

MODEL FOR ENZYME KINETICS USING PETRI NETWORKS

RADU DOBRESCU¹, ȘTEFAN A. POPA¹, VICTOR PURCAREA²,
CĂTĂLIN VASILESCU²

¹*“Politehnica” University of Bucharest, Automatic Control and Computers
Science Faculty, 313 Splaiul Independentei Bucharest, Romania*

²*University of Medicine and Pharmacy “Carol Davila”, 37 Dionisie
Lupu st., Bucharest, Romania*

**corresponding author: e-mail: radud @isis.pub.ro*

Abstract

In this paper we propose a model for single substrate enzyme kinetics based on the differential Petri network formalism. Metabolic signaling pathways imply biochemical reactions in which substrates are enzyme catalyzed and turn them into active biochemical products. The enzymatic reactions are described quantitatively through ordinary differential equations (ODEs) in the proposed Petri network model. The specificity of the biochemical reactions are captured in the proposed Petri network model. The simulation study shows qualitative validation of the dependability of the proposed Petri network model with experimental results for enzyme kinetics.

Rezumat

Lucrarea prezintă un model matematic de reprezentare și simulare a cineticii enzimatică, rețeaua Petri. Aceasta este foarte utilă pentru descrierea sistemelor dinamice și poate fi utilizată pentru modelări matematice ale reacțiilor metabolice, a mecanismelor de reglare alosterică și a mecanismelor de acțiune ale medicamentelor.

Keywords: Enzyme kinetics, dependable model, Petri network, ordinary differential equations, penicillin

Introduction

Metabolic network modelling and simulation is an important step in complex biological system development and allows the comprehension of the molecular mechanisms that take place in the cell. The metabolic network modelling breaks down metabolism pathways into their respective reactions and enzymes, and analyzes them within the perspective of the entire network [9]. Quantitative models of biochemical networks are a central component of modern systems biology. Building and managing these complex models is a major challenge that can benefit from the application of formal methods adopted from theoretical computing science [1, 11].

Many approaches exist for modelling metabolic network. Some of the approaches have constructs that are specifically tailored for modelling only the biological aspects [10]. Few of these approaches have tools which

facilitate the study and analysis of the dynamic behaviour of the system together with the discrete elements and presents dependable characteristics. Biochemical reaction systems have by their very nature three distinctive characteristics [6]: 1) they are inherently bipartite, i.e. they consist of two types of players, the species and their interactions; 2) they are inherently concurrent, i.e. several interactions can usually happen independently and in parallel and 3) they are inherently stochastic, i.e. the timing behaviour of the interactions is governed by stochastic laws. So it seems to be a natural choice to model and analyse them using a formal method, which shares exactly these distinctive characteristics: the Petri networks.

The main advantages of using Petri networks are: intuitive modeling style, mathematically founded analysis techniques, coverage of structural and behavioural properties as well as their relations, integration of qualitative and quantitative analysis techniques and reliable tool support.

This paper presents preliminary results in modelling and simulation of biochemical metabolic pathways with the Petri network formalism which translates biological and chemical processes to Petri network formalisms and uses Petri network based tools to study the behaviour of these processes. The example model is the Penicillin N production.

Petri networks formalism for studying system dynamics

The Petri networks are used extensively for the representation and the simulation of concurrent discrete-event dynamic systems. A Petri network is a directed bipartite graph, in which nodes represent transitions, which are events that may occur, and places, which are seen as conditions, and directed arcs that describe which places are pre- and/or post- conditions for which transitions [8].

Tokens that are placed in places signify that the condition that represents the place holds. The placement of tokens in the network, called marking, defines the network's state. The Petri network can be simulated by moving tokens according to a firing rule; when all the places with arcs leading to a transition have a token, the transition is enabled, and may fire, by removing a token from each input place and adding a token to each output place. The results of the simulation can be plotted as graphs, or otherwise analyzed.

The Petri networks can also be structurally examined in order to verify the desired the system properties, such as boundedness and liveness. Boundedness guarantees that in every place of the network, the number of tokens is always less than some finite number, for example there is no toxic accumulation of metabolites. Liveness guarantees that all transitions can be enabled. Another type of analysis involves determining whether we can

move from one state of the system to another state, for example there were accomplished the conditions for taking place the enzyme-substrate reaction from the specific metabolic pathway.

