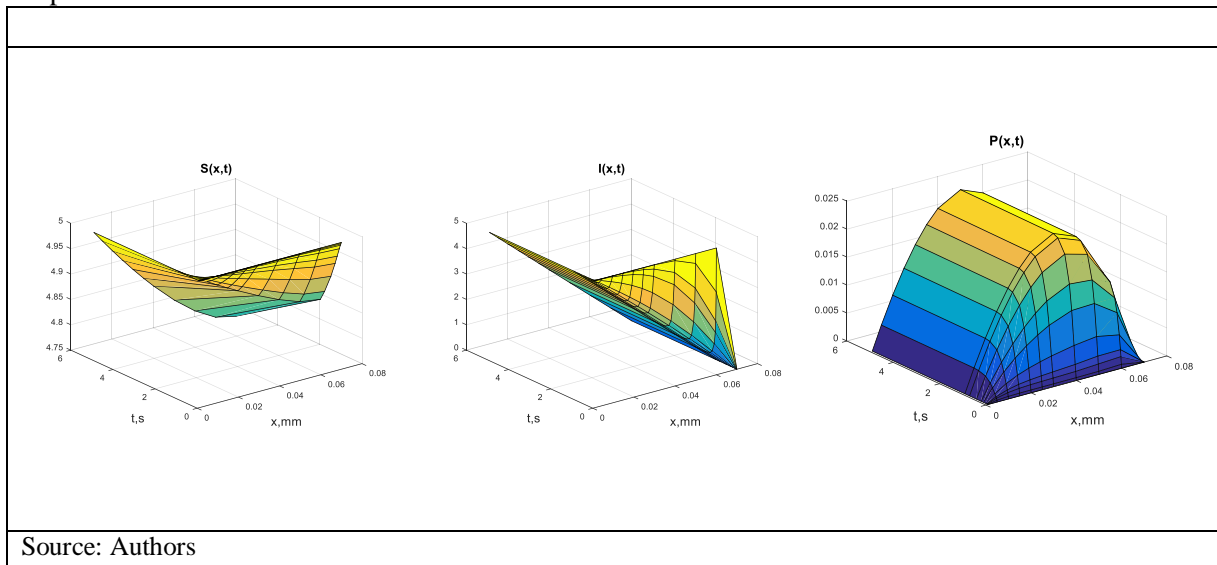
Source: Authors

Fig. 2: **Competitive inhibition.**

Concentration profiles of substrate S(x,t), inhibitor I(x,t) and product P(x,t) in active membrane, up – when concentration of the inhibitor is *I = 0mM.*, and down when concentration of the inhibitor is *I = 5mM.*, all units for comcentration profiles are in mM.

3.2. Concentration profiles of substrate *S(x,t),* inhibitor *I(x,t)* and product *P(x,t)*, for the biosensor with with non-competitive inhibitions

In fig.3 are given the same reagents   *S(x,t),   I(x,t)* and   *P(x,t)*   but for the biosensor with non-competitive inhibitions. The starting conditions are equal, so the resulting profiles have no big difference. Here the the output product reaches maximal consentration of *0,025mM* which is *4,8* times smaller then when has no inhibition the reason is, the decreasing of the maximal reaction rate V_S in the presence of inhibitor.

Source: Authors

Fig. 3: **Non - Competitive inhibition.**

Concentration profiles of substrate S(x,t), inhibitor I(x,t) and product P(x,t) in active membrane, when concentration of the inhibitor is *I = 5mM* all units for comcentration profiles are in mM.

3.3. Concentration profiles of substrate *S(x,t)*, inhibitor *I(x,t)* and product *P(x,t)*, for the biosensor with mixed inhibitions

