Stochastic Petri Net Modeling of Biosynthesis of Tetracycline

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Abstract— System biology is a rapidly developing discipline which utilizes mathematical and computer science techniques to analyze and interpret biological models. In this paper we use Petri nets to model and validate the biosynthesis of tetracycline pathways as described in KEGG (Kyoto Encyclopedia of Genes and Genomes) representation. The biosynthesis pathway of tetracycline is transformed into Petri net model and the resulting network has been validated employing p-invariance and t-invariance properties of Petri net. The Stoichiometric matrix generated in the Petri net modeling has been used to compute the change of state of the system after every reaction and time evolution of the reaction has been simulated using Gillespie's exact stochastic simulation algorithm. The plots generated during simulation explain the nature of reaction satisfactorily.

Index Terms — Petri net, Tetracycline, Biosynthesis of Tetracycline, Petri net of Tetracycline, P-invariant and T-invariant, stochastic simulation

1 INTRODUCTION

Aromatic polyketides formed by type II polyketide synthases (PKS) comprise an important and structurally diverse group of bacterial secondary metabolites. Many of these compounds that are produced by soil-born and marine Gram-positive Actinomycetes have immerged as clinically important drugs or drug leads such as tetracyclines. 7-chlorotetracycline was isolated by Duggar in 1948 [1]. Reductive dehalogenation of this compound by hydrogen over Pt catalyst to tetracycline was performed by Boothe et al [2]. The crystal structure, configuration, bond distances and conformational behavior of chlortetracycline hydrochloride, has been determined by Donohue et al [3]. The biosynthesis of tetracycline has been studied using blocked mutants of the tetracycline producer streptomyces aureofaciens [4], [5], [6], [7], [8], [9]. An investigation of the early tailoring reaction in the oxytetracycline biosynthetic pathway is reported by Zhang et al [10]. Vanek et al have reviewed the experimental evidence on control of tetracycline biosynthesis pathway during the various phases of the bacterial growth cycle. It is suggested that chlortetracycline does not fit into the category of the so- called secondary metabolites and should be regarded rather as an 'excessive metabolite produced by variation in the control of certain key primary pathways. KEGG pathways on biosynthesis of Type II polyketide backbone, type II polyketide products and tetracycline are published [11], [12], [13].

Petri Net is an important technique for modeling and simulating biological systems. These can be used for qualitative as well as quantitative modeling of biochemical networks. In quantitative modeling one can use stochastic Petri nets and employ the formulation proposed by Gillespie [15]. While in qualitative modeling formalism of simple Petri nets can be used for steady state conditions basically to glean information about the structure of the model and its behavior. Stochastic model provides information about the nature of reaction while simple Petri net model is suitable for multi step complex reaction network such as the one under consideration and provide information about correctness of the pathway, error in the representation, marking of the species involved in the reactions and presence of subnets in the overall system. An excellent review of the stochastic Petri net is written by Heiner [16] while Petri net modelings of few complex biochemical pathways have appeared recently [17], [18]. In recent times, stochastic simulation of chemical kinetics has received an increased amount of attention from the modeling community.

There are two formalism for mathematically describing the time behavior of a spatially homogeneous chemical system (1) the Gillespie method which assumes a chemical reaction to be discreet and stochastic and (2) the conventional 'reaction rate method' which assumes reactions to be deterministic and continuous. In fact basic nature of chemical reactions is stochastic, it is due to large size of chemical reactors (test tube, beaker or industrial reactors) and hence the large population that chemical reactants seem to be continuous, however, in the biochemical reaction, because of small size of reactor (cell) and comparatively small number of some of the reactants, above concept is not applicable and reaction has to be treated as discrete and probabilistic. It is therefore suggested that biochemical reaction where population of some of the reactants is very small, should be treated by stochastic method instead of reaction rate method

The stochastic simulation algorithm (SSA) allows one to numerically simulate the time evolution of a well-stirred chemically reacting system in a way that takes proper account of the randomness that is inherent in such a system. The SSA is exact in the sense that it is rigorously based on the same microphysical premise that underlies the chemical master equation (CME), thus a history of 'realization' produced by the SSA gives a more realistic representation of the systems evolution than would history inferred from the conventional deterministic reaction rate equation (RRE). The RRE can be particularly misleading if the molecular population of some critical reactant species becomes so small that microscopic fluctuation can conspire with dramatic consequences in the genetic\enzymatic reaction that go on inside a living cell.

