Theory on the rate equations of Michaelis-Menten type enzyme kinetics with competitive inhibition

Rajamanickam Murugan
Department of Biotechnology, Indian Institute of Technology Madras
Chennai, Tamil Nadu, India. Email: rmurugan@gmail.com
Phone: +91 44 2257 4116

Abstract

We derive approximate expressions under various conditions of validity over both pre- and post-steady state regimes of the velocity-substrate-inhibitor phase-spaces of fully and partially competitive enzyme kinetic schemes. Our refinement over the currently available standard quasi steady state approximations (QSSA) seems to be valid for wide range of enzyme to substrate and inhibitor ratios. Further, we show that, the enzyme-inhibitor-substrate system can exhibit a complicated dynamical behavior with non-monotonic trend in the temporal depletion of the substrate and there is also a possibility of the occurrence of two steady states with respect to enzyme-substrate and enzyme-inhibitor complexes. We define the ratios $f_S = v_{max}/(K_{MS} + e_0)$ and $f_S = u_{max}/(K_{MI} + e_0)$ as the acceleration factors with respect to the conversion dynamics of substrate and inhibitor into their respective products. Here K_{MS} and K_{MI} are the Michaelis-Menten parameters associated with the binding of substrate and inhibitor with the enzyme, v_{max} and u_{max} are the respective maximum reaction velocities. When $f_S/f_I < 1$, then the enzyme-substrate complex can reach the full-fledged steady state only after the depletion of the enzyme-inhibitor complex. On the other hand, when $f_S/f_I > 1$ then the enzyme-inhibitor complex can reach the full-fledged steady state only after the depletion of the enzyme-substrate complex. This complicated behavior of the enzyme-substrate-inhibitor system especially when $f_S/f_I \neq 1$ is the root cause of large amount of error in the estimation of various kinetic parameters both in the cases of fully and partially competitive inhibition schemes using the standard QSSA methods. Remarkably, we show that our refined expressions for the reaction velocities over enzyme-substrate-inhibitor space can control this error more significantly than the currently available standard QSSA velocity expressions.

Key words

Competitive enzyme inhibition, fully and partially competitive inhibition, steady state approximations, perturbation expansion, phase-space dynamics

1. Introduction

Enzymes catalyze various reactions of the biological and biochemical pathways [1-4]. The **M**ichaelis-**M**enten (MM) kinetics [5, 6] is the fundamental mechanistic description of the biological catalysis of enzyme reactions [7-9]. In this kinetics scheme, the enzyme reversibly binds its substrate to form the enzyme-substrate complex which subsequently decompose into free enzyme and product of substrate. Integral solution to the rate equations associated with the Michaelis-Menten scheme (**MMS**) is not expressible in terms of elementary functions. Several analytical methods were tried to obtain the approximate solution of MMS in terms of ordinary [10] and singular perturbation series [11] and perturbation expansions over slow manifolds [12, 13]. In general, the singular perturbation expansions yield a combination of inner and outer solutions which were then combined via proper matching at the boundary layer [11, 14-19].

Several steady state approximations were proposed in the light of experimental characterization of a single substrate MM enzyme. The **standard quasi steady state approximation** (sQSSA) is widely used across several fields of biochemical research to obtain the enzyme kinetic parameters such as v_{max} and K_M from the experimental datasets on velocity versus initial substrate concentrations. This approximation works well when the product formation step is a rate limiting one apart from the condition that the substrate concentration is much higher than the enzyme concentration. In general, sQSSA yields expressions which can be directly used by the experimentalists to obtain various enzyme kinetic parameters [20]. Recently, explicit closed form expressions of integrated rate equation corresponding to sQSSA were obtained in terms of **Lambert's W** functions [21-25]. The **total QSSA** (tQSSA) assumes that the amount of product formed near the steady state is much negligible compared to the total substrate concentration [26, 27]. The **reverse QSSA** (rQSSA) works very well [28, 29] when the substrate concentration is much lesser than the enzyme concentration.

Several linearization techniques such as Lineweaver-Burk representation were also proposed [30, 31] to obtain the kinetic parameters from the experimental data. Although sQSSA, rQSSA and tQSSA methods work well under *in vitro* conditions, there are several situations such as single molecule enzyme kinetics [32] and other *in vivo* experimental conditions where one cannot manipulate the ratio of substrate to enzyme concentrations much. Further, successfulness of various QSSAs in accurately obtaining the kinetic parameters is strongly dependent on the timescale separation between the pre- and post-steady state regimes of MMS [33, 34]. Particularly, when the timescale separation between pre- and post-steady states of MMS is high enough, then the standard QSSA along with **stationary reactant assumption** where one replaces the steady state substrate concentration with the total substrate concentration [25] can be used to directly obtain the kinetic parameters.

The catalytic properties of an enzyme can be manipulated by an inhibitor. Inhibitors can be competitive or allosteric in nature [2]. Competitive inhibitors (**Fig. 1**) are substrate like molecules which reversibly bind the active site of the same enzymes and hence block the further binding of the substrate molecule. This in turn deceases the catalytic efficiency of the enzyme over the corresponding substrate. In a fully competitive inhibition (**Scheme A** in **Fig. 1**), the inhibitor competes with the substrate to bind the active site of the enzyme and subsequently gets

converted into the respective product. In this case, both substrate and inhibitor will be converted into their respective products by the same enzyme. In case of partially competitive inhibition (**Scheme B** in **Fig. 1**), the reversibly formed enzyme-inhibitor complex will be a dead-end one. Understanding the dynamical behavior of the fully and partially competitive inhibition of MM enzymes is critical to understand the pharmacokinetic aspects of several drugs. Variation of v_{max} and K_M of the enzyme with respect to the concentration of an inhibitor decides the efficiency of a given drug molecule in targeting that enzyme. In the steady state experiments on the single substrate enzymes, the total substrate concentration will be iterated to obtain the respective velocities. This substrate concentration versus reaction velocity dataset will be then used to obtain the kinetic parameters such as K_M and v_{max}. To obtain the kinetic parameters related to the enzyme inhibition, one needs to conduct a series of velocity versus substrate type steady state experiments at different concentrations of the inhibitor. Using this steady state substrate, inhibitor versus reaction velocities, one obtains the kinetic parameters related to the enzyme-substrate-inhibitor system.

The successfulness of various steady state approximations in obtaining the kinetic parameters from the experimental datasets strongly depends on the occurrence of a common steady state with respect to both substrate and inhibitor binding dynamics in case of fully and partially competitive inhibition schemes. Mismatch in the steady state timescales can be resolved by setting higher substrate and inhibitor concentrations than the enzyme concentration. This condition drives the pre-steady state timescales corresponding to both the substrate and inhibitor close to zero. However, under *in vivo* conditions, one cannot manipulate the relative concentrations of the substrate, inhibitor and the enzyme. much All the quasi steady state approximations will fail when the concentration of the enzyme is comparable with that of the substrate and inhibitor. In this article, we will address this issue in detail and derive accurate expressions for the steady state reaction velocities when the concentrations of enzyme, substrate and inhibitor are comparable with each other.

2. Theory

The competitive inhibition of Michaelis-Menten enzymes can be via a full or partial mode as depicted in **Scheme A** and **B** respectively in **Fig. 1**. In fully competitive inhibition given in Scheme A, both the substrate and inhibitor molecules compete for the same active site of the target enzyme to bind and get converted into their respective products in a parallel manner. Whereas, in case of partially competitive inhibition, the reversibly formed enzyme-inhibitor will not be converted into a product and it will be dead-end complex. Particularly, several drug molecules are partially competitive inhibitors. In the following sections we will analyze various kinetic aspects of fully and partially competitive inhibition schemes in detail.

2.1. Fully competitive inhibition

The fully competitive inhibition of Michaelis-Menten enzymes that is depicted in **Scheme A** of **Fig. 1** can be quantitatively described by the following set of differential rate equations.

$$\frac{ds}{dt} = -k_1 se + k_{-1} x \qquad [1.1]$$

$$\frac{di}{dt} = -k_i ie + k_{-i}y \quad [1.2]$$

$$\frac{dx}{dt} = -\frac{de}{dt} = k_1 se - (k_2 + k_{-1})x \quad [1.3]$$

$$\frac{dy}{dt} = k_i ie - (k_{-i} + k_3)y \quad [1.4]$$

$$\frac{dp}{dt} = v = k_2 x;\ \frac{dq}{dt} = u = k_3 y \quad [1.5]$$

In **Eqs. 1**, (s, i, e, x, y, p, q) are respectively the concentrations (mol/lit, M) of substrate, inhibitor, enzyme, enzyme-substrate complex, enzyme-inhibitor complex, product of substrate and product of inhibitor. Here k_1 and k_i are the respective forward bimolecular rate constants (1/M s) , k_{-1}, k_{-i} (1/second) are respective reverse unimolecular rate constants, u, and v (M/second) are the respective reaction velocities and k_2, k_3 (1/second) are the respective unimolecular product formation rate constants along with the mass conservation laws: $e = e_0 - x - y$; $s = s_0 - x - p$; $i = i_0 - y - q$ and initial conditions (s, i, e, x, y, p, q, v, u) = (s_0, i_0, e_0, 0, 0, 0, 0, 0, 0) at t = 0. When $t \to \infty$, then the reaction ends at (s, i, e, x, y, p, q, v, u) = (0, 0, e_0, 0, 0, s_0, i_0, 0, 0). The steady states occur under the conditions $\left(\frac{dx}{dt}, \frac{dy}{dt}\right)$ = (0, 0). However, the timescale t_{CS} at which $\frac{dx}{dt} = 0$ can be different from the timescale t_{CI} at which $\frac{dy}{dt} = 0$. When there is a mismatch in the steady state timescales, then one cannot obtain a common steady state solution to **Eqs. 1** by simultaneously equating all of them to zero. This means that there exist two different steady states with respect to enzyme-substrate and enzyme-inhibitor complexes at two different time points along with four different timescales viz. two pre-steady state timescales and two different post-steady state timescales. Various definitions and symbols used in the theory section are summarized in **Table 1**.

2.1. Scaling and non-dimensionalization

To simplify the system of **Eqs. 1**, we introduce the following set of scaling transformations.

$$S = s/s_0;\ E = e/e_0;\ P = p/s_0;\ Y = y/e_0;\ X = x/e_0;\ I = \frac{i}{i_0};\ Q = \frac{q}{i_0};\ \tau = k_2 t \quad [2]$$

Here $(\mathrm{S, I, E, X, Y, P, Q}) \in (0,1)$ are dimensionless time dependent dynamical variables along with the conservation laws: $E = 1 - X - Y$; $S = 1 - \frac{e_0}{s_0}X - P$; $I = 1 - \frac{e_0}{i_0}Y - Q$. With these scaling transformations, one can reduce **Eqs. 1** into the following set of dimensionless equations.

$$\eta_S \frac{dX}{d\tau} = S(1 - X - Y) - (\eta_S + \kappa_S)X \quad [3.1]$$

$$\left(\frac{\eta_I}{\rho}\right)\frac{dY}{d\tau} = I(1 - X - Y) - (\eta_I + \kappa_I)Y \quad [3.2]$$

$$\frac{dP}{d\tau} = \varepsilon_S X = V;\ \frac{dQ}{d\tau} = \rho\varepsilon_I Y = U \quad [3.3]$$

Upon expanding S and I with their definition in the right-hand side of **Eqs. 3.1-2** and rearranging the terms, we arrive at the following form.

$$\eta_S \frac{dX}{d\tau} + \alpha_S X + P - 1 + Y = (\varepsilon_S X + P)(X + Y) \quad [3.4]$$

$$\left(\frac{\eta_I}{\rho}\right)\frac{dY}{d\tau} + \alpha_I Y + Q - 1 + X = (\varepsilon_I Y + Q)(X + Y) \quad [3.5]$$

Eqs. 3.4-5 completely characterize the dynamics of competitive inhibition scheme over (P, Q, X, Y, τ) space. Here the initial conditions are (S, I, E, X, Y, P, Q, V, U) = (1, 1, 1, 0, 0, 0, 0, 0, 0) at τ = 0. When $\tau \to \infty$, then the reaction trajectory ends at (S, I, E, X, Y, P, Q, V, U) = (0, 0, 1, 0, 0, 1, 1, 0, 0). The steady states occur under the conditions that $\left(\frac{dX}{d\tau}, \frac{dY}{d\tau}\right)$ = (0, 0). Various dimensionless parameters in **Eqs. 3** are defined as follows.

$$K_{RS} = \frac{k_2}{k_1}; K_{DS} = \frac{k_{-1}}{k_1}; K_{MS} = K_{RS} + K_{DS} \quad [4.1]$$

$$\eta_S = \frac{K_{RS}}{s_0} = \frac{k_2}{k_1 s_0};\ \ \varepsilon_S = \frac{e_0}{s_0};\ \ \kappa_S = \frac{K_{DS}}{s_0} = \frac{k_{-1}}{k_1 s_0}\ ;\ \rho = \frac{k_3}{k_2} = \frac{u_{max}}{v_{max}};\ \mu_S = \eta_S + \kappa_S = \frac{K_{MS}}{s_0} \quad [4.2]$$

$$K_{RI} = \frac{k_3}{k_i}; K_{DI} = \frac{k_{-i}}{k_i}; K_{MI} = K_{RI} + K_{DI} \quad [4.3]$$

$$\eta_I = \frac{K_{RI}}{i_0} = \frac{k_3}{k_i i_0};\ \ \varepsilon_I = \frac{e_0}{i_0};\ \ \kappa_I = \frac{K_{DI}}{i_0} = \frac{k_{-i}}{k_i i_0};\ \mu_I = \eta_I + \kappa_I = \frac{K_{MI}}{i_0} \quad [4.4]$$

$$\alpha_S = 1 + \varepsilon_S + \eta_S + \kappa_S;\ \alpha_I = 1 + \varepsilon_I + \eta_I + \kappa_I \quad [4.5]$$

In **Eqs. 3**, V and U are the dimensionless reaction velocities associated with the substrate and inhibitor conversions into their respective products (P, Q) and the mass conservation laws can be rewritten in the dimensionless velocity-substrate-product spaces as $V + P + S = 1$; $\frac{U}{\rho} + Q + I = 1$; $E + \frac{V}{\varepsilon_S} + \frac{U}{\rho\varepsilon_I} = 1$. Further, one finds the transformation rules for the reaction velocities as $V = \frac{\varepsilon_S}{v_{max}} v$ and $U = \frac{\rho\varepsilon_I}{u_{max}} u$ where we have defined $v_{max} = k_2 e_0$ and $u_{max} = k_3 e_0$. Numerically integrated sample trajectories of **Eqs. 3** are shown in **Fig. 2A-E**. Clearly, all the reaction trajectories in the (V, P, S) space fall on the plane V + P + S = 1, and all the reaction trajectories in the (U, Q, I) space will fall on the plane $\frac{U}{\rho} + Q + I = 1$ as demonstrated in **Figs. 2B** and **2C**. Parameters associated with the nonlinear system of **Eqs. 3** as defined in **Eqs. 4** can be grouped into ordinary and singular ones. Here $(\varepsilon_S, \kappa_S, \varepsilon_I, \kappa_I)$ are the ordinary perturbation parameters and (η_S, η_I, ρ) are the singular perturbation parameters since they multiply or divide the highest derivative terms. Particularly, (η_S, η_I) decide how fast the system of **Eqs. 3** attains the steady state and (κ_S, κ_I) decide how fast the enzyme-substrate / inhibitor complexes dissociate and (μ_S, μ_I) are the dimensionless Michaelis-Menten type constants which describe the summary the effects of $(\eta_S, \kappa_S, \eta_I, \kappa_I)$. The relative fastness of the reaction steps in **Scheme A** of **Figs. 1** can be characterized by the following critical ratios of reactions rates.

$$\sigma = \frac{k_1}{k_i};\ \gamma = \frac{k_{-1}}{k_{-i}};\ \rho = \frac{k_3}{k_2} \quad [4.6]$$

When $(\sigma, \gamma, \rho) = (1,1,1)$, then the dynamical aspects of the enzyme-substrate and enzyme-inhibitor complexes will be similar. The fully competitive enzyme kinetics scheme can exhibit a complex behavior depending on the relative values of the parameters (σ, γ, ρ).

2.2. Variable transformations

Using the substitutions $X = \frac{1}{\varepsilon_S}\frac{dP}{d\tau}$; $Y = \frac{1}{\rho\varepsilon_I}\frac{dQ}{d\tau}$ and noting that $V = \varepsilon_S X$ and $U = \rho\varepsilon_I Y$ which are the dimensionless reaction velocities, the system of **Eqs. 3** can be reduced to following set of coupled nonlinear second order ODEs in the (P, Q, τ) and first order ODEs in the (V, P, Q) and (U, P, Q) spaces [35].

$$\frac{d^2P}{d\tau^2} + \frac{\alpha_S}{\eta_S}\frac{dP}{d\tau} + \frac{\varepsilon_S P}{\eta_S} - \frac{\varepsilon_S}{\eta_S} = \frac{\varepsilon_S}{\eta_S}\left(\left(\frac{dP}{d\tau} + P\right)\left(\frac{1}{\varepsilon_S}\frac{dP}{d\tau} + \frac{1}{\rho\varepsilon_I}\frac{dQ}{d\tau}\right) - \frac{1}{\rho\varepsilon_I}\frac{dQ}{d\tau}\right) \quad [5.1]$$

$$\frac{d^2Q}{d\tau^2} + \frac{\rho\alpha_I}{\eta_I}\frac{dQ}{d\tau} + \frac{\varepsilon_I\rho^2 Q}{\eta_I} - \frac{\varepsilon_I\rho^2}{\eta_I} = \frac{\varepsilon_I\rho^2}{\eta_I}\left(\left(\frac{1}{\rho}\frac{dQ}{d\tau} + Q\right)\left(\frac{1}{\varepsilon_S}\frac{dP}{d\tau} + \frac{1}{\rho\varepsilon_I}\frac{dQ}{d\tau}\right) - \frac{1}{\varepsilon_S}\frac{dP}{d\tau}\right) \quad [5.2]$$

$$V\frac{dV}{dP} + \frac{\alpha_S}{\eta_S}V + \frac{\varepsilon_S}{\eta_S}P - \frac{\varepsilon_S}{\eta_S} = \frac{\varepsilon_S}{\eta_S}\left((V + P)\left(\frac{V}{\varepsilon_S} + \frac{U}{\rho\varepsilon_I}\right) - \frac{U}{\rho\varepsilon_I}\right) \quad [5.3]$$

$$U\frac{dU}{dQ} + \frac{\rho\alpha_I}{\eta_I}U + \frac{\varepsilon_I\rho^2}{\eta_I}Q - \frac{\varepsilon_I\rho^2}{\eta_I} = \frac{\varepsilon_I\rho^2}{\eta_I}\left(\left(\frac{U}{\rho} + Q\right)\left(\frac{V}{\varepsilon_S} + \frac{U}{\rho\varepsilon_I}\right) - \frac{V}{\varepsilon_S}\right) \quad [5.4]$$

When $\rho \neq 1$, $\eta_S \neq \eta_I$ and $\varepsilon_S \neq \varepsilon_I$ the system of **Eqs. 1**, **3** and **5** will have distinct and temporally well separated steady states corresponding to substrate-product and inhibitor-product conversion dynamics. Under such conditions, the system of **Eqs. 3** will not have common steady state solutions both in the (V, S, I) and (U, S, I) spaces (as demonstrated in **Fig. 2A**) as given by most of the currently proposed standard quasi steady state approximations.

2.3. Standard quasi steady state solutions

Case I: When $(\eta_S, \eta_I) \to 0$ simultaneously on **Eqs. 3.1-2**, then upon noting the fact that $V = \varepsilon_S X$ and $U = \rho\varepsilon_I Y$ one can obtain the following set of well-known quasi steady state velocity equations in the (V, S, I) and (U, S, I) spaces.

$$\lim_{(\eta_S,\eta_I)\to 0} V \cong \frac{\varepsilon_S S}{\left(S + (\kappa_S + \eta_S)\left(1 + \left(\frac{I}{\kappa_I + \eta_I}\right)\right)\right)}; \quad \lim_{(\eta_S,\eta_I)\to 0} U \cong \frac{\rho\varepsilon_I I}{\left(I + (\kappa_I + \eta_I)\left(1 + \left(\frac{S}{\kappa_S + \eta_S}\right)\right)\right)} \quad [6.1]$$

Particularly, these equations are the approximations of the post steady state dynamics of competitive inhibition scheme A in the (V, S, I) and (U, S, I) spaces. When $(\varepsilon_S, \varepsilon_I) \to 0$ along with $(\eta_S, \eta_I) \to 0$, then one finds that $(V, U) \to 0$ along with $(P, Q) \cong 0$ in the pre-steady state regime. This results in the ***reactants stationary assumption*** where we set S ≅1 and I ≅ 1 in **Eqs. 6.1** and the quasi-steady state velocities become as follows.

$$V = V_1 \cong \frac{\varepsilon_S}{\left(1 + (\kappa_S + \eta_S)\left(1 + \left(\frac{1}{\kappa_I + \eta_I}\right)\right)\right)}; \quad U = U_1 \cong \frac{\rho\varepsilon_I}{\left(1 + (\kappa_I + \eta_I)\left(1 + \left(\frac{1}{\kappa_S + \eta_S}\right)\right)\right)} \quad [6.2]$$

We denote these approximations given in **Eqs. 6. 2** as V_1 and U_1. In terms of the original velocity variables (v, u), **Eqs. 6.2** can be written as follows.

$$v = v_1 \cong \frac{v_{max}s_0}{s_0+K_{MS}\left(1+\frac{i_0}{K_{MI}}\right)};\ \ u = u_1 \cong \frac{u_{max}i_0}{\left(i_0+K_{MI}\left(1+\frac{s_0}{K_{MS}}\right)\right)} \qquad [6.3]$$

Eqs. 6.3 are generally used to obtain the kinetic parameters from the steady state based fully competitive inhibition experiments via reciprocal plotting methods under the assumptions that $(\varepsilon_S, \varepsilon_I) \to 0$ and $\rho = 1$. Similarly, when the conditions $(\eta_S, \eta_I) \to 0$ applied on **Eqs. 3.4-5**, one can arrive at the following quasi steady state velocities in the (V, P, Q) and (U, P, Q) spaces.

$$\lim_{(\eta_S,\eta_I)\to 0} V \cong \frac{-W^2\varepsilon_I + (-Q+\alpha_I)W + Q - 1}{W\varepsilon_I + Q - 1};\ \lim_{(\eta_S,\eta_I)\to 0} U \cong W \qquad [6.4]$$

In these equations, W is the appropriate real root of the cubic equation $aW^3 + bW^2 + cW + d = 0$ where the coefficients are defined as follows.

$$a = \alpha_I\varepsilon_I\varepsilon_S - \alpha_S{\varepsilon_I}^2 + {\varepsilon_I}^2 - \varepsilon_I\varepsilon_S \qquad [6.5]$$

$$b = -P\alpha_I\varepsilon_I + P{\varepsilon_I}^2 + Q\alpha_I\varepsilon_S - 2Q\alpha_S\varepsilon_I + Q\varepsilon_I\varepsilon_S - {\alpha_I}^2\varepsilon_S + \alpha_I\alpha_S\varepsilon_I + P\varepsilon_I + 2Q\varepsilon_I - Q\varepsilon_S + \alpha_I\varepsilon_S + \alpha_S\varepsilon_I - {\varepsilon_I}^2 - \varepsilon_I\varepsilon_S - 2\varepsilon_I \qquad [6.6]$$

$$c = -PQ\alpha_I + PQ\varepsilon_I - Q^2\alpha_S + Q^2\varepsilon_S + Q\alpha_I\alpha_S - 2Q\alpha_I\varepsilon_S + Q\alpha_S\varepsilon_I + PQ + P\alpha_I - P\varepsilon_I + Q^2 + Q\alpha_S - 2Q\varepsilon_I - \alpha_I\alpha_S + 2\alpha_I\varepsilon_S - \alpha_S\varepsilon_I - P - 2Q + 2\varepsilon_I - \varepsilon_S + 1 \qquad [6.7]$$

$$d = Q^2\alpha_S - Q^2\varepsilon_S - Q^2 - 2Q\alpha_S + 2Q\varepsilon_S + 2Q + \alpha_S - \varepsilon_S - 1 \qquad [6.8]$$

Case II: When $Q \to 0$ in the pre-steady state regime along with the conditions that $(\eta_S, \eta_I) \to 0$, then **Eqs. 3.4-5** can be approximated in the (X, P, S) and (Y, P, S) spaces as follows.

$$\alpha_S X + P - 1 + Y \cong (\varepsilon_S X + P)(X + Y) \qquad [6.9]$$

$$(\alpha_S - \varepsilon_S)X - S + Y \cong (1 - S)(X + Y) \qquad [6.10]$$

$$\alpha_I Y - 1 + X \cong \varepsilon_I Y(X + Y) \qquad [6.11]$$

Upon solving **Eqs. 6.10-11** for (X, Y) and then converting them into (V, U) using **Eqs. 3.3**, one finds the following expressions for the post-steady state reaction velocity in the (V, S) space.

$$\lim_{(\eta_S,\eta_I,Q)\to 0} V \cong \frac{\varepsilon_S S}{2\varepsilon_I\mu_S(S+\mu_S)}\left((S+\mu_S)\alpha_I + (S+2\mu_S)\varepsilon_I + \sqrt{{\mu_I}^2S^2 + 2\alpha_I\mu_S\mu_I S - 4\left(-\frac{{\alpha_I}^2}{4} + \varepsilon_I\right){\mu_S}^2} + S\right) \qquad [6.12]$$

Noting that V + P + S = 1, one can express P as function of S, using P = 1 – V – S where V is defined as in **Eqs. 6.12**. These two equations parametrically express the post-steady state dynamics of the fully competitive inhibition scheme in the (V, P, S) space where S acts as the parameter. When

$P \to 0$ in the pre-steady state regime along with the conditions that $(\eta_S, \eta_I) \to 0$, then Eqs**. 3.4-5** can be approximated in the (X, Q, I) and (Y, Q, I) spaces as follows.

$$\alpha_S X - 1 + Y = \varepsilon_S X(X + Y) \qquad [6.13]$$

$$\alpha_I Y + Q - 1 + X = (\varepsilon_I Y + Q)(X + Y) \qquad [6.14]$$

$$(\alpha_I - \varepsilon_I)Y - I + X = (1 - I)(X + Y) \qquad [6.15]$$

Upon solving **Eqs. 6.13** and **6.15** for (X, Y) and then converting it into (V, U), one finds the following expressions for the post-steady state reaction velocity in the (U, I) space.

$$\lim_{(\eta_S,\eta_I,P)\to 0} U \cong \frac{I\rho\varepsilon_I}{2\varepsilon_S\mu_I(I+\mu_I)}\left(-(I+\mu_I)\,\alpha_S + (I + 2\mu_I)\varepsilon_S - \sqrt{\mu_S{}^2 I^2 + 2\alpha_S\mu_S\mu_I I - 4\mu_I{}^2\left(-\frac{\alpha_S{}^2}{4} + \varepsilon_S\right)} + I\right) \qquad [6.16]$$

Noting that U/ρ + Q + I = 1, one can express Q as function of I, using Q = 1 – U/ρ – I where U is defined as in **Eqs. 6.16**. These two equations parametrically express the post-steady state dynamics in the (U, Q, I) space where I acts as the parameter.

