Nonlinear Analysis: Modelling and Control, 2006, Vol. 11, No. 4, 385–392

Biosensor Response at Mixed Enzyme Kinetics and External Diffusion Limitation in Case of Substrate Inhibition*

J. Kulys

Department of Chemistry and Bioengineering
Faculty of Fundamental Sciences
Vilnius Gediminas Technical University
Saulėtekio ave. 11, 10223 Vilnius, Lithuania
juozas.kulys@fm.vtu.lt

Received: 25.05.2006 **Revised:** 10.07.2006 **Published online:** 30.10.2006

Abstract. The action of a biosensor containing non Michaelis-Menten enzyme was modeled at mixed enzyme kinetics and external diffusion limitation in the case of substrate inhibition. The calculations show that at the electrode surface multi steady-state concentrations of substrate may be generated if diffusion module is much larger than 1 and the substrate bulk concentration is much bigger than Michaelis-Menten parameter. The multi steady-state concentration generates multi response of the biosensor. The production of the multi steady-state may cause the biosensor response oscillation.

Keywords: biosensor, non Michaelis-Menten kinetics, modelling.

1 Introduction

The response of biosensors is determined by an enzyme activity and mass transport [1]. If enzyme activity is low in comparison to mass transfer rate the response is determined by biocatalytical reaction, and a biosensor acts in “kinetic regime”. If overall process is determined by mass transport through stagnant (Nernst) layer the biosensor acts in “external diffusion limitation” regime. If process is limited by mass transport inside the biocatalytical membrane the biosensor act in “internal diffusion limitation” regime. The modelling of biosensors utilizing simple

*The research was supported by Lithuanian State Science and Studies Foundation, project No. C-03048.

Michaelis-Menten kinetics in different regimes was carried out using digital integration [2–16].

Very often the kinetics of an enzyme action is much more complicated. Inhibition, activation, allostery and other types of non Michaelis-Menten kinetics determinate the diversity of the enzymes, and finally a life [17]. The simplest scheme of non Michaelis-Menten kinetics, for example, may be produced by addition into Michaelis-Menten scheme (equation (1)) a stadium of the interaction of the enzyme substrate complex (ES) with other substrate molecule (S) (equation (2)) following the generation of non active complex (ES_2):

$$E + S \leftrightarrow ES \rightarrow E + P, \tag{1}$$

$$ES + S \leftrightarrow ES_2. \tag{2}$$

For this scheme the steady-state "initial" rate (Vst) shows a maximum on the dependence of the Vst on the substrate concentration.

To the best of our knowledge the biosensors utilizing non Michaelis-Menten enzymes still have not built. To develop new biosensors containing non Michaelis-Menten enzymes useful may be the modelling these biosensors. The work describes the results of the biosensors modelling at mixed enzyme kinetics and external diffusion limitation.

2 Mathematical model

The steady-state kinetics of an enzyme acting following the scheme (1), (2) is described by equation (3).

$$V_{st} = -ds/dt = k_{cat} e_t s/(K_m + s + s^2/K_i), \tag{3}$$

where V_{st} – steady-state "initial" enzymatic rate, k_{cat} – catalytic constant, e_t – total enzyme concentration, s – substrate concentration, K_m and K_i – Michaelis-Menten and inhibition constants, respectively.

The biosensor is considered as infinite nontransparent plate covered by thin (molecular) layer of an enzyme. The substrate flux to the layer is perpendicular to the surface of the plate. The thickness of stagnant (Nernst) layer covering the enzyme layer is δ. In real conditions it can be as large as $0.04\,$cm [18]. The

substrate concentration on the surface (s_s) is less than in the bulk (s_b) due to the enzyme action. At steady-state conditions the substrate flux (J_{st}) through the stagnant layer is equal to enzymatic rate on the surface:

$$J_{st} = D(s_b - s_s)/\delta = V_{st}, \tag{4}$$

where D – diffusion coefficient of the substrate in the stagnant layer.

The biosensor response (R_{st}) is directly proportional to substrate conversion rate:

$$R_{st} = nFJ_{st}, \tag{5}$$

where n – number of electrons ($n = 1$), F – Faraday number.

At steady-state conditions the response of biosensor can be expressed:

$$R_{st} = nFD(s_b - s_s)/\delta = nFk_{cat}e_t s_s/(K_m + s_s + s_s^2/K_i), \tag{6}$$

where concentration of enzyme on surface (e_t) is expressed in mol/cm^2. The dimensions of other parameters: F – C/mol, D – cm^2/s, k_{cat} – 1/s, s_b, s_s, K_m and K_i – mol/cm^3.

