



## THERMODYNAMIC STABILITY OF ENZYME CATALYTIC REACTIONS BY LYAPUNOV FUNCTION ANALYSIS

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### ABSTRACT

Thermodynamic stability of enzyme catalyzed reactions has been investigated using the recently proposed thermodynamic Lyapunov function. The thermodynamic Lyapunov function is the magnitude of excess rate of entropy production and hence is a positive definite quantity. In the present study we have investigated the stability of Michaelis-Menten kinetics and its associate phenomena such as cooperative activation, inhibition and catalytic poisoning. The present study reveals the domains of thermodynamic stability under the constantly acting small disturbances and asymptotic stability of the investigated reactions.

**Key words :** Irreversible Thermodynamics, Lyapunov Function, Stability, Enzyme Reactions, Cooperative Phenomenon, Inhibition

### INTRODUCTION

Lyapunov's direct method of stability of motion<sup>1, 2</sup> involves the identification of suitable sign definite Lyapunov function and then determines its total time derivative. The sign and behaviour of this time derivative of the Lyapunov function then tell us whether the dynamic system is stable, asymptotically stable, stable under constantly acting small disturbances or unstable<sup>3, 4</sup>. Since long, it has been felt that the thermodynamic stability consideration should follow the same line but remained eluded so far. In view of this, one of us<sup>5-7</sup> recently proposed the excess rate of entropy production as a thermodynamic Lyapunov function,  $L_s$ , namely,

$$L_s = |\sigma_s - \sigma_s^0| > 0 \quad \dots(1)$$

where  $\sigma_s^0$  is the entropy source strength along the real trajectory whose stability is being investigated and  $\sigma_s$  is that along the perturbed one. As per the dictates, the second law of thermodynamics the entropy source strength is a positive definite quantity that appears in Clausius-Duhem inequality<sup>8-10</sup>, namely,

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$$\rho \frac{ds}{dt} + \text{div } \mathbf{J}_s = \sigma_s \geq 0 \quad \dots(2)$$

where  $\rho$  is the mass density,  $s$  is the per unit mass entropy,  $\mathbf{J}_s$  is the entropy flux density,  $\sigma_s$  is the rate of entropy source strength and  $t$  is time. The terms involved in eq.(2) are position and time dependent but for the sake of brevity this dependence has not been shown. Further, the coordinates of perturbation,  $\alpha_i$ , are defined as

$$\alpha_i = |y_i - y_i^0| > 0, \quad \alpha_{i0} = |y_{i0} - y_{i0}^0| = \chi = \text{constant} \quad \dots(3)$$

where

$$\alpha_i(t_0) = \alpha_{i0}, \quad y_i(t_0) = y_{i0}, \quad y_i^0(t_0) = y_{i0}^0 \quad \dots(4)$$

and hence the equation of unperturbed motion are,

$$\alpha_i^0 \equiv 0, \quad \alpha_{i0}^0 \equiv 0 \quad \dots(5)$$

In eqs.(3) and (4)  $y_i$ 's are the thermodynamic coordinates of the process under investigation and they are provided by the appropriate Gibbs relation and expression of entropy source strength.

The differential equations of perturbed motion in thermodynamic perturbation space read as,

$$\frac{d\alpha_i}{dt} = f_i(t, \alpha_1, \alpha_2, \dots, \alpha_n) \quad (i = 1, 2, 3, \dots, n) \quad \dots(6)$$

The domain of thermodynamic perturbation space is determined by,

$$t \geq t_0, \quad t_0 \geq 0, \quad \alpha_i \leq \varepsilon, \quad \varepsilon > 0 \quad \dots(7)$$

where  $\varepsilon$  is a sufficiently small positive number. The functional dependence of entropy source strength within the thermodynamic perturbation space, that is on  $\alpha_i$ 's, are now obtained as,

$$\sigma_s = \sigma_s(t, \alpha_1, \alpha_2, \dots, \alpha_n) > 0 \quad \dots(8)$$

$$\sigma_s^0 = \sigma_s(t, 0, 0, \dots, 0) > 0 \quad \dots(9)$$

Correspondingly, the functional dependence of the thermodynamic Lyapunov function,  $L_s$ , are obtained as,

$$L_s = L_s(t, \alpha_1, \alpha_2, \dots, \alpha_n) > 0 \quad \text{for } t \geq t_0 \quad \dots(10)$$

$$L_s^0 = L_s(t, 0, 0, \dots, 0) \equiv 0 \quad \text{for } t \geq t_0 \quad \dots(11)$$

that is  $L_s$  has a strict minimum at the origin.

On applying Lyapunov's second method<sup>1,2</sup> the asymptotic stability is guaranteed if the result is,

$$\frac{dL_s}{dt} = \frac{\partial L_s}{\partial t} + \sum_i \frac{\partial L_s}{\partial \alpha_i} f_i \leq -\beta < 0 \quad \dots(12)$$

where  $\beta$  is a positive number that vanishes only at the origin, that is at  $\alpha_i = 0$  ( $i = 1, 2, \dots, n$ ). In addition if every  $\partial L_s / \partial \alpha_i$  is finite then the stability under the constantly acting small disturbances is guaranteed by Malkin's theorem<sup>3,4</sup>. On the other hand instead of eq.(12) if one obtains,

$$\dot{L}_s = \frac{dL_s}{dt} = \frac{\partial L_s}{\partial t} + \sum_i \frac{\partial L_s}{\partial \alpha_i} f_i \leq 0 \quad \dots(13)$$

the unperturbed motion is said to be stable. However, if the result is  $\dot{L}_s > 0$  then the unperturbed motion is unstable.

The above mathematical description reveals that our method involves the act of perturbation (bringing in of  $\alpha_i$ 's) and then observing the response of the system (determining the sign of  $L_s$ ). We stress that this very element was missing in the Glansdorff-Prigogine theory of stability of local equilibrium states<sup>11</sup> and was first pointed out by Lavenda<sup>12</sup> and one of us<sup>5-7</sup>. In view of this the above framework has been christened as comprehensive thermodynamic theory of stability of irreversible processes (CTTSIP)<sup>5-7</sup>.

## THERMODYNAMIC SPACE

The first task in stability considerations is to identify the appropriate thermodynamic space through corresponding Gibbs relation and the expression of rate of entropy production. The traditional Gibbs relation is appropriate one for a spatially uniform closed system undergoing chemical conversions at finite rates read as<sup>10, 13-15</sup>,

$$\frac{dS}{dt} = T^{-1} \frac{dU}{dt} + pT^{-1} \frac{dV}{dt} - T^{-1} \sum_k \mu_k \frac{dn_k}{dt} \quad \dots(14)$$

where  $S$  is the entropy,  $T$  is the temperature,  $p$  is the pressure,  $U$  is the internal energy,  $V$  is the volume,  $\mu_k$  is the chemical potential per mole of the component  $k$  and  $n_k$ 's are the mole numbers. Notice that the first two terms on the right-hand-side of eq.(14) are mainly due to the thermal and mechanical interactions of the system with its surrounding and the last term originates due to the occurrence of a chemical reaction at a finite rate, that is chemical interactions. Further if the irreversibility is only due to a single chemical reaction occurring at a finite rate, then from Dalton's law<sup>13</sup> we have,

$$dn_k = \nu_k d\xi \quad \dots(15)$$

where  $\xi$  is the extent of advancement of the chemical reaction and  $\nu_k$ 's are the stoichiometric coefficients and by convention are taken positive for products and negative for reactants. Further, the standard expression for chemical affinity<sup>13</sup>,  $\mathcal{A}$ , is,

$$\mathcal{A} = -\sum_k \mu_k \nu_k > 0 \quad \dots(16)$$

On substitution of eqs.(15) and (16) into eq.(14) gives

$$\frac{dS}{dt} = T^{-1} \frac{dU}{dt} + pT^{-1} \frac{dV}{dt} + \mathcal{A}T^{-1} \frac{d\xi}{dt} \quad \dots(17)$$

We recall that eq.(17) is the De Donderian equation<sup>13</sup>. Thus, in the absence of irreversibility in thermal and mechanical interactions, the rate of entropy production due to a single chemical reaction reads as,

$$\Sigma_S = \frac{\mathcal{A}}{T} \frac{d\xi}{dt} > 0 \quad \dots(18)$$

where

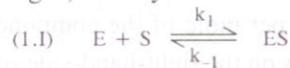
$$\Sigma_S = \int_V \sigma_s dV \quad \dots(19)$$

Notice that eq.(18) conforms to the dictates of second law of thermodynamics that is the rate of entropy production is never a negative quantity<sup>16</sup>.