Case II. When $(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I) \to 0$, then one finds that $S \cong 1 - P, I \cong 1 - Q$ and $(V, U) \to 0$, from which one can derive the following refined QSSA approximations from **Eqs. 3**. Upon setting $(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I) \to 0$ in **Eqs. 3.4-5**, one obtains the following set of equations.

$$\alpha_S X + P - 1 + Y - P(X + Y) \cong 0\,;\;\; \alpha_I Y + Q - 1 + X - Q(X + Y) \cong 0 \qquad [6.17]$$

Upon solving this system of equations for (X, Y) and transforming the solution into (V, U) using **Eq. 3.3**, one obtains the following approximations in the (V, P, Q) and (U, P, Q) spaces.

$$\lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0} V = \frac{dP}{d\tau} \cong \frac{\varepsilon_S(1-P)}{(1-P)+\tilde{\mu}_S\left(1+\frac{1-Q}{\tilde{\mu}_I}\right)};\;\; \lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0} U = \frac{dQ}{d\tau} \cong \frac{\rho\varepsilon_I(1-Q)}{(1-Q)+\tilde{\mu}_I\left(1+\frac{1-P}{\tilde{\mu}_S}\right)} \qquad [6.18]$$

Similarly, noting that $S \cong 1 - P$, $I \cong 1 - Q$ in such conditions, **Eqs. 6.18** in the (V, S, I) and (U, S, I) spaces becomes as follows.

$$\lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0} V = -\frac{dS}{d\tau} \cong \frac{\varepsilon_S S}{S+\tilde{\mu}_S\left(1+\frac{I}{\tilde{\mu}_I}\right)};\;\; \lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0} U = -\frac{dI}{d\tau} \cong \frac{\rho\varepsilon_I I}{I+\tilde{\mu}_I\left(1+\frac{S}{\tilde{\mu}_S}\right)} \qquad [6.19]$$

Here $\tilde{\mu}_S = \varepsilon_S + \mu_S$ and $\tilde{\mu}_I = \varepsilon_I + \mu_I$. Here we should note that **Eqs. 6.17-19** are similar to **Eqs. 6.1** where μ_S and μ_I are replaced with $\tilde{\mu}_S$ and $\tilde{\mu}_S$. Upon dividing the expression of V by the expression of U in **Eqs. 6.18**, one can obtain the following differential equation corresponding to the (P, Q) space in the limit $(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I) \to 0$.

$$\frac{dP}{dQ} \cong \left(\frac{\varepsilon_S(1-P)}{\rho\varepsilon_I(1-Q)}\right)\frac{(1-Q)+\tilde{\mu}_I\left(1+\frac{1-P}{\tilde{\mu}_S}\right)}{(1-P)+\tilde{\mu}_S\left(1+\frac{1-Q}{\tilde{\mu}_I}\right)} \qquad [7.1]$$

Here the initial condition is $P = 0$ at $Q = 0$. Similarly, in the (S, I) space, one obtains the following differential equation from **Eqs. 6.19**.

$$\frac{dS}{dI} \cong \left(\frac{\varepsilon_S S}{\rho \varepsilon_I I}\right) \frac{I + \tilde{\mu}_I\left(1 + \frac{S}{\tilde{\mu}_S}\right)}{S + \tilde{\mu}_S\left(1 + \frac{I}{\tilde{\mu}_I}\right)} \qquad [7.2]$$

Here the initial condition is $S = 1$ at $I = 1$. Upon solving **Eq. 7.2** with the given initial condition, one obtains the following integral solutions in the (S, I) and (P, Q) spaces [36, 37].

$$S \cong I^{\delta}; P \cong 1 - (1 - Q)^{\delta};\ Q \cong 1 - (1 - P)^{\frac{1}{\delta}};\ \delta = \frac{\tilde{\mu}_I \varepsilon_S}{\rho \tilde{\mu}_S \varepsilon_I} = \frac{v_{max}/\tilde{\mu}_S}{u_{max}/\tilde{\mu}_I} \qquad [8.1]$$

Here δ is a critical parameter which measures the relation speed by which enzyme-substrate and enzyme-inhibitor attain the steady state. The expression for V in **Eqs. 6.19** in terms of S and I along with the expression for $I \cong S^{\frac{1}{\delta}}$ from **Eqs. 8.1** parametrically describe the fully competitive enzyme kinetics in the (V, S, I) space. Similarly, expression for V in terms of P and Q as given in **Eqs. 6.18** along with the expression for Q that is given in **Eqs. 8.1** parametrically describe the dynamics in (V, P, Q) space. Upon substituting the expression for Q in terms of P obtained from **Eqs. 8.1** into the expression for $\frac{dP}{d\tau}$ and $\frac{dQ}{d\tau}$ which are given in **Eqs. 6.19** and noting that $S \cong (1 - P)$ and $I \cong (1 - Q)$ when $(\varepsilon_S, \varepsilon_I) \to 0$, so that $\frac{dP}{d\tau} \cong -\frac{dS}{d\tau}$ and $\frac{dQ}{d\tau} \cong -\frac{dI}{d\tau}$, one can obtain the following approximate differential equations corresponding to the (S, τ) and (I, τ) spaces.

$$\lim_{(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I) \to 0} V \cong -\frac{dS}{d\tau} \cong \frac{\varepsilon_S S}{\left(S + \tilde{\mu}_S\left(1 + \frac{S^{1/\delta}}{\tilde{\mu}_I}\right)\right)};\ \lim_{(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I) \to 0} U = -\frac{dI}{d\tau} \cong \frac{\rho \varepsilon_I I}{I + \tilde{\mu}_I\left(1 + \frac{I^{\delta}}{\tilde{\mu}_S}\right)} \qquad [8.2]$$

Solutions to the variable separated ODEs given in **Eqs. 8.2** can be implicitly written as follows.

$$\delta \tilde{\mu}_S S^{\frac{1}{\delta}} + \tilde{\mu}_S \tilde{\mu}_I \ln(S) - \tilde{\mu}_I - \delta \tilde{\mu}_S + \varepsilon_S \tilde{\mu}_I \tau + S \tilde{\mu}_I = 0 \qquad [8.3]$$

$$\tau \delta \varepsilon_I \tilde{\mu}_S \rho + \tilde{\mu}_I \ln(I)\, \delta \tilde{\mu}_S + I \delta \tilde{\mu}_S + \tilde{\mu}_I I^{\delta} - \delta \tilde{\mu}_S - \tilde{\mu}_I = 0 \qquad [8.4]$$

Noting that $\lim_{\delta \to 0} \delta I^{\frac{1}{\delta}} \to 0$ and $\lim_{\delta \to \infty} \delta I^{\frac{1}{\delta}} \to \delta$ when S ≤ 1, the nonlinear algebraic equation **Eq. 8.3** can be inverted for the substrate variable *S* under various limiting conditions of δ as follows.

$$\lim_{\delta \to 0} S \cong \tilde{\mu}_S \mathrm{LambertW}\left(\frac{1}{\tilde{\mu}_S} \exp\left(\frac{-\varepsilon_S \tilde{\mu}_I \tau + \delta \tilde{\mu}_S + \tilde{\mu}_I}{\tilde{\mu}_S \tilde{\mu}_I}\right)\right) \qquad [8.5]$$

$$\lim_{\delta \to 1} S \cong \left(\frac{\tilde{\mu}_S \tilde{\mu}_I}{\tilde{\mu}_S + \tilde{\mu}_I}\right) \mathrm{LambertW}\left(\left(\frac{\tilde{\mu}_S + \tilde{\mu}_I}{\tilde{\mu}_S \tilde{\mu}_I}\right) \exp\left(\frac{-\varepsilon_S \tilde{\mu}_I \tau + \tilde{\mu}_S + \tilde{\mu}_I}{\tilde{\mu}_S \tilde{\mu}_I}\right)\right) \qquad [8.6]$$

$$\lim_{\delta \to \infty} S \cong \tilde{\mu}_S \mathrm{LambertW}\left(\frac{1}{\tilde{\mu}_S} \exp\left(\frac{-\varepsilon_S \tau + 1}{\tilde{\mu}_S}\right)\right) \qquad [8.7]$$

Similarly, noting that $\lim_{\delta \to 0} I^{\delta} \to 1$ and $\lim_{\delta \to \infty} I^{\delta} \to 0$ when I ≤ 1, the inhibitor evolution with time can be given under various values of δ as follows.

$$\lim_{\delta\to 1} I \cong \left(\frac{\tilde{\mu}_S\tilde{\mu}_I}{\tilde{\mu}_S+\tilde{\mu}_I}\right)\text{LambertW}\left(\left(\frac{\tilde{\mu}_S+\tilde{\mu}_I}{\tilde{\mu}_S\tilde{\mu}_I}\right)\exp\left(-\frac{\varepsilon_I\rho\tau-\tilde{\mu}_S-\tilde{\mu}_I}{\tilde{\mu}_S\tilde{\mu}_I}\right)\right) \quad [8.8]$$

$$\lim_{\delta\to 0} I \cong \tilde{\mu}_I\text{LambertW}\left(\frac{1}{\tilde{\mu}_I}\exp\left(-\frac{\varepsilon_I\rho\tau-1}{\tilde{\mu}_I}\right)\right) \quad [8.9]$$

$$\lim_{\delta\to\infty} I \cong \tilde{\mu}_I\text{LambertW}\left(\frac{1}{\tilde{\mu}_I}\exp\left(\frac{(-\varepsilon_I\rho\tau+1)\delta\tilde{\mu}_S+\tilde{\mu}_I}{\delta\tilde{\mu}_S\tilde{\mu}_I}\right)\right) \quad [8.10]$$

Here LambertW(Z) is the solution of W exp(W) = Z [38]**.** Expressions similar to **Eqs. 8.4-8.9** were proposed earlier to obtain the kinetic parameters from the substrate depletion curves of fully competitive inhibition scheme [37]. One should note that **Eqs. 8.4-8.9** are valid only under the conditions that $(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I) \to 0$. The right-hand sides of V and U in **Eqs. 8.2** can be expanded around $\delta \cong 1$ in a Taylor series as follows.

$$\lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0} V \cong \frac{\varepsilon_S S}{\left(S+\tilde{\mu}_S\left(1+\frac{S}{\tilde{\mu}_I}\right)\right)} + \frac{\varepsilon_S S^2\tilde{\mu}_S\ln(S)(\delta-1)}{\left((\tilde{\mu}_S+\tilde{\mu}_I)S+\tilde{\mu}_S\tilde{\mu}_I\right)\left(S+\tilde{\mu}_S\left(1+\frac{S}{\tilde{\mu}_I}\right)\right)} + O((\delta-1)^2) \quad [8.11]$$

$$\lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0} U \cong \frac{\varepsilon_I\rho I}{\left(I+\tilde{\mu}_I\left(1+\frac{I}{\tilde{\mu}_S}\right)\right)} - \frac{\varepsilon_I\rho I^2\tilde{\mu}_I\ln(I)(\delta-1)}{(\tilde{\mu}_S\tilde{\mu}_I+(\tilde{\mu}_S+\tilde{\mu}_I)I)\left(I+\tilde{\mu}_I\left(1+\frac{I}{\tilde{\mu}_S}\right)\right)} + O((\delta-1)^2) \quad [8.12]$$

Case III: When $(\eta_S, \eta_I, P, Q) \to 0$ so that $S \cong (1-\varepsilon_S X)$, $I \cong (1-\varepsilon_I Y)$ in the pre-steady state regime of **Eqs. 3.1** and **3.2**, then one can arrive at the total quasi steady state approximation (tQSSA) [39, 40]. We will derive explicit expressions for tQSSA in the later sections. From **Eqs. 6.5**, we can conclude that these are the approximate trajectories of fully competitive enzyme kinetic systems over post-steady state regime in the (V, S, I) and (U, S, I) spaces strictly either under the conditions $(\varepsilon_S, \varepsilon_I) \to 0$, $\eta_S = \eta_I$, $\kappa_S = \kappa_I$ and $\rho = 1$ or $(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I) \to 0$ apart from $\rho = 1$.

2.3.1. Exact steady state solutions

When the steady state timescales associated with the enzyme-substrate and enzyme-inhibitor complexes are different from each other, then **Eqs. 3.1-3** will not have a common steady state solution with respect to both the enzyme-substrate and enzyme-inhibitor complexes. In such scenarios, one can derive exact steady state velocities as follows. Noting the fact that at the steady states $\left(\frac{dV}{dS}, \frac{dV}{dP}\right) = (0,0)$ in the (V, S) and (V, P) spaces and $\left(\frac{dU}{dI}, \frac{dU}{dQ}\right) = (0,0)$ in the (U, I) and (U, Q) spaces, one can derive the following expressions from **Eqs. 5.3-4**.

$$\frac{\alpha_S}{\eta_S}V_{CP} + \frac{\varepsilon_S}{\eta_S}(1-V_{CP}-S_{CP}) - \frac{\varepsilon_S}{\eta_S} = \frac{\varepsilon_S}{\eta_S}\left((1-S_{CP})\left(\frac{V_{CP}}{\varepsilon_S}+\frac{U_{CP}}{\rho\varepsilon_I}\right)-\frac{U_{CP}}{\rho\varepsilon_I}\right) \quad [8.13]$$

$$V_{CP} = \frac{\varepsilon_S S_{CP}}{S_{CP}+\mu_S}\left(1-\frac{U_{CP}}{\rho}\right) \quad [8.14]$$

Here (V_{CP}, S_{CP}, U_{CP}) are the corresponding values along with (P_{CP}, Q_{CP}, I_{CP}) at the steady state at which $\left(\frac{dV}{dP}, \frac{dV}{dS}\right) = (0,0)$ with respect to the enzyme-substrate complex. Similarly, one obtains the following set of equations corresponding to the (U, I) and (U, Q) spaces.

$$\frac{\rho\alpha_I}{\eta_I}U_{CQ}+\frac{\varepsilon_I\rho^2}{\eta_I}\left(1-\frac{U_{CQ}}{\rho}-I_{CQ}\right)-\frac{\varepsilon_I\rho^2}{\eta_I}=\frac{\varepsilon_I\rho^2}{\eta_I}\left(\left(1-I_{CQ}\right)\left(\frac{V_{CQ}}{\varepsilon_S}+\frac{U_{CQ}}{\rho\varepsilon_I}\right)-\frac{V_{CQ}}{\varepsilon_S}\right) \quad [8.15]$$

$$\frac{dU}{dI}=0;\ U_{CQ}=\frac{\rho\varepsilon_I I_{CQ}}{I_{CQ}+\mu_I}\left(1-\frac{V_{CQ}}{\varepsilon_S}\right) \quad [8.16]$$

Here (V_{CQ}, I_{CQ}, U_{CQ}) are the corresponding values along with (S_{CQ}, P_{CQ}, Q_{CQ}) at the steady state at which $\left(\frac{dU}{dQ},\frac{dU}{dI}\right)=(0,0)$ with respect to the enzyme-inhibitor complex. However, to find V_{CP} and U_{CQ} which are the exact steady state values, one needs to know S_{CP}, U_{CP}, I_{CQ} and V_{CQ}. When the steady state timescales corresponding to enzyme-substrate and enzyme-inhibitor complexes are the same, then $S_{CP} = S_{CQ}$, $U_{CP} = U_{CQ}$, $I_{CQ} = I_{CP}$ and $V_{CQ} = V_{CP}$ and subsequently **Eqs. 8.13-16** reduce to the standard QSSA **Eqs. 6.1**.

2.3.2. Complexity of the steady states

When $\delta=1$, then the approximate steady state reaction velocities under the conditions that $(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0$ can be given by **Eqs. 8.2** which are monotonically increasing functions of S and I. When $\delta\neq 1$, then **Eqs. 8.2** will show a turn over type behavior with extremum points at which $\left(\frac{dV}{dS},\frac{dU}{dI}\right)=(0,0)$. This dynamical behavior is demonstrated in **Figs. 3**. This means that there are at least two different points at which $\frac{dV}{dS}=0$ of $\frac{dU}{dI}=0$ depending on the value of δ and the standard QSSA equations **Eqs. 6.3** are valid only when $\delta=1$. Velocity expressions given in **Eqs. 8.2** denote the first extrema. The second extremum points can be obtained as follows. When $\delta<1$, then the reaction velocity associated with the enzyme-substrate dynamics will show two different steady state regions as demonstrated in **Fig. 3A**. One can obtain the approximate steady state velocity V_C and substrate S_C from **Eqs. 8.2** upon solving $\frac{dV}{dS}=0$ for S as follows.

$$S_C\cong\left(\frac{\delta\tilde{\mu}_I}{1-\delta}\right)^{\delta};V_C\cong\frac{\varepsilon_S S_C}{\left(S_C+\tilde{\mu}_S\left(1+\frac{{S_C}^{1/\delta}}{\tilde{\mu}_I}\right)\right)};P_C\cong 1-\left(\frac{\delta\tilde{\mu}_I}{1-\delta}\right)^{\delta}-\frac{\varepsilon_S S_C}{\left(S_C+\tilde{\mu}_S\left(1+\frac{{S_C}^{1/\delta}}{\tilde{\mu}_I}\right)\right)} \quad [8.17]$$

One can obtain P_C using the conservation relationship $V_C + S_C + P_C = 1$. When $\delta>1$, then the reaction velocity associated with the enzyme-inhibitor dynamics will show two different steady states as demonstrated in **Fig. 3B**. One can obtain the approximate steady state velocity U_C and the inhibitor concentration I_C from **Eqs. 8.2** as follows. Upon solving $\frac{dU}{dI}=0$ for I, one finds the following results.

$$I_C\cong\left(\frac{\tilde{\mu}_S}{\delta-1}\right)^{\frac{1}{\delta}};U_C\cong\frac{\rho\varepsilon_I I_C}{I_C+\tilde{\mu}_I\left(1+\frac{{I_C}^{\delta}}{\tilde{\mu}_S}\right)};\ Q_C\cong 1-I_C-\frac{U_C}{\rho}\cong 1-\left(\frac{\tilde{\mu}_S}{\delta-1}\right)^{\frac{1}{\delta}}-\frac{\varepsilon_I I_C}{I_C+\tilde{\mu}_I\left(1+\frac{{I_C}^{\delta}}{\tilde{\mu}_S}\right)} \quad [8.18]$$

One can obtain Q_C using the conservation relationship $U_C/\rho + Q_C + I_C = 1$. Here one should note that **Eqs. 8.17** will not be valid when $\delta\geq 1$ and **Eqs. 8.18** will not be valid when $\delta\leq 1$ since the steady state values can be negative or complex under such conditions. A common steady state

can occur only when $\delta = 1$ as demonstrated in **Fig. 3C** and **Eqs. 6.3**. Remarkably, for the first time in the literature we report this phenomenon and none of the earlier studies on the fully and partially competitive inhibition captured this complex dynamical behavior.

2.4. Solutions under coupled and uncoupled conditions

For the general case, the approximate steady state timescales corresponding to the enzyme-substrate and enzyme-inhibitor complexes can be obtained as follows. Using the scaling transformations $M = \frac{P}{\varepsilon_S \eta_S{}^2}$ and $N = \frac{Q}{\varepsilon_I \eta_I{}^2}$, **Eqs. 5.1** and **5.2** can be rewritten in the following form of Murugan equations [10, 35].

$$\frac{d^2M}{d\tau^2} + \frac{\alpha_S}{\eta_S}\frac{dM}{d\tau} + \frac{\varepsilon_S}{\eta_S}M - \frac{1}{\eta_S{}^3} + \frac{\eta_I{}^2}{\rho\eta_S{}^3}\frac{dN}{d\tau} = \phi_S\left(\left(\frac{dM}{d\tau} + M\right)\left(\frac{dM}{d\tau} + \frac{\eta_I{}^2}{\rho\eta_S{}^3}\frac{dN}{d\tau}\right)\right) \quad [9.1]$$

$$\frac{d^2N}{d\tau^2} + \frac{\rho\alpha_I}{\eta_I}\frac{dN}{d\tau} + \frac{\varepsilon_I\rho^2}{\eta_I}N - \frac{\rho^2}{\eta_I{}^3} + \frac{\rho^2\eta_s{}^2}{\eta_I{}^3}\frac{dM}{d\tau} = \rho^2\phi_I\left(\left(\frac{1}{\rho}\frac{dN}{d\tau} + N\right)\left(\frac{1}{\rho}\frac{dN}{d\tau} + \frac{\eta_s{}^2}{\eta_I{}^2}\frac{dM}{d\tau}\right)\right) \quad [9.2]$$

$$\phi_S = \eta_S\varepsilon_S;\ \phi_I = \eta_I\varepsilon_I;\ [M]_{\tau=0} = 0; \left[\frac{dM}{d\tau}\right]_{\tau=0} = 0;\ [N]_{\tau=0} = 0; \left[\frac{dN}{d\tau}\right]_{\tau=0} = 0 \quad [9.3]$$

Here ϕ_S and ϕ_I are the ordinary perturbation parameters which multiply the nonlinear terms. **Eqs. 9.1-2** along with the initial conditions given in **Eqs. 9.3** completely characterize the dynamical aspects of the fully competitive inhibition scheme. **Eqs. 9.1-3** are the central equations of this paper from which we will derive several approximations for the pre- and post-steady state regimes under various set of conditions.

2.4.1. Approximate solutions under coupled conditions

When $(\phi_S, \phi_I) \to 0$, then **Eqs. 9.1** and **9.2** become coupled linear system of ordinary differential equations as follows.

$$\frac{d^2M}{d\tau^2} + \frac{\alpha_S}{\eta_S}\frac{dM}{d\tau} + \frac{\varepsilon_S}{\eta_S}M - \frac{1}{\eta_S{}^3} + \frac{\eta_I{}^2}{\rho\eta_S{}^3}\frac{dN}{d\tau} \cong 0; [M]_{\tau=0} = 0; \left[\frac{dM}{d\tau}\right]_{\tau=0} = 0 \quad [9.4]$$

$$\frac{d^2N}{d\tau^2} + \frac{\rho\alpha_I}{\eta_I}\frac{dN}{d\tau} + \frac{\varepsilon_I\rho^2}{\eta_I}N - \frac{\rho^2}{\eta_I{}^3} + \frac{\rho^2\eta_s{}^2}{\eta_I{}^3}\frac{dM}{d\tau} \cong 0; [N]_{\tau=0} = 0; \left[\frac{dN}{d\tau}\right]_{\tau=0} = 0 \quad [9.5]$$

Eqs. 9.4 and **9.5** are derived here for the first time in the literature. We denote **Eqs. 9.1-5** as Murugan type II equations [35] and the ϕ-approximations given by **Eqs. 9.4-5** are exactly solvable. Interestingly, these equations can be rewritten in terms of fourth order uncoupled ODEs with constant coefficients both in the (M, τ) and (N, τ) spaces as follows (see **Appendix A** for details). In (M, τ) one can straightforwardly derive the following results.

$$\frac{d^4M}{d\tau^4} + a\frac{d^3M}{d\tau^3} + b\frac{d^2M}{d\tau^2} + c\frac{dM}{d\tau} + hM - \frac{\varepsilon_I\rho^2}{\eta_S{}^3\eta_I} \cong 0 \quad [9.6]$$

$$a = \left(\frac{\alpha_S}{\eta_S} + \frac{\rho\alpha_I}{\eta_I}\right); b = \left(\frac{\varepsilon_S}{\eta_S} + \frac{\rho\alpha_I}{\eta_I}\frac{\alpha_S}{\eta_S} + \frac{\varepsilon_I\rho^2}{\eta_I} - \frac{\rho}{\eta_s\eta_I}\right); c = \frac{\rho}{\eta_I}\left(\frac{\alpha_I\varepsilon_S}{\eta_S} + \frac{\varepsilon_I\rho\alpha_S}{\eta_S}\right); h = \frac{\varepsilon_I\rho^2}{\eta_I}\frac{\varepsilon_S}{\eta_S} \quad [9.7]$$

Upon obtaining the solution for the (M, τ) space, one can straightforwardly obtain the expression corresponding to the (N, τ) space as follows.

$$N = \frac{\rho\eta_S{}^3}{\eta_I{}^2}\int_0^\tau \left(\frac{d^2M}{d\tau^2} + \frac{\alpha_S}{\eta_S}\frac{dM}{d\tau} + \frac{\varepsilon_S}{\eta_S}M - \frac{1}{\eta_S{}^3}\right)d\tau \qquad [9.8]$$

The first two initial conditions corresponding to the fourth order uncoupled ODE given by **Eqs. 9.6** can be written as follows.

$$[M]_{\tau=0} = 0; \left[\frac{dM}{d\tau}\right]_{\tau=0} = 0 \qquad [9.9]$$

Other two initial conditions directly follow from the initial conditions corresponding to N.

$$\left[\frac{dN}{d\tau}\right]_{\tau=0} = \left[\frac{d^2M}{d\tau^2} + \frac{\alpha_S}{\eta_S}\frac{dM}{d\tau} + \frac{\varepsilon_S}{\eta_S}M - \frac{1}{\eta_S{}^3}\right]_{\tau=0} = 0 \qquad [9.10]$$

$$[N]_{\tau=0} = \left[\frac{d^3M}{d\tau^3} + \frac{\alpha_S}{\eta_S}\frac{d^2M}{d\tau^2} + \left(\frac{\varepsilon_S}{\eta_S} + \frac{\rho^2\eta_S{}^2}{\eta_I{}^3}\right)\frac{dM}{d\tau} - \frac{\rho^2}{\eta_I{}^3}\right]_{\tau=0} = 0 \qquad [9.11]$$

Similar to **Eq. 9.6**, one can also derive the following solution set corresponding to (N, τ) space.

$$\frac{d^4N}{d\tau^3} + r\frac{d^3N}{d\tau^3} + g\frac{d^2N}{d\tau^2} + m\frac{dN}{d\tau} + zN - \frac{\varepsilon_S}{\eta_S}\frac{\rho^2}{\eta_I{}^3} \cong 0 \qquad [9.12]$$

$$r = \left(\frac{\rho\alpha_I}{\eta_I} + \frac{\alpha_S}{\eta_S}\right); g = \left(\frac{\varepsilon_I\rho^2}{\eta_I} + \frac{\alpha_S}{\eta_S}\frac{\rho\alpha_I}{\eta_I} + \frac{\varepsilon_S}{\eta_S} - \frac{\rho}{\eta_S\eta_I}\right); m = \left(\frac{\alpha_S}{\eta_S}\frac{\varepsilon_I\rho^2}{\eta_I} + \frac{\varepsilon_S}{\eta_S}\frac{\rho\alpha_I}{\eta_I}\right); z = \frac{\varepsilon_S}{\eta_S}\frac{\varepsilon_I\rho^2}{\eta_I} \qquad [9.13]$$

Upon obtaining the solution for the (N, τ) space one can directly obtain the expression corresponding to the (M, τ) space as follows.

$$M = \frac{\eta_I{}^3}{\rho^2\eta_S{}^2}\int_0^\tau \left(\frac{d^2N}{d\tau^2} + \frac{\rho\alpha_I}{\eta_I}\frac{dN}{d\tau} + \frac{\varepsilon_I\rho^2N}{\eta_I} - \frac{\rho^2}{\eta_I{}^3}\right)d\tau \qquad [9.14]$$

The initial conditions corresponding to the fourth order uncoupled ODEs given by **Eqs. 9.12** can be written as follows.

$$[N]_{\tau=0} = 0; \left[\frac{dN}{d\tau}\right]_{\tau=0} = 0 \qquad [9.15]$$

$$\left[\frac{dM}{d\tau}\right]_{\tau=0} = \left[\frac{d^2N}{d\tau^2} + \frac{\rho\alpha_I}{\eta_I}\frac{dN}{d\tau} + \frac{\varepsilon_I\rho^2N}{\eta_I} - \frac{\rho^2}{\eta_I{}^3}\right]_{\tau=0} = 0 \qquad [9.16]$$

$$[M]_{\tau=0} = \left[\frac{d^3N}{d\tau^3} + \frac{\rho\alpha_I}{\eta_I}\frac{d^2N}{d\tau^2} + \left(\frac{\varepsilon_I\rho^2}{\eta_I} + \frac{\eta_I{}^2}{\rho\eta_S{}^3}\right)\frac{dN}{d\tau} - \frac{1}{\eta_S{}^3}\right]_{\tau=0} = 0 \qquad [9.17]$$

Solution to **Eqs. 9.3-9.4** can be obtained either by solving **Eqs. 9.6-9.11** or **Eqs. 9.12-9.17**. The detailed expressions for the solution are given in **Appendix A**. Upon obtaining solutions in the (M, τ) and (N, τ) spaces, one can revert back to (P, τ) and (Q, τ) spaces using the scaling transformations $P = M\varepsilon_S\eta_S{}^2$ and $Q = \varepsilon_I\eta_I{}^2N$ from which one can obtain the parametric expressions for the phase-space trajectories in the (V, P, S), (U, I, Q), (V, I, S), (U, I, S), (V, P, Q),

(U, P, Q) and (V, U) spaces using the appropriate mass conservation relationships where τ acts as the parameter here.

