Stochastic Petri net modeling of α-chymotrypsin catalyzed hydrolysis of p-nitrophenyl acetate in cationic microemulsions

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Abstract- We consider chemical reactions taking place in micro emulsions, where the mean number of reactant molecules present is small and countable because of small volume of micelles. We describe time evolution of such stochastic reactions and compare the results with traditional chemical kinetics. Petri net modeling and stochastic simulation of α-chymotrypsin catalyzed hydrolysis of p-nitrophenyl acetate in cationic microemulsion has been performed for the first time. The net has been verified using invariant properties of Petri net. A stochastic simulation of the reaction has been carried out using Gillespie hypothesis. The model conclude the nature of a typical enzymatic reaction. The study suggests the concept that enzyme catalyzed reactions in micro emulsions can mimic in vivo enzymatic reactions.

Index Terms- α-chymotrypsin, Microemulsion, p-nitrophenyl acetate, Stochastic Petri nets

I. Introduction

Recent advances in genetic and molecular biology has created large amount of genomic data. Analysis of this data to understand the functioning of genes and proteins necessitates use of computers both for modeling and data interpretation. Exponential increase in the use of computers in system biology reflects the widespread belief that computers and programming together could effectively solve the complexity of living cells. Attempts have therefore been made to produce sophisticated computer algorithm to simulate bio-molecules and bio-reactions which can help better understanding of biological phenomenon.

Traditionally reactions are modeled by monochromatic graphs and time evolution is considered to be continuous and deterministic. In monochromatic representation of reactions, there is only one type of player, i.e. chemical species and there is no representation of reaction. The representation does not provide enough detail of the reaction and can not be treated mathematically. The present study aims to model α-chymotrypsin catalyzed hydrolysis of p-nitrophenyl acetate in cationic microemulsions by Petri nets which are directed bipartite graphs used for modeling, concurrent (several interactions happening independently and in parallel) processes. Petri net is invented in 1962 by Adam Petri.[1] There have been many advances in the subject and different types of Petri nets developed, e.g. simple Petri nets [2,3], hybrid Petri net [4] and stochastic Petri nets [5]. Since biochemical reactions are inherently bipartite, concurrent and stochastic (timing behaviors of the interactions governed by stochastic laws), stochastic Petri nets offer a suitable tool for modeling biochemical reactions. In this model reactant and product molecules are represented by circles, Number of reactant/ product molecules are modeled by dots inside the circles. Reactions are represented by rectangles and stoichiometry is represented by weights on the directed edges. A formal definition of Petri nets is described in section 2. Since number of reactants and products are countable for reactions occurring in micro reactors (bio-cells), the problem of reactions in emulsions micro reactors can handled by stochastic Petri nets. The concept has been applied to many biological reactions [6-17] Although there are reports on the studies on enzymatic reaction in emulsion micro reactors [18-22], to the best of our knowledge, there is no report on the stochastic Petri net modeling of enzymatic reactions in emulsion micro reactors. Therefore we have undertaken the stochastic Petri net modeling of α-chymotrypsin catalyzed hydrolysis of p-nitrophenyl acetate in cationic microemulsion to confirm whether this model satisfactory reproduces the nature of enzymatic reactions in emulsion microreactors.

II. Formal Definition and Properties of Stochastic Petri Nets

A stochastic Petri Nets can be defined as a quintuple N= (P, T, f, λ, M_0) where P is the set of places, T is the set of transitions and N_0 is the set of natural numbers.

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.

λ: T→H is a function , which assigns a stochastic hazard function h_t to each transition t. H is the set of stochastic hazard functions and $v(t) = h_t$ for all transitions t Є T.

In stochastic Petri Net function is returning, for every possible pair the multiplicity of the arc; and that, if this is zero the arc does not exist.