Previously using above method of CTTSIP we have investigated the thermodynamic stability of some elementary chemical reactions<sup>17,18</sup>, stress relaxation in viscoelastic fluids<sup>19,20</sup> and rigid body heat conduction<sup>5</sup>. In this paper we present the study of thermodynamic stability of enzyme catalyzed reaction, cooperative phenomenon associate with it and catalytic reversible and irreversible inhibitions.

## MICHAELIS-MENTEN ENZYME KINETICS

The Michaelis-Menten kinetic scheme<sup>21-24</sup> is the simplest mechanism of enzyme catalyzed reactions. This involves two steps. In the first step enzyme, E, reacts with the substrate, S, to form intermediate complex, ES, which in the second step gets converted to product P regenerating E, namely:



where  $k_1$  and  $k_{-1}$  are the rate constants of forward and reverse reaction respectively of (1.I),  $k_2$  is the rate constant of reaction (1.II). In enzyme catalyzed reactions the molar

concentration of substrate usually is larger in excess over that of the enzyme and hence, only a very small proportion of the substrate gets bound to the enzyme. We recall that the ratio of concentration of enzyme to substrate is normally in the range<sup>21</sup> of  $10^{-3}$  to  $10^{-6}$ . It is an observed fact that after a lapse of a very short induction period the concentration of complex ES achieves a steady state<sup>22, 23</sup>. To be specific the condition of attainment of the steady state as per van't Hoff<sup>22-24</sup> is  $k_{-1} \ll k_2$ . Further as enzyme gets regenerated in step 2, its concentration during the course of reaction remains stringently constant. That is the rate of change of the concentration of enzyme as well as intermediate complex remains practically zero. Thus, from chemical kinetics<sup>22-24</sup> the rate equations are,

$$\frac{d\xi_1^0}{dt} = k_1 n_E^0 n_S^0 - k_{-1} n_{ES}^0 > 0 \quad \dots(20)$$

$$\frac{d\xi_2^0}{dt} = k_2 n_{ES}^0 > 0 \quad \dots(21)$$

where  $n_i$ 's are the mole numbers of chemical species and  $\xi_1^0$  and  $\xi_2^0$  are the extent of advancement of reactions (1.I) and (1.II), respectively. Superscript <sup>0</sup> indicates the quantities on unperturbed trajectory. Moreover, from the relevant stoichiometry and steady state approximation, the rate of change of concentrations of S, E, ES and P read as,

$$\frac{dn_S^0}{dt} = -k_1 n_S^0 n_E^0 + k_{-1} n_{ES}^0 < 0 \quad \dots(22)$$

$$\frac{dn_E^0}{dt} = -k_1 n_E^0 n_S^0 + k_{-1} n_{ES}^0 + k_2 n_{ES}^0 = 0 \quad \dots(23)$$

$$\frac{dn_{ES}^0}{dt} = k_1 n_S^0 n_E^0 - k_{-1} n_{ES}^0 - k_2 n_{ES}^0 = 0 \quad \dots(24)$$

$$\frac{dn_P^0}{dt} = k_2 n_{ES}^0 > 0 \quad \dots(25)$$

From eqs.(20)-(25), we have following identities:

$$\frac{d\xi_1^0}{dt} = \frac{d\xi_2^0}{dt}, \quad -\frac{dn_S^0}{dt} = \frac{dn_P^0}{dt} \quad \dots(26)$$

As the concentrations of enzyme and the intermediate complex are very small compared to that of substrate, the rate involving former species would be highly sensitive to even small disturbances in their concentrations making the steady state to instantly break down. Therefore, we have investigated the thermodynamic stability when mole numbers of enzyme and the intermediate complex are perturbed from their steady state values. Further in view of the complex nature of enzyme catalyzed reaction, we have not considered the cases of simultaneous perturbation in mole numbers of E and ES, which also help us in considerably pruning down the length of this paper.

**Thermodynamic stability when mole number of enzyme, E, is perturbed**

First consider the case when concentration of E is perturbed by sufficiently small amount say,  $\delta n_E$  and that is,

$$\delta \xi = \left| \xi - \xi^0 \right| > 0, \quad \delta n_E = \left| n_E - n_E^0 \right| > 0 \quad \dots(27)$$

The rate equations on perturbed trajectory would read as,

$$\frac{d\xi_1}{dt} = k_1 n_E n_S^0 - k_{-1} n_{ES}^0 > 0 \quad \dots(28)$$

$$\frac{dn_E}{dt} = -k_1 n_E n_S^0 + k_{-1} n_{ES}^0 + k_2 n_{ES}^0 \neq 0 \quad \dots(29)$$

Now on subtracting eqs.(20) and (23) respectively from eqs.(28) and (29), we obtain,

$$\left| \frac{d(\xi_1 - \xi_1^0)}{dt} \right| = k_1 \delta n_E n_S^0 > 0 \quad \dots(30)$$

$$\frac{d|n_E - n_E^0|}{dt} = \frac{d(\delta n_E)}{dt} = -k_1 \delta n_E n_S^0 < 0 \quad \dots(31)$$

Notice that eq.(21) remains valid both on unperturbed and perturbed trajectories as  $\delta \xi_{II} = 0$  and hence identically we have,

$$\left| \frac{d(\xi_{II} - \xi_{II}^0)}{dt} \right| = 0 \quad \dots(32)$$

Notice that the rate eqs.(30) and (31) vanish only on the unperturbed (real) trajectory.

Further in fairly dilute solutions the expression for chemical affinities on unperturbed trajectory is given by chemical thermodynamic<sup>9-11</sup> as,

$$\mathcal{A}_I^0 = \mathcal{A}_I^\ominus + RT \ln \left( \frac{n_S^0 n_E^0}{n_{ES}^0} \right) \quad \dots(33)$$

$$\mathcal{A}_{II}^0 = \mathcal{A}_{II}^\ominus + RT \ln \left( \frac{n_{ES}^0}{n_P^0 n_E^0} \right) \quad \dots(34)$$

where  $\mathcal{A}_I^0$  and  $\mathcal{A}_{II}^0$  are the chemical affinities of reactions (1.I) and (1.II), respectively and  $\mathcal{A}_I^\ominus$  and  $\mathcal{A}_{II}^\ominus$  are the corresponding standard state chemical affinities. Using steady state condition of eq.(23),  $\mathcal{A}_I^0$  is further simplified as,

$$\mathcal{A}_I^0 = \mathcal{A}_I^\varnothing + RT \ln \left( \frac{k_{-1} + k_2}{k_1} \right) = \text{constant} \quad \dots(35)$$

However, since  $k_2 \gg k_{-1}$  (van't Hoff condition of steady state) eq.(35) reduces to

$$\mathcal{A}_I^0 = \mathcal{A}_I^\varnothing + RT \ln \left( \frac{k_2}{k_1} \right) = \text{constant} \quad \dots(36)$$

Eq.(36) thus confirms the constant value of the chemical affinity in steady state<sup>11</sup>. In view of the assumption that the concentration of enzyme alone is perturbed by a sufficiently small amount the expression for the chemical affinity on perturbed trajectory is obtained as,

$$\mathcal{A}_I = \mathcal{A}_I^\varnothing + RT \ln \left( \frac{n_S^0 n_E}{n_{ES}^0} \right) = \mathcal{A}_{II}^\varnothing + RT \ln \left\{ \frac{n_S^0 n_E^0}{n_{ES}^0} \left( 1 \pm \frac{\delta n_E}{n_E^0} \right) \right\} \quad \dots(37)$$

$$\mathcal{A}_{II} = \mathcal{A}_{II}^\varnothing + RT \ln \left( \frac{n_{ES}^0}{n_P^0 n_E} \right) = \mathcal{A}_{II}^\varnothing + RT \ln \left\{ \frac{n_{ES}^0}{n_P^0 n_E^0} \left( 1 \pm \frac{\delta n_E}{n_E^0} \right)^{-1} \right\} \quad \dots(38)$$

Further from eqs.(33), (34), (37) and (38) the small change in chemical affinities due to perturbation in mole numbers of E, obtain as,

$$\mathcal{A}_I - \mathcal{A}_I^0 = RT \frac{\delta n_E}{n_E^0} \quad \dots(39)$$

$$\mathcal{A}_{II} - \mathcal{A}_{II}^0 = -RT \frac{\delta n_E}{n_E^0} \quad \dots(40)$$

The local time derivatives of chemical affinities,  $\mathcal{A}_I^0$  and  $\mathcal{A}_{II}^0$  read as,

$$\frac{\partial \mathcal{A}_I^0}{\partial t} = \frac{\partial}{\partial t} \left[ \mathcal{A}_I^\varnothing + RT \ln \left( \frac{k_2}{k_1} \right) \right] = 0 \quad \dots(41)$$

$$\frac{\partial \mathcal{A}_{II}^0}{\partial t} = \frac{\partial}{\partial t} \left[ \mathcal{A}_{II}^\varnothing + RT \ln \left( \frac{n_{ES}^0}{n_P^0 n_E^0} \right) \right] = -\frac{RT}{n_P^0} \frac{\partial n_P^0}{\partial t} < 0 \quad \dots(42)$$

The results of eq.(41) follows from eq.(35) and that of eq.(42) is based on the fact that  $\partial \mathcal{A}_{II}^\varnothing / \partial t$  remain identically zero as  $\mathcal{A}_{II}^\varnothing = \mathcal{A}_{II}^\varnothing(T, p)$  and from steady state approximation,  $\partial n_E^0 / \partial t = -\partial n_{ES}^0 / \partial t = 0$ . Further as on the real trajectory  $(n_E - n_E^0) = (\xi - \xi^0) = 0$  that provides the following identities, namely,

$$\frac{\partial \xi^0}{\partial t} = \frac{d\xi^0}{dt}, \quad \frac{\partial n_P^0}{\partial t} = \frac{dn_P^0}{dt}, \quad \text{etc.} \quad \dots(43)$$

That is the local time derivative of mole number of reacting species is equal to its total time derivative.