In fig.4 are given the same reagents   *S(x,t),   I(x,t)* and   *P(x,t)*   but for the biosensor with mixed inhibitions. Now 2 reaction are in action. The inhibitor bind to both *E* and *ES*, so we use two different reaction constants and their affinities for these two forms of the enzyme are different, for the enzyme *E* , $K_I = 0,6 \ mM$ - reaction constant for inhibitor, and for the complex *ES,* we chose 3 times bigger constant $K_{I'} = 2mM$ - reaction constant for inhibitor. Other parameters are the same - reaction constant for substrate, all corresponding diffusion constants, starting comcentration of substrate. . Here the the output product reaches maximal consentration of *0,004mM* which is *30* times smaller then when has no inhibition. The reason is the decreasing of the maximal reaction rate V_S in the presence of inhibitor, and it influens over the both – enzyme activity and enzyme substrate complex activity. And thw comcentration of the onhibitor stays in very big values- almost the same, in previous two types of inhibition the inhibitor almost fully exhausted.
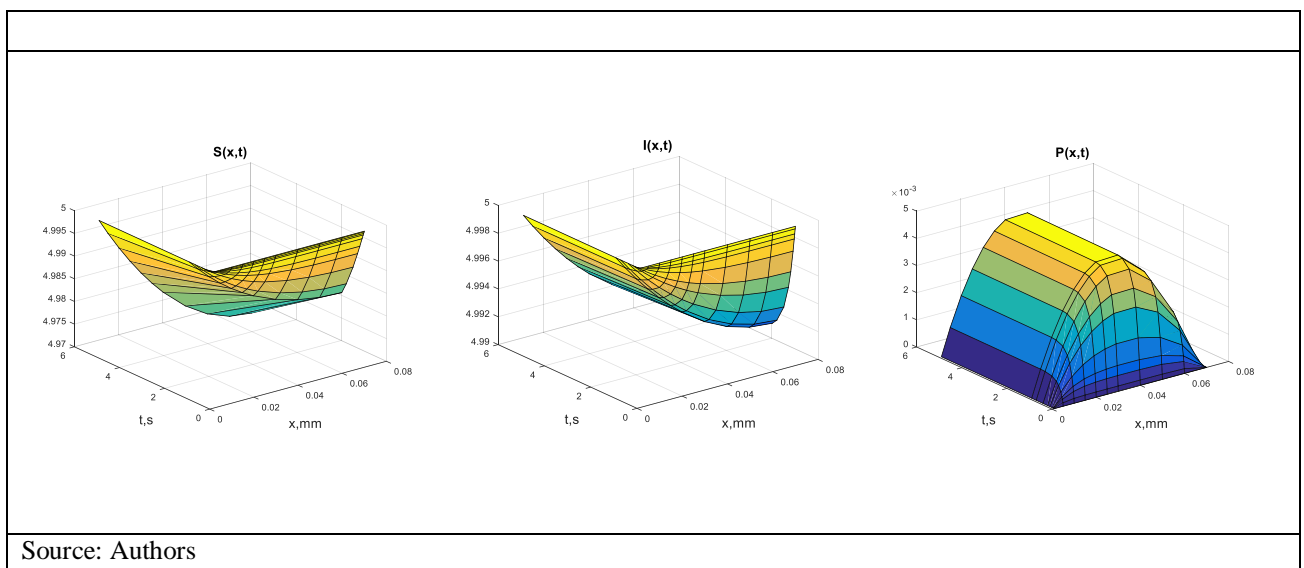

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Fig. 4: **Mixed inhibition.**
Concentration profiles of substrate S(x,t), inhibitor I(x,t) and product P(x,t) in active membrane, when concentration of the inhibitor is *I = 5mM* all units for comcentration profiles are in mM.

Now we wil investigate the influence of starting concentration *So, Io*, kinetic parameters – *Vs, Ks* and reaction constant of inhibitor K_I over output current of biosensors

3.4. Influence of starting concentration *So, Io*, kinetic parameters – *Vs, Ks* and reaction constant of inhibitor K_I over output current of biosensors For the biosensor with competitive inhibition.

The influence of starting concentration *So* of measured substrate over the output current *I* of the biosensor with competitive inhibition is given to the fig.5. a. We gave the calculated relation of *Io* to *So*. The concentration of *I = 2mM*. For the relation *Io/So = 0,001, So* is *1000* times bigger then *Io*. With increasing of *So* from *2mM* to *2000mM* the output current is increase too, which is obviously from the kinetics reaction, more substrate - bigger output current. For the *So* from *2mM* , current is *I = 34nA* and for the *So* from *2000mM* , current is *I = 58nA*. And after *20mM* the biosensor system is fully saturated so no significant changes in the output are possible. This kind of dependence, which distinguishes the enzymatic reactions from the other chemical processes, is due to the intermediate formation of enzyme-substrate complex (ES) during the enzyme-catalyzed reaction, and when substrate is in very big concentration - it reaches saturation of all active substrate centers.