Petri nets posses structural as well as behavioral properties. While structural properties depend upon the topology of the Petri net and are independent of initial marking, the behavioral properties reflect the chemical properties of the reaction and depend upon the initial markings. Details of the properties can be found elsewhere [23]. Petri net model of a typical enzymatic reaction is shown in Figure 1:

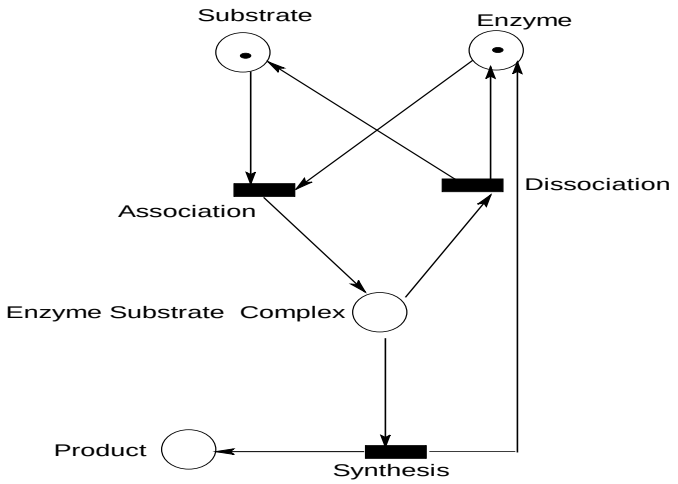

Figure 1. Petri net model for a typical enzymatic reaction

In-vitro enzymatic reactions in nanometer sized water pockets are important as they can mimic models for intracellular enzymatic reactions. Such pockets are formed in water-in-oil type microemulsions [18] (reverse micelles) due to dispersion of water droplets in low polarity bulk solvent and stabilized by a surfactant like AOT. Hydrophilic enzymes such as α-chymotrypsin remain active in water pockets of such microemulsions. Reverse micelles find applications in the fields of synthesis of nanomaterials [24], drug transport, catalysis, and liquid-liquid extraction. α-chymotrypsin catalyzed hydrolysis of p-nitrophenyl acetate in cationic microemulsions have been reported [21].

Conventional modeling of chemical reactions is based on the assumption that time evolution of a chemically reacting system is continuous and deterministic and are modeled by reaction rate equation (RRE) which is a ordinary differential equation (ODE), one for each reactant.

$$dX_i/dt = f_i (X_1 \ldots, X_N) \quad (i=1,\ldots,N) \quad (1)$$

Where *fi* are specific rate constants of the reactions. It is usually expressed in terms of the concentration variables Zi = Xi/V, where V = volume of the system.

The first assumption of reaction being continuous can be ruled out because molecular population levels can change, only by discrete integer amounts. The second assumption of population being deterministic can also be ruled out because it is impossible, either by quantum mechanics or classical mechanics to predict the exact molecular population levels at a future time. Because of large population in test tube size or bigger reactors RRE seems to work quite well. However, for nano-reactors, micelles and bio-cells where molecular population of at least some of the reactant species are not too many orders of magnitude large than one, discreteness and stochasticity may play important roles. In the present work an attempt have been made to model such reactions employing theory of Petri nets [3] and exact stochastic theory of chemical reactions proposed by Gillespie [25, 26, 27]. Although, there are reports on the stochastic modeling of few bio-reactions, there seems to be no report on the modeling of enzymatic reactions in microemulsions employing stochastic Petri nets. The present problem of the Stochastic Petri net modeling of α-chymotrypsin catalyzed hydrolysis of p-nitrophenyl acetate in cationic microemulsions [21] was therefore under taken to study the structural properties of the net, validation of the net by its behavioral properties and to simulate the reaction employing stochastic simulation algorithm.

III. Theoretical Background

Let us consider N reactants S1, S_2 …. S_n with their population $X(t_0) = X_1(t_0), X_2(t_0) \ldots Xn(t_0)$ at time t_0 , contained in a well stirred reactor of volume V in thermal equilibrium at a constant temperature. Assume further that these reactants undergo M reactions producing a change in the reactant populations. Our objective is to calculate the state of the system $X(t) = X_1(t), X_2(t) \ldots X_n(t)$ at time t [26].