The unknown value of s_s can be found by solving equation (6). The analysis of dependence of the response on substrate bulk concentration (s_b) and mass transport rate was performed assuming for simplicity $K_i = K_m$ and using dimensionless parameter (diffusion module) $\rho = k_{cat}e_t\delta/K_mD$ that corresponds to the ratio of the enzyme reaction and the diffusion rate. The concentrations of substrate were also normalized to K_m. If $c_b = s_b/K_m$ and $c_s = s_s/K_m$ was used the expression for c_s calculation was:

$$c_b - c_s = \rho c_s/(1 + c_s + c_s^2). \tag{7}$$

The equation (7) was solved using symbolic processor of "Mathcad" (MathSoft Inc., Cambridge, MA). The analytical solution of the equation (7) has not been presented in the text due to very bulky expression.

3 Results and discussion

For the calculations the enzyme concentration 10^{-11} mol/cm^2 what corresponds to monolayer of enzyme molecules adsorbed on geometrically flat surface was

used. The values of other parameters used for the calculations were: catalytic constant (10^3 1/s) corresponds to moderately active enzyme, Michaelis-Menten constant (10^{-5} mol/cm^3) is typical for many enzymes [19], diffusion coefficient of substrate 10^{-6} cm^2/s, thickness of stagnant layer 0.03 cm. For these parameters the calculated dimensionless parameter (ρ) was 30.

The solution of (7) at $0 < c_b < 9.714$ gave a single value of surface concentration that was less in comparison to c_b. At $c_b = 9.714$ two values of $c_s = 0.589$ and $c_s = 4.063$ were calculated. At $11.091 > c_b > 9.714$ three values of c_s were found. At $c_b = 11.091$ two values of c_s were calculated $(1.201, 7.688)$. At $c_b > 11.091$ a single value was calculated again. To verify correctness of the calculations a graphical solution of (7) was performed. A function $Y = c_s/(1+c_s+c_s^2)$ was plotted to show enzymatic rate, and the function $y = (c_b - c_s)/\rho$ at fixed c_b concentration – to show diffusion rate. Crossing of these function gave c_s. In Fig. 1 three approximate values of $c_s = 0.64$, $c_s = 2.8$ and $c_s = 5.5$ gave the crossing of the functions at $c_b = 10$ and $\rho = 30$. These values fitted the calculated c_s $(0.636, 2.833$ and $5.529)$.

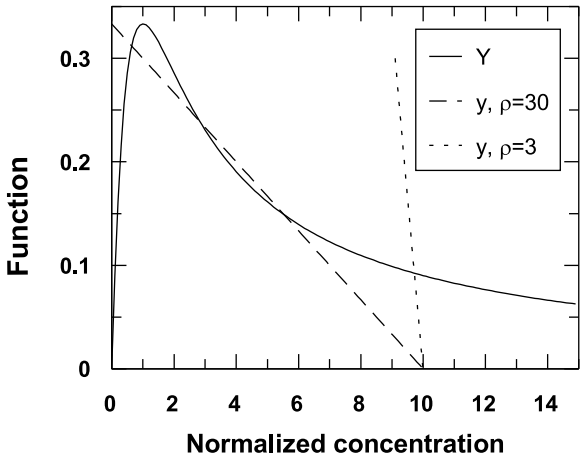

Fig. 1. Graphical surface concentration calculation. Functions: $Y = c_s/(1 + c_s + c_s^2)$, $y = (c_b - c_s)/\rho$. The parameters of calculations are written in the text.

The dependence of substrate surface concentration on bulk concentration is shown in Fig. 2. The figure shows that the surface concentration is less than the

bulk concentration. At critical bulk concentrations 9.714 and 11.091, two steady state concentrations are available whereas in the intermediate three concentrations are possible. However, an intermediate concentration is not stabile, since any perturbation of parameters produces a low or a high concentration.