In the present case there we have two chemical reactions for which De Donderian equation<sup>13</sup> reads as,

$$\frac{dS}{dt} = \frac{1}{T} \frac{dU}{dt} + \frac{p}{T} \frac{dV}{dt} + \frac{\mathcal{A}_1}{T} \frac{d\xi_1}{dt} + \frac{\mathcal{A}_2}{T} \frac{d\xi_2}{dt} \quad \dots(44)$$

and hence the rate of entropy production on unperturbed trajectory in this case, reads as,

$$\Sigma_S^0 = \frac{\mathcal{A}_1^0}{T} \frac{d\xi_1^0}{dt} + \frac{\mathcal{A}_{II}^0}{T} \frac{d\xi_{II}^0}{dt} > 0 \quad \dots(45)$$

and that on the perturbed one reads as,

$$\Sigma_S = \frac{\mathcal{A}_1}{T} \frac{d\xi_1}{dt} + \frac{\mathcal{A}_{II}}{T} \frac{d\xi_{II}}{dt} > 0 \quad \dots(46)$$

In eq.(1) we have local level thermodynamic Lyapunov function,  $L_S$ , but in view of eqs.(19), (45) and (46) the global level thermodynamic Lyapunov function,  $\mathcal{L}_S$ , in the present case is obtained as,

$$\mathcal{L}_S = \left| \Sigma_S - \Sigma_S^0 \right| = \left| \frac{\mathcal{A}_1}{T} \frac{d\xi_1}{dt} + \frac{\mathcal{A}_{II}}{T} \frac{d\xi_{II}}{dt} - \frac{\mathcal{A}_1^0}{T} \frac{d\xi_1^0}{dt} - \frac{\mathcal{A}_{II}^0}{T} \frac{d\xi_{II}^0}{dt} \right| > 0$$

This on further simplification gives,

$$\mathcal{L}_S = \left| \frac{\mathcal{A}_1^0}{T} \frac{d(\delta\xi_1)}{dt} + \frac{\mathcal{A}_{II}^0}{T} \frac{d(\delta\xi_{II})}{dt} + \frac{(\mathcal{A}_1 - \mathcal{A}_1^0)}{T} \frac{d\xi_1^0}{dt} + \frac{(\mathcal{A}_{II} - \mathcal{A}_{II}^0)}{T} \frac{d\xi_{II}^0}{dt} \right. \\ \left. \frac{(\mathcal{A}_1 - \mathcal{A}_1^0)}{T} \frac{d(\delta\xi_1)}{dt} + \frac{(\mathcal{A}_{II} - \mathcal{A}_{II}^0)}{T} \frac{d(\delta\xi_{II})}{dt} \right| > 0 \quad \dots(47)$$

Further, from eqs.(26), (39) and (40), we have,

$$\frac{(\mathcal{A}_1 - \mathcal{A}_1^0)}{T} \frac{d\xi_1^0}{dt} + \frac{(\mathcal{A}_{II} - \mathcal{A}_{II}^0)}{T} \frac{d\xi_{II}^0}{dt} = 0 \quad \dots(48)$$

Thus using eq.(48) and ignoring  $\frac{(\mathcal{A}_1 - \mathcal{A}_1^0)}{T} \frac{d(\delta\xi_1)}{dt} + \frac{(\mathcal{A}_{II} - \mathcal{A}_{II}^0)}{T} \frac{d(\delta\xi_{II})}{dt}$  as  $|\mathcal{A}_1 - \mathcal{A}_1^0| \ll \mathcal{A}_1^0$  and  $|\mathcal{A}_{II} - \mathcal{A}_{II}^0| \ll \mathcal{A}_{II}^0$  eq.(47) reduces to\*\*

\*\* Recall that in the expression of affinities  $\delta n_E$  appears in the logarithmic term. Thus, the sufficiently small magnitude of  $\delta n_E$  will have much more smaller effect on  $\mathcal{A}$ .

$$\mathcal{L}_S = \left| \frac{\mathcal{A}_1^0}{T} \frac{d(\delta\xi_1)}{dt} \right| = \frac{\mathcal{A}_1^0}{T} k_1 n_S^0 \delta n_E > 0 \quad \dots(49)$$

From eq.(49)  $\mathcal{L}_S$  has the following functional dependence:

$$\mathcal{L}_S = \mathcal{L}_S(t, \delta n_E) > 0 \quad \dots(50)$$

The total time derivative of  $\mathcal{L}_S$  in view of eq.(50) reads as,

$$\frac{d\mathcal{L}_S}{dt} = \frac{\partial \mathcal{L}_S}{\partial t} + \frac{\partial \mathcal{L}_S}{\partial(\delta n_E)} \frac{d(\delta n_E)}{dt} \quad \dots(51)$$

Note that the form of eqs.(50) and (51) remain identically same for all perturbation coordinates appear later in this paper. From eqs.(22), (25), (26) and (42) the local time derivative of  $\mathcal{L}_S$  is obtained as,

$$\frac{\partial \mathcal{L}_S}{\partial t} = \frac{\mathcal{A}_1^0}{T} \frac{\partial n_S^0}{\partial t} k_1 \delta n_E = -\frac{\mathcal{A}_1^0}{T} k_1 \frac{\partial n_P^0}{\partial t} \delta n_E = -\frac{\mathcal{A}_1^0}{T} k_1 k_2 n_{ES}^0 \delta n_E < 0 \quad \dots(52)$$

The equalities of eq.(43) have been used in obtaining eq.(52). The gradient of Lyapunov function,  $\mathcal{L}_S$ , reads as,

$$\frac{\partial \mathcal{L}_S}{\partial(\delta n_E)} = \frac{\mathcal{A}_1^0}{T} k_1 n_S^0 > 0 \text{ and finite} \quad \dots(53)$$

Thus from eqs.(31) and (53), we obtain,

$$\frac{\partial \mathcal{L}_S}{\partial(\delta n_E)} \frac{d(\delta n_E)}{dt} = -\frac{\mathcal{A}_1^0}{T} (k_1 n_S^0)^2 \delta n_E < 0 \quad \dots(54)$$

Using eqs.(51)-(54) the total time derivative of  $\mathcal{L}_S$  gets expressed as,

$$\frac{d\mathcal{L}_S}{dt} = -\frac{\mathcal{A}_1^0}{T} k_1 \left[ k_2 n_{ES}^0 + k_1 (n_S^0)^2 \right] \delta n_E \leq -\beta < 0 \quad \dots(55)$$

Thus the asymptotic stability and stability under constantly acting small disturbances is guaranteed.