2.4.2. Approximate solutions under uncoupled conditions

Interestingly, when $({\eta_S}^2, {\eta_I}^2) \to 0$ along with the conditions of ϕ-approximations as $(\phi_S, \phi_I) \to 0$, then **Eqs. 9.1** and **9.2** can be approximated by the following uncoupled set of ODEs.

$$\frac{d^2M}{d\tau^2} + \frac{\alpha_S}{\eta_S}\frac{dM}{d\tau} + \frac{\varepsilon_S}{\eta_S}M - \frac{1}{{\eta_S}^3} \cong 0 \qquad [9.18]$$

$$\frac{d^2N}{d\tau^2} + \frac{\rho\alpha_I}{\eta_I}\frac{dN}{d\tau} + \frac{\varepsilon_I\rho^2N}{\eta_I} - \frac{\rho^2}{{\eta_I}^3} \cong 0 \qquad [9.19]$$

Upon reverting these equations back into the (P, Q, τ) space of **Eqs. 5.1** and **5.2**, one obtains the following set of ODEs along with the corresponding initial conditions.

$$\frac{d^2P}{d\tau^2} + \frac{\alpha_S}{\eta_S}\frac{dP}{d\tau} + \frac{\varepsilon_S P}{\eta_S} - \frac{\varepsilon_S}{\eta_S} \cong 0; [P]_{\tau=0} = 0; \left[\frac{dP}{d\tau}\right]_{\tau=0} = 0 \qquad [9.20]$$

$$\frac{d^2Q}{d\tau^2} + \frac{\rho\alpha_I}{\eta_I}\frac{dQ}{d\tau} + \frac{\varepsilon_I\rho^2Q}{\eta_I} - \frac{\varepsilon_I\rho^2}{\eta_I} \cong 0; [Q]_{\tau=0} = 0; \left[\frac{dQ}{d\tau}\right]_{\tau=0} = 0 \qquad [9.21]$$

Along with the conditions that $({\eta_S}^2, {\eta_I}^2) \to 0$, the uncoupled **Eqs. 9.20** and **9.21** are valid only (a) when $(\phi_S, \phi_I) \to 0$ so that there is no competitive kinetics or (b) the dissociation rate constants of both the enzyme-substrate and enzyme-inhibitor complexes are high enough to uncouple the competitive kinetics i.e., $(\kappa_S, \kappa_I) \to \infty$. Both these conditions will lead to the approximation $E = (1 - X - Y) \cong 1$. We will show later that these conditions decrease the mismatch between the steady state timescales associated with the enzyme-substrate and enzyme-inhibitor complexes. Upon solving these ODEs with the given initial conditions, we can derive the approximate expressions for the dynamics of the competitive inhibition scheme (P, Q, V, U, S, I) as follows [10, 35].

$$P \cong 1 - \frac{1}{2a}\left(\left(exp\left(\frac{-\alpha_S+a}{2\eta_S}\tau\right)(\alpha_S + a)\right) - \left(exp\left(\frac{-\alpha_S-a}{2\eta_S}\tau\right)(-\alpha_S + a)\right)\right) \qquad [10.1]$$

$$Q \cong 1 - \frac{1}{2b}\left(\left(exp\left(\frac{-\alpha_I+b}{2\eta_I}\rho\tau\right)(\alpha_I + b)\right) - \left(exp\left(\frac{-\alpha_I-b}{2\eta_I}\rho\tau\right)(-\alpha_I + b)\right)\right) \qquad [10.2]$$

$$V = \frac{dP}{d\tau} \cong \frac{\varepsilon_S}{a}\left(exp\left(\frac{-\alpha_S+a}{2\eta_S}\tau\right) - exp\left(\frac{-\alpha_S-a}{2\eta_S}\tau\right)\right) \qquad [10.3]$$

$$U = \frac{dQ}{d\tau} \cong \frac{\rho\varepsilon_I}{b}\left(exp\left(\frac{-\alpha_I+b}{2\eta_I}\rho\tau\right) - exp\left(\frac{-\alpha_I-b}{2\eta_I}\rho\tau\right)\right) \qquad [10.4]$$

$$S = 1 - V - P;\ I = 1 - \frac{U}{\rho} - Q;\ a = \sqrt{\alpha_S^2 - 4\varepsilon_S\eta_S}\ ;\ b = \sqrt{\alpha_I^2 - 4\varepsilon_I\eta_I} \qquad [10.5]$$

The expressions for (P, V, S) will be valid only when $({\eta_I}^2, \phi_S) \to 0$ and the expressions for (Q, U, I) will be valid only when $({\eta_S}^2, \phi_I) \to 0$. Upon solving $\frac{dV}{d\tau} = 0$ and $\frac{dU}{d\tau} = 0$ for τ in **Eqs. 10.3** ad **10.4**, one can obtain the following approximations for the steady state timescales corresponding to substrate and inhibitor conversion dynamics.

$$\lim_{({\eta_I}^2,\phi_S)\to 0} \tau_{CS} \cong \frac{\eta_S}{\sqrt{\alpha_S^2 - 4\varepsilon_S\eta_S}} ln\left(-\frac{2\varepsilon_S\eta_S}{\alpha_S\sqrt{\alpha_S^2-4\varepsilon_S\eta_S}+\alpha_S^2-2\varepsilon_S\eta_S}\right) \quad [11.1]$$

$$\lim_{({\eta_S}^2,\phi_I)\to 0} \tau_{CI} \cong \frac{\eta_I}{\rho\sqrt{\alpha_I^2 - 4\varepsilon_I\eta_I}} ln\left(-\frac{2\varepsilon_I\eta_I}{\alpha_I\sqrt{\alpha_I^2-4\varepsilon_I\eta_I}+\alpha_I^2-2\varepsilon_I\eta_I}\right) \quad [11.2]$$

Here τ_{CS} and τ_{CI} are the approximate timescales at which the steady states with respect to the enzyme-substrate and enzyme-inhibitor complexes occur under uncoupled conditions. Upon substituting the expression for τ_{CS} into the expressions for (V, P, S) one can obtain the corresponding steady state values (V_C, P_C, S_C). In the same way, upon substituting the expression for τ_{CI} into the expressions for (U, Q, I) one can obtain the corresponding steady state values (U_C, Q_C, I_C). Clearly, the condition $\tau_{CS} \cong \tau_{CI}$ is critical for the occurrence of a common steady state with respect to the reaction dynamics of both the enzyme-substrate and enzyme-inhibitor complexes. When $\tau_{CI} > \tau_{CS}$, then the substrate depletion with respect to time will show a typical non-monotonic trend since the inhibitor reverses the enzyme-substrate complex formed before time τ_{CI} in the pre-steady state regime. In the same way, when $\tau_{CI} < \tau_{CS}$ then the inhibitor depletion will show a non-monotonic behavior since the substrate reverses the enzyme-inhibitor complex formed before time τ_{CS} in the pre-steady state regime. These phenomena eventually introduce significant amount of error in various QSSAs. Under uncoupled conditions i.e., when $({\eta_I}^2, \phi_I, {\eta_S}^2, \phi_S) \to 0$, then one can rewrite the approximations given in **Eqs. 5.3-4** over (V, P) and (U, Q) spaces as follows.

$$V\frac{dV}{dP} + \frac{\alpha_S}{\eta_S}V + \frac{\varepsilon_S}{\eta_S}P - \frac{\varepsilon_S}{\eta_S} \cong 0; [V]_{P=0} = 0 \quad [12.1]$$

$$-V\frac{dV}{dS} + \frac{\alpha_S}{\eta_S}V + \frac{\varepsilon_S}{\eta_S}(1-S) - \frac{\varepsilon_S}{\eta_S} \cong 0; [V]_{S=1} = 0;\ lim_{\varepsilon_S\to 0}\left(\frac{dS}{dP}\right) \cong -1; P \cong 1 - S \quad [12.2]$$

$$U\frac{dU}{dQ} + \frac{\rho\alpha_I}{\eta_I}U + \frac{\varepsilon_I\rho^2}{\eta_I}Q - \frac{\varepsilon_I\rho^2}{\eta_I} \cong 0; [U]_{Q=0} = 0 \quad [12.3]$$

$$-U\frac{dU}{dI} + \frac{\rho\alpha_I}{\eta_I}U + \frac{\varepsilon_I\rho^2}{\eta_I}(1-I) - \frac{\varepsilon_I\rho^2}{\eta_I} \cong 0; [U]_{I=1} = 0\ ;\ lim_{\varepsilon_I\to 0}\left(\frac{dI}{dQ}\right) \cong -1;\ Q \cong 1 - I \quad [12.4]$$

Upon considering only the linear, uncoupled portions in the (V, P) space of **Eqs. 5.3** as given in **Eqs. 12.1**, one obtains the following approximations for V and S as functions of P.

$$V \cong \frac{1}{2\eta_S}\left(\alpha_S - tan(W_P)\sqrt{-\alpha_S^2 + 4\varepsilon_S\eta_S}\right)(1-P) \quad [13.1]$$

$$S = 1 - P - V \cong (1 - P)\left(1 - \frac{1}{2\eta_S}\left(\alpha_S - tan(W_P)\sqrt{-\alpha_S^2 + 4\varepsilon_S\eta_S}\right)\right) \quad [13.2]$$

Similar expressions can also be derived in the (V, S), (U, Q) and (U, I) spaces. These approximate equations parametrically describe the dynamics of the enzyme catalyzed substrate conversion in the entire regime of (V, P, S) space from (V, P, S) = (0, 0, 1) to (V, P, S) = (0, 1, 0) including the steady states (V_C, P_C, S_C) that occurs at $\left(\frac{dV}{dP}, \frac{dV}{dS}\right) = (0,0)$. Here W_P is the solution of the following nonlinear algebraic equation.

$$-a\, ln\left(-\frac{\left(1+tan(W_P)^2\right)a^2(P-1)^2}{4\varepsilon_S\eta_S}\right) - 2\alpha_S arctan\left(\frac{\alpha_S}{a}\right) + 2\alpha_S W_P = 0;\ a = \sqrt{-\alpha_S^2 + 4\varepsilon_S\eta_S} \quad [14]$$

Further, upon solving $\frac{dV}{dP} = 0$ one obtains the steady state concentration of the product of substrate as follows.

$$P_C \cong 1 - \alpha_S\sqrt{\frac{1}{\varepsilon_S\eta_S} exp\left(\frac{2\alpha_S}{a}\left(arctanh\left(\frac{\alpha_S^2 - 2\varepsilon_S\eta_S}{\alpha_S a}\right) - arctanh\left(\frac{\alpha_S}{a}\right)\right)\right)};\ a = \sqrt{\alpha_S^2 - 4\varepsilon_S\eta_S} \quad [15]$$

Upon substituting the expression of P_C into the expressions of V and S as given in **Eqs. 13.1-2**, one can obtain the corresponding steady state expressions for V_C and S_C. In the same way, upon considering only the linear, uncoupled portions in the (U, P, Q) space of **Eqs. 5.4**, one obtains the following approximations for U and I as functions of Q.

$$U \cong \frac{\rho(1-Q)\left(\alpha_I - tan(W_Q)\sqrt{-\alpha_I^2 + 4\varepsilon_I\eta_I}\right)}{2\eta_I} \quad [16.1]$$

$$I = 1 - Q - \frac{U}{\rho} \cong (1 - Q)\left(1 - \frac{1}{2\eta_I}\left(\alpha_I - tan(W_Q)\sqrt{-\alpha_I^2 + 4\varepsilon_I\eta_I}\right)\right) \quad [16.2]$$

Here W_Q is the solution of the following nonlinear algebraic equation.

$$2W_Q\rho\alpha_I - \rho b ln\left(-\frac{\left(1+tan(W_Q)^2\right)b^2(Q-1)^2}{4\varepsilon_I\eta_I}\right) - 2\rho\alpha_I arctan\left(\frac{\alpha_I}{b}\right) = 0;\ b = \sqrt{-\alpha_I^2 + 4\varepsilon_I\eta_I} \quad [17]$$

Further upon solving $\frac{dU}{dQ} = 0$ one obtains the steady state level of the product of inhibitor as follows.

$$Q_C \cong 1 - \alpha_I\sqrt{\frac{1}{\varepsilon_I\eta_I} exp\left(\frac{2\alpha_I}{z}\left(arctan\left(\frac{\alpha_I^2 - 2\varepsilon_I\eta_I}{\alpha_I B}\right) - arctan\left(\frac{\rho\alpha_I}{B}\right)\right)\right)}; B = \sqrt{-\alpha_I^2 + 4\varepsilon_I\eta_I} \quad [18]$$

Upon substituting the expression of Q_C into the expressions of U and I as given in **Eqs. 16.1-2**, one can obtain the corresponding steady state expressions for U_C and I_C. **Eqs. 12-18** are valid only when the conditions $({\eta_I}^2, \phi_I, {\eta_S}^2, \phi_S) \to 0$ are true.

2.5. Approximate pre-steady state solutions

Using the scaling transformation $M = \frac{P}{\varepsilon_S \eta_S{}^2}$ one can rewrite the coupled nonlinear ODEs corresponding to the fully competitive inhibition given in **Eqs. 5.1** and **3.2** as follows.

$$\frac{d^2M}{d\tau^2} + \frac{\alpha_S}{\eta_S}\frac{dM}{d\tau} + \frac{\varepsilon_S}{\eta_S}M - \frac{1}{\eta_S{}^3} + \frac{1}{\eta_S{}^3}Y = \eta_S\varepsilon_S\left(\left(\frac{dM}{d\tau} + M\right)\left(\frac{dM}{d\tau} + \frac{1}{\eta_S{}^2}Y\right)\right) \quad [19.1]$$

$$\left(\frac{\eta_I}{\rho}\right)\frac{dY}{d\tau} + \eta_S{}^2\frac{dM}{d\tau} + Q + \alpha_I Y - 1 = (\varepsilon_I Y + Q)\left(\eta_S{}^2\frac{dM}{d\tau} + Y\right) \quad [19.2]$$

Case I: When $(\varepsilon_I, \eta_I, \varepsilon_S) \to 0$ and noting that $I = (1 - \varepsilon_I Y - Q) \cong 1 - \varepsilon_I Y$ and $Q \cong 0$ under such conditions in the pre-steady state regime, one can arrive at the approximation from **Eq. 19.2** that $Y \cong \frac{1}{\alpha_I}\left(1 - \eta_S{}^2\frac{dM}{d\tau}\right)$. Upon substituting this expression for Y in **Eq. 19.1** and setting the variable transformation $F = \frac{dM}{d\tau}$, one finally arrives at the following approximate ODEs in the (M, τ) and (F, M) spaces.

$$\frac{d^2M}{d\tau^2} + \frac{\alpha_S}{\eta_S}\left(1 - \frac{1}{\alpha_S\alpha_I}\right)\frac{dM}{d\tau} - \frac{1}{\eta_S{}^3}\left(1 - \frac{1}{\alpha_I}\right) \cong 0; [M]_{\tau=0} = 0; \left[\frac{dM}{d\tau}\right]_{\tau=0} = 0 \quad [20.1]$$

$$F\frac{dF}{dM} + \frac{\alpha_S}{\eta_S}\left(1 - \frac{1}{\alpha_S\alpha_I}\right)F - \frac{1}{\eta_S{}^3}\left(1 - \frac{1}{\alpha_I}\right) \cong 0; [F]_{M=0} = 0 \quad [20.2]$$

Case II: When $(\varepsilon_I, \eta_I, \phi_S) \to 0$ in **Eq. 19**, then following the same arguments with respect to **Eqs. 20**, one finds the following refined approximations in the (M, τ) and (F, M) spaces.

$$\frac{d^2M}{d\tau^2} + \frac{\alpha_S}{\eta_S}\left(1 - \frac{1}{\alpha_S\alpha_I}\right)\frac{dM}{d\tau} + \frac{\varepsilon_S}{\eta_S}M - \frac{1}{\eta_S{}^3}\left(1 - \frac{1}{\alpha_I}\right) \cong 0; [M]_{\tau=0} = 0; \left[\frac{dM}{d\tau}\right]_{\tau=0} = 0 \quad [21.1]$$

$$F\frac{dF}{dM} + \frac{\alpha_S}{\eta_S}\left(1 - \frac{1}{\alpha_S\alpha_I}\right)F + \frac{\varepsilon_S}{\eta_S}M - \frac{1}{\eta_S{}^3}\left(1 - \frac{1}{\alpha_I}\right) \cong 0; [F]_{M=0} = 0 \quad [21.2]$$

Eq. 20.1 is a linear second order ODE with constant coefficients that is exactly solvable. Upon solving the nonlinear ODE given in **Eq. 20.2** along with the initial condition, one arrives at the following integral solution particularly in the (F, M) space.

$$\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0} F \cong \frac{\left(1-\frac{1}{\alpha_I}\right)}{\alpha_S\eta_S{}^2\left(1-\frac{1}{\alpha_S\alpha_I}\right)}\left(1 + \mathrm{LambertW}\left(-exp\left(-1 - \eta_S{}^3 M\frac{\left(1-\frac{1}{\alpha_S\alpha_I}\right)^2}{\left(1-\frac{1}{\alpha_I}\right)}\right)\right)\right) \quad [22]$$

Reverting back to (V, P), (V, S) and (V, τ) spaces using the transformation $P = \varepsilon_S\eta_S{}^2 M$ and conservation relationships, we arrive at the following approximate solutions to **Eqs. 20**.

$$\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0} V \cong \frac{\varepsilon_S}{1+(\varepsilon_S+\mu_S)\left(1+\frac{1}{\varepsilon_I+\mu_I}\right)}\left(1 - exp\left(-\frac{\alpha_S}{\eta_S}\left(1 - \frac{1}{\alpha_S\alpha_I}\right)\tau\right)\right) \quad [23.1]$$

$$\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0} V \cong \frac{\varepsilon_S(\alpha_I-1)}{(\alpha_S\alpha_I-1)}\left(1+\text{LambertW}\left(-exp\left(-1-\frac{\eta_S(\alpha_S\alpha_I-1)^2}{\alpha_S{}^2\alpha_I\varepsilon_S(\alpha_I-1)}P\right)\right)\right) \quad [23.2]$$

Upon solving **Eq. 20.2** implicitly and then substituting P = 1 – V – S, one obtains the following pre-steady state solution in the (V, S) space.

$$\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0} V \cong \frac{b\varepsilon_S\eta_S{}^2}{a}\left(1-\frac{1}{(a-1)}\text{LambertW}\left((a-1)exp\left((a-1)+\frac{a^2(S-1)}{b\varepsilon_S\eta_S{}^2}\right)\right)\right) \quad [23.4]$$

The parameters *a* and *b* in **Eq. 23.4** are defined as follows.

$$a=\frac{\alpha_S}{\eta_S}\left(1-\frac{1}{\alpha_S\alpha_I}\right); b=\frac{1}{\eta_S{}^3}\left(1-\frac{1}{\alpha_I}\right) \quad [23.5]$$

Clearly, **Eqs. 23** are valid only in the pre-steady state regime. When P or τ becomes sufficiently large, then **Eqs. 23** asymptotically converge to the following limiting value that is close to the steady state reaction velocity V in the limit as $(\varepsilon_I,\eta_I,\varepsilon_S)\to 0$. We denote this approximation as V_2. We will show in the later sections that this approximation that is similar to **Eqs. 8.2** works very well in predicting the steady state reaction velocities over wide ranges of parameters.

$$V_2 \cong \lim_{P\to 1}\left(\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0} V\right) \cong \frac{\varepsilon_S(\alpha_I-1)}{(\alpha_S\alpha_I-1)} = \frac{\varepsilon_S}{1+(\varepsilon_S+\mu_S)\left(1+\frac{1}{\varepsilon_I+\mu_I}\right)} \quad [24.1]$$

One can also arrive at **Eq. 24.1** in the limit $(\varepsilon_I,\eta_I,\varepsilon_S,\eta_S)\to 0$ as in the standard QSSA given in **Eq. 6.1**. In terms of original variables, this equation can be written as follows.

$$v_2 = \lim_{p\to s_0}\left(\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0} v\right) \cong \frac{k_2e_0s_0}{s_0+(e_0+K_{MS})\left(1+\frac{i_0}{e_0+K_{MI}}\right)} \quad [24.2]$$

Similar to **Eqs. 19.1-2**, using the transformation $N=\frac{Q}{\varepsilon_I\eta_I{}^2}$ one can rewrite **Eqs. 5.2** and **3.1** as the following coupled system of ODEs.

$$\frac{d^2N}{d\tau^2}+\frac{\rho\alpha_I}{\eta_I}\frac{dN}{d\tau}+\frac{\varepsilon_I\rho^2N}{\eta_I}-\frac{\rho^2}{\eta_I{}^3}+\rho^2\frac{\varepsilon_I}{\eta_I}X=\rho^2\eta_I\varepsilon_I\left(\left(\frac{1}{\rho}\frac{dN}{d\tau}+N\right)\left(\frac{1}{\eta_I{}^2}X+\frac{1}{\rho}\frac{dN}{d\tau}\right)\right) \quad [25.1]$$

$$\eta_S\frac{dX}{d\tau}+\alpha_SX+P-1+\frac{\eta_I{}^2}{\rho}\frac{dN}{d\tau}=(\varepsilon_SX+P)\left(X+\frac{\eta_I{}^2}{\rho}\frac{dN}{d\tau}\right) \quad [25.2]$$

Case III: When $(\varepsilon_S,\eta_S,\varepsilon_I)\to 0$ and noting that $S=(1-\varepsilon_SX-P)\cong 1-\varepsilon_SX$ and $P\cong 0$ in the pre-steady state regime, one finds from **Eq. 25.2** that $X\cong\frac{1}{\alpha_S}\left(1-\frac{\eta_I{}^2}{\rho}\frac{dN}{d\tau}\right)$. Upon substituting this expression of X into **Eq. 25.1**, setting $(\varepsilon_S,\eta_S,\varepsilon_I)\to 0$ and applying the transformation $G=\frac{dN}{d\tau}$ in **Eqs. 25.1** and **25.2** one can derive the following approximate differential equations in the (N, τ) and (G, N) spaces.

$$\frac{d^2N}{d\tau^2}+\frac{\rho\alpha_I}{\eta_I}\left(1-\frac{1}{\alpha_I\alpha_S}\right)\frac{dN}{d\tau}+\frac{\rho^2}{{\eta_I}^3}\left(1-\frac{1}{\alpha_S}\right)\cong 0;\,[N]_{\tau=0}=0;\,\left[\frac{dN}{d\tau}\right]_{\tau=0}=0 \qquad [26.1]$$

$$G\frac{dG}{dN}+\frac{\rho\alpha_I}{\eta_I}\left(1-\frac{1}{\alpha_I\alpha_S}\right)G-\frac{\rho^2}{{\eta_I}^3}\left(1-\frac{1}{\alpha_S}\right)\cong 0;\,[G]_{N=0}=0 \qquad [26.2]$$

Case IV: When $(\varepsilon_S,\eta_S,\phi_I)\to 0$, then following the same arguments with respect to **Eqs. 24**, one finds the following approximations in the (N, τ) and (G, N) spaces.

$$\frac{d^2N}{d\tau^2}+\frac{\rho\alpha_I}{\eta_I}\left(1-\frac{1}{\alpha_I\alpha_S}\right)\frac{dN}{d\tau}+\frac{\varepsilon_I\rho^2}{\eta_I}N+\frac{\rho^2}{{\eta_I}^3}\left(1-\frac{1}{\alpha_S}\right)\cong 0;\,[N]_{\tau=0}=0;\,\left[\frac{dN}{d\tau}\right]_{\tau=0}=0 \qquad [27.1]$$

$$G\frac{dG}{dN}+\frac{\rho\alpha_I}{\eta_I}\left(1-\frac{1}{\alpha_I\alpha_S}\right)G+\frac{\varepsilon_I\rho^2}{\eta_I}N-\frac{\rho^2}{{\eta_I}^3}\left(1-\frac{1}{\alpha_S}\right)\cong 0;\,[G]_{N=0}=0 \qquad [27.2]$$

Eq. 26.1 is a second order linear ODE with constant coefficients that is exactly solvable. Upon solving the nonlinear ODE given in **Eq. 26.2** along with the initial condition, one can arrive at the following integral solution in the pre-steady state regime in the (G, N) space.

$$\lim_{(\varepsilon_I,\eta_S,\varepsilon_S)\to 0} G\cong\frac{\rho\left(1-\frac{1}{\alpha_S}\right)}{\alpha_I{\eta_I}^2\left(1-\frac{1}{\alpha_S\alpha_I}\right)}\left(1+\mathrm{LambertW}\left(-exp\left(-1-{\eta_I}^3N\frac{\left(1-\frac{1}{\alpha_S\alpha_I}\right)^2}{\left(1-\frac{1}{\alpha_I}\right)}\right)\right)\right) \qquad [28]$$

Reverting back to the (U, Q), (U, I) and (U, τ) spaces we find the following approximate solutions to **Eqs. 26**.

$$\lim_{(\varepsilon_S,\eta_S,\varepsilon_I)\to 0} U\cong\frac{\rho\varepsilon_I(\alpha_I-1)}{(\alpha_S\alpha_I-1)}\left(1-exp\left(-\frac{\alpha_I}{\eta_I}\left(1-\frac{1}{\alpha_S\alpha_I}\right)\tau\right)\right) \qquad [29.1]$$

$$\lim_{(\varepsilon_I,\eta_S,\varepsilon_S)\to 0} U\cong\frac{\rho\varepsilon_I(\alpha_I-1)}{(\alpha_S\alpha_I-1)}\left(1+\mathrm{LambertW}\left(-exp\left(-1-\frac{\eta_I(\alpha_S\alpha_I-1)^2}{{\alpha_I}^2\alpha_S\varepsilon_I(\alpha_I-1)}Q\right)\right)\right) \qquad [29.2]$$

Upon solving **Eq. 26.2** implicitly and then substituting Q = 1 - U/ρ - I, one obtains the following pre-steady state solution in the (U, I) space.

$$\lim_{(\varepsilon_I,\eta_S,\varepsilon_S)\to 0} U\cong\frac{h\varepsilon_I{\eta_I}^2}{g}\left(1-\frac{\rho}{(g-\rho)}\mathrm{LambertW}\left(\frac{(g-\rho)}{\rho}exp\left(\frac{(g-\rho)}{\rho}+\frac{g^2(I-1)}{h\varepsilon_I{\eta_I}^2}\right)\right)\right) \qquad [29.3]$$

The parameters *g* and *h* in **Eq. 29.3** are defined as follows.

$$g=\frac{\rho\alpha_I}{\eta_I}\left(1-\frac{1}{\alpha_I\alpha_S}\right);\,h=\frac{\rho^2}{{\eta_I}^3}\left(1-\frac{1}{\alpha_S}\right) \qquad [29.4]$$

Clearly, **Eqs. 29.1-2** are valid only in the pre-steady state regime. When Q or τ becomes sufficiently large, then **Eqs. 29.1-2** asymptotically converge to the following limiting value that is close to the steady state value of U in the limit $(\varepsilon_S,\eta_S,\varepsilon_I)\to 0$. We denote this approximation as

U_2. We will show in the later sections that this approximation works very well over wide ranges of parameter values.

$$U_2 \cong \lim_{Q\to 1}\left(\lim_{(\varepsilon_I,\eta_S,\varepsilon_S)\to 0} U\right) \cong \frac{\rho\varepsilon_I(\alpha_I-1)}{(\alpha_S\alpha_I-1)} = \frac{\rho\varepsilon_I}{1+(\varepsilon_I+\mu_I)\left(1+\frac{1}{\varepsilon_S+\mu_S}\right)} \quad [30.1]$$

In terms of original variables, this equation can be written as follows.

$$u_2 = \lim_{q\to i_0}\left(\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0} u\right) \cong \frac{k_3 e_0 i_0}{i_0+(e_0+K_{MI})\left(1+\frac{s_0}{e_0+K_{MS}}\right)} \quad [30.2]$$

2.5.1. Steady state timescales

Eqs. 23.1 and **29.1** clearly suggest the following approximate steady state timescales associated with the substrate and inhibitor conversion dynamics under coupled conditions.

$$\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0}\tau_{CS} \cong \frac{1}{\frac{\alpha_S}{\eta_S}\left(1-\frac{1}{\alpha_S\alpha_I}\right)} = \frac{\alpha_I\eta_S}{(\alpha_S\alpha_I-1)};\ \lim_{(\varepsilon_S,\eta_S,\varepsilon_I)\to 0}\tau_{CI} \cong \frac{1}{\frac{\alpha_I}{\eta_I}\left(1-\frac{1}{\alpha_S\alpha_I}\right)} = \frac{\alpha_S\eta_I}{(\alpha_S\alpha_I-1)} \quad [31.1]$$

In terms of original variables, **Eqs 31.1** can be rewritten as follows.

$$\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0} t_{CS} \cong \frac{1}{k_1}\left(\frac{\left(1+\frac{i_0}{e_0+K_{MI}}\right)}{s_0+(e_0+K_{MS})\left(1+\frac{i_0}{e_0+K_{MI}}\right)}\right);\ \lim_{(\varepsilon_S,\eta_S,\varepsilon_I)\to 0} t_{CI} \cong \frac{1}{k_i}\left(\frac{\left(1+\frac{s_0}{e_0+K_{MS}}\right)}{i_0+(e_0+K_{MI})\left(1+\frac{s_0}{e_0+K_{MS}}\right)}\right) \quad [31.2]$$

Eqs. 31.2 are similar to the equations derived in Ref. [37] (see Eqs. 34-35 of this reference) for the steady state timescales. However, the numerator terms were set to unity in their expression. Unlike those expressions for t_{CS} and t_{CI} suggested in Ref. [37] which predict that $\lim_{i_0\to\infty} t_{CS} \to 0$ and $\lim_{s_0\to\infty} t_{CI} \to 0$, **Eqs. 31.2** correctly predict the following limiting behavior of the steady state timescales when $(s_0, i_0) \to 0$ or $(s_0, i_0) \to \infty$.

$$\lim_{s_0\to\infty}\left(\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0} t_{CS}\right) \cong 0;\ \lim_{s_0\to 0}\left(\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0} t_{CS}\right) \cong \frac{1}{(e_0+K_{MS})k_1} \quad [31.3]$$

$$\lim_{i_0\to 0}\left(\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0} t_{CS}\right) \cong \frac{1}{k_1}\left(\frac{1}{s_0+e_0+K_{MS}}\right);\ \lim_{i_0\to\infty}\left(\lim_{(\varepsilon_S,\eta_I,\varepsilon_I)\to 0} t_{CS}\right) \cong \frac{1}{(e_0+K_{MS})k_1} \quad [31.4]$$

Here one should note that $s_0 \to 0$ or $i_0 \to \infty$ will have similar limiting behavior on t_{CS}. This is reasonable since setting $i_0 \to \infty$ will eventually decreases the effective substrate concentration towards zero. Similarly, one also finds the following limiting behaviors of t_{CI}.

$$\lim_{i_0\to\infty}\left(\lim_{(\varepsilon_S,\eta_S,\varepsilon_I)\to 0} t_{CI}\right) \cong 0;\ \lim_{i_0\to 0}\left(\lim_{(\varepsilon_S,\eta_S,\varepsilon_I)\to 0} t_{CI}\right) \cong \frac{1}{(e_0+K_{MI})k_i} \quad [31.5]$$

$$\lim_{s_0\to 0}\left(\lim_{(\varepsilon_S,\eta_S,\varepsilon_I)\to 0} t_{CI}\right) \cong \frac{1}{k_1}\left(\frac{1}{i_0+e_0+K_{MI}}\right);\ \lim_{s_0\to\infty}\left(\lim_{(\varepsilon_S,\eta_S,\varepsilon_I)\to 0} t_{CI}\right) \cong \frac{1}{(e_0+K_{MI})k_i} \quad [31.6]$$

Here one should note that $i_0 \to 0$ or $s_0 \to \infty$ will have similar limiting behavior on t_{CI}. **Eqs. 31.1** clearly suggest that a common steady state can occur only when $\alpha_I\eta_S \cong \alpha_S\eta_I$ or explicitly when

the ratio $\psi = \frac{(1+\varepsilon_I+\eta_I+\kappa_I)\eta_S}{(1+\varepsilon_S+\eta_S+\kappa_S)\eta_I} \cong 1$. When $\eta_S \cong \eta_I$, then this condition $\psi \cong 1$ can be achieved by setting large values for any one of the parameters $(\varepsilon_S, \eta_S, \kappa_S)$ in the numerator part and any one of the parameters $(\varepsilon_I, \eta_I, \kappa_I)$ from the denominator part so that their ratio ψ tends towards one. For example, one can consider setting $(\varepsilon_I, \eta_S) \to \infty$ or a combination $(\varepsilon_S, \kappa_I) \to \infty$ and so on. Under such conditions, the error in the estimation of the kinetic parameters using quasi steady state methods will be at minimum. In general, the condition for the minimal error can also be written in the following form.

$$\left(\frac{1+\varepsilon_I+\eta_I+\kappa_I}{1+\varepsilon_S+\eta_S+\kappa_S}\right) \cong \frac{\varepsilon_{IS}}{\rho\sigma};\ \sigma = \frac{k_1}{k_i};\ \gamma = \frac{k_{-1}}{k_{-i}};\ \varepsilon_{IS} = \frac{i_0}{s_0} = \frac{\varepsilon_S}{\varepsilon_I} \qquad [31.7]$$

The parameters (σ, γ, ρ) are defined as in **Eq. 4.6**. Here one should note that the parameters $(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I, \rho)$ are connected via $\rho\frac{\varepsilon_I\eta_S}{\varepsilon_S\eta_I} = \frac{1}{\sigma}$ so that one finds that $\eta_S = \eta_I\frac{\varepsilon_S}{\rho\varepsilon_I\sigma}$. Similarly, the set of parameters $(\kappa_S, \kappa_I, \varepsilon_S, \varepsilon_I, \gamma, \sigma)$ are connected via $\frac{\kappa_S}{\kappa_I} = \frac{\varepsilon_S}{\varepsilon_I}\frac{\gamma}{\sigma}$ so that $\kappa_S = \kappa_I\frac{\varepsilon_S}{\varepsilon_I}\frac{\gamma}{\sigma}$. These mean that $\sigma = \frac{\eta_I\varepsilon_S}{\rho\eta_S\varepsilon_I}$, $\gamma = \frac{\eta_I\kappa_S}{\rho\eta_S\kappa_I}$, $\frac{\gamma}{\sigma} = \frac{\varepsilon_I\kappa_S}{\varepsilon_S\kappa_I} = \frac{K_{DS}}{K_{IS}}$ and $\sigma\rho = \frac{K_{RI}}{K_{RS}}$. In this background, the conditional **Eqs. 31.7** can be rewritten as follows.

$$(1+\varepsilon_I+\eta_I+\kappa_I) \cong \frac{\varepsilon_S}{\rho\varepsilon_I\sigma}\left(1+\varepsilon_S+\eta_I\frac{\varepsilon_S}{\rho\varepsilon_I\sigma}+\kappa_S\right) \qquad [31.8]$$

In terms of original variables this conditional equation **Eqs. 31.7** can be written as follows.

$$\left(\frac{s_0+e_0+K_{MS}}{i_0+e_0+K_{MI}}\right) \cong \frac{1}{\rho\sigma}\left(\frac{\varepsilon_S}{\varepsilon_I}\right)^2 \qquad [31.9]$$

In this equation, ρ is defined as in **Eqs. 4.2**. Since in most of the *in vitro* quasi-steady state experiments one sets large values as $(s_0, i_0) \to \infty$ so that the left-hand side of **Eq. 31.9** tends towards unity. In this situation, the conditional statement given in **Eq. 31.9** will be true when the right hand side $\left(\frac{\eta_S}{\eta_I}\frac{\varepsilon_S}{\varepsilon_I}\right) \to 1$ which is essential (but not sufficient) condition to minimize the error of such quasi steady state approximations as suggested [36, 37] by most of the earlier studies in a slightly different form as $\rho \cong 1$, $\sigma \cong 1$ and $\varepsilon_{IS} \cong 1$.