Each reaction channel R_j is associated with a state change vector $\nu_j \equiv (\nu_{1j}, \nu_{2j} \ldots \nu_{Nj})$, where ν_{ij} is the change in the Si reactant population due to R_j reaction and a propensity function which gives the probability a_j of R_j in V in the time interval (t , t+dt) , The state change vector ν_j can be calculated with the help of stoichiometric matrix U of Petri net. which is defined as

$$U = u_{ij} = U^+ - U^- \quad (2)$$

Here U^+ is the matrix of weights on outgoing arcs (reactions to places) and U^- is the matrix of weights of incoming arcs (places to reactions). All three matrices are P x T. The population change after one reaction is :

$$X_{t_1} = X_{t_0} + U^{(j)} \quad (3)$$

Where $_U{}^{(j)}$ is the j^{th} column of U. The stoichiomatrix U for the Reaction under consideration is:

$$U = U^+ - U^- = \begin{bmatrix} 0 & 1 & 1 \\ 0 & 1 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 1 \end{bmatrix} - \begin{bmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 0 & 1 & 1 \\ 0 & 0 & 0 \end{bmatrix} = \begin{bmatrix} -1 & 1 & 1 \\ -1 & 1 & 0 \\ 1 & -1 & -1 \\ 0 & 0 & 1 \end{bmatrix}$$

The propensity function a_j is the probability that jth reaction will occurring in time interval (t, t+dt) and is related to the constant $c_j dt$, which defines the probability of reaction between a pair of molecules of jth reaction in time dt [26].

$$a_j dt = h_j c_j dt \quad (4)$$

Here h_j is number of all possible combinations of reactant molecule pairs involved in the jth reaction. If reaction is unimolecular of the type $S_1 \rightarrow$ product and x_1 is the population of S_1 reactants undergoing the reaction, , then $h_j = x_1$. If reaction is of the type $S_1 + S_2 \rightarrow$ Product then $hj = X_1.X_2$ where X_1 and X_2 are the populations of S_1 and S_2. If reaction is of the type $S_1 + S_1 \rightarrow$ Product, the $h_j = \frac{1}{2} x_1(x_1-1).c_j$

can be accurately computed from the physical parameters of the molecules such as mass, relative velocity and diameter of the molecules.

The first stochastic formulation of chemical reactions is popularly known as chemical master equation (CME) [26] which is time derivative of the grand probability function P(X; t) = the probability that there are Xi molecules of reactant Si in volume V at time t and X= (X_1, X_2,… Xn) is a vector of molecular species populations. Knowledge of this function can lead to the complete understanding of the probability distribution of all the states at any time. The CME is given by the relation:

$$\frac{\delta P(X,t)}{\delta t} = \sum_{j=1}^{M}[a_j(X- \nu_j)P(X- \nu_j, t)- aj(X)P(X,t)] \quad (5)$$

Where ν_j is the stoichiometric vector defining the change in the state vector X due to occurrence of jth reaction, i.e. X = X+ νj. CME is a linear ordinary differential equation, one ODE for each state. The number of ODE's will increase with increase in the number of states. It is not easy to solve CME except for simplest of the chemical reaction.

Stochastic simulation algorithm is another rigorous method for study of stochastic chemical reactions. Given X(t) = X, SSA is based on the definition of joint probability function P (τ, j) defined by

P (τ, j) dτ = The probability that (A) the next reaction will be j^{th} reaction and (B) will occur in the time interval (t + τ, t + τ+ dτ). (6)

= probability of no occurrence of any reaction in the time interval (t, t+ τ) multiplied by Probability of occurrence of j th reaction in the time interval
(t + τ, t + τ+ dτ)

= P' (t) . $a_j(x)dt$; where P' (t) is the probability of (A) (7)

P'(t) can be proved [26] to be = $e^{-a_{sum}(x)\tau}$

Therefore P (τ, j) = $a_j(x)e^{-a_{sum}(x)\tau}$

= $\frac{a_j(x)}{a_0(x)}\ a_{0\,(x)}e^{-a_0(x)\tau}$

Here from eq.(4) $a_j = h_j c_j$ (j=1, …M)

And $a_0 = \sum_{j=1}^{M} a_j = \sum_{j=1}^{M} c_j h_j$

The pair (τ, j) can be obtained by applying standard Monte Carle inversion generation rule for random numbers. The method generates two independent samples r_1 and r_2 from the unit interval uniform distribution U (0,1) and then
τ = $1/a_0(x)$. ln $(1/r_1)$ and
j = the smallest integer such that $\sum_{j-1}^{j} a_j(x) > r_2 a_0(x)$

Bartholomay [29] was one of the first biochemist to examine enzyme catalyzed reaction within the framework of statistical kinetics. Since then a large number of reactions have been investigated including the Michaelis-Menten mechanism. In recent years, the SSA has been successfully applied to λ– phase [30] and circadian rhythms [31]

IV. Petri Net Modeling and Stochastic Simulation of α-Chymotrypsin Catalyzed Hydrolysis of p-Nitrophenyl Acetate in Cationic Microemulsions.