#### Thermodynamic stability when mole number of complex, ES, is perturbed

In this case, we have,

$$\delta n_{ES} = |n_{ES} - n_{ES}^0| > 0, \quad \delta \xi = |\xi - \xi^0| > 0 \quad \dots(56)$$

where  $n_{ES}^0$  is the mole number of ES on unperturbed trajectory and  $n_{ES}$  that on perturbed one. Thus the rate equations on the perturbed trajectory read as,

$$\frac{d\xi_I}{dt} = k_1 n_E^0 n_S^0 - k_{-1} n_{ES} > 0 \quad \dots(57)$$

$$\frac{d\xi_{II}}{dt} = k_2 n_{ES} > 0 \quad \dots(58)$$

$$\frac{dn_{ES}}{dt} = -k_1 n_E^0 n_S^0 + k_{-1} n_{ES} + k_2 n_{ES} \neq 0 \quad \dots(59)$$

and those on unperturbed trajectory are given by eqs.(20), (21) and (24). On subtracting eqs.(20), (21) and (24) respectively from eqs.(57), (58) and (59), we obtain,

$$\frac{d(\delta\xi_I)}{dt} = -k_{-1} \delta n_{ES} < 0 \quad \dots(60)$$

$$\frac{d(\delta\xi_{II})}{dt} = k_2 \delta n_{ES} > 0 \quad \dots(61)$$

$$\frac{d(\delta n_{ES})}{dt} = -(k_{-1} + k_2) \delta n_{ES} < 0 \quad \dots(62)$$

The expression for chemical affinities,  $\mathcal{A}_I^0$  and  $\mathcal{A}_{II}^0$  of unperturbed state are given by eqs.(33) and (34). However, on perturbed trajectory, we have,

$$\mathcal{A}_I = \mathcal{A}_I^\varnothing + RT \ln \left( \frac{n_S^0 n_E^0}{n_{ES}} \right) = \mathcal{A}_I^\varnothing + RT \ln \left\{ \frac{n_S^0 n_E^0}{n_{ES}^0} \left( 1 \pm \frac{\delta n_{ES}}{n_{ES}^0} \right)^{-1} \right\} \quad \dots(63)$$

$$\mathcal{A}_{II} = \mathcal{A}_{II}^\varnothing + RT \ln \left( \frac{n_{ES}}{n_P^0 n_E^0} \right) = \mathcal{A}_{II}^\varnothing + RT \ln \left\{ \frac{n_{ES}^0}{n_P^0 n_E^0} \left( 1 \pm \frac{\delta n_{ES}}{n_{ES}^0} \right) \right\} \quad \dots(64)$$

Further, from eqs.(33), (34), (63), (64) and identity of eq.(26) we obtain eq.(48), which remains valid in this case too. Thus on the line of eq.(47) and help of eq.(48) we obtain following operative expression for  $\mathcal{L}_S$ , namely:

$$\mathcal{L}_S = \left| -\frac{\mathcal{A}_I^0}{T} k_{-1} + \frac{\mathcal{A}_{II}^0}{T} k_2 \right| \delta n_{ES} > 0 \quad \dots(65)$$

From eqs.(41), (42) and (65), the local time derivative of  $\mathcal{L}_S$  is obtained as,

$$\frac{\partial \mathcal{L}_S}{\partial t} = \frac{\partial}{\partial t} \left| -\frac{\mathcal{A}_I^0}{T} k_{-1} + \frac{\mathcal{A}_{II}^0}{T} k_2 \right| \delta n_{ES} = \frac{k_2}{T} \frac{\partial \left| \mathcal{A}_{II}^0 \right|}{\partial t} = -Rk_2 \frac{\delta n_{ES}}{n_P^0} \frac{\partial n_P^0}{\partial t} < 0 \quad \dots(66)$$

since  $\mathcal{A}_I^0$  is constant. Similarly from eq.(65) the gradient of Lyapunov function,  $\mathcal{L}_S$ , reads as,

$$\frac{\partial \mathcal{L}_S}{\partial (\delta n_{ES})} = \left| -\frac{\mathcal{A}_I^0}{T} k_{-1} + \frac{\mathcal{A}_{II}^0}{T} k_2 \right| > 0 \text{ and finite} \quad \dots(67)$$

Thus from eqs.(62) and (67), we obtain,

$$\frac{\partial \mathcal{L}_S}{\partial(\delta n_{ES})} \frac{d(\delta n_{ES})}{dt} = - \left| -\frac{\mathcal{A}_I^0}{T} k_{-1} + \frac{\mathcal{A}_{II}^0}{T} k_2 \right| (k_{-1} + k_2) \delta n_{ES} < 0 \quad \dots(68)$$

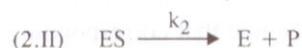
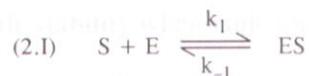
From eqs.(66) and (67) the expression for  $d\mathcal{L}_S/dt$ , is obtained as,

$$\frac{d\mathcal{L}_S}{dt} = -R \left\{ \frac{(k_2)^2}{n_p^0} n_{ES}^0 + \left| -\frac{\mathcal{A}_I^0}{T} k_{-1} + \frac{\mathcal{A}_{II}^0}{T} k_2 \right| (k_{-1} + k_2) \right\} \delta n_{ES} \leq -\beta < 0 \quad \dots(69)$$

Thus the asymptotic stability and stability under constantly acting small disturbances is guaranteed.

## COOPERATIVE PHENOMENON IN ENZYME REACTION

The cooperative phenomenon<sup>21,23</sup> occurs when enzyme possesses more than one binding sites for substrate molecules. In this event a simple mechanism that operates is a four steps one. Both ES and ES<sub>2</sub>, the intermediate complexes, produce the product P. This mechanism reads as,



where ES<sub>2</sub> is the intermediate complex composed of one enzyme molecule bound with two substrate molecules. If this intermediate complex ES<sub>2</sub> remains inactive then the reaction step (2.IV) is absent and the cooperative phenomenon leads to a noncompetitive inhibition<sup>23</sup>.

Notice that above model is the extension of Michaelis-Menten mechanism. Herein too the rate equations and the expressions for affinities on unperturbed trajectory for reaction steps (2.I) and (2.II) remain identically the same as in the preceding section (c.f. eqs.(20), (21), (33) and (34)). Rate equations for steps (2.III) and (2.IV) on the unperturbed trajectory read as,

$$\frac{d\xi_{III}^0}{dt} = k_3 n_S^0 n_{ES}^0 - k_{-3} n_{ES_2}^0 > 0 \quad \dots(70)$$

$$\frac{d\xi_{IV}^0}{dt} = k_4 n_{ES_2}^0 > 0 \quad \dots(71)$$

Note that in the case of inhibition reaction step (2.IV) is absent. On applying steady state approximation to the species E, ES and ES<sub>2</sub> the following rate expressions for cooperative phenomenon, are obtained,

$$\frac{dn_S^0}{dt} = -k_1 n_S^0 n_E^0 + k_{-1} n_{ES}^0 - k_3 n_S^0 n_{ES}^0 + k_{-3} n_{ES_2}^0 < 0 \quad \dots(72)$$

$$\frac{dn_{ES}^0}{dt} = k_1 n_S^0 n_E^0 - k_{-1} n_{ES}^0 - k_2 n_{ES}^0 - k_3 n_S^0 n_{ES}^0 + k_{-3} n_{ES_2}^0 + k_4 n_{ES_2}^0 = 0 \quad \dots(73)$$

$$\frac{dn_{ES_2}^0}{dt} = k_3 n_S^0 n_{ES}^0 - k_{-3} n_{ES_2}^0 - k_4 n_{ES_2}^0 = 0 \quad \dots(74)$$

$$\frac{dn_P^0}{dt} = k_2 n_{ES}^0 + k_4 n_{ES_2}^0 > 0 \quad \dots(75)$$

and eq.(23) gives the steady state condition for enzyme, E. This is also implied by the validity of eqs.(73) and (74). However, in case of inhibition we have

$$\frac{dn_{ES}^0}{dt} = k_1 n_S^0 n_E^0 - k_{-1} n_{ES}^0 - k_2 n_{ES}^0 - k_3 n_S^0 n_{ES}^0 + k_{-3} n_{ES_2}^0 = 0 \quad \dots(76)$$

$$\frac{dn_{ES_2}^0}{dt} = k_3 n_S^0 n_{ES}^0 - k_{-3} n_{ES_2}^0 > 0 \quad \dots(77)$$

as equilibrium state can not be assumed for ES<sub>2</sub>. Notice that corresponding to eqs.(76) and (77) we have,

$$\frac{dn_E^0}{dt} = -k_1 n_S^0 n_E^0 + k_{-1} n_{ES}^0 + k_2 n_{ES}^0 < 0 \quad \dots(78)$$

That is no steady state is possible for the enzyme in case of inhibition. Further from eqs.(73), (73) and (76) we also have,

$$\frac{d\xi_I^0}{dt} = \frac{d\xi_{II}^0}{dt}, \quad \frac{d\xi_{III}^0}{dt} = \frac{d\xi_{IV}^0}{dt} \quad \dots(79)$$

$$-\frac{dn_S^0}{dt} = \frac{dn_P^0}{dt}, \quad \frac{dn_{ES}^0}{dt} = -\frac{dn_E^0}{dt} = \frac{dn_{ES_2}^0}{dt} = 0 \quad \dots(80)$$

Because in the case of cooperative phenomenon, eqs.(23) and (29) hold and hence the stability aspects for this case remains the same as that in section 3 (c.f. eq.(55)). However, in the case of inhibition the rate of eq.(23) is not zero (i.e. eq.(78) in the present section) and hence, we reconsider this case in this section again. Besides, we also discuss the case of the thermodynamic stability when the mole numbers of intermediate complexes ES and ES<sub>2</sub> are perturbed.