2.6. Minimization of error in the quasi steady state approximations

The essential conditions required to minimize the error in various standard QSSAs of fully competitive inhibition schemes with stationary reactants assumption can be obtained as follows. From **Eqs. 23.1**, **29.1** and **Eqs. 6.2** corresponding to the standard QSSA with stationary reactants assumption, one can define the error estimate of standard QSSAs in the pre-steady state regime as follows [35].

$$\Lambda_S \cong \frac{\varepsilon_S}{1+(\varepsilon_S+\mu_S)\left(1+\frac{1}{\varepsilon_I+\mu_I}\right)}\int_0^{\tau_{CS}} exp\left(-\frac{\alpha_S}{\eta_S}\left(1-\frac{1}{\alpha_S\alpha_I}\right)\tau\right)d\tau \cong \frac{\eta_S\varepsilon_S\alpha_I}{\left(1+(\varepsilon_S+\mu_S)\left(1+\frac{1}{\varepsilon_I+\mu_I}\right)\right)(\alpha_S\alpha_I-1)} \qquad [31.10]$$

$$\Lambda_I \cong \frac{\rho\varepsilon_I}{1+(\varepsilon_I+\mu_I)\left(1+\frac{1}{\varepsilon_S+\mu_S}\right)}\int_0^{\tau_{CI}} exp\left(-\frac{\alpha_I}{\eta_I}\left(1-\frac{1}{\alpha_S\alpha_I}\right)\tau\right)d\tau \cong \frac{\rho\varepsilon_I\eta_I\alpha_S}{\left(1+(\varepsilon_I+\mu_I)\left(1+\frac{1}{\varepsilon_S+\mu_S}\right)\right)(\alpha_S\alpha_I-1)} \quad [31.10]$$

Here Λ_S and Λ_I are the overall errors on the standard QSSAs on enzyme-substrate and enzyme-inhibitor complexes respectively. Upon solving $\frac{d\Lambda_S}{d\varepsilon_S}=0$ for ε_S and $\frac{d\Lambda_I}{d\varepsilon_I}=0$ for ε_I and using the approximate expressions for τ_{CS} and τ_{CI} from **Eqs. 31.1**, one obtains the following expressions for $\varepsilon_{S,max}$ and $\varepsilon_{I,max}$ at which the error due to QSSAs attain maxima.

$$\varepsilon_{S,max} \cong \frac{(\mu_S+1)\kappa_I+(\eta_S+\varepsilon_I+1)\kappa_S+(\eta_S+1)\eta_I+(1+\varepsilon_I)\eta_S+\varepsilon_I}{1+\varepsilon_I+\mu_I} \quad [31.11]$$

$$\varepsilon_{I,max} \cong \frac{(1+\varepsilon_S+\mu_S)\kappa_I+(\eta_I+1)\kappa_S+(1+\varepsilon_S+\eta_S)\eta_I+\eta_S+\varepsilon_S}{1+\varepsilon_S+\mu_S} \quad [31.12]$$

Clearly, the following generalized conditions i.e., $\varepsilon_S \ll \varepsilon_{S,max}$ and $\varepsilon_I \ll \varepsilon_{I,max}$ are essential to minimize the error in QSSAs (**Eqs. 6.2**) associated with the enzyme-substrate and enzyme-inhibitor dynamics since $(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0$ are the preconditions for the validity of QSSAs. One can write these sufficient conditions (we denote them as $\mathbf{E_1}$ and $\mathbf{E_2}$) explicitly as follows.

$$\boldsymbol{E_1}: \quad \left(\frac{\varepsilon_S(1+\varepsilon_I+\mu_I)}{(\mu_S+1)\kappa_I+(\eta_I+\varepsilon_I+1)\kappa_S+(\eta_S+1)\eta_I+(1+\varepsilon_I)\eta_S+\varepsilon_I}\right) \ll 1 \quad [31.12]$$

$$\boldsymbol{E_2}: \quad \left(\frac{\varepsilon_I(1+\varepsilon_S+\mu_S)}{(1+\varepsilon_S+\mu_S)(\kappa_I+\eta_I)+(\eta_I+1)\kappa_S+\eta_S+\varepsilon_S}\right) \ll 1 \quad [31.13]$$

We will show later that there is strong correlation between **Eqs. 31.12-13** and the overall error in the standard QSSA methods.

2.7. Approximate solutions under the conditions that $(\boldsymbol{\varepsilon_S},\boldsymbol{\eta_S},\boldsymbol{\phi_I})\to\mathbf{0}$

From **Eqs. 21.1** and **27.1**, one can derive the refined expressions for the reaction velocities V, U and products P, Q in the limit $(\varepsilon_S,\eta_S,\phi_I)\to 0$ as follows.

$$\lim_{(\varepsilon_I,\eta_I,\phi_S)\to 0} V \cong \frac{c\varepsilon_S\eta_S^2}{\sqrt{(a^2-4b)}}\left(\exp\left(-\frac{\left(a-\sqrt{(a^2-4b)}\right)\tau}{2}\right)-\exp\left(-\frac{\left(a+\sqrt{(a^2-4b)}\right)\tau}{2}\right)\right) \quad [32.1]$$

$$\lim_{(\varepsilon_I,\eta_I,\phi_S)\to 0} P \cong \frac{c\varepsilon_S\eta_S^2}{2(a^2-4b)b}\left(-\exp\left(\frac{\left(-a+\sqrt{(a^2-4b)}\right)\tau}{2}\right)\left(a^2-4b+\sqrt{(a^2-4b)}a\right)-\right.$$
$$\left.\exp\left(-\frac{\left(a+\sqrt{(a^2-4b)}\right)\tau}{2}\right)\left(-\sqrt{(a^2-4b)}a+a^2-4b\right)\right)+\frac{c}{b} \quad [32.2]$$

Here the parameters *a*, *b* and *c* are defined as follows.

$$a=\frac{\alpha_S}{\eta_S}\left(1-\frac{1}{\alpha_S\alpha_I}\right);\ b=\frac{\varepsilon_S}{\eta_S};\ c=\frac{1}{\eta_S{}^3}\left(1-\frac{1}{\alpha_I}\right) \quad [32.3]$$

$$\lim_{(\varepsilon_S,\eta_S,\phi_I)\to 0} U \cong \frac{q\varepsilon_I\eta_I^2}{\sqrt{(r^2-4g)}}\left(\exp\left(-\frac{\left(r-\sqrt{(r^2-4g)}\right)\tau}{2}\right)-\exp\left(-\frac{\left(r+\sqrt{(r^2-4g)}\right)\tau}{2}\right)\right) \quad [32.4]$$

$$\lim_{(\varepsilon_S,\eta_S,\phi_I)\to 0} Q \cong \frac{d\varepsilon_S\eta_S^2}{2(r^2-4g)g}\left(-\exp\left(\frac{\left(-r+\sqrt{(r^2-4g)}\right)\tau}{2}\right)\left(r^2-4g+\sqrt{(r^2-4b)}r\right)-\right.$$
$$\left.\exp\left(-\frac{\left(r+\sqrt{(r^2-4g)}\right)\tau}{2}\right)\left(-\sqrt{(r^2-4g)}r+r^2-4g\right)\right)+\frac{d}{g} \qquad [32.5]$$

Here the parameters *r*, *g* and *d* are defined as follows.

$$r=\frac{\rho\alpha_I}{\eta_I}\left(1-\frac{1}{\alpha_I\alpha_S}\right);\ g=\frac{\varepsilon_I\rho^2}{\eta_I};\ d=\frac{\rho^2}{{\eta_I}^3}\left(1-\frac{1}{\alpha_S}\right) \qquad [32.6]$$

Clearly, there exist four different timescales viz. pre-steady state and post steady state timescales corresponding to enzyme-substrate and enzyme-inhibitor conversions. We denote them as $(\tau_{S1},\tau_{S2},\tau_{I1},\tau_{I2})$. From **Eqs. 32.1** and **32.4** one can define these timescales as follows.

$$\tau_{S1}\cong\frac{2}{\left(a+\sqrt{(a^2-4b)}\right)};\ \tau_{S2}\cong\frac{2}{\left(a-\sqrt{(a^2-4b)}\right)};\ \tau_{I1}\cong\frac{2}{\left(r+\sqrt{(r^2-4g)}\right)};\ \tau_{I2}\cong\frac{2}{\left(r-\sqrt{(r^2-4g)}\right)} \qquad [32.7]$$

Here τ_{S1} and τ_{S2} are the pre-steady state and post steady state timescales corresponding to enzyme-substrate dynamics and τ_{I1} and τ_{I2} are the pre-steady state and post steady state timescales associated with the enzyme-inhibitor dynamics. Parameters *a*, *b*, *c*, *r*, *g* and *d* are defined as in **Eqs. 32.3** and **32.6**. The error in various QSSAs will be low when the timescale separation ratios tend towards zero.

$$\Delta\tau_S\cong\frac{\tau_{S1}}{\tau_{S2}}=\frac{a-\sqrt{(a^2-4b)}}{a+\sqrt{(a^2-4b)}};\ \Delta\tau_I\cong\frac{\tau_{I1}}{\tau_{I2}}=\frac{r-\sqrt{(r^2-4g)}}{r+\sqrt{(r^2-4g)}} \qquad [32.8]$$

Eqs. 32.1 and **32.4** can approximately describe the dynamics of fully competitive enzyme kinetics scheme over the entire (V, U) space in the parametric form when the conditions given in **Eqs. 31.12-13** and **Eqs. 32.8** are true.

2.8. Partially competitive inhibition

The differential rate equations corresponding to the Michaelis-Menten type partially competitive inhibition **Scheme B** can be written as follows.

$$\frac{dx}{dt}=-\frac{de}{dt}=k_1se-(k_2+k_{-1})x \qquad [33.1]$$

$$-\frac{ds}{dt}=k_1se-k_{-1}x \qquad [33.2]$$

$$\frac{dy}{dt}=-\frac{di}{dt}=k_iie-k_{-i}y \qquad [33.3]$$

$$\frac{dp}{dt}=k_2x \qquad [33.4]$$

Here the dynamical variables (s, e, x, y, i, p) denote respectively the concentrations of substrate, enzyme, enzyme-substrate complex, enzyme-inhibitor complex and inhibitor. Further k_1 and k_i are the respective bimolecular forward rate constants and k_{-1} and k_{-i} are the respective reverse rate constants, k_2 is the product formation rate constant. Here the initial conditions are (s, e, x,

y, i, p) = (s_0, e_0, 0, 0, i_0, 0) at t = 0. The mass conservation laws are $e = e_0 - x - y$; $s = s_0 - x - p$; $i = i_0 - y$. When $t \to \infty$, then the reaction ends at $(\mathrm{s, e, x, y, i, p}) = (0, \mathrm{e}_\infty, 0, \mathrm{y}_\infty, \mathrm{i}_\infty, \mathrm{s}_0)$ where $(\mathrm{e}_\infty, \mathrm{y}_\infty, \mathrm{i}_\infty)$ are the final equilibrium concentrations of the enzyme, enzyme-inhibitor complex and free inhibitor. The reaction ends at Similar to the scaling transformation in **Eqs. 2** and **3**, **Eqs. 33** can be reduced to the following set of dimensionless equations.

$$\eta_S \frac{dX}{d\tau} = (1 - \varepsilon_S X - P)(1 - X - Y) - (\eta_S + \kappa_S)X \qquad [34.1]$$

$$\chi_I \frac{dY}{d\tau} = (1 - \varepsilon_I Y)(1 - X - Y) - \kappa_I Y \qquad [34.2]$$

$$\frac{dP}{d\tau} = V = \varepsilon_S X \qquad [34.3]$$

$$\chi_I = \frac{k_2}{k_i i_0};\ \varepsilon_I = \frac{e_0}{i_0};\ \kappa_I = \frac{k_{-i}}{k_i i_0} \qquad [34.4]$$

The mass conservation relations in the dimensionless space are $I = (1 - \varepsilon_I Y)$, E + X + Y = 1 and V + P + S = 1. Other dimensionless parameters are defined similar to the case of fully competitive inhibition scheme as given in **Eqs. 4**. The initial conditions are (S, E, X, Y, I, P) = (1, 1, 0, 0, 1, 0) at τ = 0. When τ → ∞, then the reaction ends at $(\mathrm{S, E, X, Y, I, P}) = (0, \mathrm{E}_\infty, 0, \mathrm{E}_\infty, \mathrm{I}_\infty, 1)$ where the terms $(\mathrm{E}_\infty, \mathrm{Y}_\infty, \mathrm{I}_\infty)$ are the final equilibrium concentrations of the enzyme, enzyme-inhibitor complex and free inhibitor. When $(\chi_I, \eta_S) \to 0$, then **Eqs. 34.1-2** can be equated to zero and solved for (X, Y). Upon converting X into the velocity using $V = \varepsilon_S X$, one obtains the following standard QSSA result.

$$\lim_{(\eta_S, \chi_I) \to 0} V \cong \frac{\varepsilon_S S}{S + \mu_S\left(1 + \frac{I}{\kappa_I}\right)};\ \lim_{(\eta_S, \chi_I) \to 0} Y \cong \frac{I}{I + \kappa_I\left(1 + \frac{S}{\mu_S}\right)} \qquad [34.5]$$

Upon applying the stationary reactants assumption under the condition that $(\varepsilon_S, \varepsilon_I) \to 0$ so that $(S, I) \to (1,1)$, one finally obtains the following expressions.

$$\lim_{(\eta_S, \varepsilon_S, \varepsilon_I, \chi_I) \to 0} V \cong \frac{\varepsilon_S}{1 + \mu_S\left(1 + \frac{1}{\kappa_I}\right)};\ \lim_{(\eta_S, \varepsilon_S, \varepsilon_I, \chi_I) \to 0} Y \cong \frac{1}{1 + \kappa_I\left(1 + \frac{1}{\mu_S}\right)} \qquad [34.6]$$

In terms of original variables, the steady state reaction velocity defined in **Eqs. 34.6** can be rewritten as follows.

$$\lim_{(\eta_S, \varepsilon_S, \varepsilon_I, \chi_I) \to 0} v \cong \frac{v_{max} s_0}{s_0 + K_{MS}\left(1 + \frac{i_0}{K_{DI}}\right)} \qquad [34.7]$$

Eq. 34.7 is generally used to estimate the kinetic parameters from the experimental datasets on partially competitive inhibition scheme using double reciprocal plotting methods.

2.8.1. Variable transformations

Using the transformation $X = \frac{1}{\varepsilon_S}\frac{dP}{d\tau}$ along with the other scaling transformations given in **Eqs. 2**, **Eqs. 34** can be reduced to the following set of coupled nonlinear ODEs in the (P, Y, τ) space.

$$\eta_S \frac{d^2P}{d\tau^2} + \alpha_S \frac{dP}{d\tau} + \varepsilon_S P - \varepsilon_S + \varepsilon_S Y = \left(\frac{dP}{d\tau} + P\right)\left(\frac{dP}{d\tau} + \varepsilon_S Y\right) \quad [35.1]$$

$$\chi_I \frac{dY}{d\tau} + \beta_I Y + \frac{1}{\varepsilon_S}\frac{dP}{d\tau} - 1 = \varepsilon_I Y \left(\frac{1}{\varepsilon_S}\frac{dP}{d\tau} + Y\right) \quad [35.2]$$

Eqs. 35.1-2 can transformed into the (V, Y, P) and (V, Y, S) spaces using the substitution $V = \frac{dP}{d\tau}$ as follows.

$$\eta_S \frac{dV}{d\tau} + \alpha_S V + \varepsilon_S P - \varepsilon_S + \varepsilon_S Y = (V + P)(V + \varepsilon_S Y) \quad [35.3]$$

$$\eta_S \frac{dV}{d\tau} + \alpha_S V + \varepsilon_S (1 - V - S) - \varepsilon_S + \varepsilon_S Y = (1 - S)(V + \varepsilon_S Y) \quad [35.4]$$

$$\chi_I \frac{dY}{d\tau} + \beta_I Y + \frac{1}{\varepsilon_S} V - 1 = \varepsilon_I Y \left(\frac{1}{\varepsilon_S} V + Y\right) \quad [35.5]$$

$$\beta_I = 1 + \varepsilon_I + \kappa_I \quad [35.6]$$

2.8.2. Post-steady state approximations

Case I: When $(\eta_S, \chi_I, \varepsilon_S, \varepsilon_I) \to 0$, then **Eqs. 34.1-2** can be approximated as follows.

$$\alpha_S X - P + 1 - Y - P(X + Y) \cong 0\,;\;\; \beta_I Y - 1 + X \cong 0 \quad [35.7]$$

Upon solving **Eqs. 35.**7 for (X, Y) and noting that $S \cong 1 - P$, $I \cong 1$ in such conditions, we obtain the following results similar to **Eqs. 6.18** corresponding to the fully competitive inhibition scheme in the (V, S, I) and (Y, S, I) spaces.

$$\lim_{(\eta_S,\chi_I,\varepsilon_S,\varepsilon_I)\to 0} V = -\frac{dS}{d\tau} \cong \frac{\varepsilon_S S}{S + \tilde{\mu}_S\left(1 + \frac{1}{\tilde{\kappa}_I}\right)}\,;\;\; \lim_{(\eta_S,\chi_I,\varepsilon_S,\varepsilon_I)\to 0} Y = -\frac{1}{\varepsilon_I}\frac{dI}{d\tau} \cong \frac{1}{1 + \tilde{\kappa}_I\left(1 + \frac{S}{\tilde{\mu}_S}\right)} \quad [35.8]$$

Here we have defined $\tilde{\kappa}_I = \varepsilon_I + \kappa_I$. The expression for V in **Eqs. 35.8** are similar to **Eqs. 34.5** where μ_S and κ_I are replaced with $\tilde{\mu}_S$ and $\tilde{\kappa}_I$. The approximation of the phase-space trajectory in the (S, I) space in the post steady state regime can be expressed in a parametric form using $I = 1 - \varepsilon_I Y$. Here Y is given in terms of S as in **Eq. 35.8** where S acts as the parameter. Upon applying the transformation $M = \frac{P}{\varepsilon_S {\eta_S}^2}$ in **Eqs. 35**, one finally arrives at the following set of coupled nonlinear second order ODEs.

$$\frac{d^2M}{d\tau^2} + \frac{\alpha_S}{\eta_S}\frac{dM}{d\tau} + \frac{\varepsilon_S M}{\eta_S} - \frac{1}{{\eta_S}^3} + \frac{1}{{\eta_S}^3} Y = \eta_S \varepsilon_S \left(\left(\frac{dM}{d\tau} + M\right)\left(\frac{dM}{d\tau} + \frac{1}{{\eta_S}^2} Y\right)\right) \quad [36.1]$$

$$\chi_I \frac{dY}{d\tau} + \beta_I Y + {\eta_S}^2 \frac{dM}{d\tau} - 1 = \varepsilon_I Y \left({\eta_S}^2 \frac{dM}{d\tau} + Y\right) \quad [36.2]$$

Eqs. 36.1-2 are the central equations corresponding to the Michaelis-Menten enzyme kinetics with partially competitive inhibition. Using **Eqs. 36.1-2**, one can derive several approximations under various limiting conditions.

Case II: When $(\chi_I, \varepsilon_I) \to 0$, then one finds from **Eq. 36.2** that $\alpha_I Y + {\eta_S}^2 \frac{dM}{d\tau} - 1 \cong 0$. Using this, one obtains the approximation for Y as $Y \cong \frac{1}{\beta_I}\left(1 - {\eta_S}^2 \frac{dM}{d\tau}\right)$. Upon substituting this approximate expression for Y in **Eqs. 36.1**, and setting $\phi_S = \eta_S \varepsilon_S \to 0$ in **Eq. 36.1** one can derive the following linear ODE in the (M, τ) space.

$$\frac{d^2M}{d\tau^2} + \frac{\alpha_S}{\eta_S}\left(1 - \frac{1}{\alpha_S \beta_I}\right)\frac{dM}{d\tau} + \frac{\varepsilon_S}{\eta_S}M - \frac{1}{{\eta_S}^3}\left(1 - \frac{1}{\beta_I}\right) \cong 0; [M]_{\tau=0} = 0; \left[\frac{dM}{d\tau}\right]_{\tau=0} = 0 \qquad [37]$$

Upon solving this second order ODE for M with respect to the given initial conditions and then reverting back to V using the transformation $V = \varepsilon_S \eta_S^2 \frac{dM}{d\tau}$, one can obtain the following expression for the approximate reaction velocity of the partially competitive inhibition scheme in the (V, τ) space.

$$\lim_{(\varepsilon_I, \eta_I, \phi_S) \to 0} V \cong \varepsilon_S \eta_S^2 \frac{c}{\sqrt{(a^2 - 4b)}}\left(\exp\left(\frac{\left(-a + \sqrt{(a^2 - 4b)}\right)\tau}{2}\right) - \exp\left(-\frac{\left(a + \sqrt{(a^2 - 4b)}\right)\tau}{2}\right)\right) \qquad [38.1]$$

The terms *a*, *b* and *c* in **Eq. 38.1** are defined as follows.

$$a = \frac{\alpha_S}{\eta_S}\left(1 - \frac{1}{\alpha_S \beta_I}\right);\ b = \frac{\varepsilon_S}{\eta_S};\ c = \frac{1}{{\eta_S}^3}\left(1 - \frac{1}{\beta_I}\right) \qquad [38.2]$$

Upon solving $\frac{dV}{d\tau} = 0$ for τ, one can obtain the following expression for the steady state timescale associated with the enzyme-substrate complex of the partially competitive inhibition scheme.

$$\lim_{(\varepsilon_I, \chi_I, \phi_S) \to 0} \tau_{CS} \cong \frac{\eta_S}{\sqrt{w}} \ln\left(\frac{-2\varepsilon_S \eta_S {\beta_I}^2}{{\beta_I}^2 \alpha_S \sqrt{w} - {\alpha_S}^2 {\beta_I}^2 + 2\varepsilon_S \eta_S {\beta_I}^2 - \sqrt{w}\beta_I + 2\alpha_S \beta_I - 1}\right) \qquad [39.1]$$

In this equation, w is defined as follows.

$$w = \frac{1 + ({\alpha_S}^2 - 4\varepsilon_S \eta_S){\beta_I}^2 - 2\alpha_S \beta_I}{{\beta_I}^2} \qquad [39.2]$$

2.8.3. Pre-steady state approximations

Case III: When $\eta_S \to 0$, then one obtains the uncoupled equation in the (Y, τ) space from **Eq. 36.2** as $\chi_I \frac{dY}{d\tau} + \beta_I Y - 1 \cong \varepsilon_I Y^2$. The integral solution of this first order nonlinear ODE with the initial condition $[Y]_{\tau=0} = 0$ can be written as follows.

$$\lim_{\eta_S \to 0} Y \cong \frac{\chi_I}{2\varepsilon_I}\left(1 - \frac{\sqrt{{\beta_I}^2 - 4\varepsilon_I}}{\chi_I}\tanh\left(\operatorname{arctanh}\left(\frac{\eta_I}{\sqrt{{\beta_I}^2 - 4\varepsilon_I}}\right) + \frac{\sqrt{{\beta_I}^2 - 4\varepsilon_I}}{2\chi_I}\tau\right)\right) \qquad [40]$$

Eq. 40 suggests the following expression for the timescale associated with the enzyme-inhibitor complex to attain the (pseudo) steady state.

$$\lim_{\eta_S \to 0} \tau_{CY} \cong \frac{2\chi_I}{\sqrt{{\beta_I}^2 - 4\varepsilon_I}} \qquad [41]$$

Clearly, **Eqs. 34.1** and **34.2** can have common steady states only when $\tau_{CY} = \tau_C$. We will show in the later sections that when $\tau_{CY} > \tau_C$, then the substrate depletion with respect to time will show a typical non-monotonic trend since the inhibitor reverses the enzyme-substrate complex formed before time τ_{CY} in the pre-steady state regime with respect to the enzyme-substrate complex. This phenomenon introduces significant amount of error in various the quasi steady state approximations. Using the transformation $F = \frac{dM}{d\tau}$, **Eqs. 36.1** and **36.2** can be rewritten in the (F, M) space as follows.

$$F\frac{dF}{dM} + \frac{\alpha_S}{\eta_S}F + \frac{\varepsilon_S}{\eta_S}M - \frac{1}{\eta_S{}^3} + \frac{1}{\eta_S{}^3}Y = \eta_S\varepsilon_S\left((F+M)\left(F + \frac{1}{\eta_S{}^2}Y\right)\right) \quad [42.1]$$

$$\chi_I\frac{dY}{d\tau} + \beta_I Y + \eta_S{}^2F - 1 = \varepsilon_I Y(\eta_S{}^2F + Y) \quad [42.2]$$

Case IV: When $(\varepsilon_S, \chi_I, \varepsilon_I) \to 0$, then one can derive the approximate differential rate equations governing the pre-steady state dynamics in the (F, M) space as follows.

$$F\frac{dF}{dM} + \frac{\alpha_S}{\eta_S}F - \frac{1}{\eta_S{}^3} + \frac{1}{\eta_S{}^3}Y \cong 0;\ \beta_I Y + \eta_S{}^2F - 1 \cong 0; Y \cong \frac{1-\eta_S{}^2F}{\beta_I} \quad [43.1]$$

Upon substituting the expression for Y into the differential equation corresponding to the (F, M) space one finally obtains the following approximation.

$$F\frac{dF}{dM} + \frac{\alpha_S}{\eta_S}\left(1 - \frac{1}{\alpha_S\beta_I}\right)F - \frac{1}{\eta_S{}^3}\left(1 - \frac{1}{\beta_I}\right) \cong 0;\ [F]_{M=0} = 0 \quad [43.2]$$

Upon solving **Eq. 43.2** with the given initial conditions, and then reverting back to (V, P) space using the transformations $V = \varepsilon_S\eta_S^2F$ and $P = \varepsilon_S\eta_S^2M$, one finally obtains the following approximate expressions for the reaction velocity in the pre-steady state regime of the (V, P) and (V, S) spaces of the partially competitive inhibition scheme.

$$\lim_{(\varepsilon_S,\chi_I,\varepsilon_I)\to 0} V \cong \frac{\varepsilon_S(\beta_I-1)}{(\alpha_S\beta_I-1)}\left(1 + \text{LambertW}\left(-exp\left(-1 - \frac{\eta_S(\alpha_S\beta_I-1)^2}{\alpha_S{}^2\beta_I\varepsilon_S(\beta_I-1)}P\right)\right)\right) \quad [44.1]$$

To obtain the expression for the (V, S) space one needs to first solve **Eqs. 43.2** implicitly in the (V, P) space. Then upon inverting the solution for V after the substitution P = 1 – V – S, one finally arrives at the following expression.

$$\lim_{(\varepsilon_S,\chi_I,\varepsilon_I)\to 0} V \cong \frac{b\varepsilon_S\eta_S{}^2}{a(a-1)}\left((a-1) - \text{LambertW}\left((a-1)exp\left((a-1) + \frac{a^2(S-1)}{b\eta_S{}^2\varepsilon_S}\right)\right)\right) \quad [44.2]$$

In this equation, the terms *a* and *b* are defined as follows.

$$a = \frac{\alpha_S}{\eta_S}\left(1 - \frac{1}{\alpha_S\beta_I}\right); b = \frac{1}{\eta_S{}^3}\left(1 - \frac{1}{\beta_I}\right) \quad [44.3]$$

One can directly translate the (V, S) space approximation given in **Eq. 44.2** into (Y, S) space using **Eqs. 43.1**. However, there will be a mismatch in the initial condition i.e., Y = 0 at S = 1 in such

case. Particularly, **Eqs. 43.1** sets $Y \cong \frac{1}{\beta_I}$ at S = 1 (at which V = 0 and therefore F = 0) that is inconsistent since $Y \neq 0$. Numerical analysis suggests the following approximate expression i.e., $Y \cong \frac{V}{\varepsilon_S \beta_I}$ for the phase-space trajectory in the pre-steady state regime of the (Y, S) space.

$$\lim_{(\varepsilon_S,\chi_I,\varepsilon_I)\to 0} Y \cong \frac{b\eta_S{}^2}{a\beta_I(a-1)}\left((a-1) - \text{LambertW}\left((a-1)exp\left((a-1) + \frac{a^2(S-1)}{b\eta_S{}^2\varepsilon_S}\right)\right)\right) \qquad [44.4]$$