The influence of starting concentration of *Io* is given to the fig.5.b. For the two values of inhibitor, *I = 0mM and I = 5mM* the output current decrease from *54nA* to *40* nA, due to the presence of

inhibitor. Naturaly those results depend significally and from values of the other reagents, but in common the result is decreasing the output current.

The influence of kinetic parameters - **maximal reaction velocity** *Vs* is given to the fig.5.c. Again in relation , so for the *So/Vs = 50* , *So* is *50* times bigger then *Vs*. With increasing the reaction velocity *Vs* from *0,1 mM/s* to *5 mM/s* the output current is increasing too,   because the enzyme reaction velocity is proportional to maximal reaction velocity. For the values of *5 mM/s* is seen the appiarance of maximal resonse like in a second order oscillating unit with a decay factor of less than 1. It is only for the transient time, untill is reached the steady state line. So the biosensor system looks like a oscillating unit with a decay factor.

The influence of kinetic parameters - **reaction constant for substrate** *Ks* is given to the fig.5.d.

The bigger value of maximal reaction velocity *Vs* leads to bigger output current, the bigger value of Michaelis-Menten constant for given substrate *Ks* leads to smaller output current. It is because we sad

in the beginning that the enzyme kinetic has the reaction velocity of the kind $v = \dfrac{V_S S}{K_S + S}$.
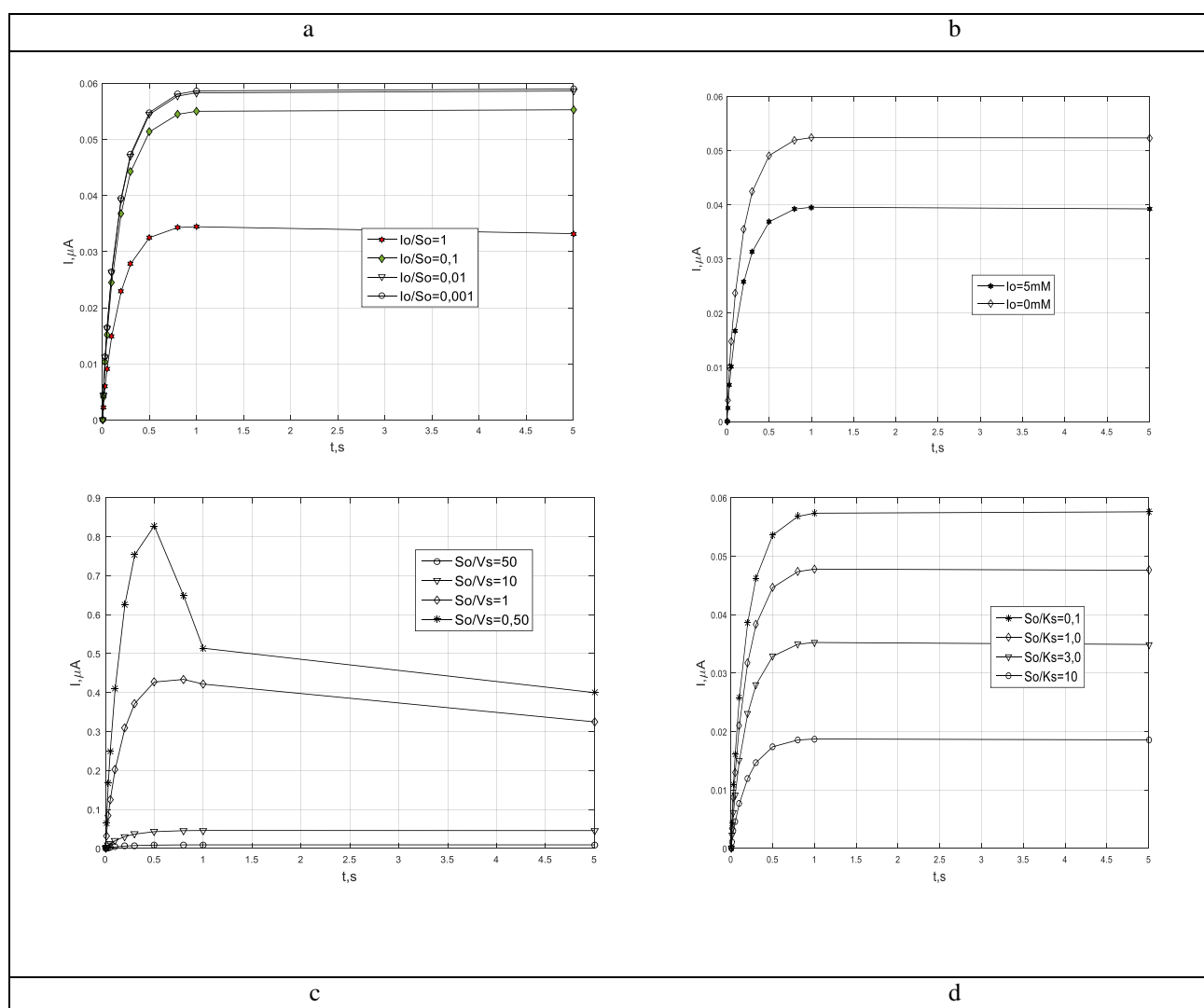