Particular models for enzyme kinetics

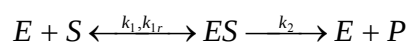
Almost all processes in a biological cell need enzymes to occur at significant rates. Since enzymes are selective for their substrates and speed up only a few reactions from among many possibilities, the set of enzymes in a cell determines which metabolic pathways occur in that cell. This study is limited to the Michaelian enzymes.

In biochemistry, a metabolic pathway represents the series of chemical reactions that occur in a cell. In each pathway, a principal chemical is modified by chemical reactions. Enzymes catalyze these reactions, and often require additional minerals, vitamins and other factors in order the reactions to take place. Pathways may be very complex because of the many chemicals that are involved. The entire collection of pathways that exists in the cell is called the metabolic network. The importance of the pathways is that they maintain the homeostasis of the organism.

The metabolism modifies the initial molecule and shapes it into an end product. The end product can be stored by the cell, used as a metabolic product or used to initiate another metabolic pathway. A molecule called substrate enters a metabolic pathway depending on the needs of the cell and the availability of the substrate. An increase in concentration of anabolic and catabolic end products would slow the metabolic rate for that particular pathway.

Enzyme kinetics modelling with ordinary differential equations

Enzyme kinetics is the investigation of how enzymes (E) bind substrates (S) and turn them into products (P). Equation 1 shows most of the enzyme kinetics characterized by single-substrate reactions based on Michaelis–Menten–Henri kinetics [7].



Equation 1. Single-substrate mechanism for enzyme kinetics

Enzymatic reactions take place in two stages. In the first stage, the substrate S binds reversibly to the enzyme E, which forms the enzyme-substrate complex ES. The enzyme-substrate complex ES is sometimes called the Michaelis complex. The enzyme E then catalyzes the chemical step in the reaction and releases the product P. k_1 represents the constant rate at which the enzyme-substrate complex ES is obtained. k_{1r} represents the constant rate at which the enzyme-substrate complex ES dissociates back in enzyme E and substrate S. k_2 represents the constant rate at which the

enzyme-substrate complex ES dissociates in enzyme E and product P. k_2 , also called k_{cat} or the turnover number, is the maximum number of enzymatic reactions catalyzed per second.

The set of ordinary differential equations used to model the enzyme kinetics described in the above paragraph are presented below in Equation 2.

$$\begin{aligned}\frac{dE(t)}{dt} &= -k_1 \cdot E(t) \cdot S(t) + (k_{1r} + k_2) \cdot ES(t) \\ \frac{dS(t)}{dt} &= -k_1 \cdot E(t) \cdot S(t) + k_{1r} \cdot ES(t) \\ \frac{dES(t)}{dt} &= k_1 \cdot E(t) \cdot S(t) - (k_{1r} + k_2) \cdot ES(t) \\ \frac{dP(t)}{dt} &= k_2 \cdot ES(t)\end{aligned}$$

Equation 2. Ordinary differential equations for enzyme kinetics

Michaelis-Menten kinetics relies on the law of mass action, which is derived from the assumptions of free diffusion and thermodynamically-driven random collision. However, many biochemical or cellular processes deviate significantly from these conditions, due to macromolecular crowding, phase-separation of the enzyme/substrate/product, or one or two-dimensional molecular movement. This is why the time variation of the enzyme-substrate product concentration is not assumed in the differential equations presented in Equation 2 to be zero.

Several enzymes can work together in a specific order, creating metabolic pathways. In a metabolic pathway, one enzyme takes the product of another enzyme as a substrate. After the catalytic reaction, the product is then passed on to another enzyme. Sometimes more than one enzyme can catalyze the same reaction in parallel, this allowing a more complex regulation: with, for example, a low constant activity being provided by one enzyme but an inducible high activity from a second enzyme.

Enzymes determine the steps of in these pathways. Without enzymes, metabolism would neither progress through the same steps, nor be fast enough to serve the needs of the cell.