To the best of our knowledge there is no report on the Petri net modeling and stochastic simulation of biosynthesis of tetracycline. The present problem of Petri net based description and modeling of biosynthesis of tetracycline was therefore undertaken with the view (1) to give a bipartite graph theoretical model to the conventional monochromatic representation (2) to high light the superiority of such models over conventional one (3) to validate the model and to utilize the incident matrix obtained from the Petri net consideration, to simulate the process employing stochastic simulation algorithm.

2 PETRI NETS

Petri nets are bipartite digraphs containing two types of nodes called places (P_i) and transitions (T_i). Places are represented by circles and transitions are represented by rectangles. In the language of chemistry places can be mapped to molecular species (reactants or products) and transitions can be mapped to reactions. Places may contain tokens, represented by black dotes within the circles. Tokens are the dynamic elements and represent movable objects residing in places. Tokens correspond usually to number of molecules, number of moles, concentration levels etc. distribution of tokens in different places defines the state of a Petri net and is called marking, M, of the state. State of the net, before execution of any transition, is called initial marking M_0. Directed edges, called arcs, connect places to transitions and transitions to places. Edges may contain weights. Weights over an edge from reactant to reaction represent number of tokens (molecules) to be removed from reactants during a transition and weights over an edge from reaction to product place represent tokens (molecules) to be added to the product. Fig. 1 represents the Petri net for famous reaction between hydrogen and oxygen to produce water.

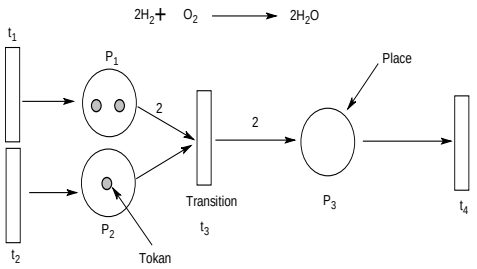

Fig 1. **Petri net model for synthesis of water from hydrogen and oxygen M0 = (2, 1, 0)**

A transition is enabled when the number of tokens in its input places is greater than or equal to the weights on the arc connecting the places to the transition.

A transition with no input places, called a source transition, is always enabled. The transition t_1 and t_2 in Fig.1 are source transitions. An enabled transition can fire, depositing tokens in its output places, again their number determined by the arc weights. A transition with no output places, called sink transition, can fire when enabled consuming the tokens from its input places. Transition t_4 in Fig.1 is a sink transition.

A firing itself is timeless, i.e. frequency of firing per unit time and duration of firing are not taken into account in simple Petri nets, and however these are important in timed Petri nets. The firing is atomic as well, which means that only integral number of tokens (not fractions) can be transferred from pre places (reactants) to post places (products). Fig.2 shows the same PN after the enabled transitions are fired. Thus the firing of a sequence of transition may change the marking of the net.

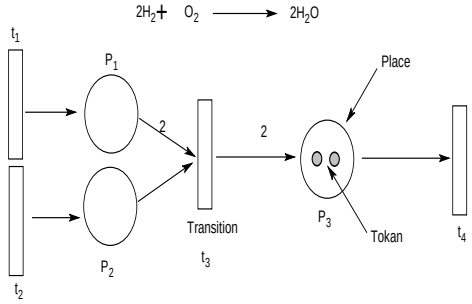

Fig.2. **Petri net model for synthesis of water from hydrogen and oxygen after firing t_3, M1 = (0, 0, 2)**

3 FORMAL DEFINITION AND PROPERTIES OF PETRI NETS

A Petri Nets can be defined as a quadruple N= (P, T, F, M_0) where P is the set of places and T is the set of transitions.