### Thermodynamic stability when mole number of E is perturbed

As mention above we consider only a case of inhibition. In this case the expression for Lyapunov function is given by eq. (49). The local time derivative of  $\mathcal{L}_S$  however is obtained as,

$$\frac{\partial \mathcal{L}_S}{\partial t} = Rk_1 \left[ \left( 1 + \frac{\mathcal{A}_1^0}{RT} \right) \frac{\partial n_S^0}{\partial t} + \left( \frac{n_S^0}{n_E^0} \right) \frac{\partial n_E^0}{\partial t} \right] \delta n_E < 0 \quad \dots(81)$$

$$\text{since } \frac{\partial n_S^0}{\partial t} = \frac{dn_S^0}{dt} < 0, \quad \frac{\partial n_E^0}{\partial t} = \frac{dn_E^0}{dt} < 0$$

The gradient of  $\mathcal{L}_S$  in this case remains identically same as given in eq.(53). Thus using eqs.(53), (54) and (81) the total time derivative of Lyapunov function,  $\mathcal{L}_S$ , is obtained as,

$$\frac{d\mathcal{L}_S}{dt} = -Rk_1 \left\{ \frac{\mathcal{A}_1^0}{RT} k_1 (n_S^0)^2 - \left( 1 + \frac{\mathcal{A}_1^0}{RT} \right) \frac{dn_S^0}{dt} + \left( \frac{n_S^0}{n_E^0} \right) \frac{dn_E^0}{dt} \right\} \delta n_E \leq -\beta < 0 \quad \dots(82)$$

Thus once again the asymptotic stability and stability under constantly acting small disturbances is guaranteed in the case of noncompetitive inhibition.

### Thermodynamic stability when mole number of ES is perturbed

In this case the rate equations on perturbed trajectory, read as,

$$\frac{d\xi_{III}}{dt} = k_3 n_S^0 n_{ES} - k_{-3} n_{ES}^0 > 0 \quad \dots(83)$$

$$\frac{dn_{ES}}{dt} = k_1 n_S^0 n_E^0 - k_{-1} n_{ES} - k_2 n_{ES} - k_3 n_S^0 n_{ES} + k_{-3} n_{ES}^0 + k_4 n_{ES}^0 \neq 0 \quad \dots(84)$$

whereas in the case of inhibition eq.(83) holds on perturbed trajectory but instead of eq.(84) there we have,

$$\frac{dn_{ES}}{dt} = k_1 n_S^0 n_E^0 - k_{-1} n_{ES} - k_2 n_{ES} - k_3 n_S^0 n_{ES} + k_{-3} n_{ES}^0 \neq 0 \quad \dots(85)$$

The expressions for  $d\xi_I/dt$  and  $d\xi_{II}/dt$  are that given in eqs.(28) and (29) respectively in both the cases. On subtraction of eqs.(70) and (73) from eqs.(83), and (84), we obtain,

$$\frac{d(\delta\xi_{III})}{dt} = k_3 n_S^0 \delta n_{ES} > 0 \quad \dots(86)$$

$$\frac{d(\delta n_{ES})}{dt} = -(k_{-1} + k_2 + k_3 n_S^0) \delta n_{ES} < 0 \quad \dots(87)$$

But the rate  $d\xi_{IV}/dt$  remains identically same on both; unperturbed and perturbed trajectories and hence, we have,

$$\frac{d(\delta\xi_{IV})}{dt} = 0 \quad \dots(88)$$

The expressions on unperturbed trajectory of chemical affinities of reaction steps (2.III) and (2.IV), read as,

$$\mathcal{A}_{III}^0 = \mathcal{A}_{III}^\ominus + RT \ln \left( \frac{n_S^0 n_{ES}^0}{n_{ES_2}^0} \right) \quad \dots(89)$$

$$\mathcal{A}_{IV}^0 = \mathcal{A}_{IV}^\ominus + RT \ln \left( \frac{n_{ES_2}^0}{n_P^0 n_{ES}^0} \right) \quad \dots(90)$$

where  $\mathcal{A}_{III}^\ominus$  and  $\mathcal{A}_{IV}^\ominus$  are the chemical affinity of standard state of reaction steps (2.III) and (2.IV). Further in case of steady state of  $ES_2$ , eq.(89) reduces to

$$\mathcal{A}_{III}^0 = \mathcal{A}_{III}^\ominus + RT \ln \left( \frac{k_{-3} + k_4}{k_3} \right) = \text{constant} \quad \dots(91)$$

Notice that eq.(91) is valid only for cooperative mechanism. On the perturbed trajectory the chemical affinities are given by following expressions:

$$\mathcal{A}_{III} = \mathcal{A}_{III}^\ominus + RT \ln \left( \frac{n_S^0 n_{ES}^0}{n_{ES_2}^0} \right) = RT \ln \left\{ \frac{k_3 n_S^0 n_{ES}^0}{k_{-3} n_{ES_2}^0} \left( 1 \pm \frac{\delta n_{ES}}{n_{ES}^0} \right) \right\} \quad \dots(92)$$

$$\mathcal{A}_{IV} = \mathcal{A}_{IV}^\ominus + RT \ln \left( \frac{n_{ES_2}^0}{n_P^0 n_{ES}^0} \right) = \mathcal{A}_{IV}^\ominus + RT \ln \left\{ \frac{n_{ES_2}^0}{n_P^0 n_{ES}^0} \left( 1 \pm \frac{\delta n_{ES}}{n_{ES}^0} \right)^{-1} \right\} \quad \dots(93)$$

The expressions for  $\mathcal{A}_I^0$ ,  $\mathcal{A}_{II}^0$ ,  $\mathcal{A}_I$  and  $\mathcal{A}_{II}$  are still given by eqs.(33), (34), (63) and (64). Further from (89), (90), (92) and (93), we have,

$$\mathcal{A}_{III} - \mathcal{A}_{III}^0 = RT \frac{\delta n_{ES}}{n_{ES}^0} \quad \dots(94)$$

$$\mathcal{A}_{IV} - \mathcal{A}_{IV}^0 = -RT \frac{\delta n_{ES}}{n_{ES}^0} \quad \dots(95)$$

The local time derivative of  $\mathcal{A}_{III}^0$  and  $\mathcal{A}_{IV}^0$  are obtained from eqs.(89) and (90), read as,

$$\frac{\partial \mathcal{A}_{III}^0}{\partial t} = RT \left( \frac{1}{n_S^0} \frac{\partial n_S^0}{\partial t} - \frac{1}{n_{ES_2}^0} \frac{\partial n_{ES_2}^0}{\partial t} \right) < 0 \quad \dots(96)$$

$$\frac{\partial \mathcal{A}_{IV}^0}{\partial t} = \frac{\partial}{\partial t} \left[ \mathcal{A}_{IV}^{\circ} + RT \ln \left( \frac{n_{ES_2}^0}{n_p^0 n_{ES}^0} \right) \right] = - \frac{RT}{n_p^0} \frac{\partial n_p^0}{\partial t} < 0 \quad \dots(97)$$

However, in the case of cooperative phenomenon the local derivative of  $\mathcal{A}_{III}^0$  is obtained from eq.(91) as,

$$\frac{\partial \mathcal{A}_{III}^0}{\partial t} = \frac{\partial}{\partial t} \left[ \mathcal{A}_{III}^{\circ} + RT \ln \left( \frac{k_{-3} + k_4}{k_3} \right) \right] = 0 \quad \dots(98)$$

On the lines of eqs.(47)-(49) the thermodynamic Lyapunov function,  $\mathcal{L}_S$ , for both cooperative and inhibition mechanism gets expressed as,

$$\mathcal{L}_S = \left| \Sigma_S - \Sigma_S^0 \right| = \left| - \frac{\mathcal{A}_I^0}{T} k_{-1} + \frac{\mathcal{A}_{II}^0}{T} k_2 + \frac{\mathcal{A}_{III}^0}{T} k_3 n_S^0 \right| \delta n_{ES} > 0 \quad \dots(99)$$