Enzyme kinetics modelling with Petri networks

Differential Petri networks are a kind of Petri networks which contain differential places and differential transitions [3].

If all the markings and all the arc weights were non-negative, the behaviour of a differential Petri network could be modelled by a hybrid Petri network with special speeds for continuous transitions [2].

Differential predicate transition Petri networks are a special type of Differential Petri networks that can monitor sets of differential equations associated with specific places. In particular, when a token is produced in a place p , the corresponding set of differential equations is activated and continuous variables associated with the produced token can evolve.

Enabling functions are associated with transitions and depend on the continuous values associated to the tokens located in the input places of the corresponding transition.

Junction functions, associated with transitions too, allow modifying and communicating the values of the continuous variables to the tokens produced in the output places of the fired transitions.

A differential predicate transition Petri network is composed of the following elements:

- $N = \langle P, T, Pre, Pos \rangle$ is a Petri network, where P is the set of places, T the set of transitions, Pre and Pos represent the matrix that defines arcs connecting places to transitions and transitions to places.

- $X = \{X_{p_1}, X_{p_2}, X_{p_3}, \dots\}$ is the set of continuous variables.

- X_{p_i} is a vector of variables (which belongs to X) associated with each place p_i . Each marking in the place p_i is actually an instance of X_{p_i} .

- e_i is an enabling function associated with each transition t_i . This function enables or not the firing of t_i according to the values of X_{p_i} which are in the input places of t_i .

- j_i is a junction function associated with each transition t_i . When considering the firing of transition t_i , this function defines the values of vectors X_{p_i} which belong to the output places of t_i .

- f_i is a differential equation system associated with each place p_i . This system defines the variables evolution associated with tokens in the place p_i .

- M_0 is the initial marking, specifying the number of tokens in each place and the variables values associated to each token for the initial time ($t = 0$ s).

The proposed Petri network model which uses the differential Petri networks is presented below.

Variables vector for the places p_1 and p_2 for the enzyme kinetics model is defined as follows

- For the place p_1 , the variables vector $X_{p_1} = (E_1, S_1, ES_1)$ contains the enzyme E_1 , substrate S_1 and enzyme-substrate complex ES_1 concentrations.

- For the place p_2 , the variables vector $X_{p_2} = (P_2, ES_2)$ contains the product P_2 and enzyme-substrate complex ES_2 concentrations.

System's equations are defined by f_1 and f_2 , based on the ordinary differential equations presented in Equation 2 and are partitioned as follows:

$$\begin{aligned}
 - f_1: \quad & \frac{dE_1(t)}{dt} = -k_1 \cdot E_1(t) \cdot S_1(t) + (k_{1r} + k_2) \cdot ES_1(t) \\
 & \frac{dS_1(t)}{dt} = -k_1 \cdot E_1(t) \cdot S_1(t) + k_{1r} \cdot ES_1(t) \\
 & \frac{dES_1(t)}{dt} = k_1 \cdot E_1(t) \cdot S_1(t) - (k_{1r} + k_2) \cdot ES_1(t) \\
 - f_2: \quad & \frac{dP_2(t)}{dt} = k_2 \cdot ES_2(t)
 \end{aligned}$$

The enabling function e_1 associated with the fire's enabling of the transition t_1 is defined as upper or lower bounds for the enzyme, substrate and enzyme-substrate complex concentration values as follows:

$$\begin{aligned}
 E_1(t) &\leq threshold_1 \\
 S_1(t) &\leq threshold_2 \\
 ES_1(t) &\geq threshold_3
 \end{aligned}$$

The threshold values, $threshold_1$, $threshold_2$ and $threshold_3$, depend on the metabolic pathway characteristics.

The junction function j_1 associated with the firing of the transition t_1 is defined as the same value concentration for enzyme-substrate complex in each place p_1 and p_2 :

$$ES_1(t) = ES_2(t)$$

Petri network modelling of the enzyme kinetics is presented in Figure 1. In this model, the place p_1 models the first dynamic step of the single-substrate mechanism for enzyme kinetics from Equation 1, which is the forming of the enzyme-substrate complex ES in the presence of enzyme and substrate levels of concentration. The place p_2 models the second dynamic step of the single-substrate mechanism for enzyme kinetics from Equation 1, which is the forming of the product P in the presence of the enzyme-substrate complex level of concentration obtained from the previous place, place p_1 .