Here *a* and *b* are defined as in **Eqs. 44.3**. Similarly, in (F, τ) space the differential equation **Eq. 43.1** can be written as follows.

$$\frac{dF}{d\tau} + \frac{\alpha_S}{\eta_S}\left(1 - \frac{1}{\alpha_S\beta_I}\right)F - \frac{1}{\eta_S{}^3}\left(1 - \frac{1}{\beta_I}\right) \cong 0;\ [F]_{\tau=0} \qquad [45]$$

Upon solving **Eq. 45** with the given initial condition and then reverting back to (V, τ) space using the transformation $V = \varepsilon_S\eta_S^2 F$, one obtains the following integral solution corresponding to the reaction velocity in the pre-steady state regime of the partially competitive inhibition scheme.

$$\lim_{(\varepsilon_S,\chi_I,\varepsilon_I)\to 0} V \cong \frac{\varepsilon_S}{1+(\varepsilon_S+\mu_S)\left(1+\frac{1}{\varepsilon_I+\kappa_I}\right)}\left(1 - exp\left(-\frac{\alpha_S}{\eta_S}\left(1 - \frac{1}{\alpha_S\beta_I}\right)\tau\right)\right) \qquad [46.1]$$

This equation along with **Eq. 41** suggest the following expressions for the steady state timescale associated with the enzyme-substrate and enzyme inhibitor complexes.

$$\lim_{(\varepsilon_I,\chi_I,\varepsilon_S)\to 0} \tau_{CS} \cong \frac{\eta_S\beta_I}{(\alpha_S\beta_I-1)};\ \lim_{(\varepsilon_I,\chi_I)\to 0} \tau_{CY} \cong \frac{2\chi_I}{\beta_I};\ \xi = \frac{\eta_S\beta_I{}^2}{2\chi_I(\alpha_S\beta_I-1)} \qquad [46.2]$$

Clearly, common steady states between enzyme-substrate and enzyme-inhibitor complexes can occur only when the ratio in **Eqs. 46.2** becomes as $\xi \cong 1$ at which the error associated with various quasi steady state approximations will be at minimum. Upon solving $\xi \cong 1$ for β_I, one obtains the following two possible roots.

$$\beta_I = \frac{\alpha_S\chi_I \mp \sqrt{\alpha_S{}^2\chi_I{}^2 - 2\chi_I\eta_S}}{\eta_S};\ \lim_{\eta_I\to 0}\beta_I \cong \frac{2\alpha_S\chi_I}{\eta_S} \qquad [46.3]$$

When $\eta_I \to 0$ then the ratio ξ in **Eq. 46.2** becomes as follows.

$$\xi \cong \frac{\beta_I\eta_S}{2\alpha_S\chi_I} = \frac{(1+\varepsilon_I+\kappa_I)\eta_S}{2(1+\varepsilon_S+\eta_S+\kappa_S)\chi_I} \qquad [46.4]$$

When $\eta_S \cong 2\chi_I$, then **Eq. 46.4** suggests that the condition $\xi \cong 1$ can be achieved by settings sufficiently large values for any one of the parameters in the numerator $(\varepsilon_I, \kappa_I)$ and any one of the parameters in the denominator $(\varepsilon_S, \chi_I, \kappa_S)$. When P, S and τ become sufficiently large, then **Eqs. 44** and **46** asymptotically converge to the following limiting value that is close to the steady state reaction velocity V associated with the partially competitive enzyme kinetics described in **Scheme B** in the limit $(\varepsilon_S, \chi_I, \varepsilon_I) \to 0$. We denote this approximation as V_3. We will show in the

later sections that this approximation works very well in predicting the steady state reaction velocity of the partially competitive inhibition **Scheme B** over wide ranges of parameters.

$$V_3 = \lim_{P\to 1}\left(\lim_{(\varepsilon_S,\chi_I,\varepsilon_I)\to 0} V\right) \cong \frac{\varepsilon_S(\beta_I-1)}{(\alpha_S\beta_I-1)} = \frac{\varepsilon_S}{1+(\varepsilon_S+\mu_S)\left(1+\frac{1}{\varepsilon_I+\kappa_I}\right)} \quad [47.1]$$

2.8.4 Error in the standard QSSA of the partially competitive inhibition scheme

The overall error in the standard QSSA with stationary reactant assumption as given in **Eqs. 35.8** with $S \cong 1$ corresponding to the partially competitive inhibition can be written as follows [35].

$$\Gamma_S \cong \frac{\varepsilon_S}{1+(\varepsilon_S+\mu_S)\left(1+\frac{1}{\varepsilon_I+\kappa_I}\right)} \int_0^{\tau_{CS}} exp\left(-\frac{\alpha_S}{\eta_S}\left(1-\frac{1}{\alpha_S\beta_I}\right)\tau\right) d\tau \cong \frac{\varepsilon_S\eta_S\beta_I}{\left(1+(\varepsilon_S+\mu_S)\left(1+\frac{1}{\varepsilon_I+\kappa_I}\right)\right)(\alpha_S\beta_I-1)} \quad [47.2]$$

Upon solving $\frac{d\Gamma_S}{d\varepsilon_S} = 0$ for ε_S and using the approximate expressions for τ_{CS} of the partially competitive inhibition scheme as in **Eqs. 46.2**, one obtains the following expression for $\varepsilon_{S,max}$ at which the error due to the standard QSSA attains maximum. This means that the sufficient condition to minimize such error will be given by the inequality $\varepsilon_S \ll \varepsilon_{S,max}$ as follows.

$$\varepsilon_S \ll \varepsilon_{S,max} \cong \left(\frac{(\mu_S+1)(\varepsilon_I+\kappa_I)+\mu_S}{1+\varepsilon_I+\kappa_I}\right) \quad [47.3]$$

Inequality in **Eq. 47.3** (we denote this by E_3) can be explicitly written in the following form.

$$\boldsymbol{E_3}:\ \left(\frac{\varepsilon_S(1+\varepsilon_I+\kappa_I)}{(\mu_S+1)(\varepsilon_I+\kappa_I)+\mu_S}\right) \ll 1 \quad [47.4]$$

In terms of original variables, **Eq. 47.1** can be written as follows.

$$v_3 = \lim_{P\to 1}\left(\lim_{(\varepsilon_S,\chi_I,\varepsilon_I)\to 0} v\right) \cong \frac{k_2 e_0 s_0}{s_0+(e_0+K_{MS})\left(1+\frac{i_0}{e_0+K_{DI}}\right)} \quad [47.5]$$

Case IV: When $(\eta_S, \chi_I) \to 0$, then **Eqs. 35.3-4** reduce to the following form in the (V, Y, P) and (V, Y, S) spaces.

$$\alpha_S V + \varepsilon_S P - \varepsilon_S + \varepsilon_S Y \cong (V+P)(V+\varepsilon_S Y) \quad [48.1]$$

$$(\alpha_S - \varepsilon_S)V - \varepsilon_S S + \varepsilon_S Y \cong (1-S)(V+\varepsilon_S Y) \quad [48.2]$$

$$\beta_I Y + \frac{1}{\varepsilon_S}V - 1 \cong \varepsilon_I Y\left(\frac{1}{\varepsilon_S}V + Y\right) \quad [48.3]$$

Upon solving **Eqs. 48.2-3** for (V, Y), one obtains the following expressions for V, Y, I and P in terms of S as follows.

$$\lim_{(\eta_S,\chi_I)\to 0} V \cong \frac{\varepsilon_S S}{(S+\mu_S)}\left(\frac{1}{2\varepsilon_I\mu_S}\left(-\kappa_I S + \mu_S\big(\varepsilon_I - (1+\kappa_I)\big) + \sqrt{\kappa_I{}^2 S^2 - 2(1+\varepsilon_I+\kappa_I)\mu_S\kappa_I S - 4\mu_S^2\left(-\frac{(1+\varepsilon_I+\kappa_I)^2}{4}+\varepsilon_I\right)}\right)\right) \quad [48.4]$$

Noting that V + P + S = 1, one can derive the approximate expression for P in terms of S as follows.

$$\lim_{(\eta_S,\chi_I)\to 0} P \cong 1 - S - \frac{\varepsilon_S S}{(S+\mu_S)}\left(\frac{1}{2\varepsilon_I\mu_S}\left(-\kappa_I S + \mu_S\big(\varepsilon_I - (1+\kappa_I)\big) + \sqrt{\kappa_I{}^2 S^2 - 2(1+\varepsilon_I+\kappa_I)\mu_S\kappa_I S - 4\mu_S^2\left(-\frac{(1+\varepsilon_I+\kappa_I)^2}{4}+\varepsilon_I\right)}\right)\right) \quad [48.5]$$

The post-steady state approximation in the (Y, S) space can be written as follows.

$$\lim_{(\eta_S,\chi_I)\to 0} Y \cong \frac{(S+\mu_S)\alpha_I - S(1+\varepsilon_I) - \sqrt{\kappa_I{}^2 S^2 - 2(1+\varepsilon_I+\kappa_I)\mu_S\kappa_I S - 4\mu_S^2\left(-\frac{(1+\varepsilon_I+\kappa_I)^2}{4}+\varepsilon_I\right)}}{2\varepsilon_I\mu_S} \quad [48.6]$$

Noting the fact that $I = (1-\varepsilon_I Y)$, one can obtain the following approximate expression for the inhibitor concentration in terms of S.

$$\lim_{(\eta_S,\chi_I)\to 0} I \cong 1 - \frac{\varepsilon_I}{2\varepsilon_I\mu_S}\left((S+\mu_S)\alpha_I - S(1+\varepsilon_I) - \sqrt{\kappa_I{}^2 S^2 - 2(1+\varepsilon_I+\kappa_I)\mu_S\kappa_I S - 4\mu_S^2\left(-\frac{(1+\varepsilon_I+\kappa_I)^2}{4}+\varepsilon_I\right)}\right) \quad [48.7]$$

Integral solutions obtained under the conditions that $(\eta_S, \chi_I) \to 0$ can approximate the original trajectory in the (V, S) space very well only in the post-steady state regime.

2.8. Total quasi steady state approximations (tQSSA)

When the conditions $(\eta_S, \eta_I, P, Q) \to 0$ are true so that $S \cong (1-\varepsilon_S X)$, $I \cong (1-\varepsilon_I Y)$ in the pre-steady state regime of fully competitive inhibition scheme described by **Eqs. 3.1** and **3.2**, the one can arrive at the total quasi steady state approximation [39, 40]. In this situation, **Eqs. 3** can be approximated as follows.

$$(1-\varepsilon_S X)(1-X-Y) - (\kappa_S+\eta_S)X \cong 0 \quad [49.1]$$

$$(1-\varepsilon_I Y)(1-X-Y) - (\kappa_I+\eta_I)Y \cong 0 \quad [49.2]$$

The approximate steady state reaction velocities can be derived from the solution of this system of coupled equations for (X, Y) as follows.

$$\lim_{(\eta_S,\eta_I,P,Q)\to 0} V \cong \varepsilon_S\left(-\frac{\varepsilon_I W^2 - \varepsilon_I W - (\kappa_I+\eta_I)W - W + 1}{\varepsilon_I W - 1}\right); \quad \lim_{(\eta_S,\eta_I,P,Q)\to 0} U \cong \rho\varepsilon_I W \quad [50.1]$$

Here W is the appropriate real solution of the following cubic equation.

$$aW^3 + bW^2 + cW - (\kappa_S + \eta_S) = 0 \quad [50.2]$$

In this cubic equation, the coefficients *a*, *b* and *c* are defined as follows.

$$a = \varepsilon_I{}^2(\kappa_S + \eta_S) - \varepsilon_I\varepsilon_S(\kappa_I + \eta_I) \quad [51.1]$$

$$b = \left(-\varepsilon_I{}^2(\kappa_S + \eta_S) + \varepsilon_I\varepsilon_S(\kappa_I + \eta_I) - \varepsilon_I(\kappa_I + \eta_I)(\kappa_S + \eta_S) + \varepsilon_S(\kappa_I + \eta_I)^2 - \varepsilon_I(\kappa_I + \eta_I) - 2\varepsilon_I(\kappa_S + \eta_S) + \varepsilon_S(\kappa_I + \eta_I)\right) \quad [51.2]$$

$$c = \left(2\varepsilon_I(\kappa_S + \eta_S) - \varepsilon_S(\kappa_I + \eta_I) + (\kappa_I + \eta_I)(\kappa_S + \eta_S) + (\kappa_I + \eta_I) + (\kappa_S + \eta_S)\right) \quad [51.3]$$

The real positive root of the cubic equation can be written as follows.

$$W = \frac{H^{\frac{1}{3}}}{6a} - 2\frac{3ac - b^2}{3aH^{\frac{1}{3}}} - \frac{b}{3a} \quad [52.1]$$

The term H in **Eq. 52.1** is defined as follows.

$$H = \left(12\sqrt{3}Za + 108(\kappa_S + \eta_S)a^2 + 36cba - 8b^3\right) \quad [52.2]$$

Here Z is defined as follows.

$$Z = \sqrt{27a^2(\kappa_S + \eta_S)^2 + 18abc(\kappa_S + \eta_S) + 4ac^3 - 4b^3(\kappa_S + \eta_S) - b^2c^2} \quad [53]$$

In case of partially competitive inhibition scheme, **Eq. 48.2** becomes in the limit $(\eta_S, \eta_I, P) \to 0$ as $(1 - \varepsilon_I Y)(1 - X - Y) - \kappa_I Y \cong 0$ and therefore **Eqs. 49-53** need to be modified accordingly.

3. Simulation Methods

We use the following Euler iterative scheme to numerically integrate the nonlinear rate equations given in **Eqs. 3** corresponding to the fully competitive inhibition **Scheme A**.

$$X_{n+1} = X_n + \frac{[(\varepsilon_S X_n + P)(X_n + Y_n) - (\alpha_S X_n + P_n - 1 + Y_n)]}{\eta_S}\Delta\tau \quad [54.1]$$

$$Y_{n+1} = Y_n + \left(\frac{\rho}{\eta_I}\right)[(\varepsilon_I Y_n + Q_n)(X_n + Y_n) - (\alpha_I Y_n + Q_n - 1 + X_n)]\Delta\tau \quad [54.2]$$

$$P_{n+1} = P_n + \varepsilon_S X_n\,\Delta\tau \quad [54.3]$$

$$Q_{n+1} = Q_n + \rho\varepsilon_I Y_n \Delta\tau \quad [54.4]$$

Here the initial conditions are set as (X_0, Y_0, P_0, Q_0) = (0, 0, 0, 0) at τ = 0. From the trajectories of (X, Y, P, Q) the trajectories of (S, I, E) can be computed using the mass conservation equations E = 1 – X – Y, S = 1 – ε_SX – P and I = 1 – ε_IY – Q. We use the following Euler iterative scheme to numerically integrate the nonlinear rate equations given in **Eqs. 34-35** corresponding to the partially competitive inhibition **Scheme B**.

$$X_{n+1} = X_n + \left[\frac{(1-\varepsilon_S X_n - P_n)(1-X_n-Y_n)-(\eta_S+\kappa_S)X_n}{\eta_S}\right]\Delta\tau \quad [55.1]$$

$$Y_{n+1} = Y_n + \left[\frac{(1-\varepsilon_I Y_n)(1-X_n-Y)-\kappa_I Y_n}{\eta_I}\right]\Delta\tau \quad [55.2]$$

$$P_{n+1} = P_n + \varepsilon_S X_n \Delta\tau \quad [55.3]$$

Here the initial conditions are set as (X_0, Y_0, P_0) = (0, 0, 0) at τ = 0. From the trajectories of (X, Y, P) the trajectories of (S, I, E) can be computed using the mass conservation equations E = 1 – X – Y, S = 1 – ε_SX – P and I = 1 – ε_IY. Here we further set $\Delta\tau < 10^{-5}$ so that the dynamics with respect to the shortest timescale can be captured. We use the following scheme to numerically integrate the coupled nonlinear ODE given in **Eqs. 9.1**-**2** under various parameter settings.

$$F_{n+1} = F_n - \left(\frac{\alpha_S}{\eta_S}F_n + \frac{\varepsilon_S}{\eta_S}M_n - \frac{1}{{\eta_S}^3} + \frac{{\eta_I}^2}{\rho{\eta_S}^3}G_n - \phi_S\left((F_n+M_n)\left(F_n + \frac{{\eta_I}^2}{\rho{\eta_S}^3}G_n\right)\right)\right)\Delta\tau \quad [56.1]$$

$$M_{n+1} = M_n + F_n\Delta\tau \quad [56.2]$$

$$G_{n+1} = G_n - \left(\frac{\rho\alpha_I}{\eta_I}G_n + \frac{\varepsilon_I\rho^2}{\eta_I}N_n - \frac{\rho^2}{{\eta_I}^3} + \frac{\rho^2{\eta_S}^2}{{\eta_I}^3}\frac{dM}{d\tau} - \rho^2\phi_I\left(\left(\frac{1}{\rho}G_n + N_n\right)\left(\frac{1}{\rho}G_n + \frac{{\eta_S}^2}{{\eta_I}^2}F_n\right)\right)\right)\Delta\tau \quad [56.3]$$

$$N_{n+1} = N_n + G_n\Delta\tau \quad [56.4]$$

Here the initial conditions are set as (F_0, M_0, G_0, N_0) = (0, 0, 0, 0) at τ = 0. Using the variable transformations $P = \varepsilon_S{\eta_S}^2 M$, $Q = \varepsilon_I{\eta_I}^2 N$, $V = \varepsilon_S{\eta_S}^2 F$, and $U = \varepsilon_I{\eta_I}^2 G$ one can revert back to the original dynamical variables V, U, P and Q. The time at which the steady states occur was numerically computed from the integral trajectories by looking at the time point at which the first derivative of (X, Y) with respect to τ changes the sign. The trajectories corresponding to the ϕ-approximations can also be computed by setting $(\phi_S, \phi_I) = 0$ in **Eqs. 56.1** and **56.3** apart from using the integral solutions to **Eqs. 9.4-5** as given in **Appendix A**.

4. Results and Discussion

Competitive inhibition of the Michaelis-Menten enzymes plays critical roles in designing drug molecules against the nodal enzymes of various pathogenic organisms. The relative efficiency of an inhibitor type drug depends on the parameters K_{MS}, v_{max}, u_{max} and K_{MI}. Estimation of these parameters for a given enzyme-substrate-inhibitor system from the experimental data is critical for the screening of various inhibitor type drug molecules against a given enzyme of a pathogen both under *in vitro* as well as *in vivo* conditions. Almost all the current experimental methods use the expression derived from standard QSSA with stationary reactant assumption i.e., approximation under the conditions that $(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I) \to 0$ (we denote these conditions as $\mathbf{C_1}$). Under these conditions along with the stationary reactants assumption that the steady state values of $(S, I) \cong (1,1)$ (condition $\mathbf{C_2}$), one can approximate the steady state reaction velocities

as $v \cong \frac{v_{max}s_0}{s_0+K_{MS}\left(1+\frac{i_0}{K_{MI}}\right)}$; $u \cong \frac{u_{max}i_0}{\left(i_0+K_{MI}\left(1+\frac{s_0}{K_{MS}}\right)\right)}$ as given in **Eqs. 6.3** for the fully competitive inhibition and $v \cong \frac{v_{max}s_0}{s_0+K_{MS}\left(1+\frac{i_0}{K_{DI}}\right)}$ for the partially competitive inhibition as given in **Eqs. 34.7.**

Reciprocal plots of the dataset on the iteration of s_0 and i_0 over the steady state reaction velocities combined with linear least square fitting procedures will eventually reveal the required kinetic parameter values. Clearly, the overall error associated with this procedure can be minimized only when the inequality conditions E_1 and E_2 given by **Eqs. 31.12-13**, C_1 and C_2 are true. These mean that **Eqs. 6.3** will be valid only when conditions C_1, C_2, E_1, and E_2 are all true. In the same way, the inequality condition E_3 given by **Eqs. 47.4** should be true to minimize the error in the standard QSSA based estimation of kinetic parameters of partially competitive inhibition scheme. In this context, we have obtained here several approximate solutions to the fully as well as partially competitive inhibition schemes over both pre- and post-steady state regimes. These phase-space approximations are summarized in **Table 2** and **Table 3** along with the respective conditions of validity.

4.1. Refined expressions for the standard QSSA

Upon observing the asymptotic behavior of the pre-steady state regime approximations, we obtained refined expressions for the standard QSSA with stationary reactant assumption i.e., $v \cong \frac{v_{max}s_0}{s_0+(e_0+K_{MS})\left(1+\frac{i_0}{e_0+K_{MI}}\right)}$; $u \cong \frac{u_{max}i_0}{\left(i_0+(e_0+K_{MI})\left(1+\frac{s_0}{e_0+K_{MS}}\right)\right)}$ as given in **Eqs. 24.2** and **30.2** for the fully competitive scheme and $v \cong \frac{v_{max}s_0}{s_0+(e_0+K_{MS})\left(1+\frac{i_0}{e_0+K_{DI}}\right)}$ for the partially competitive inhibition scheme as given in **Eq. 47.5**. These approximations seem be applicable even when the condition C1 is violated. Particularly, these refined expressions can approximate the steady state reaction velocities even at high values of $(\varepsilon_S, \varepsilon_I)$. Upon using of these refined expressions, one can significantly reduce the error in the estimation of various enzyme kinetic parameters from the experimental datasets.

4.2. Error in the standard QSSA of the fully competitive inhibition scheme

The conditions given in C_1 can be set to a required value under *in vitro* scenarios by manipulating the relative concentrations of the enzyme, substrate and inhibitor. Remarkably, **Eqs. 8.17** which deals with the critical parameter δ reveal the validity of the stationary reactant assumptions associated with the condition C_2. From **Eqs. 8.17** one finds that the steady state substrate level can be approximated as $S \cong 1$ only when $\delta \to 0$. This means that the error in the standard QSSA of substrate conversion velocity will be negatively correlated with δ. On the other hand, from **Eqs. 8.18** one finds that the steady state inhibitor level can be approximated as $I \cong 1$ only when $\delta \to \infty$. This means that the error in the QSSA of inhibitor conversion velocity will be positively correlated with δ. However, when $\sigma \cong 1$ and $\delta < 0$ or $\delta > 0$, then the steady states show a

complex behavior as demonstrated in the **Section 2.4.1**. This means that the widely used standard QSSA equations along with the stationary reactant assumption as given in **Eqs. 6.3** will be valid only when $\delta \cong 1$. When $\delta < 0$ or $\delta > 0$, then there is a possibility of multiple steady states and **Eqs. 6.3** can capture only the transient first occurring steady state point. One needs to use the velocity equations given in **Eqs. 8.17** and **8.18** to capture the actual second prolonged steady state point. This phenomenon is demonstrated in **Figs. 3** and **Figs. 4**.

When $\sigma \cong 1$ and $\delta < 1$, then the temporal evolution of the enzyme-substrate complex level will show two different time points (denoted as phases **I** and **II** in **Fig. 3A**) at which the trajectory attains maxima where $\frac{dX}{d\tau} = 0$ with a local minimum (denoted as phase **III** in **Fig. 3A**) in between these two maxima. Similarly, when $\sigma \cong 1$ and $\delta > 1$, then the temporal evolution of the enzyme-inhibitor complex will show up two different time points at which the trajectory attains maxima where $\frac{dY}{d\tau} = 0$ with a local minimum (**Fig. 3B**) in between these two maxima. Common steady states corresponding to the enzyme-substrate and enzyme inhibitor complexes can occur only when $\delta \cong 1$ as demonstrated in **Fig. 3C**. One can interpret these results as follows. In most of the experimental scenarios, binding of substrate or inhibitor with the respective enzyme will be a diffusion-controlled bimolecular collision process. As a result, one can assume that $k_1 \cong k_i$ which means that $\sigma \cong 1$. However, the rate of dissociation and conversion in to the respective products will depend on the specific bonding interactions at the protein-ligand interfaces of the enzyme-substrate and enzyme-inhibitor complexes. In this context, $\delta = \frac{\tilde{\mu}_I \varepsilon_S}{\rho \tilde{\mu}_S \varepsilon_I} = \frac{v_{max}/\tilde{\mu}_S}{u_{max}/\tilde{\mu}_I}$ represents the relative speed of binding, dissociation and conversion into the respective products of the enzyme-inhibitor and enzyme-substrate complexes. Let us define the ratios $f_S = v_{max}/\tilde{\mu}_S$ and $f_I = u_{max}/\tilde{\mu}_I$ as the acceleration factors with respect to the conversion dynamics of substrate and inhibitor into their respective products. When $\delta = \frac{f_S}{f_I} < 1$, then the speed of conversion of enzyme-inhibitor complex into the product will be faster than the conversion speed of enzyme-substrate complex. As a result, when $\sigma \cong 1$, then the enzyme-substrate complex can reach the full-fledged steady state only after the depletion of the enzyme-inhibitor complex as shown in **Fig. 3A**. On the other hand, when $\delta = \frac{f_S}{f_I} > 1$ and $\sigma \cong 1$ then the enzyme-inhibitor complex can reach the full-fledged steady state value only after the depletion of the enzyme-substrate complex as shown in **Fig. 3B**.

In term of original variables, $\delta = \frac{s_0 v_{max}/(K_{MS}+e_0)}{i_0 u_{max}/(K_{MI}+e_0)}$ from which the one obtains the limiting condition as $\lim_{e_0 \to \infty} \delta \to 1$. This is a reasonable observation since the fully competitive inhibition scheme will be uncoupled under such condition. The phase-space behavior of those trajectories described in **Figs. 3** under the conditions that $\sigma \cong 1$ and $\delta < 1$ or $\delta > 1$ over (V, S) and (U, I) spaces are shown in **Figs. 4** and over (V, P) and (U, Q) spaces are shown in **Figs. 5**. When $\delta < 1$, then **Figs. 4A-B** and **Figs. 5A-B** suggest that the first occurring steady state point in the evolution

of enzyme-substrate complex will be a transient one and it will be observed as a spike in the (V, S) phase-space plot. As a result, we consider the second occurring prolonged steady state as the original steady state with respect to the enzyme-substrate and enzyme-inhibitor complexes. Clearly, the expression obtained from standard QSSA with the reactants stationary assumption as given by **Eqs. 6.3** can be used to obtain the steady state velocities only when $\sigma \cong 1$ and $\delta \cong 1$. When $\delta < 1$, then using the standard QSSA one can obtain only the steady state velocity of the enzyme-inhibitor complex as shown in **Figs. 4B** and **5B**. Similarly, when $\delta > 1$, then only the steady state velocity associated with enzyme-substrate complex can be obtained as shown in **Figs. 4C** and **5C**. When $\delta \cong 1$, then one can obtain the steady state velocities associated with both the enzyme-substrate and enzyme-inhibitor complexes as demonstrated in **Figs. 4E-F** and **5E-F**. Irrespective of the value of δ, the expressions corresponding to the pre-steady state dynamics in the (V, S) space in the limit as $(\eta_I, \varepsilon_S, \varepsilon_I) \to 0$ (**Eqs. 23.4-5**) and in the (U, I) space in the limit as $(\eta_S, \varepsilon_S, \varepsilon_I) \to 0$ (**Eq. 29.3-4**) can approximate the simulated trajectory very well in all the cases **Figs. 4A-F** and **5A-F**. Interestingly, when $\delta < 1$, then the approximation in the limit $(\eta_S, \eta_I, P) \to 0$ that is given by **Eq. 6.16** accurately predicts the post-steady state reaction velocity associated with the enzyme-inhibitor complex in the (U, I) space as shown in **Fig. 4B** and **5B**. Similarly, when $\delta > 1$, then the approximation in the limit $(\eta_S, \eta_I, Q) \to 0$ can accurately predict the post-steady state reaction velocity associated with the enzyme-substrate complex in the (V, S) space as shown in **Fig. 4C** and **5C**.

4.3. Minimization of error in the standard QSSA on (V, S) and (U, I) spaces

The overall error associated with the standard QSSA with stationary reactants assumption of the fully competitive enzyme kinetics used in the literature in the velocity-substrate (V, S), velocity-inhibitor spaces (U, I) at different values of $(\varepsilon_S, \varepsilon_I)$ and other parameters are shown in **Figs. 6**. Similarly, the error characteristics of the standard QSSA in the (V, P) and (U, Q) spaces are shown in **Figs. 7**. **Figs. 6** and **Figs. 7** clearly demonstrate the poor performance of **Eqs. 6.2-3** which are widely used in the literature to obtain the kinetic parameters of fully competitive inhibition systems especially at when $(\kappa_S, \kappa_I) \ll 1$. We summarize the following essential conditions for the validity of the standard QSSA given in **Eqs. 6.2-3** and the refined QSSA given in **Eqs. 6.19**. Apparently, these conditions are mandatory to minimize the error in the standard QSSAs.

C1: $(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I) \to 0$ [57.1]

C2: $(S, I) \cong (1,1)$

C3: $\left(\frac{\varepsilon_S(1+\varepsilon_I+\mu_I)}{(\mu_S+1)\kappa_I+(\eta_I+\varepsilon_I+1)\kappa_S+(\eta_S+1)\eta_I+(1+\varepsilon_I)\eta_S+\varepsilon_I}\right) \ll 1$ [57.2]

C4: $\left(\frac{\varepsilon_I(1+\varepsilon_S+\mu_S)}{(1+\varepsilon_S+\mu_S)(\kappa_I+\eta_I)+(\eta_I+1)\kappa_S+\eta_S+\varepsilon_S}\right) \ll 1$ [57.3]

C5: $\sigma \cong 1$ and $\delta \cong 1$ [57.4]

The condition C1 ensure the occurrence of similar steady state timescales associated with the enzyme-substrate and enzyme-inhibitor complexes. C2 is the stationary reactant assumption that

is required to approximate the unknown steady state substrate and inhibitor levels. C3 and C4 (following from **Eqs. 31.12-13**) are required to minimize the deviations occurring in the pre-steady state regime. C5 is required to avoid the complex multiple steady state dynamics of enzyme-substrate and enzyme-inhibitor complexes. The error levels of the refined standard QSSA given in **Eqs. 6.19** are demonstrated in **Figs. 7** and **Figs. 8**. **Figs. 7** show the error levels under the conditions that $\kappa_S \neq \kappa_I$ and **Figs. 7** demonstrate the error levels when $\kappa_S = \kappa_I$. **Figs. 7** and **Figs. 8** clearly show the error control capability of the refined QSSA expressions given by **Eqs. 6.19** in estimating the steady state reaction velocities over **Eqs. 6.2-3**. Remarkably, there is a strong correlation between the error levels in the estimated steady state reaction velocities and δ. The error level associated with the steady state velocity of the enzyme-substrate complex is positively correlated with δ. On the other hand, the error levels associated with the steady state velocity of the enzyme-inhibitor complex seem to be negatively correlated with δ. These means that to obtain the accurate estimate of the steady state velocity of enzyme-substrate complex using **Eqs. 6.3**, one needs to set $\delta > 1$. To obtain the accurate estimate of the steady state velocity of the enzyme-inhibitor complex, one needs to set $\delta < 1$. On the overall basis, we find that the conditions C1, C5 are critical to minimize the error in the refined standard QSSA.