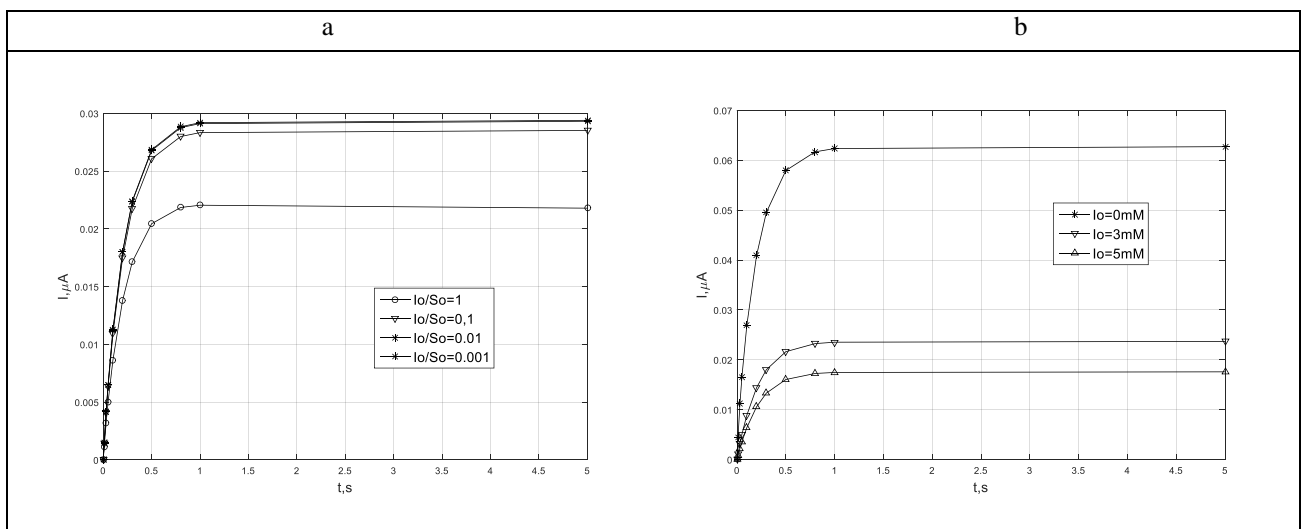Fig.5. **Competitive inhibition** a-Influence of So,b -Influence of Io, c-Influence of Vs,d-Influence of Ks.

3.5. Influence of starting concentration *So, Io*, kinetic parameters – *Vs, Ks* and reaction constant of inhibitor *K_I* over output current of biosensors For the biosensor with non - competitive inhibition.

The influence of starting concentration *So* of measured substrate over the output current *I* of the biosensor with non-competitive inhibition is given to the fig.6. a. We use the same values of all parameters only the equations are different. And form graph is seen that output current icreases but in much values, due to te inhibition of the both complexes – enzyme and enzyme-substrate. For the *So* from *2mM* , current is *I = 24nA* and for the *So* from *2000mM* , current is *I = 28nA*. Here the max deviation is only *16,6%,* but for the competittive inhibition it was *70%.*

The influence of starting concentration of *Io* is given to the fig.6.b. For the three values of inhibitor concentration, *I = 0mM, I = 3mM and I = 5mM* the output current decrease from *62nA* to *23nA*, to *18nA* , due to the presence of inhibitor. Here for the starting concentration of the inhibitor *I = 0mM,* the output current is slightly bigger - *62nA,* but for the presence of the inhibitor *I = 5mM* where we do the research it values is lower - *18nA* versus *39nA* for non-competative inhibition.