The Petri net model for the reaction is shown in Figure 2. The resulting incident matrix is presented in Table 1. The model posses the basic structural properties of Petri net like PUR, ORD, HOM and SC which are necessary for preliminary consistency of the net and its correctness. The net is pure (PUR) as there are no place and transition connected in both directions. So the net structure is fully represented by the incidence matrix, which is used for the calculation of the P- and T-invariants. If all arc weights are one the net is ordinary (ORD). The net is homogenous as the outgoing arcs for each place have the same multiplicity (HOM). The net posses directed paths between all pairs of nodes which prove it to be strongly connected (SC). This net is without source places and sink transitions. Invariant properties were computed with the help of free download software "Platform independent Petri net Editor' (Pipe) [28]. The two P-invariant vectors corresponding to four places were found to be:

P-Invariants 1: x = (1010) ; P-Invariants 2: x = (0111) ;Support of the P -invariants (non zero elements) were as follows. P-Invariants 1: {α-chymotrypsin, α-chymotrypsin- p-nitrophenyl acetate complex}; P-Invariants 2: {p-nitrophenyl acetate, p-nitrophenol, α-chymotrypsin -p-nitrophenyl acetate complex}.

According to the definition of p-invariant, the weighted sum of tokens over invariant places should remain constant and should be independent of any firing. It is worth noting that according to P-1, the sum of molecules of α-chymotrypsin and α-chymotrypsin–p-nitrophenol complex should always remain constant and is a characteristic of an enzymatic reaction. Similarly, according to P-2, the sum (p-nitrophenyl acetate, p-nitrophenol, α-chymotrypsin -p-nitrophenyl acetate complex) should remain constant. The support of p invariants 1 is not a subset of P-invariant 2, and vice versa. The greatest divisor of the non zero elements in both p invariants is 1. Thus, both P-invariants are minimal. Each place contains in at least one of the two P- invariants. Thus the Petri net of our example is covered by p- invariants.

The only T-invariant vectors corresponding to three transitions was found to be:

T-invariants 1:x = (110)

The support of the T-invariants (non zero elements) are: T-invariants 1 (Association, Dissociation).

The greatest divisor of non zero elements in T-invariants is equal to one. Thus, the T-invariants is minimal. Transitions synthesis is not contained in the T-invariants. The Petri net of our example is not covered by T-invariants. The Reachability graph shown in Figure 3 is the graph when the initial marking of the net only includes a single molecule of the substrate.

Table-1. Incident matrix for Petri net model of α-chymotrypsin Catalyzed hydrolysis of p- nitrophenyl acetate in cationic microemulsion