To obtain eq.(99) we used the facts that

$$\frac{(\mathcal{A}_{III} - \mathcal{A}_{III}^0)}{T} \frac{d\xi_{III}^0}{dt} + \frac{(\mathcal{A}_{IV} - \mathcal{A}_{IV}^0)}{T} \frac{d\xi_{IV}^0}{dt} = 0 \quad \dots(100)$$

in case of cooperative phenomenon and

$$\frac{(\mathcal{A}_I - \mathcal{A}_I^0)}{T} \frac{d\xi_I^0}{dt} + \frac{(\mathcal{A}_{II} - \mathcal{A}_{II}^0)}{T} \frac{d\xi_{II}^0}{dt} + \frac{(\mathcal{A}_{III} - \mathcal{A}_{III}^0)}{T} \frac{d\xi_{III}^0}{dt} = 0 \quad \dots(101)$$

for case of inhibition. From eq.(99) the local derivative of  $\mathcal{L}_S$  for cooperative mechanism obtain as,

$$\frac{\partial \mathcal{L}_S}{\partial t} = \left( -k_2 \frac{R}{n_p^0} \frac{\partial n_p^0}{\partial t} + k_3 \frac{\mathcal{A}_3^0}{T} \frac{\partial n_S^0}{\partial t} \right) \delta n_{ES} < 0 \quad \dots(102)$$

and that in case of inhibition we obtained,

$$\begin{aligned} \frac{\partial \mathcal{L}_S}{\partial t} = R \left[ 2k_3 \left( 1 + \frac{\mathcal{A}_{III}^0}{RT} \right) + \frac{k_3 n_S^0}{n_{ES_2}^0} - \frac{(k_2 + k_{-1})}{n_E^0} \right] \frac{\partial n_E^0}{\partial t} \delta n_{ES}^0 \\ - R \left[ k_3 \left( 1 + \frac{\mathcal{A}_{III}^0}{RT} \right) + \frac{k_2}{n_p^0} \right] \frac{\partial n_p^0}{\partial t} \delta n_{ES}^0 \quad \dots(103) \end{aligned}$$

The gradient of Lyapunov function,  $\mathcal{L}_S$ , for both cooperative and inhibition mechanism reads as,

$$\frac{\partial \mathcal{L}_S}{\partial (\delta n_{ES})} = \left| - \frac{\mathcal{A}_I^0}{T} k_{-1} + \frac{\mathcal{A}_{II}^0}{T} k_2 + \frac{\mathcal{A}_{III}^0}{T} k_3 n_S^0 \right| > 0 \text{ and finite} \quad \dots(104)$$

and thus from eq.(87) and (104), we have,

$$\frac{\partial \mathcal{L}_S}{\partial (\delta n_{ES})} \frac{d(\delta n_{ES})}{dt} = - \left| -\frac{\mathcal{A}_I^0}{T} k_{-1} + \frac{\mathcal{A}_{II}^0}{T} k_2 + \frac{\mathcal{A}_{III}^0}{T} k_3 n_S^0 \right| (k_{-1} + k_2 + k_3 n_S^0) \delta n_{ES} < 0 \quad \dots(105)$$

Using eqs.(102) and (105) the total time derivative of  $\mathcal{L}_S$  for cooperative mechanism is obtained as,

$$\begin{aligned} \frac{d\mathcal{L}_S}{dt} = & - \left| -\frac{\mathcal{A}_I^0}{T} k_{-1} + \frac{\mathcal{A}_{II}^0}{T} k_2 + \frac{\mathcal{A}_{III}^0}{T} k_3 n_S^0 \right| (k_{-1} + k_2 + k_3 n_S^0) \delta n_{ES} \\ & + \left( -k_2 \frac{R}{n_p^0} \frac{\partial n_p^0}{\partial t} + k_3 \frac{\mathcal{A}_{III}^0}{T} \frac{\partial n_S^0}{\partial t} \right) \delta n_{ES} \leq -\beta < 0 \end{aligned} \quad \dots(106)$$

and in the case of inhibition we obtained from eqs.(103) and (104) as,

$$\begin{aligned} \frac{d\mathcal{L}_S}{dt} = & -R \left\{ \left[ -\frac{\mathcal{A}_I^0}{RT} k_{-1} + \frac{\mathcal{A}_{III}^0}{RT} k_3 n_S^0 \right] (k_{-1} + k_2 + k_3 n_S^0) + k_2 \frac{\mathcal{A}_{II}^0}{RT} \frac{k_{-3} n_{ES_2}^0}{n_{ES}^0} \right\} \delta n_{ES} \\ & - R \left\{ \left[ \frac{\mathcal{A}_{II}^0}{RT} \frac{k_2}{n_{ES}^0} - \frac{(k_2 + k_{-1})}{n_E^0} \right] k_1 n_S^0 n_E^0 - \left[ 2k_3 \left( 1 + \frac{\mathcal{A}_{III}^0}{RT} \right) + \frac{k_3 n_S^0}{n_{ES_2}^0} \right] \frac{\partial n_E^0}{\partial t} \right\} \delta n_{ES} \\ & - R \left\{ k_3 \left( 1 + \frac{\mathcal{A}_{III}^0}{RT} \right) + \frac{k_2}{n_p^0} + \frac{(k_2 + k_{-1})}{n_E^0} \right\} \frac{\partial n_p^0}{\partial t} \delta n_{ES} \leq -\beta < 0 \end{aligned} \quad \dots(107)$$

since  $n_{ES}^0 \ll n_E^0$ ,  $k_{-1} \ll k_2$ ,  $\partial n_E^0 / \partial t = dn_E^0 / dt < 0$ . Thus in both the cases of cooperative and inhibition mechanism of enzyme catalyzed reactions the asymptotic stability and the stability under constantly acting small disturbances of real trajectory is guaranteed when mole number of intermediate complex ES is perturbed.

### Thermodynamic stability when mole number of $ES_2$ is perturbed

We consider the perturbation in mole numbers of dual bounded substrate-enzyme complex,  $ES_2$  by small amount say,  $\delta n_{ES_2}$  and then we have,

$$\delta n_{ES_2} = |n_{ES_2} - n_{ES_2}^0| > 0, \quad \delta \xi = |\xi - \xi^0| > 0 \quad \dots(108)$$

In this case the rate equations on perturbed trajectory, read as,

$$\frac{d\xi_{III}}{dt} = k_3 n_S^0 n_{ES}^0 - k_{-3} n_{ES_2} > 0 \quad \dots(109)$$

$$\frac{d\xi_{IV}}{dt} = k_4 n_{ES_2} > 0 \quad \dots(110)$$

$$\frac{dn_{ES_2}}{dt} = k_3 n_S^0 n_{ES}^0 - k_{-3} n_{ES_2} - k_4 n_{ES_2} \neq 0 \quad \dots(111)$$

Notice that eq.(109) is valid in both cooperative and inhibition mechanisms, however for later case instead of eq.(110) we have,

$$\frac{dn_{ES_2}}{dt} = k_3 n_S^0 n_{ES}^0 - k_{-3} n_{ES_2} \neq 0 \quad \dots(112)$$

On subtracting eqs.(70), (71) and (74) respectively from eqs.(109), (110) and (111) we obtain,

$$\frac{d(\delta\xi_{III})}{dt} = -k_{-3} \delta n_{ES_2} < 0 \quad \dots(113)$$

$$\frac{d(\delta\xi_{IV})}{dt} = k_4 \delta n_{ES_2} > 0 \quad \dots(114)$$

$$\frac{d(\delta n_{ES_2})}{dt} = -(k_{-3} + k_4) \delta n_{ES_2} < 0 \quad \dots(115)$$

In case of inhibition eq.(115) reduces to

$$\frac{d(\delta n_{ES_2})}{dt} = -k_{-3} \delta n_{ES_2} < 0 \quad \dots(116)$$

Further the terms  $d(\delta\xi_I)/dt=0$  and  $d(\delta\xi_{II})/dt=0$  as the rates of  $\xi_I$  and  $\xi_{II}$  both on perturbed and unperturbed trajectory remain identically the same. The expression for chemical affinities on perturbed trajectory read as,

$$\mathcal{A}_{III} = \mathcal{A}_{III}^{\circ} + RT \ln \left( \frac{n_S^0 n_{ES}^0}{n_{ES_2}} \right) = \mathcal{A}_{III}^{\circ} + RT \ln \left\{ \frac{n_S^0 n_{ES}^0}{n_{ES_2}^0} \left( 1 \pm \frac{\delta n_{ES}}{n_{ES_2}^0} \right)^{-1} \right\} \quad \dots(117)$$

$$\mathcal{A}_{IV} = \mathcal{A}_{IV}^{\circ} + RT \ln \left( \frac{n_{ES_2}}{n_P^0 n_{ES}^0} \right) = \mathcal{A}_{IV}^{\circ} + RT \ln \left\{ \frac{n_{ES_2}^0}{n_P^0 n_{ES}^0} \left( 1 \pm \frac{\delta n_{ES_2}}{n_{ES_2}^0} \right) \right\} \quad \dots(118)$$