4.4. The ϕ-approximations

The performance of ϕ-approximations described by the set of coupled linear ODEs given by **Eqs. 9.4-5** over the (V, I, S) and (U, I, S) spaces is demonstrated along with the standard QSSA in **Figs. 9**. These parametric solutions were generated with τ as the parameter as given in the integral solutions of **Eqs. 9.4-5** in **Appendix A**. Clearly, the ϕ-approximations accurately fit both the pre- and post-steady state regimes especially at large values of $(\varepsilon_S, \varepsilon_I)$. Whereas, the standard QSSAs work only in the post steady state regime. Behavior of ϕ-approximations in the (V, P, Q) and (U, P, Q) spaces are demonstrated in **Figs. 10** and the performance of ϕ-approximations in the (V, U) space a various parameter values are demonstrated in **Figs. 11**.

4.5 Partially competitive inhibition

Most of the inhibitory drug molecules and the respective experimental systems are partially competitive ones following the **Scheme B** of **Fig. 1**. These inhibitor molecules competitively bind the active site of the target enzyme against the substrate and form reversible dead-end complexes. Upon complete depletion of the substrate, the enzyme-inhibitor complex attains an equilibrium state as demonstrated in **Fig. 12A**. Similar to the fully competitive inhibition scheme, depending on the steady state timescales of the enzyme-substrate and enzyme-inhibitor complexes, partially completive inhibition scheme can also exhibit a complex behavior as demonstrated in **Fig. 12A**. Sample phase-space trajectories over (V, S), (Y, S), (I, S), (V, I, S) and (V, P, S) spaces are shown in **Figs. 12B-D** along with the pre- and post-steady state approximations under the conditions $(\varepsilon_S, \chi_I, \varepsilon_I) \to 0$ and $(\eta_S, \chi_I) \to 0$ respectively. Similar to the fully competitive inhibition, one can approximate the steady state reaction velocity associated with the enzyme-substrate compelx using $\lim_{(\eta_S,\chi_I)\to 0} V \cong \frac{\varepsilon_S S}{S+\tilde{\mu}_S\left(1+\frac{1}{\tilde{\kappa}_I}\right)}$ in the (V, S) space and the level of

enzyme-inhibitor compelx using $\lim_{(\eta_S,\chi_I)\to 0} Y \cong \frac{1}{1+\tilde{\kappa}_I\left(1+\frac{S}{\tilde{\mu}_S}\right)}$ in the (Y, S) space. In the derivation of these equations, we have applied stationary reactant assumption on the inhibitor level as $I \cong 1$. Upon applying the stationary reactant assumption that $S \cong 1$ at the steady state of enzyme-substrate compelx under the conditions $(\varepsilon_S, \varepsilon_I) \to 0$, one finally arives at the expression $\lim_{(\eta_S,\chi_I,\varepsilon_S,\varepsilon_I)\to 0} V \cong \frac{\varepsilon_S}{1+\tilde{\mu}_S\left(1+\frac{1}{\tilde{\kappa}_I}\right)}$. In terms of the original variables, this refined reaction velocity equaiton for the partially competitive inhbition scheme can be rewriten as in **Eqs. 47.5** as follows.

$$\lim_{(\eta_S,\chi_I,\varepsilon_S,\varepsilon_I)\to 0} v \cong \frac{v_{max}s_0}{s_0+(K_{MS}+e_0)\left(1+\frac{i_0}{K_{DI}+e_0}\right)} \qquad [58]$$

In the standard QSSA equation used in the literatures and textbooks, the term e_0 will not be added up to K_{MS} and K_{DI}. Similar to **Eqs. 24.2**, **Eq. 58** will be valid over wide range of parameters $(\varepsilon_S, \varepsilon_I)$ as demonstrated in **Figs. 12B-D**. As in **Eqs. 57**, the error in the steady state reaction velocity **Eqs. 58** can be minimized using the following conditions.

G1: $(\eta_S, \chi_I, \varepsilon_S, \varepsilon_I) \to 0$ [59.1]

G2: $(S, I) \cong (1,1)$ [59.2]

G3: $\left(\frac{\varepsilon_S(1+\varepsilon_I+\kappa_I)}{(\mu_S+1)(\varepsilon_I+\kappa_I)+\mu_S}\right) \ll 1$ [59.3]

Here the condition G1 is required for the occurrence of common steady state with respect to both the enzyme-substrate and enzyme-inhibitor complexes. Th condition G2 is the stationary reactant assumption that is required to approximate the unknown steady state levels of (S, I). The condition G3 ensure the minimum error in the pre-steady state regime.

5. Conclusion

Fully and the partially competitive inhibition of the Michaelis-Menten kinetics play critical role in designing drug molecules against the nodal enzymes of various harmful pathogens. Designing of such drug molecules involves screening of various substrate like small molecules which can act as potential inhibitors of the target enzymes. Estimation of various kinetic parameters associated with the competitive inhibition is essential for such comparative studies and evaluation of various potential drug candidates. The currently available standard quasi steady state approximation with stationary reactant assumption is applicable only in the post-steady state regime of the velocity-substrate-inhibitor space and it is significantly limited by the vast number of conditions of validity. Particularly, this approximation will not work when the concentration of the enzyme is equal to or higher than the substrate.

In this context, we have derived several approximations under various conditions of validity over both pre- and post-steady state regimes of the velocity-substrate-inhibitor spaces of fully and partially competition schemes. Our detailed analysis yielded refined expressions for the currently

available standard quasi steady state approximation with stationary reactants assumption. We have shown that these refined expressions are valid for wide ranges of the ratio of enzyme to substrate and inhibitor levels. Further, for the first time in the literature, we have shown that under certain conditions, the enzyme-inhibitor-substrate system can exhibit a complicated dynamical behavior with non-monotonic trend in the temporal depletion of the substrate. This means that there is a chance of occurrence of two steady states with respect to both enzyme-substrate and enzyme-inhibitor complexes. When the total or initial substrate and inhibitor levels are equal, then one can define $\delta = \frac{v_{max}/(K_{MS}+e_0)}{u_{max}/(K_{MI}+e_0)}$ which plays critical role in controlling the phase-space dynamics associated with the relative speed of binding, dissociation and conversion into the products of the enzyme-inhibitor and enzyme-substrate complexes of the fully competitive inhibition scheme. The ratios $f_S = v_{max}/(K_{MS} + e_0)$ and $f_S = u_{max}/(K_{MI} + e_0)$ are the acceleration factors with respect to the conversion dynamics of substrate and inhibitor into their respective products. When $\delta = \frac{f_S}{f_I} < 1$, then the speed of conversion of enzyme-inhibitor complex into the product will be faster than the conversion speed of enzyme-substrate complex. As a result, the enzyme-substrate complex can reach the full-fledged steady state value only after the depletion of the enzyme-inhibitor complex. On the other hand, when $\delta = \frac{f_S}{f_I} > 1$, then the enzyme-inhibitor complex can reach the full-fledged steady state value only after the depletion of the enzyme-substrate complex.

This complicated behavior of the enzyme-substrate-inhibitor system especially when $\delta \neq 1$ poses enormous difficulty in estimating the steady state velocity levels from the experimental datasets and introduces large amount of error in the estimation of various kinetic parameters both the cases of fully and partially competitive inhibition schemes. Remarkably, our refined expressions for the reaction velocities over enzyme-substrate-inhibitor space can control this error more significantly than the currently available standard QSSA velocity expressions.

Appendix A

Consider the following set of coupled linear second order ODEs.

$$\frac{d^2M}{d\tau^2}+\frac{\alpha_S}{\eta_S}\frac{dM}{d\tau}+\frac{\varepsilon_S}{\eta_S}M-\frac{1}{\eta_S{}^3}+\frac{\eta_I{}^2}{\rho\eta_S{}^3}\frac{dN}{d\tau}\cong 0;\ [M]_{\tau=0}=0;\ \left[\frac{dM}{d\tau}\right]_{\tau=0}=0 \qquad \text{[A1]}$$

$$\frac{d^2N}{d\tau^2}+\frac{\rho\alpha_I}{\eta_I}\frac{dN}{d\tau}+\frac{\varepsilon_I\rho^2N}{\eta_I}-\frac{\rho^2}{\eta_I{}^3}+\frac{\rho^2\eta_s{}^2}{\eta_I{}^3}\frac{dM}{d\tau}\cong 0;\ [N]_{\tau=0}=0;\ \left[\frac{dN}{d\tau}\right]_{\tau=0}=0 \qquad \text{[A2]}$$

This system can be uncoupled as follows. Upon differentiating **Eqs. A2** with respect to τ, one arrives at the following third order ODE.

$$\frac{d^3N}{d\tau^3}+\frac{\rho\alpha_I}{\eta_I}\frac{d^2N}{d\tau^2}+\frac{\varepsilon_I\rho^2}{\eta_I}\frac{dN}{d\tau}+\frac{\rho^2\eta_s{}^2}{\eta_I{}^3}\frac{d^2M}{d\tau^2}\cong 0 \qquad \text{[A3]}$$

From **Eqs. A1**, one can derive the following expressions.

$$\frac{dN}{d\tau}=-\frac{\rho\eta_S{}^3}{\eta_I{}^2}\left(\frac{d^2M}{d\tau^2}+\frac{\alpha_S}{\eta_S}\frac{dM}{d\tau}+\frac{\varepsilon_S}{\eta_S}M-\frac{1}{\eta_S{}^3}\right) \qquad \text{[A4]}$$

By repeated differentiation of **Eq. A4** with respect to τ, we obtain the following relationships.

$$\frac{d^2N}{d\tau^2}=-\frac{\rho\eta_S{}^3}{\eta_I{}^2}\left(\frac{d^3M}{d\tau^3}+\frac{\alpha_S}{\eta_S}\frac{d^2M}{d\tau^2}+\frac{\varepsilon_S}{\eta_S}\frac{dM}{d\tau}\right) \qquad \text{[A5]}$$

$$\frac{d^3N}{d\tau^3}=-\frac{\rho\eta_S{}^3}{\eta_I{}^2}\left(\frac{d^4M}{d\tau^4}+\frac{\alpha_S}{\eta_S}\frac{d^3M}{d\tau^3}+\frac{\varepsilon_S}{\eta_S}\frac{d^2M}{d\tau^2}\right) \qquad \text{[A6]}$$

Upon substituting **Eqs. A4-A6** into **Eq. A3**, one obtains the following uncoupled fourth order ODE corresponding to (M, τ) space.

$$\frac{d^4M}{d\tau^4}+a\frac{d^3M}{d\tau^3}+b\frac{d^2M}{d\tau^2}+c\frac{dM}{d\tau}+hM-\frac{\varepsilon_I\rho^2}{\eta_S{}^3\eta_I}\cong 0 \qquad \text{[A7]}$$

In this equation, various parameters a, b, c and h are defined as follows.

$$a=\left(\frac{\alpha_S}{\eta_S}+\frac{\rho\alpha_I}{\eta_I}\right);\ b=\left(\frac{\varepsilon_S}{\eta_S}+\frac{\rho\alpha_I}{\eta_I}\frac{\alpha_S}{\eta_S}+\frac{\varepsilon_I\rho^2}{\eta_I}-\frac{\rho}{\eta_s\eta_I}\right);\ c=\frac{\rho}{\eta_I}\left(\frac{\alpha_I\varepsilon_S}{\eta_S}+\frac{\varepsilon_I\rho\alpha_S}{\eta_S}\right);\ h=\frac{\varepsilon_I\rho^2}{\eta_I}\frac{\varepsilon_S}{\eta_S} \qquad \text{[A8]}$$

The initial conditions associated with **Eq. A7** are as follows.

$$[M]_{\tau=0}=0;\ \left[\frac{dM}{d\tau}\right]_{\tau=0}=0 \qquad \text{[A9]}$$

Other two initial conditions directly follow from the initial conditions corresponding to N.

$$\left[\frac{dN}{d\tau}\right]_{\tau=0}=\left[\frac{d^2M}{d\tau^2}+\frac{\alpha_S}{\eta_S}\frac{dM}{d\tau}+\frac{\varepsilon_S}{\eta_S}M-\frac{1}{\eta_S{}^3}\right]_{\tau=0}=0 \qquad \text{[A10]}$$

$$[N]_{\tau=0}=\left[\frac{d^3M}{d\tau^3}+\frac{\alpha_S}{\eta_S}\frac{d^2M}{d\tau^2}+\left(\frac{\varepsilon_S}{\eta_S}+\frac{\rho^2\eta_s{}^2}{\eta_I{}^3}\right)\frac{dM}{d\tau}-\frac{\rho^2}{\eta_I{}^3}\right]_{\tau=0}=0 \qquad \text{[A11]}$$

Upon obtaining the solution for the (M, τ) space one can directly obtain the expression corresponding to the (N, τ) space as follows.

$$N = \frac{\rho\eta_S{}^3}{\eta_I{}^2}\int_0^\tau \left(\frac{d^2M}{d\tau^2} + \frac{\alpha_S}{\eta_S}\frac{dM}{d\tau} + \frac{\varepsilon_S}{\eta_S}M - \frac{1}{\eta_S{}^3}\right)d\tau \qquad \text{[A12]}$$

Similar to **Eqs. A3-A12**, one can also derive the following solution set. From **Eqs. A2**, one can derive the following expression.

$$\frac{dM}{d\tau} = -\frac{\eta_I{}^3}{\rho^2\eta_s{}^2}\left(\frac{d^2N}{d\tau^2} + \frac{\rho\alpha_I}{\eta_I}\frac{dN}{d\tau} + \frac{\varepsilon_I\rho^2N}{\eta_I} - \frac{\rho^2}{\eta_I{}^3}\right) \qquad \text{[A13]}$$

By repeated differentiation of **Eq. A13** with respect to τ, we obtain the following relationships.

$$\frac{d^2M}{d\tau^2} = -\frac{\eta_I{}^3}{\rho^2\eta_s{}^2}\left(\frac{d^3N}{d\tau^3} + \frac{\rho\alpha_I}{\eta_I}\frac{d^2N}{d\tau^2} + \frac{\varepsilon_I\rho^2}{\eta_I}\frac{dN}{d\tau}\right) \qquad \text{[A14]}$$

$$\frac{d^3M}{d\tau^3} = -\frac{\eta_I{}^3}{\rho^2\eta_s{}^2}\left(\frac{d^4N}{d\tau^4} + \frac{\rho\alpha_I}{\eta_I}\frac{d^3N}{d\tau^3} + \frac{\varepsilon_I\rho^2}{\eta_I}\frac{d^2N}{d\tau^2}\right) \qquad \text{[A15]}$$

Upon differentiating **Eq. A1** with respect to τ, one obtains the following expression.

$$\frac{d^3M}{d\tau^3} + \frac{\alpha_S}{\eta_S}\frac{d^2M}{d\tau^2} + \frac{\varepsilon_S}{\eta_S}\frac{dM}{d\tau} + \frac{\eta_I{}^2}{\rho\eta_S{}^3}\frac{d^2N}{d\tau^2} \qquad \text{[A16]}$$

Upon substituting derivatives from **Eqs. A13-A15** into **Eq. A16**, one obtains the following uncoupled fourth order ODE corresponding to (N, τ) space.

$$\frac{d^4N}{d\tau^4} + r\frac{d^3N}{d\tau^3} + g\frac{d^2N}{d\tau^2} + m\frac{dN}{d\tau} + zN - \frac{\varepsilon_S}{\eta_S}\frac{\rho^2}{\eta_I{}^3} \cong 0 \qquad \text{[A17]}$$

Here the parameters r, *g*, m, and z are defined as follows.

$$r = \left(\frac{\rho\alpha_I}{\eta_I} + \frac{\alpha_S}{\eta_S}\right); g = \left(\frac{\varepsilon_I\rho^2}{\eta_I} + \frac{\alpha_S}{\eta_S}\frac{\rho\alpha_I}{\eta_I} + \frac{\varepsilon_S}{\eta_S} - \frac{\rho}{\eta_S\eta_I}\right); m = \left(\frac{\alpha_S}{\eta_S}\frac{\varepsilon_I\rho^2}{\eta_I} + \frac{\varepsilon_S}{\eta_S}\frac{\rho\alpha_I}{\eta_I}\right); z = \frac{\varepsilon_S}{\eta_S}\frac{\varepsilon_I\rho^2}{\eta_I} \qquad \text{[A18]}$$

Upon obtaining the solution for the (N, τ) space one can directly obtain the expression corresponding to the (M, τ) space as follows.

$$M = \frac{\eta_I{}^3}{\rho^2\eta_s{}^2}\int_0^\tau \left(\frac{d^2N}{d\tau^2} + \frac{\rho\alpha_I}{\eta_I}\frac{dN}{d\tau} + \frac{\varepsilon_I\rho^2N}{\eta_I} - \frac{\rho^2}{\eta_I{}^3}\right)d\tau \qquad \text{[A19]}$$

The initial conditions corresponding to the fourth order uncoupled ODE given by **Eqs. A17** can be written as follows.

$$[N]_{\tau=0} = 0; \left[\frac{dN}{d\tau}\right]_{\tau=0} = 0 \qquad \text{[A20]}$$

Other two initial conditions directly follow from the initial conditions corresponding to M.

$$\left[\frac{dM}{d\tau}\right]_{\tau=0} = \left[\frac{d^2N}{d\tau^2} + \frac{\rho\alpha_I}{\eta_I}\frac{dN}{d\tau} + \frac{\varepsilon_I\rho^2N}{\eta_I} - \frac{\rho^2}{\eta_I{}^3}\right]_{\tau=0} = 0 \qquad \text{[A21]}$$

$$[M]_{\tau=0} = \left[\frac{d^3N}{d\tau^3} + \frac{\rho\alpha_I}{\eta_I}\frac{d^2N}{d\tau^2} + \left(\frac{\varepsilon_I\rho^2}{\eta_I} + \frac{{\eta_I}^2}{\rho{\eta_S}^3}\right)\frac{dN}{d\tau} - \frac{1}{{\eta_S}^3}\right]_{\tau=0} = 0 \qquad \text{[A22]}$$

Solution to **Eqs. A1**-**A2** can be written as follows.

$$M = \left(\frac{1}{\varepsilon_S{\eta_S}^2} + C_1 exp(r_1\tau) + C_2 exp(r_2\tau) + C_3 exp(r_3\tau) + C_4 exp(r_4\tau)\right) \qquad \text{[A23]}$$

From this equation N can be obtained using **Eq. A12**. Here r_1, r_2, r_3, and r_4 are the roots of the following fourth degree polynomial in W.

$$\eta_I\eta_S W^4 + (\rho\alpha_I\eta_S + \alpha_S\eta_I)W^3 + (\eta_S\varepsilon_I\rho^2 + \rho\alpha_I\alpha_S + \eta_I\varepsilon_S - \eta_S\rho)W^2 + (\alpha_S\varepsilon_I\rho^2 + \rho\alpha_I\varepsilon_S)W + \rho^2\varepsilon_I\varepsilon_S = 0 \qquad \text{[A24]}$$

Upon using the initial conditions given by **Eqs. A9**-**11**, one can obtain the expressions for various constant terms C_1-C_4 in **Eqs. A23** as follows.

$$C_1 = \frac{(r_2r_3r_4{\eta_S}^2 - \varepsilon_S(r_2 + r_3 + r_4)\eta_S - \varepsilon_S\alpha_S){\eta_I}^3 + {\eta_S}^4\varepsilon_S\rho^2}{(r_1 - r_4)(r_1 - r_3){\eta_I}^3(r_1 - r_2){\eta_S}^4\varepsilon_S} \qquad \text{[A25]}$$

$$C_2 = \frac{(-r_1r_3r_4{\eta_S}^2 + \varepsilon_S(r_1 + r_3 + r_4)\eta_S + \varepsilon_S\alpha_S){\eta_I}^3 - {\eta_S}^4\varepsilon_S\rho^2}{{\eta_S}^4\varepsilon_S(r_1 - r_2)(r2 - r_4){\eta_I}^3(r_2 - r_3)} \qquad \text{[A26]}$$

$$C_3 = \frac{(r_1r_2r_4{\eta_S}^2 - \varepsilon_S(r_1 + r_2 + r_4)\eta_S - \varepsilon_S\alpha_S){\eta_I}^3 + {\eta_S}^4\varepsilon_S\rho^2}{{\eta_S}^4\varepsilon_S(r_2 - r_3)(r_1 - r_3)(r_3 - r_4){\eta_I}^3} \qquad \text{[A27]}$$

$$C_4 = \frac{(-r_1r_2r_3{\eta_S}^2 + \varepsilon_S*(r_1 + r_2 + r_3)\eta_S + \varepsilon_S\alpha_S){\eta_I}^3 - {\eta_S}^4\varepsilon_S\rho^2}{{\eta_I}^3{\eta_S}^4\varepsilon_S(r_3 - r_4)(r_2 - r_4)(r_1 - r_4)} \qquad \text{[A28]}$$

Using the transformations $P = \varepsilon_S{\eta_S}^2M$, $Q = \varepsilon_I{\eta_I}^2N$, $V = \varepsilon_S{\eta_S}^2F$, and $U = \varepsilon_I{\eta_I}^2G$ one can revert back to the original dynamical variables V, U, P and Q.

References

1. Alberts B. Molecular biology of the cell. New York: Garland Science; 2002.
2. Stryer L. Biochemistry. New York: W.H. Freeman; 1988.
3. Voet D, Voet JG. Biochemistry. New York: J. Wiley & Sons; 1995.
4. Fersht A. Enzyme Structure and Mechanism: W.H. Freeman; 1985.
5. Michaelis L, Menten ML. {Die kinetik der invertinwirkung}. Biochem Z. 1913;49(333-369):352. doi: citeulike-article-id:5936552.
6. Briggs GE, Haldane JBS. A Note on the Kinetics of Enzyme Action. Biochemical Journal. 1925;19(2):338-9. PubMed PMID: PMC1259181.
7. Johnson KA, Goody RS. The Original Michaelis Constant: Translation of the 1913 Michaelis-Menten Paper. Biochemistry. 2011;50(39):8264-9. doi: 10.1021/bi201284u. PubMed PMID: PMC3381512.
8. Cornish-Bowden A. One hundred years of Michaelis–Menten kinetics. Perspectives in Science. 2015;4:3-9. doi: http://dx.doi.org/10.1016/j.pisc.2014.12.002.
9. Deichmann U, Schuster S, Mazat J-P, Cornish-Bowden A. Commemorating the 1913 Michaelis–Menten paper Die Kinetik der Invertinwirkung: three perspectives. FEBS Journal. 2014;281(2):435-63. doi: 10.1111/febs.12598.
10. Murugan R. Solution to Michaelis-Menten enzyme kinetic equation via undetermined gauge functions: Resolving the nonlinearity of Lineweaver-Burk plot. Journal of Chemical Physics. 2002;117(9):4178-83. doi: 10.1063/1.1496459. PubMed PMID: WOS:000177439300010.
11. Murray JD. Mathematical biology. New York: Springer; 2002.
12. Fraser SJ. Slow manifold for a bimolecular association mechanism. J Chem Phys. 2004;120(7):3075-85. doi: 10.1063/1.1634555. PubMed PMID: 15268460.
13. Roussel MR, Fraser SJ. Invariant manifold methods for metabolic model reduction. Chaos. 2001;11(1):196-206. doi: 10.1063/1.1349891. PubMed PMID: 12779453.
14. Dingee JW, Anton AB. A new perturbation solution to the Michaelis-Menten problem. AIChE Journal. 2008;54(5):1344-57. doi: 10.1002/aic.11461.
15. Vogt D. A simplified perturbation solution of Michaelis-Menten kinetics equations in a “total” framework. Journal of Mathematical Chemistry. 2013;51(9):2257-63. doi: 10.1007/s10910-013-0221-0.
16. Dell’Acqua G, Bersani AM. A perturbation solution of Michaelis–Menten kinetics in a “total” framework. Journal of Mathematical Chemistry. 2012;50(5):1136-48. doi: 10.1007/s10910-011-9957-6.
17. Seshadri MS, Fritzsch G. The time evolution of sequential enzyme reactions: a singular perturbation approach. J Theor Biol. 1981;93(1):197-205. PubMed PMID: 7334820.
18. Segel LA, Slemrod M. The Quasi-Steady-State Assumption: A Case Study in Perturbation. SIAM Review. 1989;31(3):446-77. doi: 10.1137/1031091.
19. Dell’Acqua G, Bersani AM. On the appropriate use of asymptotic expansions in enzyme kinetics. Journal of Mathematical Chemistry. 2014;52(10):2475-81. doi: 10.1007/s10910-014-0403-4.
20. Golicnik M. The integrated Michaelis-Menten rate equation: deja vu or vu jade? J Enzyme Inhib Med Chem. 2013;28(4):879-93. doi: 10.3109/14756366.2012.688039. PubMed PMID: 22630075.
21. Golicnik M. Explicit reformulations of time-dependent solution for a Michaelis-Menten enzyme reaction model. Anal Biochem. 2010;406(1):94-6. doi: 10.1016/j.ab.2010.06.041. PubMed PMID: 20599638.
22. Golicnik M. Explicit reformulations of the Lambert W-omega function for calculations of the solutions to one-compartment pharmacokinetic models with Michaelis-Menten elimination kinetics. Eur J Drug Metab Pharmacokinet. 2011;36(3):121-7. doi: 10.1007/s13318-011-0040-2. PubMed PMID: 21533844.

23. Golicnik M. Explicit analytic approximations for time-dependent solutions of the generalized integrated Michaelis-Menten equation. Anal Biochem. 2011;411(2):303-5. doi: 10.1016/j.ab.2011.01.016. PubMed PMID: 21241654.
24. Schnell S, Mendoza C. Closed Form Solution for Time-dependent Enzyme Kinetics. Journal of Theoretical Biology. 1997;187(2):207-12. doi: http://dx.doi.org/10.1006/jtbi.1997.0425.
25. Stroberg W, Schnell S. On the estimation errors of KM and V from time-course experiments using the Michaelis-Menten equation. Biophys Chem. 2016;219:17-27. doi: 10.1016/j.bpc.2016.09.004. PubMed PMID: 27677118.
26. Kargi F. Generalized rate equation for single-substrate enzyme catalyzed reactions. Biochem Biophys Res Commun. 2009;382(1):157-9. doi: 10.1016/j.bbrc.2009.02.155. PubMed PMID: 19265680.
27. Bajzer Z, Strehler EE. About and beyond the Henri-Michaelis-Menten rate equation for single-substrate enzyme kinetics. Biochem Biophys Res Commun. 2012;417(3):982-5. doi: 10.1016/j.bbrc.2011.12.051. PubMed PMID: 22206668.
28. Tzafriri AR. Michaelis-Menten kinetics at high enzyme concentrations. Bull Math Biol. 2003;65(6):1111-29. doi: 10.1016/S0092-8240(03)00059-4. PubMed PMID: 14607291.
29. Rami Tzafriri A, Edelman ER. Quasi-steady-state kinetics at enzyme and substrate concentrations in excess of the Michaelis-Menten constant. J Theor Biol. 2007;245(4):737-48. doi: 10.1016/j.jtbi.2006.12.005. PubMed PMID: 17234216.
30. Atkins GL, Nimmo IA. A comparison of seven methods for fitting the Michaelis-Menten equation. Biochem J. 1975;149(3):775-7. PubMed PMID: 1201002; PubMed Central PMCID: PMCPMC1165686.
31. Lineweaver H, Burk D. The Determination of Enzyme Dissociation Constants. Journal of the American Chemical Society. 1934;56(3):658-66. doi: 10.1021/ja01318a036.
32. Grima R, Walter NG, Schnell S. Single-molecule enzymology a la Michaelis-Menten. FEBS J. 2014;281(2):518-30. doi: 10.1111/febs.12663. PubMed PMID: 24289171.
33. Borghans JM, De Boer RJ, Segel LA. Extending the quasi-steady state approximation by changing variables. Bulletin of Mathematical Biology. 1996;58(1):43-63. doi: http://dx.doi.org/10.1016/0092-8240(95)00306-1.
34. Li B, Shen Y, Li B. Quasi-Steady-State Laws in Enzyme Kinetics. The Journal of Physical Chemistry A. 2008;112(11):2311-21. doi: 10.1021/jp077597q.
35. Murugan R. Theory on the rate equation of Michaelis-Menten type single-substrate enzyme catalyzed reactions. Journal of Mathematical Chemistry. 2018;56(2):508-56. doi: 10.1007/s10910-017-0791-3. PubMed PMID: WOS:000419943100009.
36. Rubinow SI, Lebowitz JL. Time-dependent Michaelis-Menten kinetics for an enzyme-substrate-inhibitor system. J Am Chem Soc. 1970;92(13):3888-93. PubMed PMID: 4986734.
37. Schnell S, Mendoza C. Time-dependent closed form solutions for fully competitive enzyme reactions. Bull Math Biol. 2000;62(2):321-36. doi: 10.1006/bulm.1999.0156. PubMed PMID: 10824432.
38. Corless RM, Gonnet GH, Hare DEG, Jeffrey DJ, Knuth DE. On the LambertW function. Advances in Computational Mathematics. 1996;5(1):329-59. doi: 10.1007/BF02124750.
39. Pedersena MG, Bersani AM, Bersani E. The total quasi-steady-state approximation for fully competitive enzyme reactions. Bull Math Biol. 2007;69(1):433-57. Epub 2006/07/20. doi: 10.1007/s11538-006-9136-2. PubMed PMID: 16850351.
40. Tzafriri AR, Edelman ER. The total quasi-steady-state approximation is valid for reversible enzyme kinetics. J Theor Biol. 2004;226(3):303-13. Epub 2003/12/04. doi: 10.1016/j.jtbi.2003.09.006. PubMed PMID: 14643644.