F: ((P x T) U (T x P)) → N_0 defines the set of directed edges containing nonnegative weights and M_0: P →N_0 gives the initial marking

Petri nets posse's structural as well as behavioral properties which are helpful in the analysis of the model under study. The Petri net structure reflects the reaction topology and follows the elementary graph properties. These properties are listed here and detailed can be found else where [1]. The Petri net is ordinary (all arc weights should be 1), homogeneous (all outgoing arcs of a given place posses same weights), pure (no two nodes should be connected in both directions, such as in case of a reversible reaction), conservative (all transitions fire token-preserving), connected (direction of arcs is immaterial), strongly connected (all arcs are directed), free from boundary nodes (all transitions posses pre-/post places and all places posses pre-/post transitions) and free from static conflicts (no two transitions should share the same pre-place). These properties can be used as a check to the correct ness of the net. Some of the behavioral properties are given below

3.1. **Reachability**

A marking M_k is reachable from initial marking M_0 if there exists some firing sequence σ = (U1, U2, U_3 ….Un) that accomplishes this changes. Mathematically we can write

$$M_k = M_0 + A\sum_{k=1}^{n} U_k \quad (1)$$

M_k is the marking after k^{th} transition and is an m x 1 vector, where m is the number of places.

U_k is a control vector indicating the transition fired at k^{th} firing.

A is the m x n incident matrix. An element a_{ij} of A denotes a change in number of tokens in place I due to j^{th} firing (transition)

If we define $x = \sum_{k=1}^{n} U_k$ we obtain

$$M_n - M_0 = Ax$$

Or $A x = \Delta M \quad (2)$

These equations can be used to calculate the particular state of the net after certain number of firing. In chemical reactions, these equations can be used to know the number of molecules of different species (Reactants and products) after certain number of reactions. Thus, reachability criteria provides a quantitative method for validation of the model

3.2. Liveness

In a complex reaction network, liveness means that, each reaction will happen and products will be formed till there is enough supply of reactants. A break down (deadlock) in the net means an error in the model. Liveness can be associated with a particular transition, a particular marking or with whole Petri net. A transition is dead in a marking M, if it is not enabled in any marking M′ reachable from M. A transition is live, if it is not dead in any markings reachable from M_0. A marking is dead if`, there is no enabled transition in it. A Petri net is live if every transition in it is live.

3.3. Boundedness and safeness

Places are frequently used to represent information storage area in communication and computer systems, product and tool storage areas in manufacturing systems, etc. It is important therefore to determine whether proposed control strategies prevent form the overflows of these storage areas. The information storage areas can hold, without corruption, only a restricted number of pieces of data. In manufacturing systems, attempts to store more chemicals, for instance, in the chemical storage area may result in the equipment damage. The Petri net property which helps to identify in the modeled system the existence of overflows is the concept of boundedness. A Petri net is said to be k- bounded if the number of tokens in any place p, where p ϵ P, is always less or equal to k (k is a nonnegative integer number) for every marking M reachable from the initial marking M_0. A Petri net shown in fig. 3 (A) is safe. In this net no place can contain more then one token. An example of a Petri net which is unbounded is shown in fig. 3(B). This net is unbounded because place p_4 can hold an arbitrarily large number of tokens.

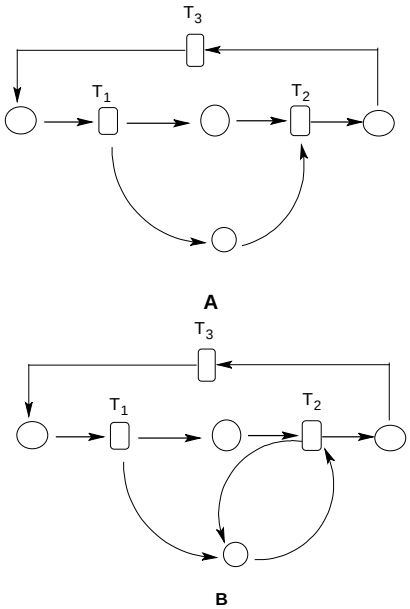

Fig 3 **(A) Safe Petri net and (B) Unbounded (unsafe) Petri net**

3.4. The incident matrix and state equations

An important aspect of Petri nets is that, matrix equations can be used to represent and analyzed its dynamic behavior. The fundamental to this approach is m x n incident matrix, A. An element a_{ij} of A denotes a change in number of tokens in place i due to j^{th} firing (transition). The entries a_{ij} can be defined as $a_{ij} = aij^{+} - aij^{-}$. Thus when a j^{th} transition fires, a_{ij}^{+} represents the number of tokens deposited to its output place and, a_{ij}^{-} represents the number of tokens removed from its input place. Therefore, a transition *t*, is said to be enabled in marking *M* if