	T_1	T_2	T_3
P_1	-1	1	1
P_2	-1	1	0
P_3	1	-1	-1
P_4	0	0	1

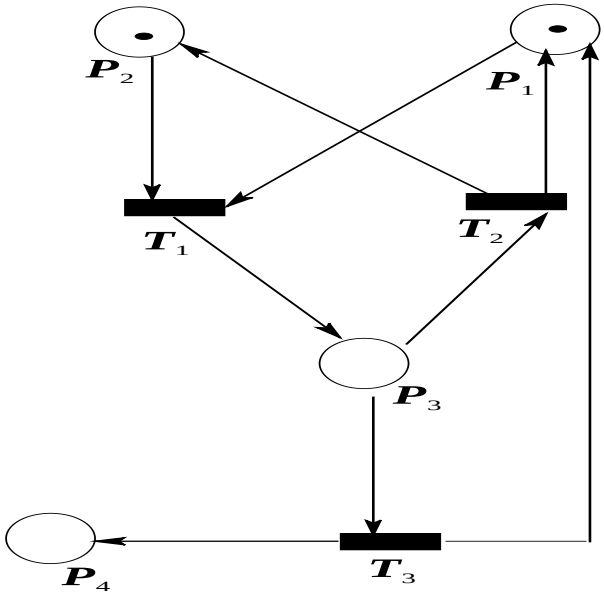

Figure 2. Petri net model for α-chymotrypsin catalyzed hydrolysis of p-nitrophenyl acetate in Cationic microemulsion. P_1= α-chymotrypsin, P_2 = p- nitrophenyl acetate P_3 =α-chymotrypsin-p-nitrophenyl acetate complex, P_4 = p-nitrophenol, T_1= association, T_2= dissociation, T_3= synthesis

The stochastic simulation using Gillespie SSA algorithm were performed employing a Matlab computer program written by Feres [5]. Input to the program were number of tokens for α-chymotrypsin, p-nitrophenyl acetate, rate constants for three reactions, total number of steps and pre- and post matrices for the reactions. The constants were transferred from [21] and [22].

The results for reaction between α-chymotrypsin, p-nitrophenyl acetate is shown in Figure 4 for 100 molecules of substrate and 100 molecules of enzyme Figure 5 represents the same simulation results with 500 molecules of substrate and the enzyme. Curves in Figure 5 are smother compared to Figure 4. This can be attributed to the increase in population level of reactant.

The Effect of head group of cationic surfactants on the α-chymotrypsin catalyzed hydrolysis of p-nitrophenyl acetate with α-chymotrypsin = 100 and p-nitrophenyl acetate = 100 is shown in Figure 6. Similar simulation with α-chymotrypsin = 500, p-nitrophenyl acetate = 500, Total number of molecules = 1000. c_1 = 3.1, c_2 = 2.08 and c_3 = 3.03 is shown in Figure 7. In this case also the smoothness of the curves increases with increasing population.

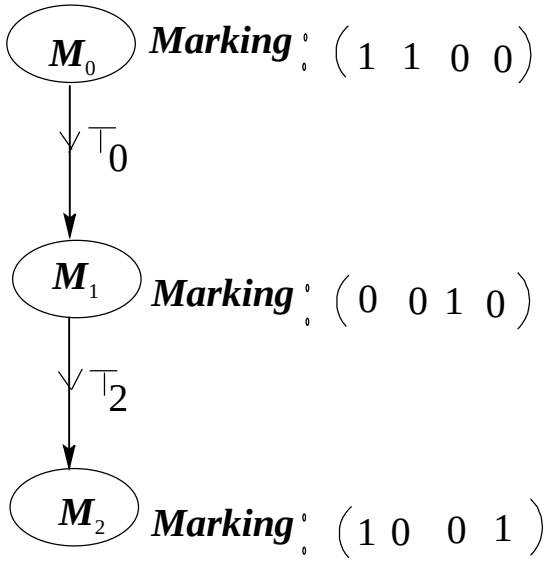

Figure 3. Reachability graph for α-chymotrypsin catalyzed hydrolysis of p-nitrophenyl acetate in cationic microemulsion. In the initial marking only 1 molecule of reactants are present.