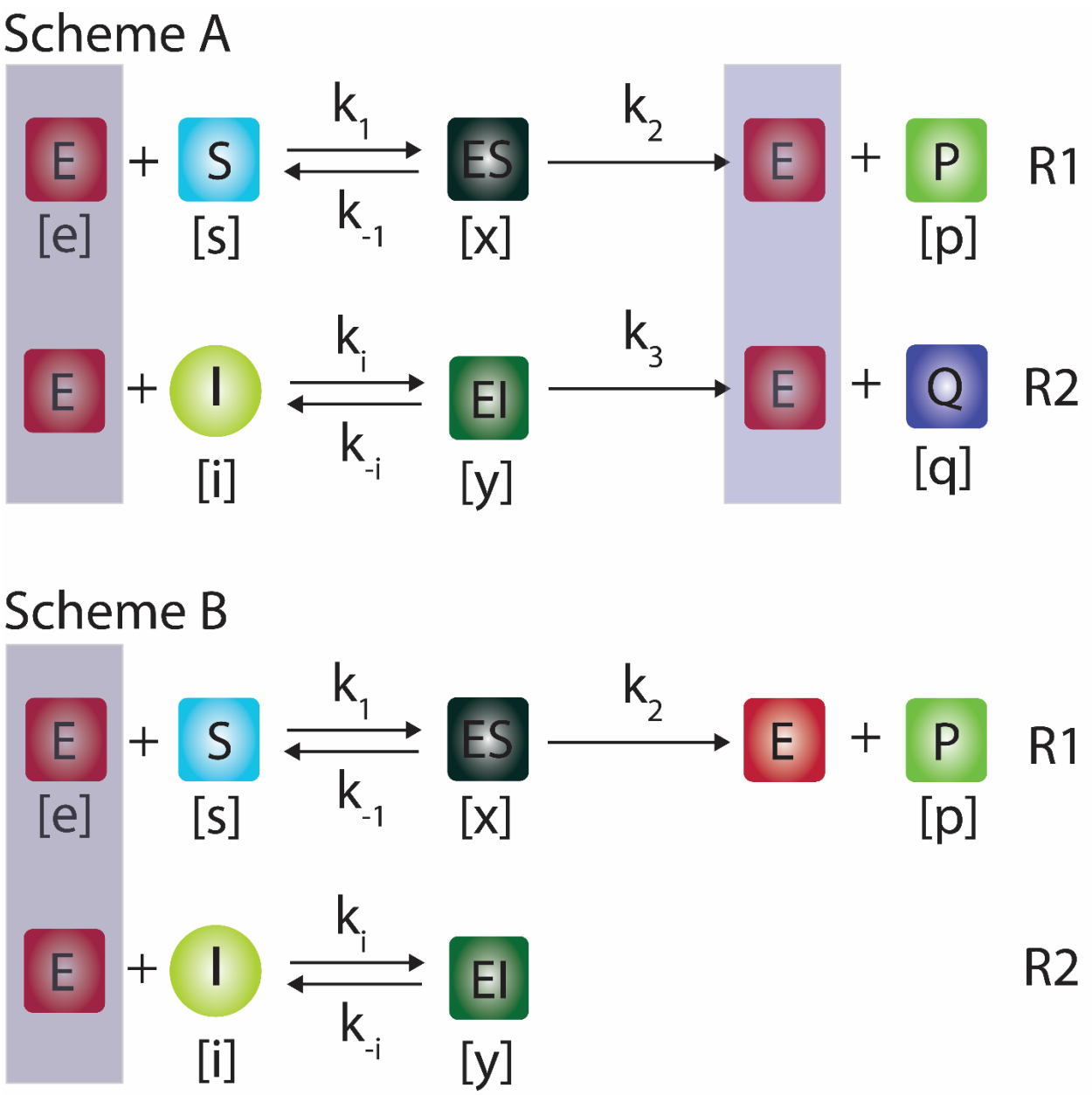

FIGURE 1. Fully (Scheme A) and partially (Scheme B) competitive inhibition schemes of Michaelis-Menten type enzyme kinetics. In fully competitive inhibition, both substrate and inhibitor compete for the same active site of enzyme to bind and get converted into their respective products. Whereas, in partially competitive inhibition, the reversibly formed enzyme-inhibitor is a dead-end complex. Here (e, s, i, x, y, p, q) are respectively the concentrations of enzyme, substrate, inhibitor, enzyme-substrate, enzyme-inhibitor, product of substrate and product of inhibitor. Further, k_1 and k_i are the respective forward rate constants, k_{-1} and k_{-i} are the reverse rate constants and, k_2 and k_3 are the respective product formation rates.

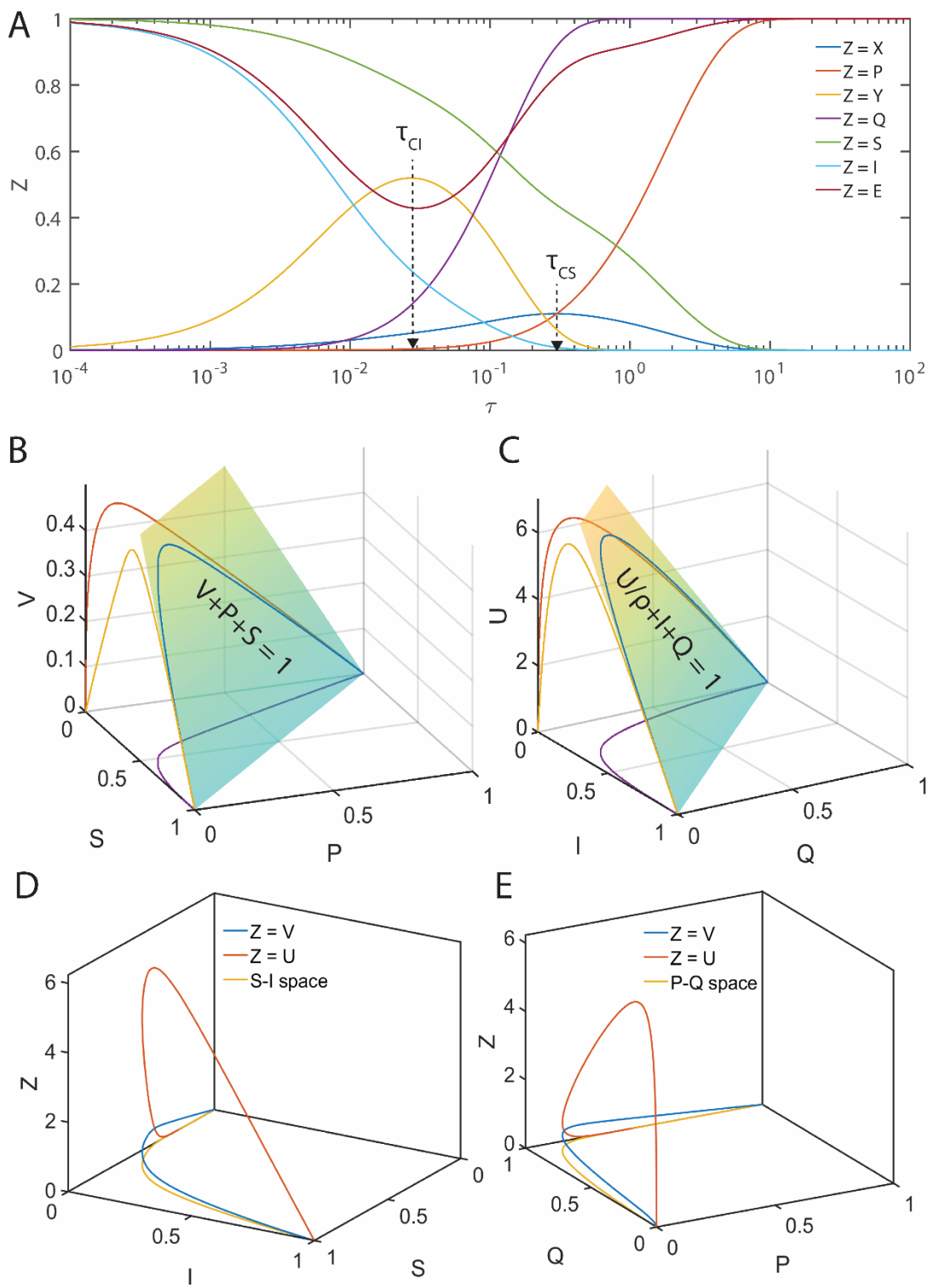

FIGURE 2. Occurrence of distinct steady state timescales with respect to enzyme-substrate (X) and enzyme-inhibitor (Y) complexes. Here S, I, E, X, Y, P, Q are the dimensionless concentrations of substrate, inhibitor, enzyme, enzyme-substrate, enzyme-inhibitor, product of substrate and product of inhibitor. Trajectories are from numerical integration of **Eqs. 3** with the parameters $\eta_S = 0.2$, $\varepsilon_S = 4.1$, $\kappa_S = 3.1$, $\rho = 10$, $\eta_I = 0.1$, $\varepsilon_I = 1.2$, $\kappa_I = 0.1$ along with the initial

conditions $(S, I, E, X, Y, P, Q) = (1,1,1,0,0,0,0)$ at τ = 0 for the simulation of **Eqs. 3.1-3**. Further upon fixing ρ, one finds that $\delta = 0.05$, $\gamma = 1.55$ and $\sigma = 0.17$. Here V = $\varepsilon_S X$ and U = $\rho\varepsilon_I Y$ are the dimensionless reaction velocities corresponding to the conversion of the substrate and inhibitor into their products. **A**. The steady states corresponding to the enzyme-inhibitor and enzyme-substrate complexes occur at τ_{CI} = 0.03, τ_{CS} = 0.31 respectively. We should note that τ_{CI} is the time at which $\frac{dY}{d\tau} = 0$ and τ_{CS} is the time at which $\frac{dX}{d\tau} = 0$. Since $\tau_{CS} \neq \tau_{CI}$ with the current parameter settings, one cannot obtain a common steady state solution to **Eqs. 3**. **B**. All the trajectories in the velocity-substrate-product (VPS) space fall within the plane V + P + S = 1. **C**. All the trajectories in the velocity-inhibitor-product (UQI) space fall within the plane U/ρ + Q + I = 1. **D**. Sample trajectories in the velocities-inhibitor-substrate (VIS, UIS) and velocities-products spaces (U, P, Q) and (V, P, Q).

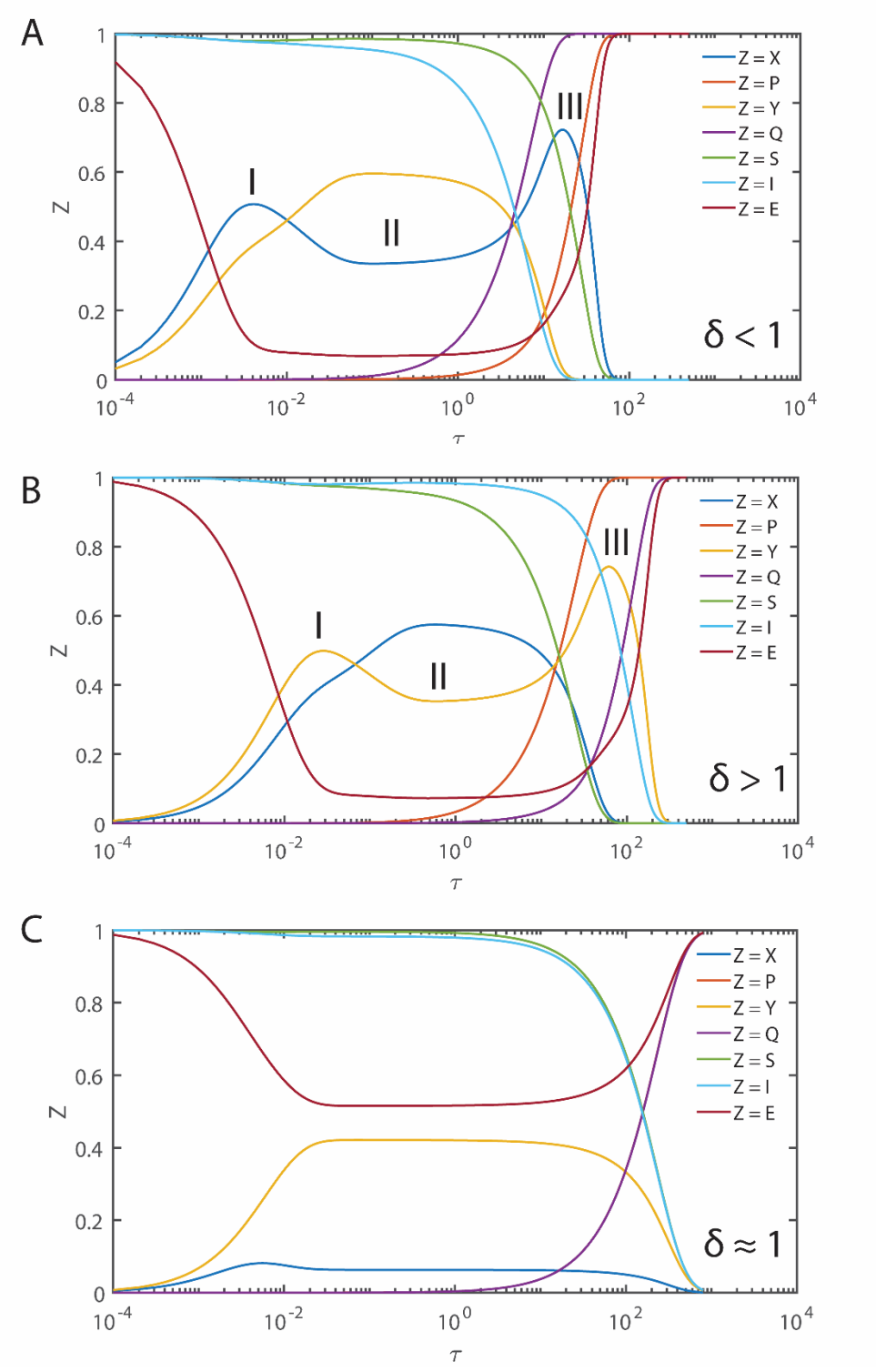

FIGURE 3. Trajectories of the fully competitive enzyme kinetics at different values of δ. The common initial conditions for the simulation of **Eqs. 3.1-3** as $(S, I, E, X, Y, P, Q) = (1,1,1,0,0,0,0)$ at τ = 0. **A-B**. Here the simulation settings are $\eta_S = 0.002, \varepsilon_S = 0.04,\ \kappa_S = 0.2,\ \eta_I = 0.01, \varepsilon_I = 0.06, \kappa_I = 0.1$ and ρ = 3.333, σ = 1, δ = 0.1405, ϒ = 3. When δ < 1 and the steady state timescale

of the enzyme-substrate complex is lower than the enzyme-inhibitor complex i.e., $\tau_{CS} < \tau_{CI}$, then the evolution of enzyme-substrate complex shows a bimodal type curve with respect to time. Particularly, when σ = 1 and δ > 1 or δ < 1, the temporal evolution of the enzyme-substrate and enzyme-inhibitor complexes show a complex behavior with multiple steady states. **C-D**. Here the simulation settings are $\eta_S = 0.02, \varepsilon_S = 0.06,\ \kappa_S = 0.1,\ \eta_I = 0.003, \varepsilon_I = 0.04, \kappa_I\ = 0.2$ and ρ = 0.225, σ = 1, δ = 9, ϒ = 0.33. When δ > 1 and the steady state timescales of enzyme-substrate complex is higher than the enzyme-inhibitor complex i.e. $\tau_{CS} > \tau_{CI}$, then the evolution of enzyme-inhibitor complex shows a bimodal type curve with respect to time. **E-F**. Here the simulation settings are $\eta_S = 0.02, \varepsilon_S = 0.06,\ \kappa_S = 8.1,\ \eta_I = 0.003, \varepsilon_I = 0.04, \kappa_I\ = 1.2$ and ρ = 0.225, σ = 1, δ = 1.013, ϒ = 4.5.

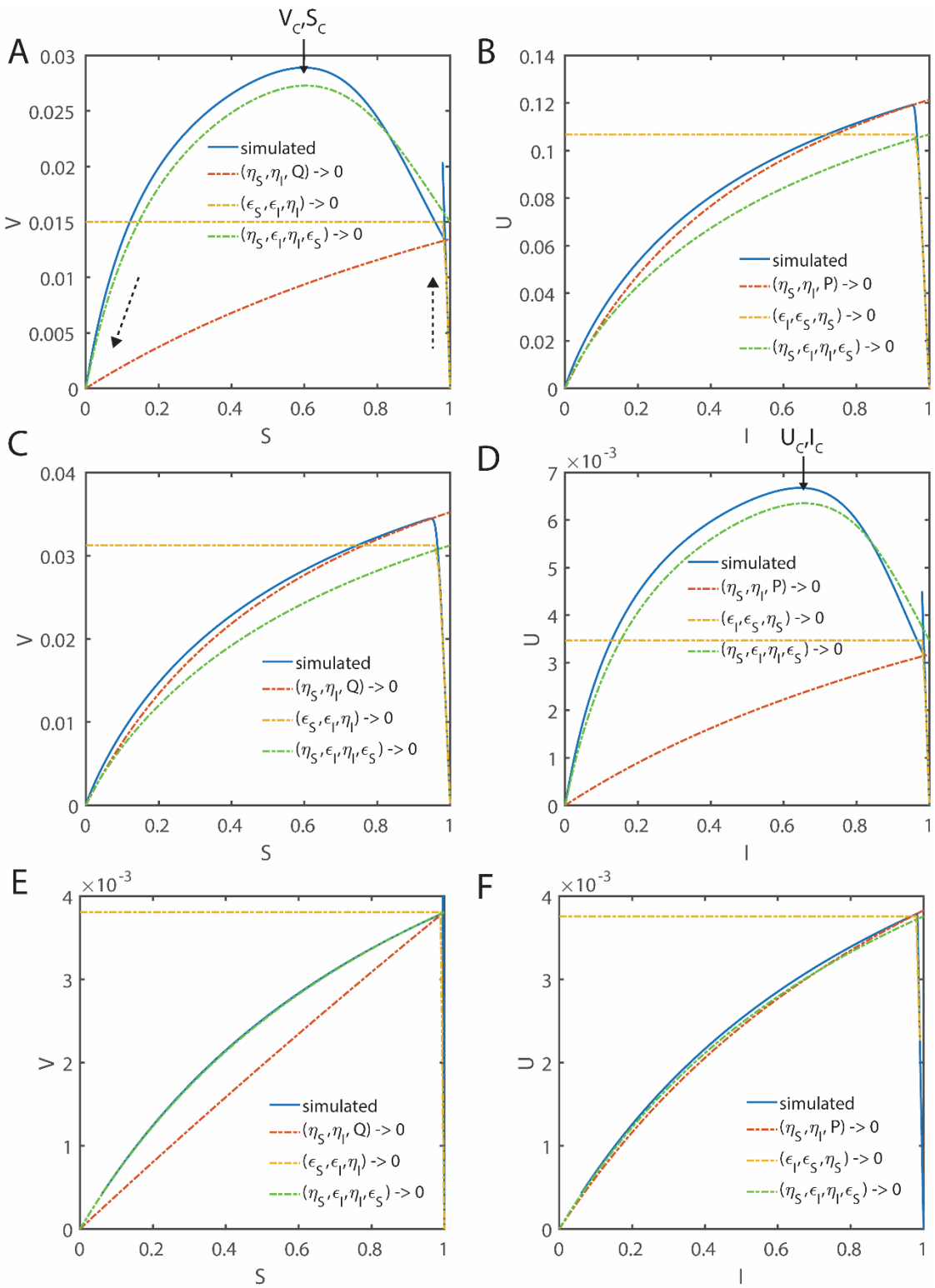

FIGURE 4. Pre-steady state and post-steady state approximations of the fully competitive enzyme kinetics in the velocity-substrate (V, S), velocity-inhibitor spaces (U, I) at different values of δ. The phase-space trajectories start at S = 1 and I = 1, and end at S = 0 and I = 0 with maxima at the

steady state. We considered the approximations viz. $\lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0}(V,U)$ which is the standard QSSA, $\lim_{(\eta_I,\varepsilon_S,\varepsilon_I)\to 0} V$ and $\lim_{(\eta_S,\eta_I,Q)\to 0} V$ in the (V, S) space as given by **Eqs. 6.12** and 23.4 respectively and, $\lim_{(\eta_S,\varepsilon_S,\varepsilon_I)\to 0} U$ and $\lim_{(\eta_S,\eta_I,P)\to 0} U$ in the (U, I) space as given by the **Eqs. 6.16** and **Eqs. 29.3-4** respectively. Here the common initial conditions for the numerical simulation of **Eqs. 3.1-3** are $(S,I,E,X,Y,P,Q) = (1,1,1,0,0,0,0)$ at τ = 0. **A-B**. The simulation settings are $\eta_S = 0.002, \varepsilon_S = 0.04,\ \kappa_S = 0.2,\ \eta_I = 0.01, \varepsilon_I = 0.06, \kappa_I\ = 0.1$ and ρ = 3.333, σ = 1, δ = 0.1405, ϒ = 3. **C-D**. Here the simulation settings are $\eta_S = 0.02, \varepsilon_S = 0.06,\ \kappa_S = 0.1,\ \eta_I = 0.003, \varepsilon_I = 0.04, \kappa_I\ = 0.2$ and ρ = 0.225, σ = 1, δ = 9, ϒ = 0.33. **E-F**. Here the simulation settings are $\eta_S = 0.02, \varepsilon_S = 0.06,\ \kappa_S = 8.1,\ \eta_I = 0.003, \varepsilon_I = 0.04, \kappa_I\ = 1.2$ and ρ = 0.225, σ = 1, δ = 1.013, ϒ = 4.5.

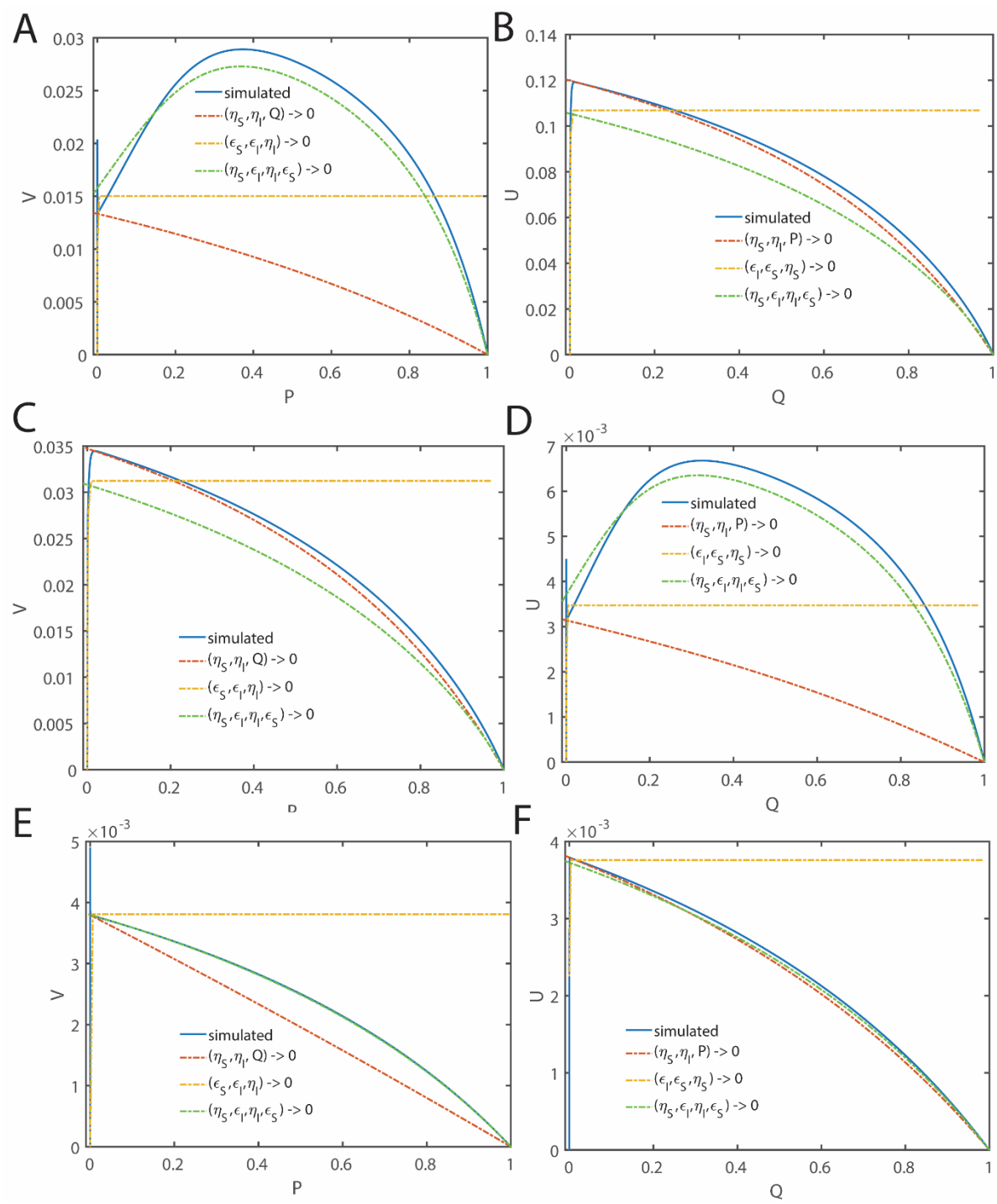

FIGURE 5. Pre-steady state and post-steady state approximations of the fully competitive enzyme kinetics in the velocity-product of substrate (V, P), velocity-product of inhibitor spaces (U, Q) at different values of δ. The phase-space trajectories start at P = 0 and Q = 0, and end at P = 1 and Q = 1 with maxima at the steady state. We considered the approximations under various limiting conditions viz. $\lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0} (V, U)$ which is the standard QSSA, $\lim_{(\eta_I,\varepsilon_S,\varepsilon_I)\to 0} V$ and $\lim_{(\eta_S,\eta_I,Q)\to 0} V$ in the pre-steady state regime of the (V, P) space obtained from **Eqs. 6.12** and **23.4** respectively using

mass conservation relationship V + P + S = 1 and expressing V and P in terms of S in a parametric form, $\lim_{(\eta_S,\varepsilon_S,\varepsilon_I)\to 0} U$ and $\lim_{(\eta_S,\eta_I,P)\to 0} U$ in the pre-steady state regime of the (U, Q) space obtained from **Eqs. 6.16** and **Eqs. 29.3-4** respectively using mass conservation relationship U/ρ + Q + I = 1 and expressing U and Q in terms of I in a parametric form. Here the common initial conditions for the simulation of **Eqs. 3.1-3** are $(S, I, E, X, Y, P, Q) = (1,1,1,0,0,0,0)$ at τ = 0. **A-B**. Here the simulation settings are $\eta_S = 0.002, \varepsilon_S = 0.04,\ \kappa_S = 0.2,\ \eta_I = 0.01, \varepsilon_I = 0.06, \kappa_I\ = 0.1$ and ρ = 3.333, σ = 1, δ = 0.1405, ϒ = 3. **C-D**. Here the simulation settings are $\eta_S = 0.02, \varepsilon_S = 0.06,\ \kappa_S = 0.1,\ \eta_I = 0.003, \varepsilon_I = 0.04, \kappa_I\ = 0.2$ and ρ = 0.225, σ = 1, δ = 9, ϒ = 0.33. **E-F**. Here the simulation settings are $\eta_S = 0.02, \varepsilon_S = 0.06,\ \kappa_S = 8.1,\ \eta_I = 0.003, \varepsilon_I = 0.04, \kappa_I\ = 1.2$ and ρ = 0.225, σ = 1, δ = 1.013, ϒ = 4.5.