The novelty of this Petri network for enzyme kinetics is the approach of partitioning the ODEs (ordinary differential equations) to

places, which holds actually the dynamic information of the biochemical process.

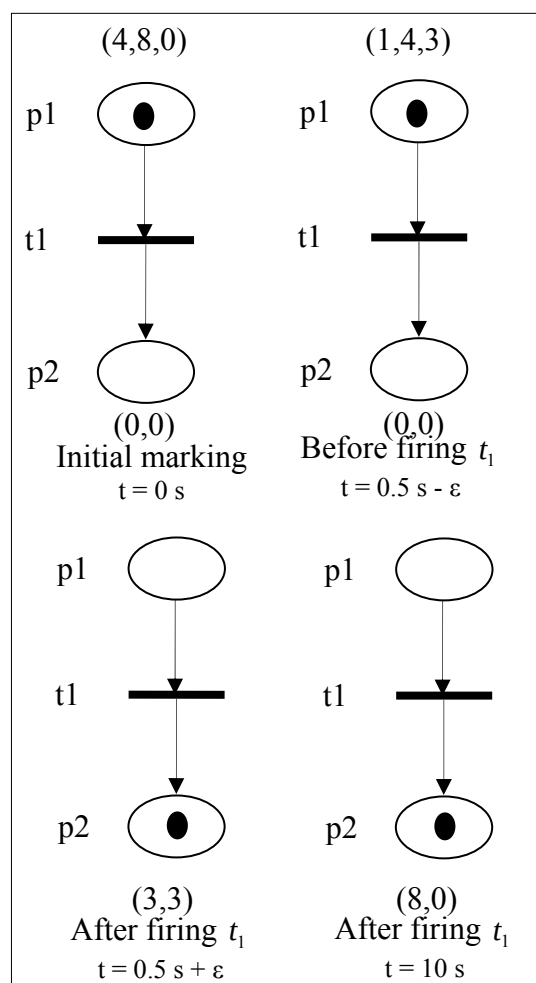


Figure 1
Petri network model of single substrate enzyme kinetics

Using the Petri network model for penicillin and cephalosporin biosynthesis

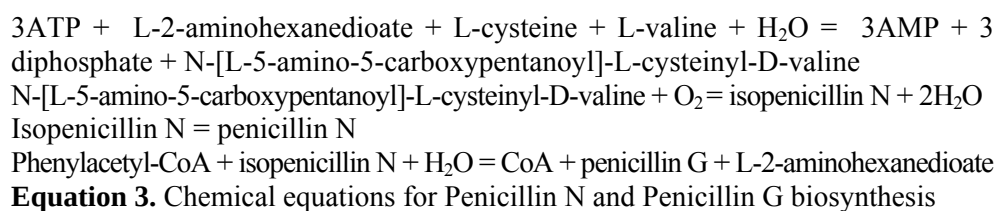
Penicillin is a large group of natural or semi synthetic antibacterial antibiotics derived directly or indirectly from strains of fungi of the genus *Penicillium* and other soil-inhabiting fungi grown on special culture media. *Penicillins* exert a bactericidal as well as a bacteriostatic effect on susceptible bacteria by interfering with the final stages of the synthesis of peptidoglycan, a substance in the bacterial cell wall. Despite their relatively

low toxicity for the host, they are active against many bacteria, especially gram-positive pathogens like *streptococci*, *staphylococci*, *pneumococci*; *clostridia*; certain gram-negative forms like *gonococci* and *meningococci*; certain spirochetes like *Treponema pallidum* and *T. pertenue*; and certain fungi. Certain strains of some target species, for example *staphylococci*, secrete the enzyme penicillinase, which inactivates penicillin and confers resistance to the antibiotic. Some newer penicillins, such as methicillin, are more effective against penicillinase-producing organisms. A class of extended-spectrum penicillins includes piperacillin and mezlocillin [5].