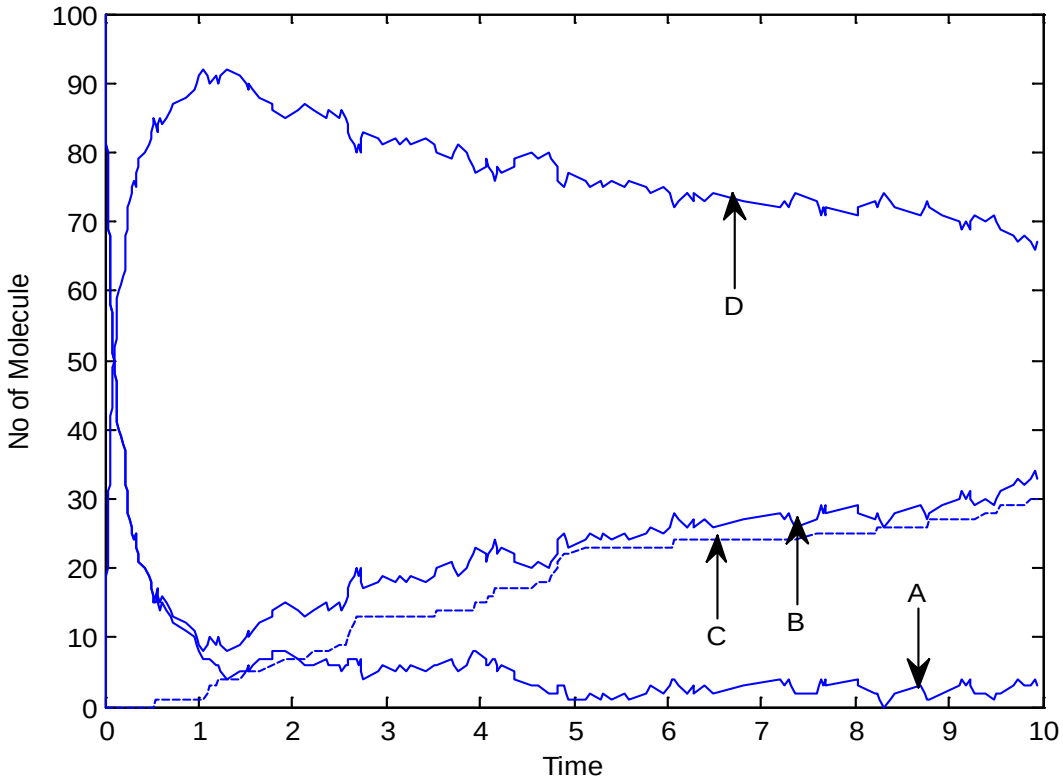

Figure 4. stochastic simulation results of α-chymotrypsin catalyzed hydrolysis of p-nitrophenyl acetate. α-chymotrypsin = 100, p-nitrophenyl acetate= 100 Total number of steps = 230, c_1 = 0 .1, c_2 = 0.06 and c_3 = 0.04. A= P-nitrophenyl acetate, B = α-Chymotrypsin, C= p-nitrophenyl acetate- α-Chymotrypsin complex, D = p-nitrophenol.