The influence of kinetic parameters - **maximal reaction velocity** *Vs* is given to the fig.6.c. Again in relation , so for the *So/Vs = 50* , *So* is *50* times bigger then *Vs*. With increasing the reaction velocity *Vs* from *0,1 mM/s* to *5 mM/s* the output current is increasing too,   because the enzyme reaction velocity is proportional to maximal reaction velocity. For the values of *5 mM/s* is seen the appiarance of maximal resonse. Its value is *620nA*, for the competititve inhibition it is *820nA,* and it is obvious from this enzymatic type of action of inhibitor.

The influence of kinetic parameters - **reaction constant for substrate** *Ks* is given to the fig.6.d.
The bigger value of maximal reaction velocity *Vs* leads to bigger output current, the bigger value of Michaelis-Menten constant for given substrate *Ks* leads to smaller output current.
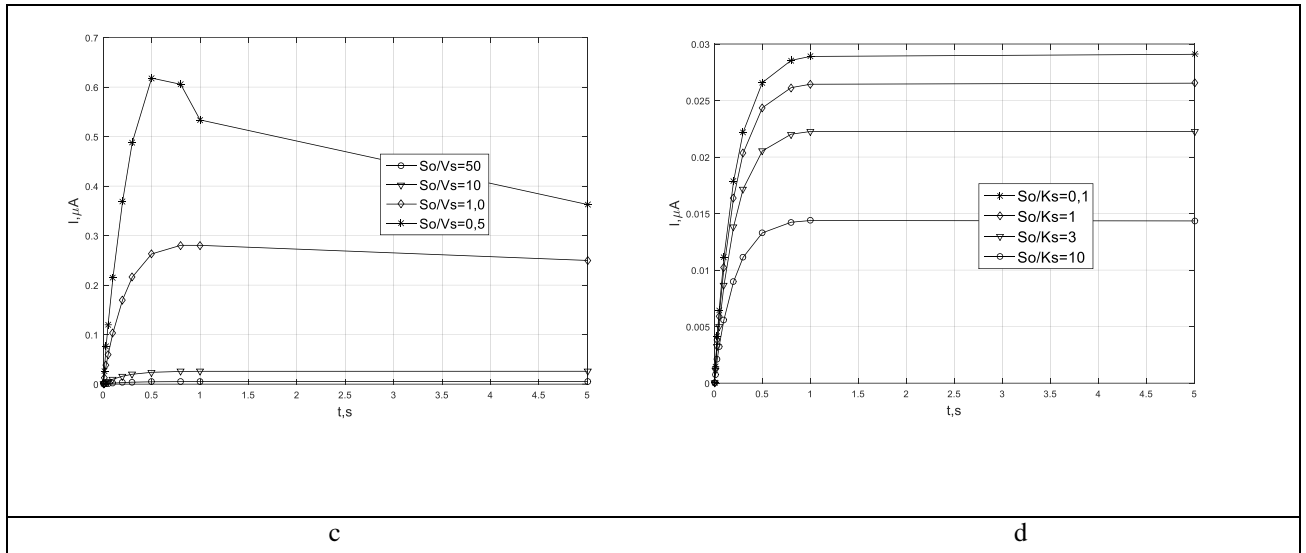
Fig.6. **Non - Competitive inhibition**. a **-** Influence of So, b -Influence of Io, c -Influence of Vs, d - Influence of Ks.

3.6. Influence of starting concentration *So, Io*, kinetic parameters – *Vs, Ks* and reaction constant of inhibitor K_I over output current of biosensors For the biosensor with mixed inhibition.

The influence of starting concentration   *So* of measured substrate over the output current *I* of the biosensor with mixed inhibition is given to the fig.7. a. What is seen form the graph here – for increasing of measured substrate *So* from *2mM* to *2000mM* , the output current has not any deviation, or it is so small that will not measured at all. Or will be with the range of measurement error *3-4 nA*.