Penicillin N is a secondary metabolite of fungus *Penicillium* that is produced when growth of the fungus is inhibited by stress. It is not produced during active growth. Production is also limited by feedback in the synthesis pathway of penicillin.

Cephalosporins are a large group of broad-spectrum antibiotics obtained from *Acremonium*, formerly *Cephalosporium*, a genus of soil-inhabiting fungi. Cephalosporins are similar in structure and antimicrobial action to penicillin. The cephalosporins have been classified according to general features of antimicrobial activity, with successive generations having increasing activity against gram-negative organisms and decreasing activity against gram-positive organisms [5].

The core set of chemical reactions that generate penicilins and cephalosporins are presented in Equation 3.



A N-(5-amino-5-carboxypentanoyl)-L-cysteinyl-D-valine synthase or EC 6.3.2.26 is an enzyme that catalyzes the first chemical reaction from Equation 3. The 5 substrates of this enzyme are ATP ($\text{C}_{10}\text{H}_{16}\text{N}_5\text{O}_{13}\text{P}_3$), L-2-aminohexanedioate ($\text{C}_6\text{H}_{11}\text{NO}_4$), obtained through Lysine degradation), L-cysteine ($\text{C}_3\text{H}_7\text{NO}_2\text{S}$), L-valine ($\text{C}_5\text{H}_{11}\text{NO}_2$), and water (H_2O), whereas its 3 products are AMP ($\text{C}_{10}\text{H}_{14}\text{N}_5\text{O}_7\text{P}$), diphosphate ($\text{P}_2\text{H}_4\text{O}_7$) and N-[L-5-amino-5-carboxypentanoyl]-L-cysteinyl-D-valine ($\text{C}_{14}\text{H}_{25}\text{N}_3\text{O}_6\text{S}$). This enzyme belongs to the family of ligases, specifically those forming carbon-nitrogen bonds as acid-D-amino-acid ligases (peptide synthases). The systematic name of this enzyme class is L-2-aminohexanedioate:L-cysteine:L-valine ligase (AMP-forming, valine-inverting). Other names in the common use include L-

delta-(alpha-aminoadipoyl)-L-cysteinyl-D-valine synthetase, ACV synthetase, and L-alpha-aminoadipyl-cysteinyl-valine synthetase. This enzyme participates in the penicillin and cephalosporin biosynthesis.

Isopenicillin-N synthase (IPNS) is a non-heme iron-dependent enzyme belonging to the oxidoreductase family. This enzyme catalyzes the formation of isopenicillin-N from δ -(L- α -aminoadipoyl)-L-cysteinyl-D-valine as it can be seen in the second chemical reaction from Equation 3. This reaction is a key step in the biosynthesis of penicillin and cephalosporin antibiotics. The active site of most isopenicillin-N synthases contains an iron ion.

An isopenicillin-N epimerase or EC 5.1.1.17 is an enzyme that catalyzes the third chemical reaction from Equation 3. This enzyme contains pyridoxal phosphate as cofactor. Epimerization at C-5 of the 5-amino-5-carboxypentanoyl group to form penicillin N is required, in order to make a substrate for EC 1.14.20.1, deacetoxycephalosporin-C synthase, to produce cephalosporins. It is part of the penicillin biosynthesis pathway. Hence, this enzyme has one substrate, isopenicillin N, and one product, penicillin N ($C_{14}H_{21}N_3O_6S$). This equation is used for the simulation with the proposed Petri network model for single substrate enzyme kinetics. This enzyme belongs to the family of isomerases, specifically those racemases and epimerases acting on amino acids and derivatives. The systematic name of this enzyme class is penicillin-N 5-amino-5-carboxypentanoyl-epimerase. This enzyme participates in the penicillin and cephalosporin biosynthesis.