$$a_{ij} \le M(p_j), i = 1, 2....., m. \quad (3)$$

For Petri nets with self-loops, $a_{ij=0}$ for a place p_j and transition t_i which belong to a self-loop. For this reason, in order to make sure that the incidence matrix properly reflects the structure of Petri net the net is assumed to be pure, or made pure by introducing two additional places. The state equation for a Petri net represents a change in the distribution of tokens on places as a result of a transition firing. This equation is defined as follows:

$$M_k = M_{k-1} + AU_k, k = 1, 2 \quad (4)$$

M_k is an m x 1 column vector representing a marketing M_k immediately reachable from a marketing M_{k-1} after firing transition t_i. The k-th firing vector U_k, an n x 1 column vector, has only one nonzero entry. This entry, a1 in the i^{th} position, represents a transition t_i firing in the k-th firing of the net firing sequence starting with an initial marketing Mo. This entry corresponds to the i^{th} row of the incidence matrix, A, which represents a change of a marking as a result of a firing transition t_i. The matrix equation is useful in studying the reachability problem.

3.5. Invariant properties

P-invariant and T-invariant are the two additional concepts useful in model validation. A transition vector is called t-

invariant if it is a non-trivial nonnegative integer solution of the homogeneous linear equation system A.Y = 0. The nonzero entries in a T-invariant represent the firing counts of the corresponding transitions which belong to a firing sequence, transforming a marking M_0 back to M_0. Although a T- invariant states the transitions comprising the firing sequence transforming a marking M_0 into M_0, and the number of times these transitions appear in this sequence, it does not specify the order of transitions firings.

A place vector is called a P-invariant, if it is a non-trivial nonnegative integer solution of the homogeneous linear equation system X.A = 0. The nonzero entries in a P-invariant represent weights associated with the corresponding places so that the weighted sum of tokens on these places is constant for all markings reachable from an initial marking.

The subset of places (transitions) corresponding to the nonzero entries of a T- invariant (P-invariant) is called the support of an invariant, and denoted by ||x|| (|| y ||). A support is said to be minimal if no proper nonempty subset of the support is also a support**.**

4 BIOSYNTHESIS OF TETRACYCLINE

The formation of polyketides is very similar to the synthesis of long chain fatty acids [19]. The enzymes responsible for synthesis of polyketides backbone can be categorized as type I and type II polyketide synthases complexes (type I PKS and type II PKS). The type I PKS gene clusters produce type I polyketide such as erythromycin A while type II PKS gene clusters produce aromatic polyketides such as tetracycline. The sequencing of type II PKS consists of three to six separate mono or bifunctional proteins. The three genes KSα, KSβ and ACP together are called minimal type II PKS. The growth of the polyketide chain is initiated by condensation of a starter unit (acetyl-CoA) with an extender unit (malonyl-CoA) present in the host as Coenzyme A thioesters. Condensation is followed by decarboxylation of the extended unit. Since ketoreductase, dehydratase and enoylreductase are absent in the PKS, fatty acid formation does not proceed and the diketide immediately undergoes another round of condensation with extender to produce a unreduced triketide and higher polyketide backbone (nonaketide). These scaffolds react with 2-oxosuccinamide to introduce amido group and produce malonamyl-CoA, which gives rise to key intermediate, 6-methylpretetramide. 2-oxosuccinamide (Malonamoyl) is produced from L-asparagine by enzymatic action of asparagine-oxo-add transaminase. The complete biosynthesis is shown in Fig 4.