The influence of starting concentration of *Io* is given to the fig.7.b. Here the same subordination continues, output values of the current began smaller and smaller, and smaller then previous two types of kinetics. They now are several *nA*, from *2* to *9nA*.

The influence of kinetic parameters - maximal reaction velocity *Vs* is given to the fig.7.c. With increasing the reaction velocity *Vs* from *0,1 mM/s* to *5 mM/s* the output current is increasing too, because the enzyme reaction velocity is proportional to maximal reaction velocity, but now due to the influence of the both complexes, there has no maximal resonse like in a second order oscillating unit. And the transient proces for the biosensor is like in a first order oscillating unit.

The influence of kinetic parameters - reaction constant for substrate *Ks* is given to the fig.7.d.

The bigger value of maximal reaction velocity *Vs* leads to bigger output current, the bigger value of Michaelis-Menten constant for given substrate *Ks* leads to smaller output current. Again the output current is in very small values – *4-5 nA*.
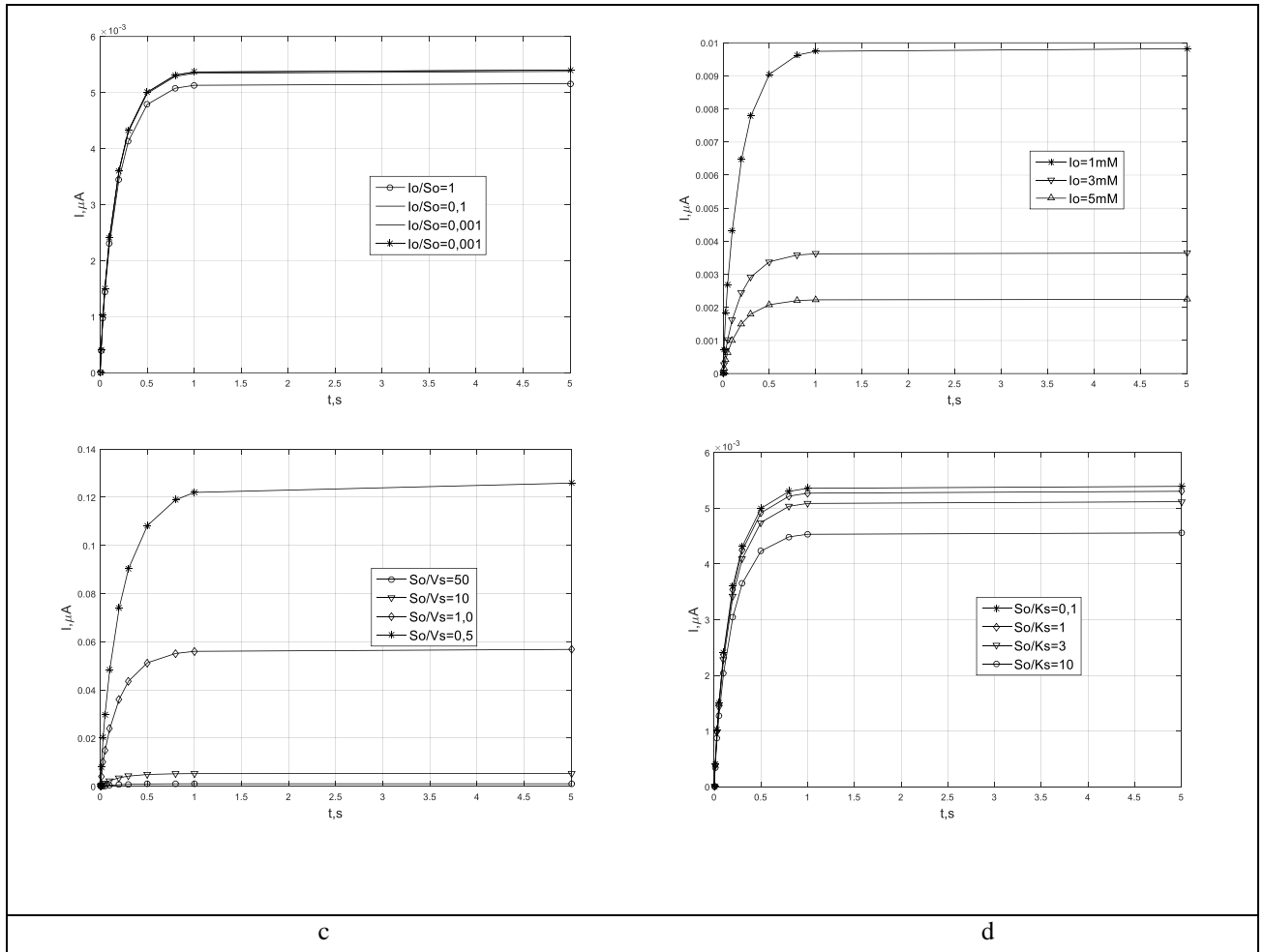
a