An isopenicillin-N: N-acyltransferase or EC 2.3.1.164 is an enzyme that catalyzes the fourth chemical reaction from Equation 3. The 3 substrates of this enzyme are phenylacetyl-CoA ($C_{29}H_{42}N_7O_{17}P_3S$), isopenicillin N, and water (H_2O) whereas its 3 products are CoA ($C_{21}H_{36}N_7O_{16}P_3S$), penicillin G ($C_{16}H_{18}N_2O_4S$), and L-2-aminohexanedioate ($C_6H_{11}NO_4$). This enzyme belongs to the family of transferases, specifically those acyltransferases transferring groups other than aminoacyl groups. The systematic name of this enzyme class is acyl-CoA:isopenicillin N N-acyltransferase. Other names in the common use include acyl-coenzyme A:isopenicillin N acyltransferase, and isopenicillin N:acyl-CoA: acyltransferase. This enzyme participates in the penicillin and cephalosporin biosynthesis.

There are two different categories of penicillin. Biosynthetic penicillin is natural penicillin that is harvested from the mould itself through fermentation. The other form of penicillin is known as semi-synthetic. There are many kinds of the so-called semisynthetic derivatives of penicillin - like Ampicillin, Penicillin V, Carbenicillin, Oxacillin, Methicillin, etc. These compounds have the basic Penicillin structure, but have been modified

chemically by removing the acyl group to leave 6-aminopenicillanic acid and then adding acyl groups that induce new properties. These modern semi-synthetic penicillins have various specific properties such as resistance to stomach acids so that they can be taken orally, a degree of resistance to penicillinase (or β -lactamase) (a penicillin-destroying enzyme produced by some bacteria) and an extended range of activity against some Gram-negative bacteria.

Penicillin G (or benzylpenicillin) is the most widely used form and the same one we get in a shot (hypodermic) form.

Results and discussion

The third chemical reaction from Equation 3 is modelled with the Petri network model proposed above. The enzyme E is the isopenicillin-N epimerase, the single substrate catalyzed enzymatic S is the isopenicillin N and the obtained product P is penicillin N ($C_{14}H_{21}N_3O_6S$).

The differential equations from the Petri network model for enzyme kinetics are solved using MATLAB library functions, for example solver ode113. The solver uses a variable order Adams-Bashforth-Moulton PECE solver (Predictor-Evaluation-Corrector-Evaluation). It is more efficient than the one-step solver based on explicit Runge-Kutta formula, which uses the Dormand-Prince pair, at stringent tolerances and when the function is particularly expensive to evaluate. The used Adams-Bashforth-Moulton PECE solver is a multistep solver, which means that it needs the solutions from several preceding time points to compute the current solution.

The absolute tolerance used is 10^{-6} and the relative tolerance used is 0.0010 for a time horizon of 10 seconds.

The simulation used the proposed Petri net model from above with the initial marking $M_0 = (4, 8, 0)$ and the threshold values: $threshold_1 = 1$, $threshold_2 = 4$, $threshold_3 = 3$. All these concentration values are expressed in Molarity (M). The values used for the constants involved in the reactions are $k_1 = 2 (M \cdot s)^{-1}$, $k_{1r} = 1 s^{-1}$ and $k_2 = 1.5 s^{-1}$. These values depend on the reaction parameters.

In the Figure 2 it is represented the concentration-time variation of the enzyme E, substrate S, enzyme-substrate complex ES and product P. It can be seen that the initial degradation of the enzyme and substrate generates the enzyme-substrate complex, modelled in the Petri network from Figure 1 as place p_1 with ODEs f_1 . The maximal concentration value of the enzyme-substrate complex corresponds to the minimal

concentration value of the enzyme, which corresponds to the enabling function e_1 . This is the moment when the transition t_1 is fired in conformance with the junction function j_1 . After this point the concentration value of the enzyme increases before setting to its steady state and the concentration value of the enzyme-product complex decreases exponentially to zero, modelled in the Petri network from Figure 1 as place p_2 and ODE f_2 . The occurrence of the enzyme-substrate complex ES boosts the increase of the concentration value for the product P.