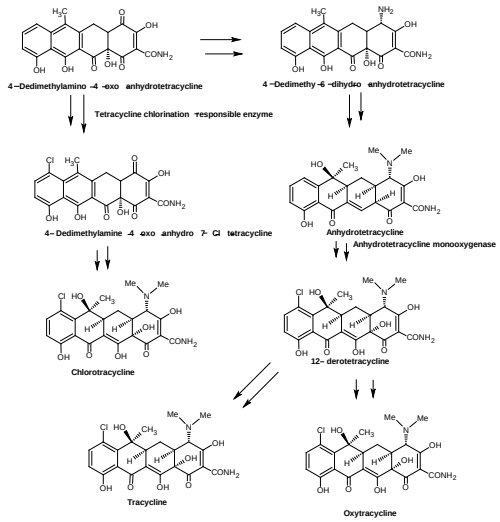

Fig.4. Mechanism of Biosynthesis of tetracycline

5 RESULT AND DISCUSSION

The biosynthesis of tetracycline is shown in Fig.4 and KEGG pathway is shown in Fig.5. The abbreviation used in the present work are shown in Table 1, Fig.4 and Fig.5 are different representations of biosynthesis of tetracycline with different focus but complementary to each other.

TABLE 1
Some important compounds and their abbreviations and place designation

	Compound name	Abbreviation	Places
1.	Acetylco-A	AcoA	P1
2.	Nonaketamide	NKD	P2
3.	6 – Methylpretetramide	6MPT	P3
4.	4 –Keto-anhydrotetracycline	4KATC	P4
5.	Anhydrotetracycline	ATC	P5
6.	5a-11a- Dehydrotetracycline	5a-11a-DTC	P6
7.	Tetracycline	Tc	P7
8.	Oxytetracycline	Otc	P8
9.	Chlorotetracycline	ClTc	P9
10.	4 –Keto-anhydrochlorotetracycline	4-KAClTc	P10

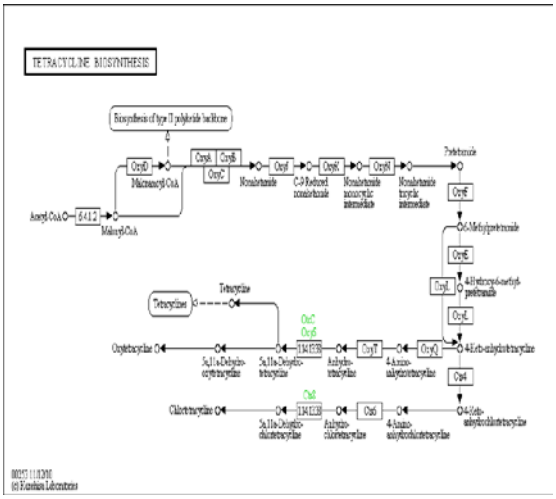

Fig.5. KEGG pathways for biosynthesis of tetracycline

These figures are schematic and informal, because they need additional verbose explanations how to read them. One of the basic draw back of such representations is that, although they represent reactions, there is no notation to represent reactions. This type of monochromatic graphical representation cannot be treated mathematically and computationally and therefore can not be validated. Another inherent draw back in monochromatics graphs is multiple interpretations of branching (node connections to several successors) to and joining (several predecessors joining at a node). Consider for example synthesis of tetracycline and oxytetracycline from 12-dehydrotetracycline in KEGG pathways for biosynthesis of tetracycline shown in Fig.6. This branching in Fig. 6a can have three interpretations (1) 12-dehydrotetracycline triggers the activation of either tetracycline or oxytetracycline (2) 12-dehydrotetracycline triggers the activation of tetracycline as well as oxytetracycline with transition state formation and (3) 12-dehydrotetracycline triggers the simultaneous activation of tetracycline and oxytetracycline as shown in Fig. 6 b, c and d respectively.