Further, from eqs.(89), (90), (117) and (118) we obtain eq.(99) which too valid in the case of cooperative mechanism and thus Lyapunov function obtained as,

$$\mathcal{L}_S = \left| \Sigma_S - \Sigma_S^0 \right| = \left| -\frac{\mathcal{A}_{III}^0}{T} k_{-3} + \frac{\mathcal{A}_{IV}^0}{T} k_4 \right| \delta n_{ES_2} > 0 \quad \dots(119)$$

and in case of inhibition instead of eq.(99), we have,

$$\frac{(\mathcal{A}_{III} - \mathcal{A}_{III}^0)}{T} \frac{d\xi_{III}^0}{dt} = -\frac{R \delta n_{ES_2}}{n_{ES_2}^0} (k_3 n_S^0 n_{ES}^0 - k_{-3} n_{ES_2}^0) \quad \dots(120)$$

With help of eq.(120) we obtained following Lyapunov function:

$$\mathcal{L}_S = \left| \Sigma_S - \Sigma_S^0 \right| = k_{-3} \frac{\mathcal{A}_{III}^0}{T} + \frac{R}{n_{ES_2}^0} \left( k_3 n_S^0 n_{ES}^0 - k_{-3} n_{ES_2}^0 \right) \delta n_{ES_2} > 0 \quad \dots(121)$$

The local time derivative of Lyapunov function,  $\mathcal{L}_S$ , for cooperative phenomenon, obtain as,

$$\frac{\partial \mathcal{L}_S}{\partial t} = R \frac{k_4}{n_p^0} \frac{\partial n_S^0}{\partial t} \delta n_{ES_2} = -R \frac{k_4}{n_p^0} \frac{\partial n_p^0}{\partial t} \delta n_{ES_2} < 0 \quad \dots(122)$$

and that in case of inhibition, we have,

$$\frac{\partial \mathcal{L}_S}{\partial t} = R \left( \frac{k_3 n_S^0 n_{ES}^0}{n_{ES_2}^0} + k_{-3} \right) \left( \frac{1}{n_S^0} \frac{\partial n_S^0}{\partial t} - \frac{1}{n_{ES_2}^0} \frac{\partial n_{ES_2}^0}{\partial t} \right) \delta n_{ES_2} < 0 \quad \dots(123)$$

In case of cooperative mechanism the gradient of Lyapunov function,  $\mathcal{L}_S$  obtain as,

$$\frac{\partial \mathcal{L}_S}{\partial (\delta n_{ES_2})} = \left| -\frac{\mathcal{A}_{III}^0}{T} k_{-3} + \frac{\mathcal{A}_{IV}^0}{T} k_4 \right| > 0 \text{ and finite} \quad \dots(124)$$

and that in case of inhibition, we have,

$$\frac{\partial \mathcal{L}_S}{\partial (\delta n_{ES_2})} = \frac{\mathcal{A}_{III}^0}{T} k_{-3} > 0 \text{ and finite} \quad \dots(125)$$

Thus from eqs.(114) and (123) for cooperative mechanism, we have,

$$\frac{\partial \mathcal{L}_S}{\partial (\delta n_{ES_2})} \frac{d(\delta n_{ES_2})}{dt} = - \left| -\frac{\mathcal{A}_{III}^0}{T} k_{-3} + \frac{\mathcal{A}_{IV}^0}{T} k_4 \right| (k_{-3} + k_4) \delta n_{ES_2} < 0 \quad \dots(126)$$

and from eqs.(115) and (124) for inhibition, we obtain,

$$\frac{\partial \mathcal{L}_S}{\partial (\delta n_{ES_2})} \frac{d(\delta n_{ES_2})}{dt} = -\frac{\mathcal{A}_{III}^0}{T} (k_{-3})^2 \delta n_{ES_2} < 0 \quad \dots(127)$$

Using eqs.(122) and (126) the total time derivative of Lyapunov function,  $\mathcal{L}_S$ , for cooperative mechanism thus gets expressed as,

$$\frac{d\mathcal{L}_S}{dt} = - \left\{ \left| -\frac{\mathcal{A}_{III}^0}{T} k_{-3} + \frac{\mathcal{A}_{IV}^0}{T} k_4 \right| (k_{-3} + k_4) + \frac{k_4}{n_p^0} (k_2 n_{ES}^0 + k_4 n_{ES_2}^0) \right\} \delta n_{ES_2} \leq -\beta < 0 \quad \dots(128)$$

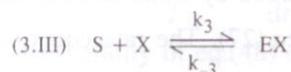
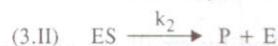
and that in case of inhibition mechanism, we obtained from eqs.(123) and (127) as,

$$\frac{d\mathcal{L}_S}{dt} = -R \left\{ \frac{\mathcal{A}_{III}^0}{RT} (k_{-3})^2 - \left( \frac{k_3 n_S^0 n_{ES}^0}{n_{ES_2}^0} + k_{-3} \right) \left( \frac{1}{n_S^0} \frac{\partial n_S^0}{\partial t} - \frac{1}{n_{ES_2}^0} \frac{\partial n_{ES_2}^0}{\partial t} \right) \right\} \delta n_{ES_2} \leq -\beta < 0 \dots(129)$$

Thus in both cases of enzyme catalyzed reaction, asymptotic stability and the stability under constantly acting small disturbances is guaranteed.

## CATALYTIC INHIBITION BY FOREIGN MATERIAL

In case of competitive inhibition inhibitor directly binds to enzyme molecule and form inactive enzyme-inhibitor complex. Inhibitor molecules may be a foreign species or reactant or product or substrate molecules. In preceding section we have considered the reversible inhibition by substrate molecules. In the present section we discuss inhibition by foreign material, say X. The reaction scheme for catalytic inhibition mechanism<sup>23</sup>, namely:



This type of mechanism is known as reversible competitive inhibition<sup>23</sup>. If the reaction step (3.III) is irreversible (or reverse rate is insignificant), the above inhibition leads to irreversible inhibition<sup>23</sup> (catalytic poisoning). In this case, the effective concentration of enzyme no more remains time independent.

The rate equations for reaction steps (3.I) and (3.II) are given by eqs.(20) and (21). However, the rate equation for step (3.III), reads as,

$$\frac{d\xi_{III}^0}{dt} = k_3 n_E^0 n_X^0 - k_{-3} n_{EX}^0 \geq 0 \quad \dots(130)$$

That is we have two cases of reversible inhibition: one corresponds to  $d\xi_{III}^0/dt = 0$ , in the event of chemical equilibrium for reaction (3.III) and other  $d\xi_{III}^0/dt > 0$ , that is for chemical reaction (3.III) is not in equilibrium but proceeding at a finite rate towards right side. However, in case of catalytic poisoning eq.(130) reduces to –

$$\frac{d\xi_{III}^0}{dt} = k_3 n_E^0 n_X^0 > 0 \quad \dots(131)$$

as no reverse reaction occurs appreciably. The rates of change of concentrations of substrate, complex and product remain identically same as in preceding section. The rate of change of concentration of E, X and EX, however, read as,

$$\frac{dn_E^0}{dt} = -k_1 n_S^0 n_E^0 + k_{-1} n_{ES}^0 + k_2 n_{ES}^0 - k_3 n_X^0 n_E^0 + k_{-3} n_{EX}^0 \leq 0 \quad \dots(132)$$

$$\frac{dn_X^0}{dt} = -k_3 n_E^0 n_X^0 + k_{-3} n_{EX}^0 \leq 0 \quad \dots(133)$$

$$\frac{dn_{EX}^0}{dt} = k_3 n_E^0 n_X^0 - k_{-3} n_{EX}^0 \geq 0 \quad \dots(134)$$

The steady state equation of intermediate complex ES is given by eq.(24). Further from steady state approximation and relevant aspects of stoichiometry we have,

$$\frac{d\xi_I^0}{dt} = \frac{d\xi_{II}^0}{dt}, \quad \frac{dn_E^0}{dt} = \frac{dn_X^0}{dt} = -\frac{dn_{EX}^0}{dt} \quad \dots(135)$$

Notice that in the present case, on perturbed trajectory eq.(59) holds and hence the relevant aspects of thermodynamic stability when mole number of ES is perturbed remains identically same described in last section. However, in this case the inhibitor being other than the substrate and hence the rate of change of concentration of enzyme gets modified. This case is discussed in the following subsections.