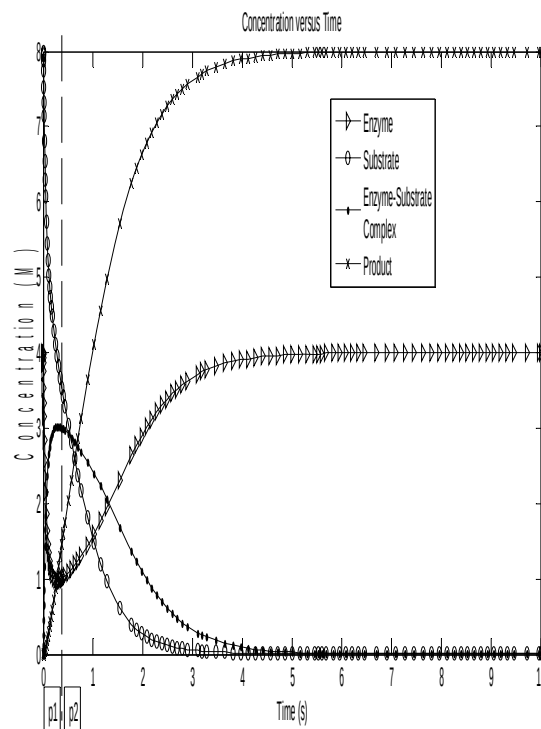


Figure 2

Concentration-time variance of single substrate enzyme kinetics

The partitioning of the graph from Figure 2 captures the Petri net modelling of the Michaelian enzyme kinetics in the p_1 place and in the p_2 place, presented in Figure 1.

Conclusions

The novelty of this Petri network model for enzyme kinetics is the approach of partitioning the ODEs to places, which holds actually the

dynamic information of the biochemical reaction and the dependability assessment of the model.

The proposed dependable Petri network model for enzyme kinetics may be used in metabolic signaling pathway modeling. The model may be extended in order to incorporate exceptions of the single substrate enzyme reactions, like cooperative binding of substrate to the active site in allosteric regulation, and also to incorporate multi-strata enzyme reactions like ternary complex or ping-pong.

The dependence of the proposed Petri network model for single substrate enzyme kinetics may be increased by incorporating fault tolerance mechanisms and ensuring more experimental results compliance. Also fractal approaches may be employed for modelling enzyme kinetics [4]

References

1. Breitling, R., Gilbert, D., Heiner, M., and Orton, R., A structured approach for the engineering of biochemical network models, illustrated for signalling pathways. *Briefings in Bioinformatics*, 2008, 9(5), 404-421
2. David, R. and Alla, H., Discrete, Continuous and Hybrid Petri Nets. Springer Verlag, Berlin Heidelberg New York, 2005
3. Demongodin, I. and Koussoulas, N.T., Differential Petri Nets: Representing Continuous Systems in a Discrete-Event World. *IEEE Transactions on Automatic Control*, 1998, 43, 4, 573-579
4. Dobrescu, R., Ichim, L., Mocanu, S. and Popa, S., A Fractal Approach to Pattern Formation in Biological Systems. *WSEAS Transactions on Biology and Biomedicine*, 2008, 6, 5, 107-116
5. Dorland's Illustrated Medical Dictionary (2007), Elsevier
6. Heiner, M., Gilbert, D. and Donaldson, R., Petri Nets for Systems and Synthetic Biology. In M Bernardo, P Degano, and G Zavattaro (Eds.): *SFM 2008*, Springer LNCS 5016, 215-264
7. Michaelis, L. and Menten, M.L., Kinetik der Invertinwirkung. *Biochemische Zeitschrift*, 1913, 49:333-369. Springer Verlag, Berlin/Heidelberg
8. Petri, C. A., Kommunikation mit Automaten. Ph.D Thesis. *Schriften des Instituts für instrumentelle Mathematik Nr.2*, 1962, University of Bonn, Germany.
9. Mohora M., Greabu M., Totan A., Mitrea N., Battino M., Redox-sensitive signaling factors and antioxidants, *Farmacia* 2009, 57(4), 399-411
10. Sandulovici R., Prasacu I., Mircioiu C., Voicu V., Medvedovici A., Anuța V., Mathematical and phenomenological criteria in selection of pharmacokinetic model for m1 metabolite of pentoxifylline, *Farmacia* 2009, 57(2), 235-246
11. Antonescu A., Mureșan M., Micle O., Micle L., Dobjanschi L., Vicaș L., Dorofteiu M., Serum oxidative stress parameters and homocysteine levels in patients with renal transplant, *Farmacia* 2008, 56(3), 352-358