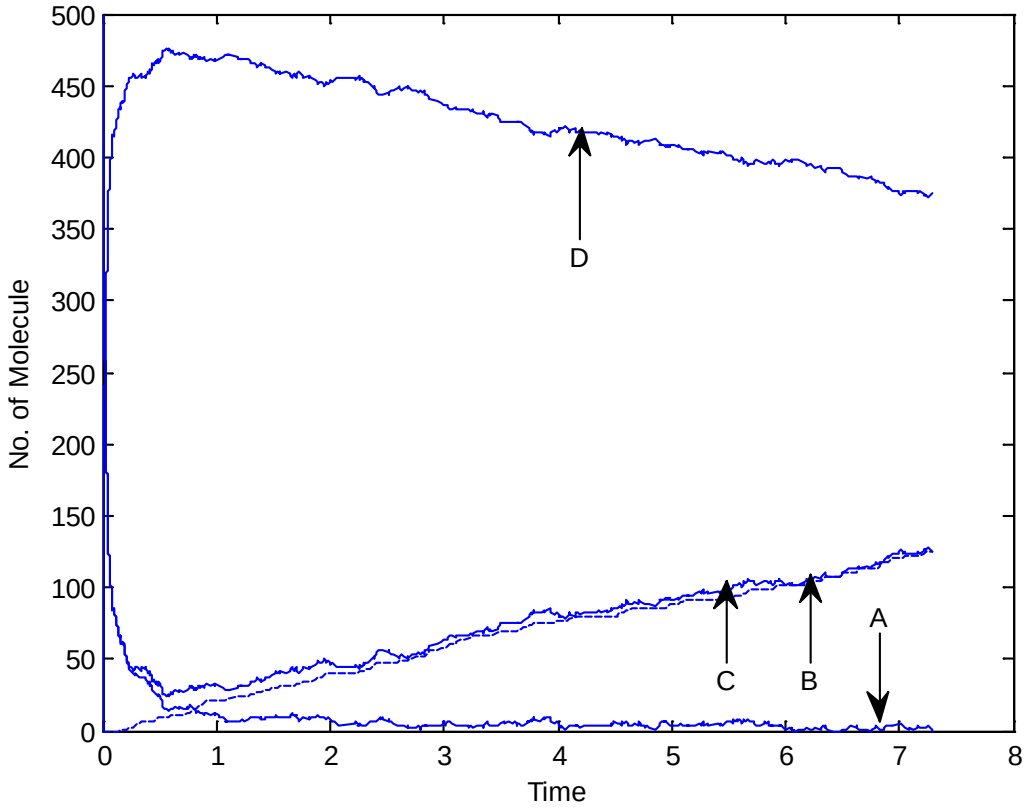

Figure 5. Stochastic simulation results of α-chymotrypsin catalyzed hydrolysis of P-nitrophenyl acetate. α-chymotrypsin = 500. p-nitrophenlyl acetate= 500 Total number of molecules = 1000. c_1 = 0 .1, c_2 = 0.06 and c_3 = 0.04. A= p-nitrophenyl acetate, B= α-Chymotrypsin, C= p-nitrophenyl acetate- α-Chymotrypsin complex, D = p-nitrophenol

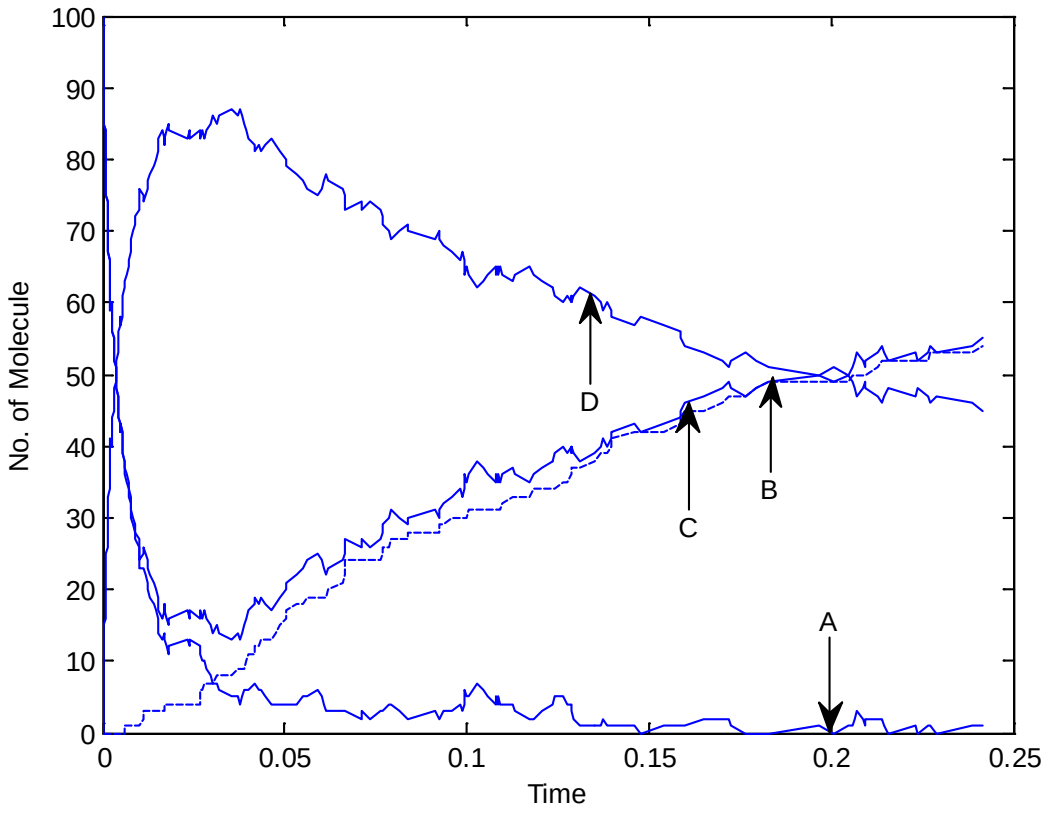

Figure 6. Effect of head group of cationic surfactants on the α-chymotrypsin catalyzed hydrolysis of P-nitrophenyl acetate. α-chymotrypsin = 100, p-nitrophenyl acetate= 100, Total number of molecules = 230. c_1 = 3.1, c_2 = 2.08 and c_3 = 3.03. A= p-nitrophenyl acetate, B= α-Chymotrypsin, C= p-nitrophenyl acetate- α-Chymotrypsin complex, D = p-nitrophenol.