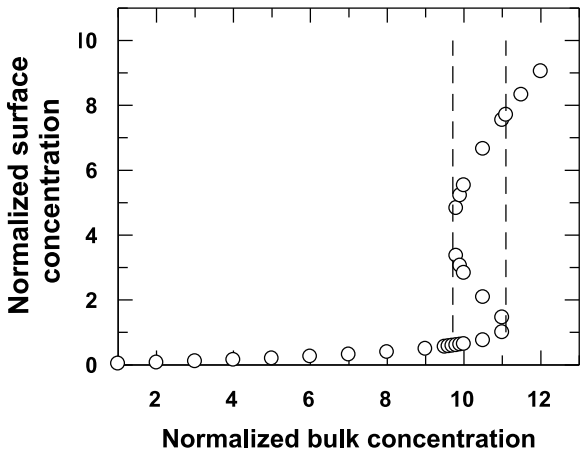

Fig. 2. The dependence of normalized surface concentration on normalized bulk concentration at $\rho = 30$. Vertical dot lines marks the zone of multi steady-state concentrations, other parameters are written in the text.

The biosensor response is related to surface concentration of substrate (equation (5)). The generation of different surface concentrations produces multi response of biosensor (Fig. 3). The multi response can be achieved at $11.091 \geq c_b \geq 9.714$.

The modelling shows that multi surface concentration is possible at large diffusion parameter (ρ). The decrease the ρ value up to 3, generates just one value of concentration $c_s = 9.7$ at $c_b = 10$ (Fig. 1). It is worth to notice that many interfaces used for the enzyme adsorption have a surface much more than geometrical. Therefore the enzyme concentration and consequently ρ may increase many orders of magnitude.

At $\rho = 0.3$ the biosensor acts in kinetic regime. The surface concentration of substrate is little less than the bulk concentration. The response of biosensor drops down almost 10–100 times, and the decrease of the response in the concentration range 1–12 is associated with enzyme activity decrease (Fig. 3).

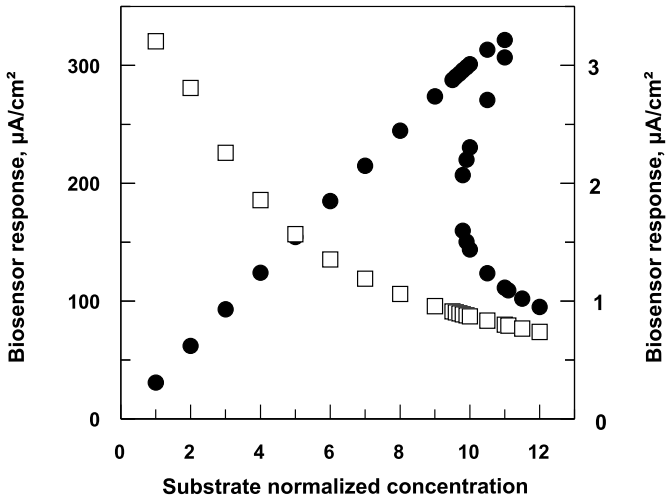

Fig. 3. The dependence of biosensor response on normalized bulk concentration of substrate. The data calculated with $\rho = 30$ (•) belongs to left hand y axis, with $\rho = 0.3$ (□) – to right hand y axis.

It is follows from the calculations that all parameters which change the diffusion module (ρ) perturb the multi steady-state zone. The thickness of stagnant layer (δ) is the most difficult controllable parameter. Using rotating disk electrode or precious flow rate may help to control the thickness of this layer [1, 18].

Multi steady-state surface concentration has many interesting consequences not only for biosensors action. It can generate oscillations of the concentration and the response of biosensor if the negligible perturbation of enzyme activity or mass transport occurs. The suggested model can be applied for modelling the enzymes action inside a cell. The most of the cellular enzymes are adsorbed or incorporated into membranes, and they act in high viscous media. These factors are beneficial for multi state generation. General consideration shows that similar effects may be expected if immobilized enzymes act in the regime of internal diffusion limitation. The digital modelling of these processes is under consideration.

4 Conclusions

The modelling of the biosensor response at mixed enzyme kinetics and external diffusion limitation in the case of substrate inhibition showed that multi steady-

state concentrations of substrate at the electrode surface may be generated if diffusion module is larger than 1, and substrate bulk concentration is much bigger than Michaelis-Menten parameter. A critical ρ value is determined by K_m and K_i ratio. The multi steady-state concentrations may generate the multi response of the biosensor. The production of the multi steady-state may cause the biosensor response oscillation. These results are principally different from the response of the biosensor acting in the kinetic regime with the same non Michaelis-Menten kinetics. In this case the biosensor response may have a maximum on the calibration curve, however, only a single value of the response may be obtained (Fig. 3).