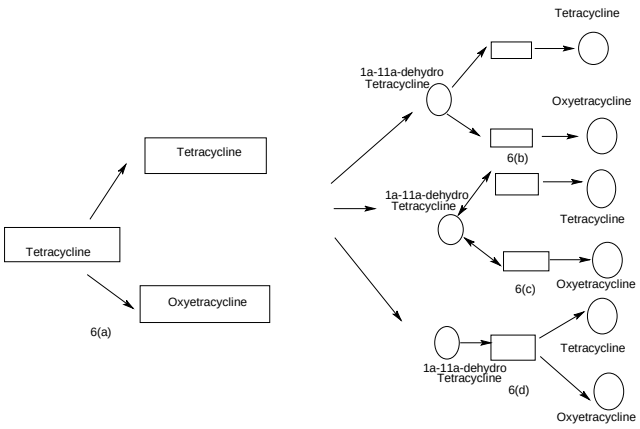

Fig. 6(a), 6(b), 6(c), 6(d). Multiple interpretation of branching in a monochromatic graph representation

Let us again consider the monochromatic representation of activation of 6-methylpretetramide from Malonamoyl-CoA and malonyl-CoA in KEGG pathways for biosynthesis of tetracycline shown in Fig. 7, as an example of joining in a monochromatic graph. Possible interpretations of monochromatic joining can be (1) the activation of 6-methylpretetramide is triggered by Malonamoyl-CoA and / or by malonyl-CoA, one of them is sufficient (Fig. 7a) (2) formation of 6-methylpretetramide is only triggered if Malonamoyl-CoA as well as malonyl-CoA are triggered and both are necessary conditions (Fig. 7b).

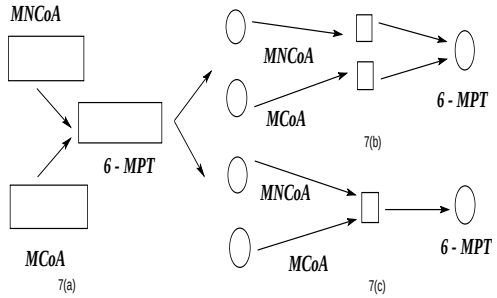

Fig. 7 (a), 7 (b), 7(c). Multiple interpretations of joining in a monochromatic graph representation

Contrary to these shortcomings with monochromatic graphs, Petri nets bipartite graphs represent complete stochiometry of the reaction and contain notations for reactants, products, as well as reactions. There are dynamic elements in the representations and animation of the graph can lead to real time visualization of the reactions. Such graphs comprise of definitions of structural and behavioral properties and sound mathematical techniques have been developed to determine them making it possible to validate the model. We have applied the criteria of P and T linear invariants to validate the net for biosynthesis of tetracycline. It is worth noting that P-invariant and T-invariants are natural (non-zero nonnegative) solutions of the equations X.A = 0 and A.Y = 0 respectively and are orthogonal to the columns and rows of A respectively. Since A is known X and Y can be generated. Algorithm for computation of P-invariants and T-invariants are available [17]. It is obvious from the definition of P-invariant that X is a row vector of places and Y is a column vector of transitions. It is easy to understand that product of X vectors (P-invariants) over each column of A (each transition) is zero. Thus the total effect of each transition on the P-invariant is zero. Same can be verified from The A matrix for biosynthesis of tetracycline and P-invariants shown in Table 2

TABLE 2
Combined incidence matrix, A, for biosynthesis of Tetracycline

	T0	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
P0	1	-1	0	0	0	0	0	0	0	0	0
P1	0	1	-1	0	0	0	0	0	0	0	0
P2	0	0	1	-1	0	0	0	0	0	0	0
P3	0	0	0	1	-1	0	0	0	0	0	0
P4	0	0	0	0	1	-1	0	0	0	0	0
P5	0	0	0	0	0	1	-1	0	0	0	0
P6	0	0	0	0	0	0	1	-1	0	0	0
P7	0	0	0	0	0	0	0	1	0	0	0
P8	0	0	0	0	0	0	0	1	0	0	0
P9	0	0	0	0	0	0	0	0	1	-1	0
P10	0	0	0	0	0	0	0	0	0	1	-1
P11	0	0	0	0	0	0	0	0	0	0	1

Solution of the equations X.A = 0 and A.Y = 0 for biosynthesis of tetracycline Petri net model Fig 8 leads to 2 solutions for P-invariants and 6 solutions for T invariants. The solutions are vectors having the length of number of places and number of reactions respectively.