b
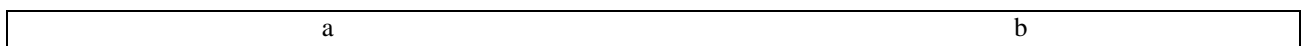
Fig.7. **Mixed inhibition**. a **-** Influence of So, b -Influence of Io, c -Influence of Vs, d -Influence of Ks.

In the fig.8 is given the output current I in *uA* in one graph - for biosensor with competitive inhibitions – the upper 3 lines, for the biosensor with non-competitive inhibitions – the middle three lines, and for the biosensor with mixed inhibitions – the down three lines.
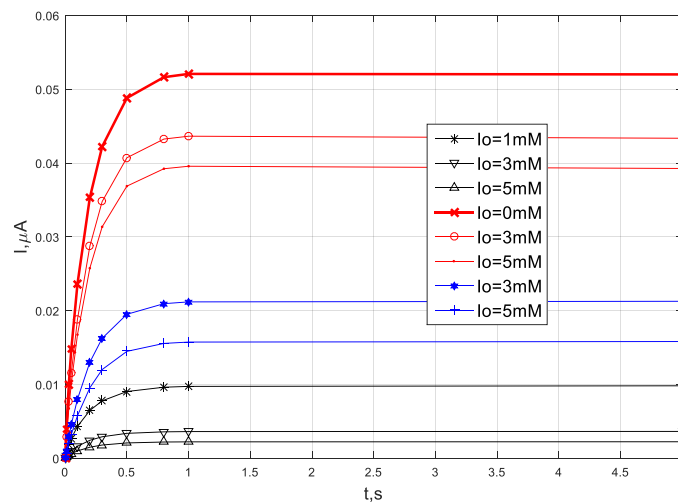
Figure 8: The output current I in uA for biosensor with competitive inhibitions – the upper 3 lines, biosensor with non-competitive inhibitions – the middle three lines, and biosensor with mixed inhibitions – the down three lines.

4. Conclusion

Generally described electrochemical amperometric biosensors work in steady state mode. In this paper has done an attempt to investigate the dynamic mode of the specific typeamperometric biosensors – those with reversible inhibition. Mathematical modeling of the acting equations were performed and solution of those in the MATLAB environment. The results show that simulations are adequate, the concentration profiles of the three reagent can be seen. In addition are done investigation of the influence of starting concentration of substrate measured So, and inhibitor Io, kinetic parameters – Vs, Ks and reaction constant of inhibitor K_I over output current of biosensors. For the futher could be in vestigatre the influence of diffusion parameters - Diffusion coefficient of substrate, Diffusion coefficient of inhibitor and Diffusion coefficient of product.

Notations

$n = 2$, Number of electrons taking part in the electrochemical reaction on the electrode surface

So – Starting concentration of the measured substrate

Io - Starting concentration of the inhibitor

V_S - Maximal reaction velocity, mM/s

d - Thickness of active membrane, μm

F - Faraday's number, F= $96,5$A.s / mmol

A – Area of the cathode of the indicator electrode, m^2

Ks - Reaction constant for substrate S, mM

K_I - Reaction constant for inhibitor toward E, mM

$K_{I'}$ - Reaction constant for inhibitor toward ES, mM

Kp - Reaction constant for product, mM

Ds - Diffusion coefficient of substrate, m^2/s.

D_I - Diffusion coefficient of inhibitor, m^2/s.

Dp- Diffusion coefficient of product, m^2/s.

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