Acknowledgments

The author expresses sincere thanks to prof's Romas Baronas and Feliksas Ivanauskas for their contribution into the digital modelling of the amperometric biosensors.

References

1. J. Kulys, *Analytical systems based on immobilized enzymes*, Mokslas, Vilnius, 1981 (in Russian).
2. R. Baronas, F. Ivanauskas, J. Kulys, Modelling of a microreactor on heterogeneous surface and an influence of geometry to microreactor operation, *Nonlinear Analysis: Modelling and Control*, **3**, pp. 19–30, 1998.
3. R. Baronas, F. Ivanauskas, J. Kulys, Modelling a biosensor based on the heterogeneous microreactor, *Journal of Mathematical Chemistry*, **25**(2–3), pp. 245–252, 1999.
4. R. Baronas, F. Ivanauskas, J. Kulys, M. Sapagovas, A. Survila, The influence of diffusion space geometry on behavior of some processes in biochemistry and electrochemistry, *Nonlinear Analysis: Modelling and Control*, **5**, pp. 3–38, 2000.
5. R. Baronas, F. Ivanauskas, J. Kulys, Modelling dynamics of amperometric biosensors in batch and flow injection analysis, *Journal of Mathematical Chemistry*, **32**(2), pp. 225–237, 2002.
6. R. Baronas, J. Christensen, F. Ivanauskas, J. Kulys, Computer simulation of amperometric biosensor response to mixtures of compounds, *Nonlinear Analysis: Modelling and Control*, **7**(2), pp. 3–14, 2002.
7. R. Baronas, F. Ivanauskas, J. Kulys, Computer Simulation of the Response of Amperometric Biosensors in Stirred and non Stirred Solution, *Nonlinear Analysis: Modelling and Control*, **8**(1), pp. 3–18, 2003.

8. R. Baronas, F. Ivanauskas, J. Kulys, The Influence of the Enzyme Membrane Thickness on the Response of Amperometric Biosensors, *Sensors,* **3**(7), pp. 248–262, 2003.

9. R. Baronas, F. Ivanauskas, J. Kulys, M. Sapagovas, Modelling of amperometric biosensors with rough surface of the enzyme membrane, *Journal of Mathematical Chemistry,* **34**(3–4), pp. 227–242, 2003.

10. F. Ivanauskas, R. Baronas, J. Kulys, M. Sapagovas, Fermento sluoksnio nelygumų amperometriniame biojutiklyje modeliavimas (Modelling of surface roughness of enzyme layer of amperometric biosensors), *Lietuvos matematikos rinkinys (Lithuanian Mathematical Journal),* **43**, pp. 625–629, 2003.

11. R. Baronas, F. Ivanauskas, J. Kulys, M. Sapagovas, Computational Modelling of a Sensor based on an Array of Enzyme Microreactors, *Nonlinear Analysis: Modelling and Control,* **9**(3), pp. 203–218, 2004.

12. R. Baronas, J. Kulys, F. Ivanauskas, Modelling amperometric enzyme electrode with substrate cyclic conversion, *Biosensors and Bioelectronics,* **19**(8), pp. 915–922, 2004.

13. R. Baronas, F. Ivanauskas, J. Kulys, The effect of diffusion limitations on the response of amperometric biosensors with substrate cyclic conversion, *Journal of Mathematical Chemistry,* **35**(3), pp. 199–213, 2004.

14. R. Baronas, J. Kulys, F. Ivanauskas, Mathematical Model of the Biosensors Acting in a Trigger Mode, *Sensors*, **4**(4), pp. 20–36, 2004.

15. R. Baronas, J. Kulys, F. Ivanauskas, Computational Modelling of Biosensors with Perforated and Selective Membranes, *Journal of Mathematical Chemistry,* **39**(2), pp. 345–362, 2006.

16. R. Baronas, F. Ivanauskas, J. Kulys, Mathematical Modelling of Biosensors Based on an Array of Enzyme Microreactors, *Sensors*, **6**(4), pp. 453–465, 2006.

17. H. Gufreund, *Kinetics for the life sciences,* Cambridge University Press, 1995.

18. J. Kulys, V. Razumas, *Bioamperometry,* Mokslas, Vilnius, 1986 (in Russian).

19. `http://www.chem.qmul.ac.uk/iubmb/enzyme`.