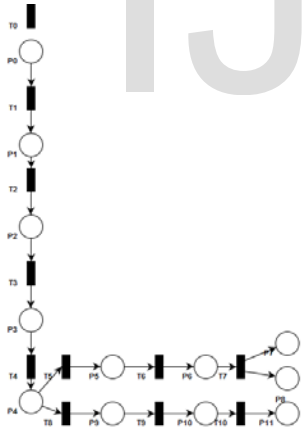

Fig.8. Petri net modeling of biosynthesis of Tetracycline

The solutions for P-invariants are:

P-invariants are:

X0 = (0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0, 0) = (P10)

X1 = (0, 0, 0, 0, 0, 1, 0, 0, 0, 0, 0, 0) = (P11)

T-invariants are:

Y0 = (1, 0, 0, 0, 0, 0, 0, 0, 0, 0) = (T1)

Y1 = (0, 1, 0, 0, 0, 1, 0, 0, 0, 0) = (T2)

Y2 = (0, 0, 1, 0, 0, 0, 0, 0, 0, 0) = (T3)

Y3 = (0, 0, 0, 1, 0, 0, 0, 0, 0, 0) = (T4)

Y4 = (0, 0, 0, 0, 0, 0, 0, 1, 0, 0) = (T8)

Y5 = (0, 0, 0, 0, 0, 0, 0, 0, 1, 0) = (T9)

It is easy to verify that the sum of products of P invariants with columns of A matrix and sum of products of T-invariants with the rows of A are zero confirming the orthogonality of P-invariants and T-invariants to the columns and rows of A respectively.

Supports are the non-zero entries in the P-invariants and T-invariants. The supports for P invariants are:

$|| x_0 || = \{P_5\}$, $|| x_1 || = \{P_6\}$

Supports for T-invariants are

$|| y_0 || = \{T_1\}$, $|| y_1 || = \{T_1\}$, $|| y_2 || = \{T_2\}$, $|| y_3 || = \{T_3\}$, $|| y_8 || = \{T_8\}$, $|| y_9 || = \{T_9\}$

All calculations were made using a computer program PIPE (Platform independent Petri net Editor) 14. The two T-invariants correspond to separate nets on this system producing two different stable compounds, one subunit corresponds to production of oxytetracycline and other subunit corresponds to chlortetracycline and all reactions are unimolecular. Stochastic simulation of the problem was performed employing stochastic simulation algorithm (SSA). Complete descrpition of the algorithm can be found else where [15]. Calculations were made by employing a modified program and the initial population ar of pyruvate was taken to be 300 and the population of rest of the entities where taken to be 0. All rate constant where taken to be 1. The total population was 1000. The plot of time vs population for reactant and product with source transition is shown in fig. 9.

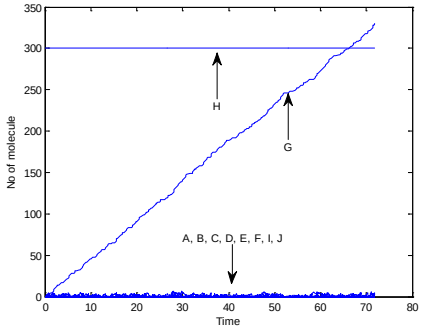

Fig.9 Tetracycline Biosynthesis in the stochastic model of Matlab program with the help of source transition. In this model each graph (A to H) represents the value of each transition and they are standing like as G = (Tc), D = (4 KATS), A =

(Acetyl coA), C = (6 MPT), H = (OxTc), F = (5a 11a DTS), J = (CLTc), E = (ATS), B = (MncoA), I = (4 KOxTc), H = (Py)

It can be seen that consentration of pyruvate remains constant while con of tetracycline rises continuously. The time, consentration profile for the reaction without sourse transition is shown in fig.10.