### Thermodynamic stability when mole number of E is perturbed

Let us consider that the mole number of E is perturbed by small amount say  $\delta n_E$ . For this case the relevant expression for  $\delta n_E$  is given by eq.(27). The rate equations on perturbed trajectory in case of reversible inhibition, read as,

$$\frac{d\xi_{III}^0}{dt} = k_3 n_E n_X^0 - k_{-3} n_{EX}^0 > 0 \quad \dots(136)$$

$$\frac{dn_E}{dt} = -k_1 n_S n_E + k_{-1} n_{ES}^0 + k_2 n_{ES}^0 - k_3 n_X^0 n_E + k_{-3} n_{EX}^0 \quad \dots(137)$$

and that in case of irreversible inhibition, read as,

$$\frac{d\xi_{III}^0}{dt} = k_3 n_E n_X^0 \quad \dots(138)$$

$$\frac{dn_E}{dt} = -k_1 n_S n_E + k_{-1} n_{ES}^0 + k_2 n_{ES}^0 - k_3 n_X^0 n_E \quad \dots(139)$$

On subtracting eqs.(131) and (132) from eqs.(136) and (137), we obtain,

$$\frac{d(\delta\xi_{III}^0)}{dt} = k_3 \delta n_E n_X^0 > 0 \quad \dots(140)$$

$$\frac{d(\delta n_E)}{dt} = -(k_1 n_S^0 + k_3 n_X^0) \delta n_E < 0 \quad \dots(141)$$

Eqs.(140) and (141) valid for both the cases of inhibition. The expression for chemical affinity,  $\mathcal{A}_{III}^0$ , reads as,

$$\mathcal{A}_{III}^0 = \mathcal{A}_{III}^\ominus + RT \ln \left( \frac{n_E^0 n_X^0}{n_{EX}^0} \right) \geq 0 \quad \dots(142)$$

The vanishing of  $\mathcal{A}_{III}^0$  corresponds to chemical equilibrium for the reaction (3.III). The expression for  $\mathcal{A}_I^0$  and  $\mathcal{A}_{II}^0$  are given by eqs.(33) and (34) of section 3. Further the expression of  $\mathcal{A}_{III}^0$  on perturbed trajectory, reads as,

$$\mathcal{A}_{III} = \mathcal{A}_{III}^{\circ} + RT \ln \left( \frac{n_E n_X^0}{n_{EX}^0} \right) = \mathcal{A}_{III}^{\circ} + RT \ln \left\{ \frac{n_E^0 n_X^0}{n_{EX}^0} \left( 1 \pm \frac{\delta n_E}{n_E^0} \right) \right\} \quad \dots(143)$$

The local time derivative of chemical affinity,  $\mathcal{A}_{III}^0$ , in both cases of inhibition, reads as,

$$\frac{\partial \mathcal{A}_{III}^0}{\partial t} = RT \left( \frac{1}{n_E^0} + \frac{1}{n_X^0} + \frac{1}{n_{EX}^0} \right) \frac{\partial n_E^0}{\partial t} < 0 \quad \dots(144)$$

Further, from eqs.(142) and (141) and using eq.(48), we obtain,

$$R \frac{\delta n_E}{n_E^0} \frac{d\xi_{III}^0}{dt} = \frac{(\mathcal{A}_{III} - \mathcal{A}_{III}^0)}{T} \frac{d\xi_{III}^0}{dt} \neq 0 \quad \dots(145)$$

In this case too the validity of eq.(48) can be easily demonstrated using eqs.(39), (40) and (135). Thus in view of eq.(145) the expression of Lyapunov function,  $\mathcal{L}_S$ , for both the case of inhibition is obtained as,

$$\mathcal{L}_S = \left| \frac{\mathcal{A}_I^0}{T} k_1 n_S^0 + \frac{\mathcal{A}_{III}^0}{T} k_3 n_X^0 + \frac{R}{n_E^0} \frac{d\xi_{III}^0}{dt} \right| \delta n_E > 0 \quad \dots(146)$$

However, in case of equilibrium of (3.III) the expression for  $\mathcal{L}_S$  is given by eq.(48). From eq.(146) the local time derivative of  $\mathcal{L}_S$  for both the cases of inhibitions, obtained as,

$$\begin{aligned} \frac{\partial \mathcal{L}_S}{\partial t} = R \left\{ k_1 \frac{\mathcal{A}_I^0}{RT} \frac{\partial n_S^0}{\partial t} + k_3 \left( \frac{\mathcal{A}_{III}^0}{RT} + 1 \right) \frac{\partial n_X^0}{\partial t} \right\} \delta n_E \\ + R \left( \frac{n_{EX}^0}{n_E^0} k_{-3} + k_3 \right) \left( \frac{1}{n_E^0} + \frac{1}{n_X^0} + \frac{1}{n_{EX}^0} \right) \frac{\partial n_X^0}{\partial t} \delta n_E < 0 \end{aligned} \quad \dots(147)$$

and that in case of equilibrium of (3.III), we have eq.(52). The gradient of Lyapunov function,  $\mathcal{L}_S$ , for both the cases of inhibitions, reads as,

$$\frac{\partial \mathcal{L}_S}{\partial (\delta n_E)} = \left| \frac{\mathcal{A}_I^0}{T} k_1 n_S^0 + \frac{\mathcal{A}_{III}^0}{T} k_3 n_X^0 + \frac{R}{n_E^0} \frac{d\xi_{III}^0}{dt} \right| > 0 \quad \dots(148)$$

and that in case of equilibrium is given by eq.(53).

Thus from eqs.(141) and (148), we have,

$$\frac{\partial \mathcal{L}_S}{\partial (\delta n_E)} \frac{d(\delta n_E)}{dt} = - \left| \frac{\mathcal{A}_I^0}{T} k_1 n_S^0 + \frac{\mathcal{A}_{III}^0}{T} k_3 n_X^0 + \frac{R}{n_E^0} \frac{d\xi_{III}^0}{dt} \right| (k_1 n_S^0 + k_3 n_X^0) \delta n_E < 0 \quad \dots(149)$$

and that in case of equilibrium of (3.III) from eqs.(54) and (141), we obtain,

$$\frac{\partial \mathcal{L}_S}{\partial(\delta n_E)} \frac{d(\delta n_E)}{dt} = -\frac{\mathcal{A}_1^0}{T} k_1 n_S^0 (k_1 n_S^0 + k_3 n_X^0) \delta n_E < 0 \quad \dots(150)$$

From eqs.(147) and (149) the total time derivative  $\mathcal{L}_S$  for both the cases of inhibition, obtain as,

$$\begin{aligned} \frac{d\mathcal{L}_S}{dt} = & -R \left\{ \left[ \frac{\mathcal{A}_1^0}{RT} k_1 n_S^0 + \frac{\mathcal{A}_3^0}{RT} k_3 n_X^0 + \frac{1}{n_E^0} \frac{d\xi_{III}^0}{dt} \right] (k_1 n_S^0 + k_3 n_X^0) \right\} \delta n_E \\ & + R \left\{ k_1 \frac{\mathcal{A}_1^0}{RT} \frac{\partial n_S^0}{\partial t} + k_3 \left( \frac{\mathcal{A}_{III}^0}{RT} + 1 \right) \frac{\partial n_X^0}{\partial t} \right\} \delta n_E \\ & + R \left( \frac{n_{EX}^0}{n_E^0} k_{-3} + k_3 \right) \left( \frac{1}{n_E^0} + \frac{1}{n_X^0} + \frac{1}{n_{EX}^0} \right) \frac{\partial n_X^0}{\partial t} \delta n_E \leq -\beta < 0 \quad \dots(151) \end{aligned}$$

and that for equilibrium of (3.III) we obtained from eqs.(52) and (150) as,

$$\frac{d\mathcal{L}_S}{dt} = -\frac{\mathcal{A}_1^0}{T} k_1 \left[ k_2 n_{ES}^0 + n_S^0 (k_1 n_S^0 + k_3 n_X^0) \right] \delta n_E \leq -\beta < 0 \quad \dots(152)$$

Thus the asymptotic stability and the stability of both reversible and irreversible inhibition mechanisms in enzyme catalyzed reaction under constantly acting small disturbances is guaranteed

## CONCLUDING REMARKS

In this paper, the systematic use of recently proposed thermodynamic Lyapunov function,  $\mathcal{L}_S$ , to investigate the stability of enzyme reactions has been described. This includes Michaelis-Menten kinetics and all its possible off shoots such as cooperative phenomenon and reversible and irreversible inhibitions. We investigated the stability against the perturbation in mole numbers of enzyme and enzyme-substrate complexes, as the substrate concentration always remains large in excess over the catalyst. The systems were considered as isothermal and spatially uniform. The  $\mathcal{L}_S$  was computed using production expression given by Gibbs relation. In all cases studied, the result is asymptotic stability and the stability under constantly acting small disturbances. This result is particularly revealing and confirms the immunity of enzymic reactions towards concentration perturbation leading to extraordinary efficiency of enzymic activity in vivo.

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