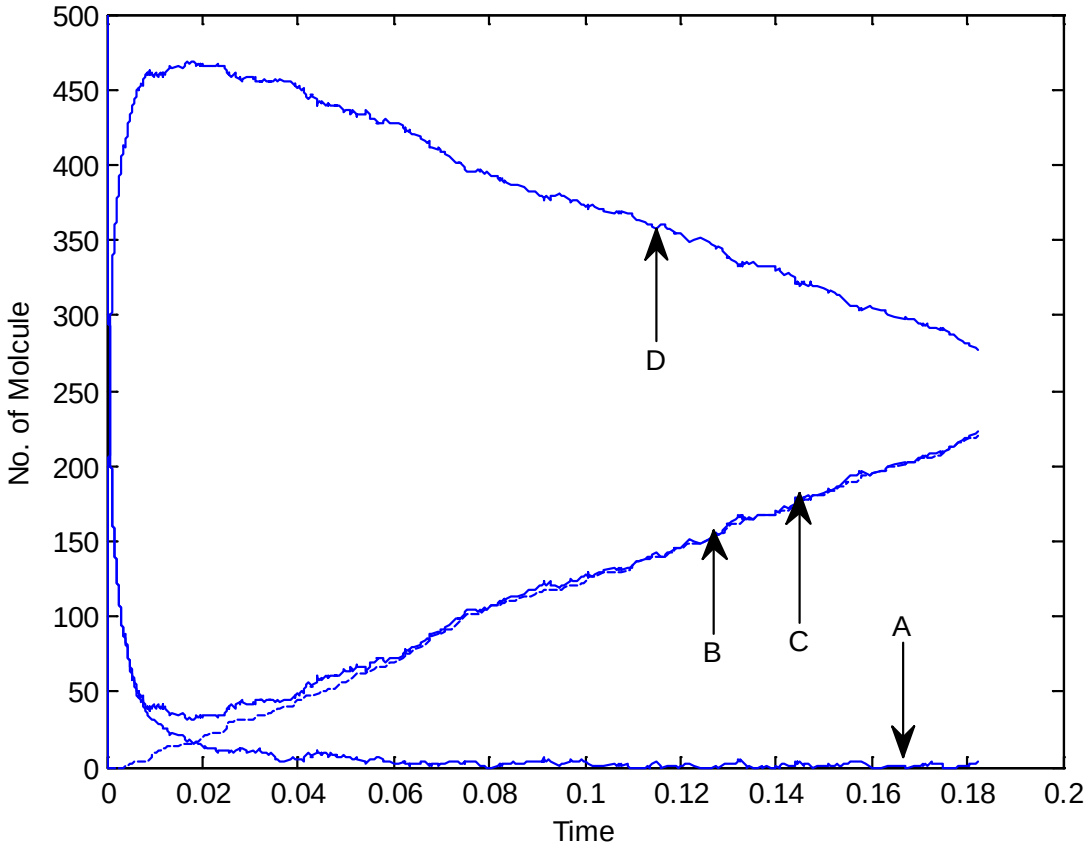

Figure 7. Effect of head group of cationic surfactants on α-chymotrypsin catalyzed hydrolysis of p-nitrophenyl acetate. α-chymotrypsin = 500, p-nitrophenyl acetate= 500, Total number of molecules = 1000. c_1 = 3.1, c_2 = 2.08 and c_3 = 3.03. A= p-nitrophenyl acetate, B= α-Chymotrypsin, C= p-nitrophenyl acetate- α-Chymotrypsin complex, D = p-nitrophenol

V. Conclusion

A stochastic modeling of α-Chymotrypsin catalyzed hydrolysis of p-nitrophenyl acetate in cationic microemulsion has been performed. Structural as well as behavioral properties of the net have been verified. This Petri net modeling shows the nature of the reaction satisfactory.

The reaction was simulated employing Gillespie SSA algorithm. These simulations confirm the reaction to follow a typical deterministic enzymatic reaction. This is the result for a typical enzymatic reaction.

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