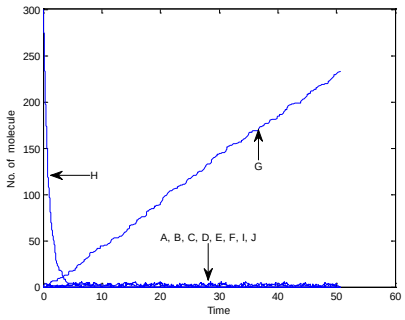

Fig.10. Tetracycline Biosynthesis in the stochastic model with the help of Matlab program. In this model each graph (A to G) represents the value of each transition and they are standing like as G = (Tc), D = (4 KATS), A = (Acetyl coA), C = (6 MPT), H = (OxTc), F = (5a 11a DTS), J = (CLTc), E = (ATS), B = (MncoA), I = (4 KOxTc), H = (Py)

In this simulation the population of pyruvate decreases with time and population of tetracycline increase with time. The population of intermediate is remains constant, from these we conclude that the prediction of SSA are satisfactory for a complex reaction like this Biosynthesis of tetracycline. The computational time was nearly 6 hrs

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7 References

[1] B. M. Duggar, "A Clinical Value of Aureomycin", Ann N. Y. Aced Sci., vol. 51, 177-181, November 1948.
[2] J. H. Boothe, J. Morton, J. P. Petisi, R. G. Wilkinson, Williams, "A Robust Platform For The Synthesis Of New Tetracycline Antibiotics', J. Am. Chem Soc, vol. 75, 4622-4623, December 1953.
[3] J. Donohue, J. D. Dunitz, K. N. Trueblood and M. S. Webster, "The Crystal Structure Of The Tetracycline Hydrochloride. Configuration, Bond Distance and Confirmation". J. Am. Chem. Soc, vol 85, 851-856, 1963.
[4] McCormick, J. R. D. and Jensen, "Investigation of Early Tailoring Reactions in the Oxytetracycline Biosynthetic Pathway", J. Am. Chem Soc, vol 87, 1793, 1965.
[5] McCormick, J. R. D. and Jensen, "Biosynthesis of Tetracyclines.X.Protetrone", J. Am. Chem Soc, vol 90, 7126-7127, December 1968.
[6] McCormick, J. R. D. and Jensen, E. R, Arnold, N. H, Corey, H. S, Joachim U. H, Johnson, S., Miller, P. A, and Sjolande, "Biosynthesis of Tetracycline.XI.methylanthrone analog of Protetrone", J. Am. Chem Soc, vol 90, 7127-7129, December 1968.
[7] McCormick, J. R. D, Jensen, E. R. Johnson, S., and M. G. Charest, D. R. Siegel and A. G. Myers , "A convergent Enantioselective Route to Structurally Diverse Tetracycline", J. Am. Chem Soc, vol 127, 8292, 2005.
[8] Sjolande, "Biosynthesis of the Tetracycline Antibiotics" J. Am. Chem Soc, vol 90, 2201, September 1968.
[9] McCormick, J. R. D, Joachim, U. H., Jensen, E. R. Johnson, S., and Sjolande, "Biosynthesis of the Tetracycline Antibiotic", J. Am. Chem Soc, vol 87, 1793, September 1965.
[10]W. Zhang, K. Watanabe, Wang Clay CC and Y. Tang, Investigation of Early Tailoring Reactions in the Oxytetracycline Biosynthetic Pathway J. Bio. Chem Soc, vol 282, 25717-25725, August 2007.
[11] http:/www.kegg jp/kegg-bin/show_pathway? map00253
[12] http:/www.kegg jp/kegg-bin/show_pathway? map01056
[13] http:/www.kegg jp/kegg-bin/show_pathway?map01057
[14] Plateform independent Petri net Editor2 (PIPE2), http://pipe2 sourceforge.net/
[15] Denial T. Gillespie, "Exact stochastic simulation of coupled chemical reactions", J.Phy. Chem, vol 81, 2340, 1977.
[16] Monika Heiner, Ina Koch and Jürgen Wil, "Model validation of Biological pathways using Petrinet-Demostrated for Apoptosis", BioSystem, vol 75, 15, July 2004.
[17] J. W. Pinney, D. N. R. Westhead and G. A. McConkey, "Petri net representations in system biology", December 2003.
[18] C. Girualt and R. Valk, "Petri nets for system engineering", Springer, 2002.
[19] D. Hranueli et al, Biotechnal, "Molecular Biology of Polyketide Biosynthesis", vol 39(3), 203-213, 2001.