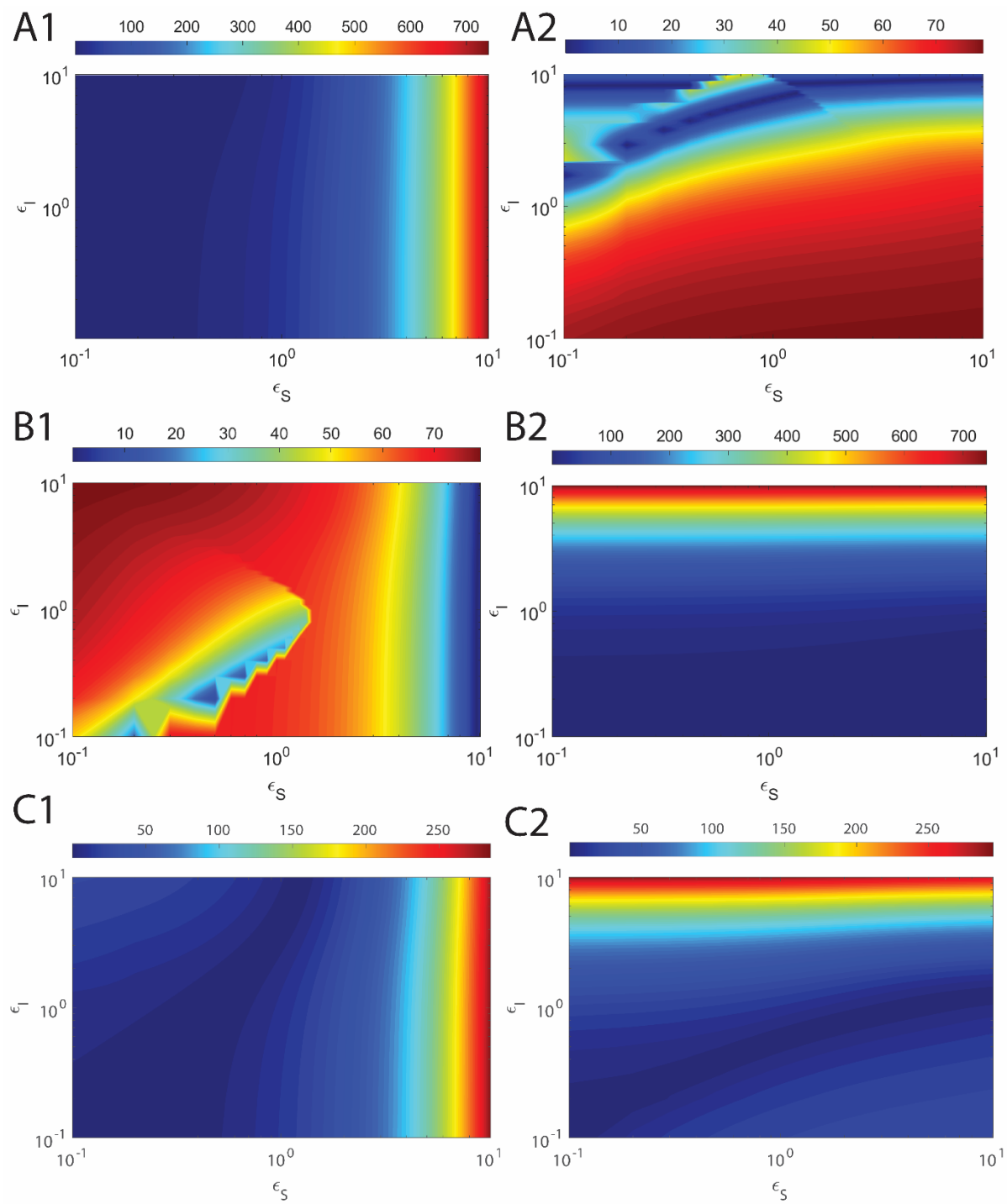

FIGURE 6. Error associated with the standard quasi steady state approximation with stationary reactant assumption of the fully competitive enzyme kinetics used in the literature over the velocity-substrate (V, S), velocity-inhibitor spaces (U, I) at different values of $\varepsilon_S, \varepsilon_I$. Here δ will vary with respect to each iteration. We considered the error in the approximations of the reaction velocities under various limiting conditions viz. $\lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0}(V,U)$ and stationary reactant assumption as defined in **Eqs. 6.2**. The error (measured in %) was computed as error (%) = (steady

state velocities from simulation – approximated velocities) / simulated velocities. Here the simulation settings are $\eta_S = 0.02$, $\eta_I = 0.01$ and σ = 1. With these settings, upon fixing σ one finds that $\rho = \frac{\eta_I \varepsilon_S}{\sigma \eta_S \varepsilon_I}$ and $\delta = \frac{\widetilde{\mu}_I \varepsilon_S}{\rho \widetilde{\mu}_S \varepsilon_I}$ as defined in **Eqs. 8.1**. **A1, B1, C1**. Error % in QSSA of V. **A2**, **B2, C2**. Error % in QSSA of U. **A1-2.** $\kappa_S = 0.1, \kappa_I = 1$. **B1-2**. $\kappa_S = 1, \kappa_I = 0.1$. **C1-2**. $\kappa_S = 1, \kappa_I = 1$.

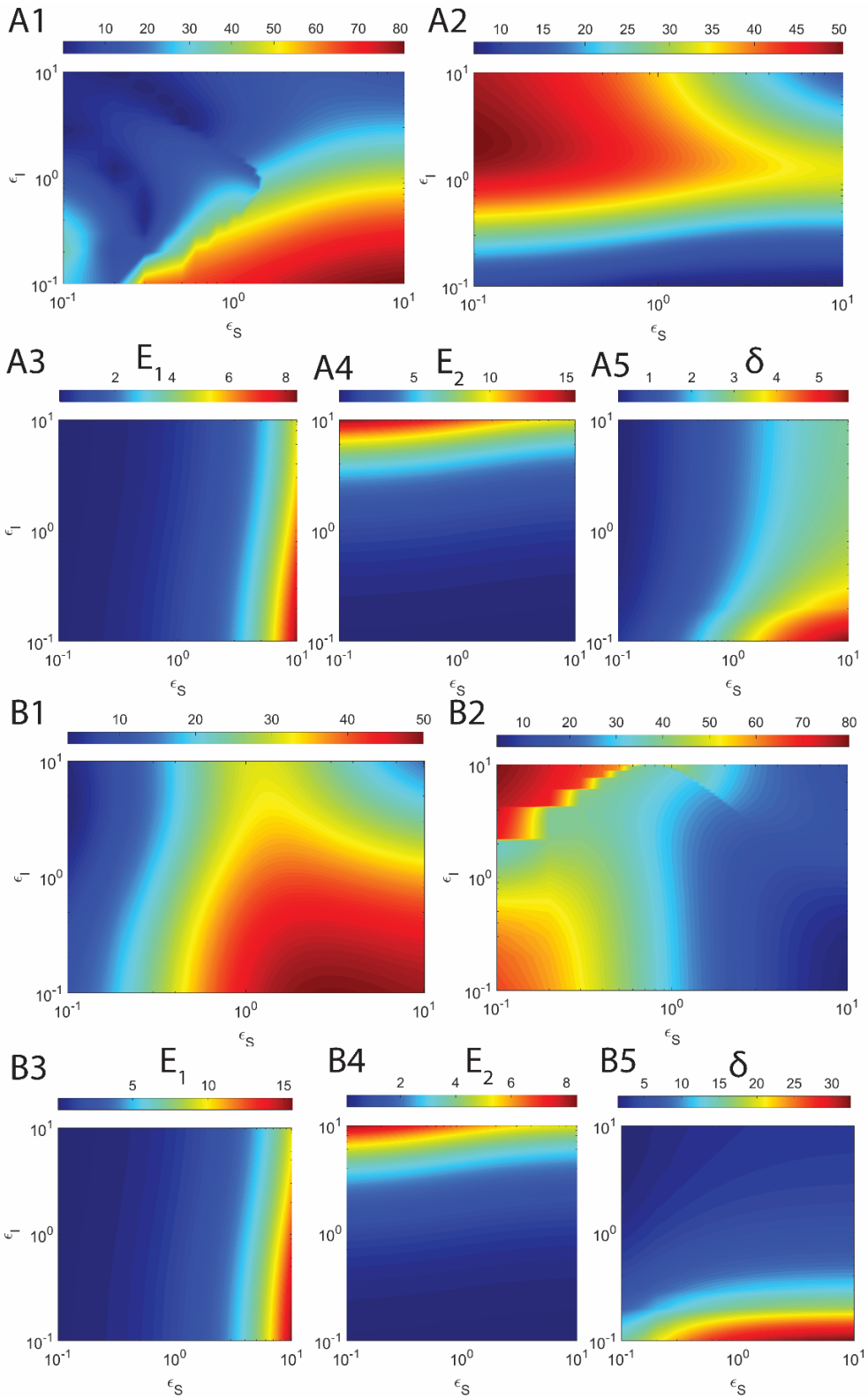

FIGURE 7. Error associated with the refined standard quasi steady state approximation with stationary reactant assumption of the fully competitive enzyme kinetics in the velocity-substrate

(V, S), velocity-inhibitor spaces (U, I) at different values of $\varepsilon_S, \varepsilon_I$ under the conditions that $\kappa_S \neq \kappa_I$. Here E_1, E_2 and δ will vary with respect to each iteration. We considered the error in the approximations of the reaction velocities under various limiting conditions viz. $\lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0}(V,U)$ as defined in **Eqs. 6.19** with (S, I) = (1, 1). The error (measured in %) was computed as error (%) = (steady state velocities from simulation – approximated velocities) / simulated velocities. Here the simulation settings for **A1-5** are $\eta_S = 0.02$, $\kappa_S = 1$, $\eta_I = 0.01, \kappa_I = 0.1$ and σ = 1. Simulation settings for **B1-5** are $\eta_S = 0.02$, $\kappa_S = 0.1$, $\eta_I = 0.01, \kappa_I = 1$ and σ = 1. With these settings, upon fixing σ one finds that $\rho = \frac{\eta_I \varepsilon_S}{\sigma \eta_S \varepsilon_I}$ and $\delta = \frac{\tilde{\mu}_I \varepsilon_S}{\rho \tilde{\mu}_S \varepsilon_I}$ as defined in **Eqs. 8.1** along with the inequality conditions E_1, and E_2 as defined in **Eqs. 31.12-13**. **A1, B1**. Error % in QSSA of V. **A2**, **B2**. Error % in QSSA of U. **A3, B3**. E_1 (**Eq. 31.12**). **A4, B4**. E_2 (**Eq. 31.13**). **A5, B5**. δ as defined in **Eqs. 8.1**.

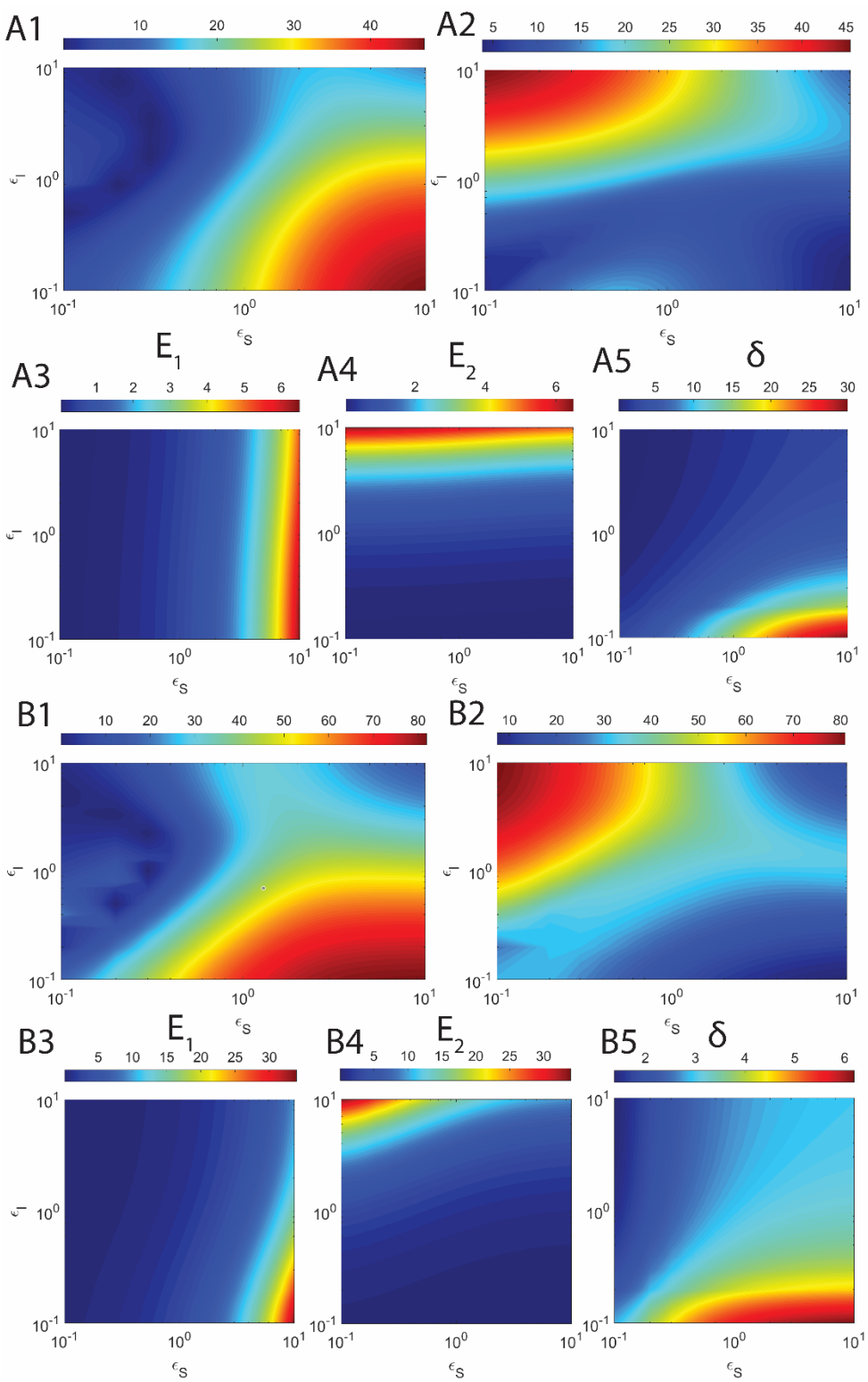

FIGURE 8. Error associated with the standard quasi steady state approximation with stationary reactant assumption of the fully competitive enzyme kinetics in the velocity-substrate (V, S), velocity-inhibitor spaces (U, I) at different values of $\varepsilon_S, \varepsilon_I$ under the condition that $\kappa_S = \kappa_I$. Here

E_1, E_2 and δ will vary with respect to each iteration. We considered the error in the approximations of the reaction velocities as defined in **Eqs. 6.19** with (S, I) = (1, 1). The error (measured in %) was computed as error (%) = (steady state velocities from simulation – approximated velocities) / simulated velocities. Here the simulation settings for **A1-5** are $\eta_S = 0.02$, $\kappa_S = \kappa_I = 1, \eta_I = 0.01$ and σ = 1. Similar simulation settings for **B1-5** with $\kappa_S = \kappa_I = 0.1$. With these settings, upon fixing σ one finds that $\rho = \frac{\eta_I \varepsilon_S}{\sigma \eta_S \varepsilon_I}$ and $\delta = \frac{\widetilde{\mu}_I \varepsilon_S}{\rho \widetilde{\mu}_S \varepsilon_I}$ as defined in **Eqs. 8.1** along with the inequality conditions E_1, and E_2 as defined in **Eqs. 31.12-13**. **A1, B1**. Error % in QSSA of V. **A2**, **B2**. Error % in QSSA of U. **A3, B3**. E_1 (**Eq. 31.12**). **A4, B4**. E_2 (**Eq. 31.13**). **A5, B5**. δ as defined in **Eqs. 8.1**.

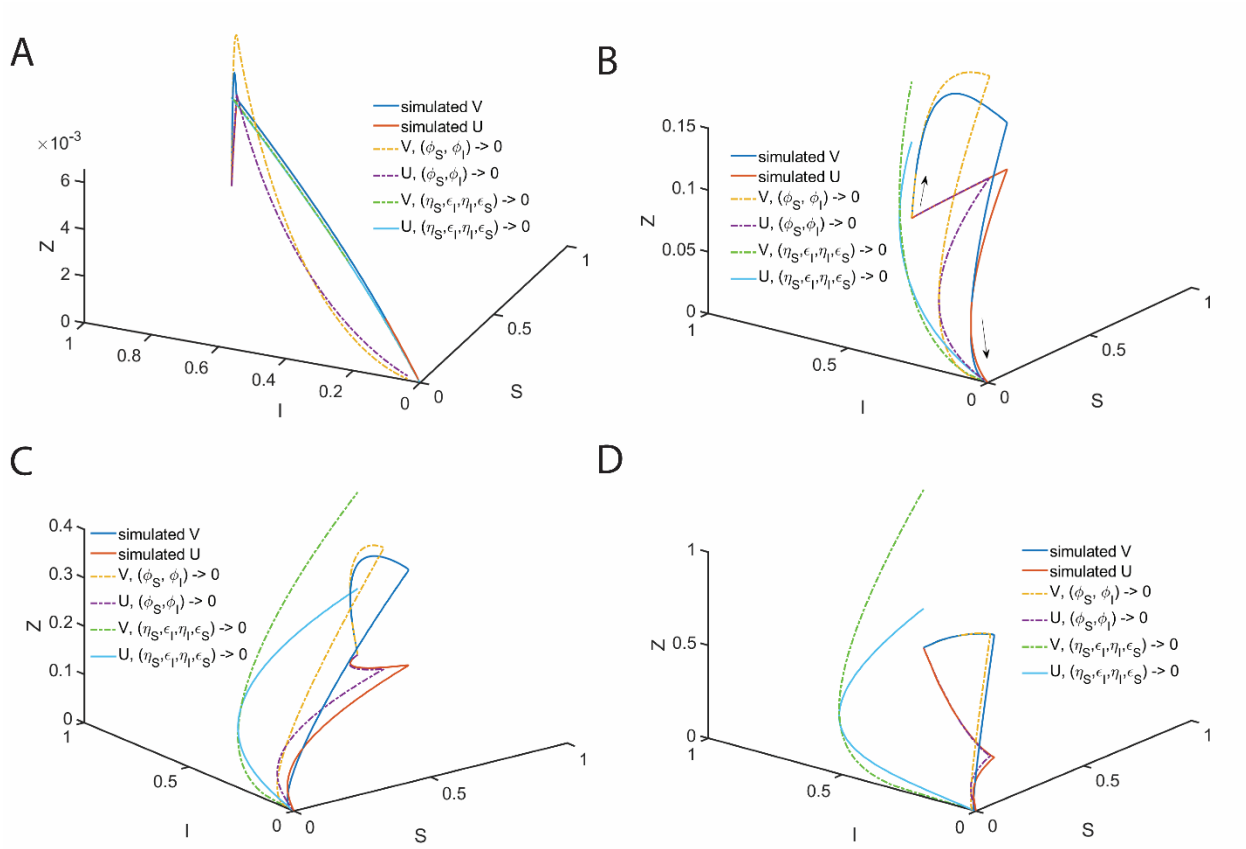

FIGURE 9. Approximate solutions in the velocity-substrate-inhibitor spaces (V, I, S) and (U, I, S). We considered the approximations of the reaction velocities (Z = V and U) under various limiting conditions viz. $\lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0}(V,U)$ which is the refined standard QSSA as given in **Eqs. 6.19** and **Eqs. 8.1** (for the relationship between S and I) and $\lim_{(\phi_I,\phi_S)\to 0} V$, $\lim_{(\phi_I,\phi_S)\to 0} U$ as given by the solutions of the coupled approximate linear ODEs **Eqs. 9.4** and **9.5** as given in **Appendix A** in a parametric form where τ act as the parameter Here the common initial conditions for the numerical simulation of **Eqs. 3.1-3** are $(S,I,E,X,Y,P,Q) = (1,1,1,0,0,0,0)$ at τ = 0 and other simulation settings are $\eta_S = 0.02$, $\kappa_S = 8.1$, $\eta_I = 0.003, \kappa_I = 1.2$ and σ = 1. **A**. $\varepsilon_S = 0.06, \varepsilon_I = 0.04$, ρ = 0.1, δ = 1.013, ϒ = 4.5. **B**. $\varepsilon_S = 0.6, \varepsilon_I = 0$, ρ = 0.1, δ = 1.41, ϒ = 10.12. **C**. $\varepsilon_S = 6, \varepsilon_I = 4$, ρ = 0.1, δ = 3.96, ϒ = 10.12. **D**. $\varepsilon_S = 60, \varepsilon_I = 40$, ρ = 0.1, δ = 8.48, ϒ = 10.13.

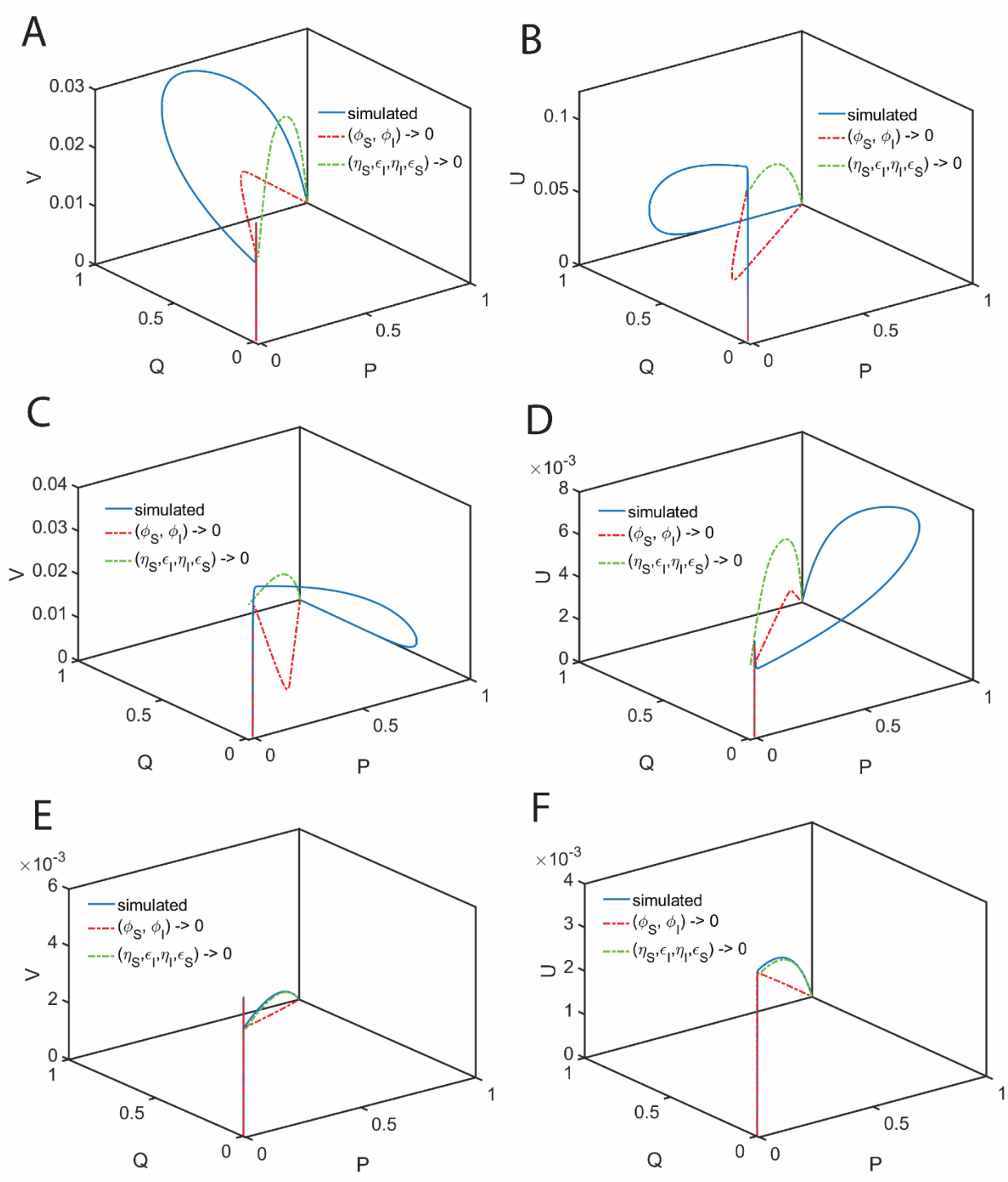

FIGURE 10. The ϕ-approximations of the fully competitive enzyme kinetics in the velocity-products spaces (V, P, Q) and (U, P, Q) at different values of δ. The phase-space trajectories start at P = 0 and Q = 0, and end at P = 1 and Q = 1 with maxima at the steady state. We considered the ϕ-approximations which are the solutions of **Eqs. 9.4-5** as given in **Appendix A** in a parametric form where τ act as the parameter and standard QSSA solutions in the limit $\lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0}(V,U)$

in a parametric form where S acts as the parameter as given in **Eqs. 6.19** and **Eqs. 8.1**. Common initial conditions for the numerical simulation of **Eqs. 3.1-3** are $(S, I, E, X, Y, P, Q) = (1,1,1,0,0,0,0)$ at τ = 0. **A-B**. The simulation settings are $\eta_S = 0.002, \varepsilon_S = 0.04,\ \kappa_S = 0.2,\ \eta_I = 0.01, \varepsilon_I = 0.06, \kappa_I = 0.1$ and ρ = 3.333, σ = 1, δ = 0.1405, ϒ = 3. **C-D**. Here the simulation settings are $\eta_S = 0.02, \varepsilon_S = 0.06,\ \kappa_S = 0.1,\ \eta_I = 0.003, \varepsilon_I = 0.04, \kappa_I = 0.2$ and ρ = 0.225, σ = 1, δ = 9, ϒ = 0.33. **E-F**. Here the simulation settings are $\eta_S = 0.02, \varepsilon_S = 0.06,\ \kappa_S = 8.1,\ \eta_I = 0.003, \varepsilon_I = 0.04, \kappa_I = 1.2$ and ρ = 0.225, σ = 1, δ = 1.013, ϒ = 4.5.

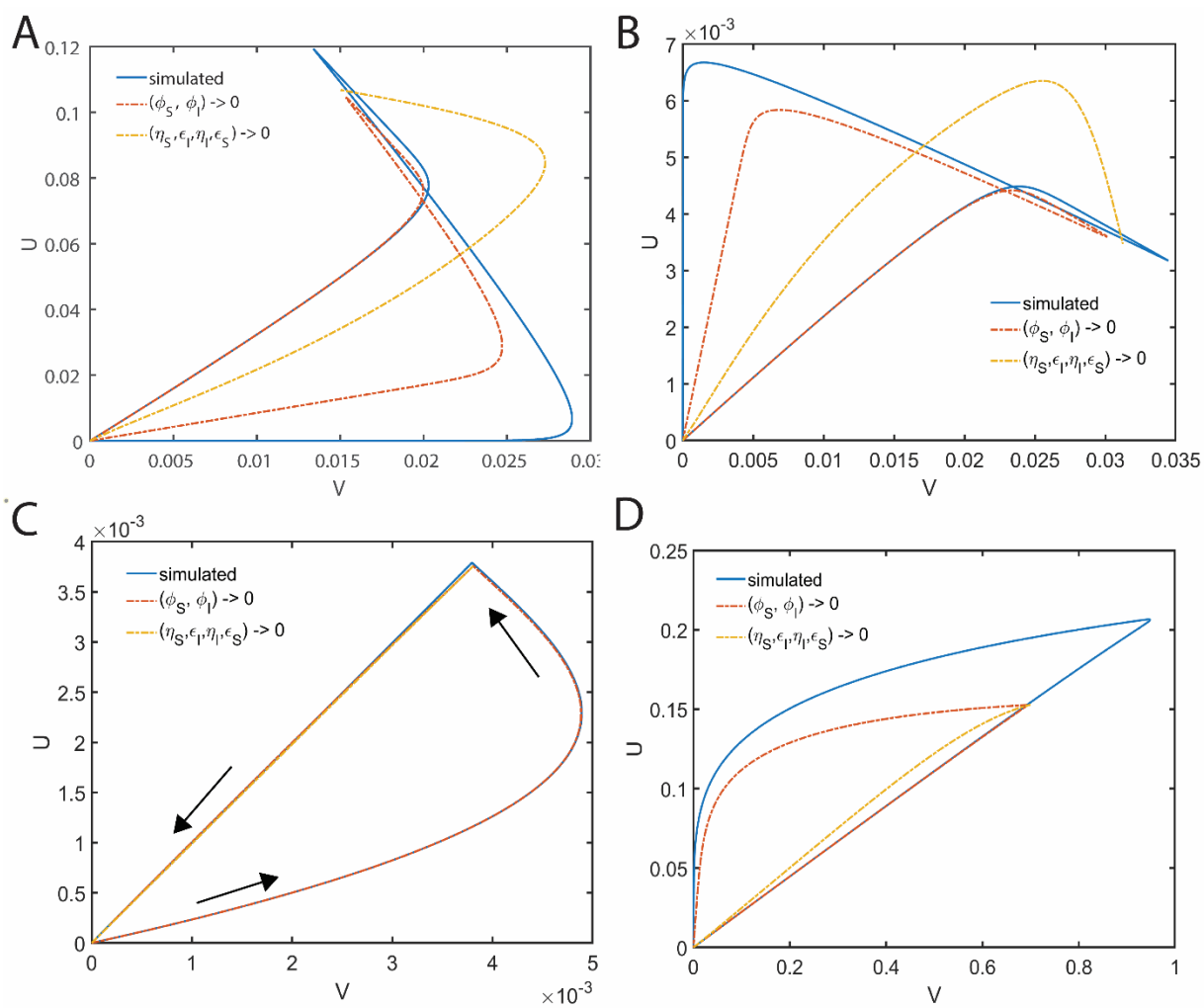

FIGURE 11. The ϕ-approximations of the fully competitive enzyme kinetics in the velocity spaces (V, U) at different values of δ. The phase-space trajectories start at V = 0 and V = 0, and end at V = 0 and U = 0. We considered the ϕ-approximations which are the solutions of **Eqs. 9.4-5** as given in **Appendix A** in a parametric form where τ act as the parameter and standard QSSA solutions in the limit $\lim_{(\eta_S,\eta_I,\varepsilon_S,\varepsilon_I)\to 0}(V,U)$ in a parametric form where S acts as the parameter as given in **Eqs. 6.19** and **Eqs. 8.1**. Common initial conditions for the numerical simulation of **Eqs. 3.1-3** are $(S,I,E,X,Y,P,Q) = (1,1,1,0,0,0,0)$ at τ = 0. The trajectory in the (V, U) space starts at (V, U) = (0, 0) and ends at (V, U) = (0, 0). Arrow in **C** indicates the direction of the trajectory evolution. **A**. The simulation settings are $\eta_S = 0.002, \varepsilon_S = 0.04,\ \kappa_S = 0.2,\ \eta_I = 0.01, \varepsilon_I = 0.06, \kappa_I = 0.1$ and ρ = 3.333, σ = 1, δ = 0.1405, ϒ = 3. **B**. Here the simulation settings are $\eta_S = 0.02, \varepsilon_S = 0.06,\ \kappa_S = 0.1,\ \eta_I = 0.003, \varepsilon_I = 0.04, \kappa_I = 0.2$ and ρ = 0.225, σ = 1, δ = 9, ϒ = 0.33. **C**. Here the simulation settings are $\eta_S = 0.02, \varepsilon_S = 0.06,\ \kappa_S = 8.1,\ \eta_I = 0.003, \varepsilon_I = 0.04, \kappa_I = 1.2$ and ρ = 0.225, σ = 1, δ = 1.013, ϒ = 4.5. **D**. Here the simulation settings are $\eta_S = 0.02, \varepsilon_S = 6,\ \kappa_S = 0.1,\ \eta_I = 0.003, \varepsilon_I = 4, \kappa_I = 0.2$ and ρ = 0.225, σ = 1, δ = 4.58, ϒ = 0.33

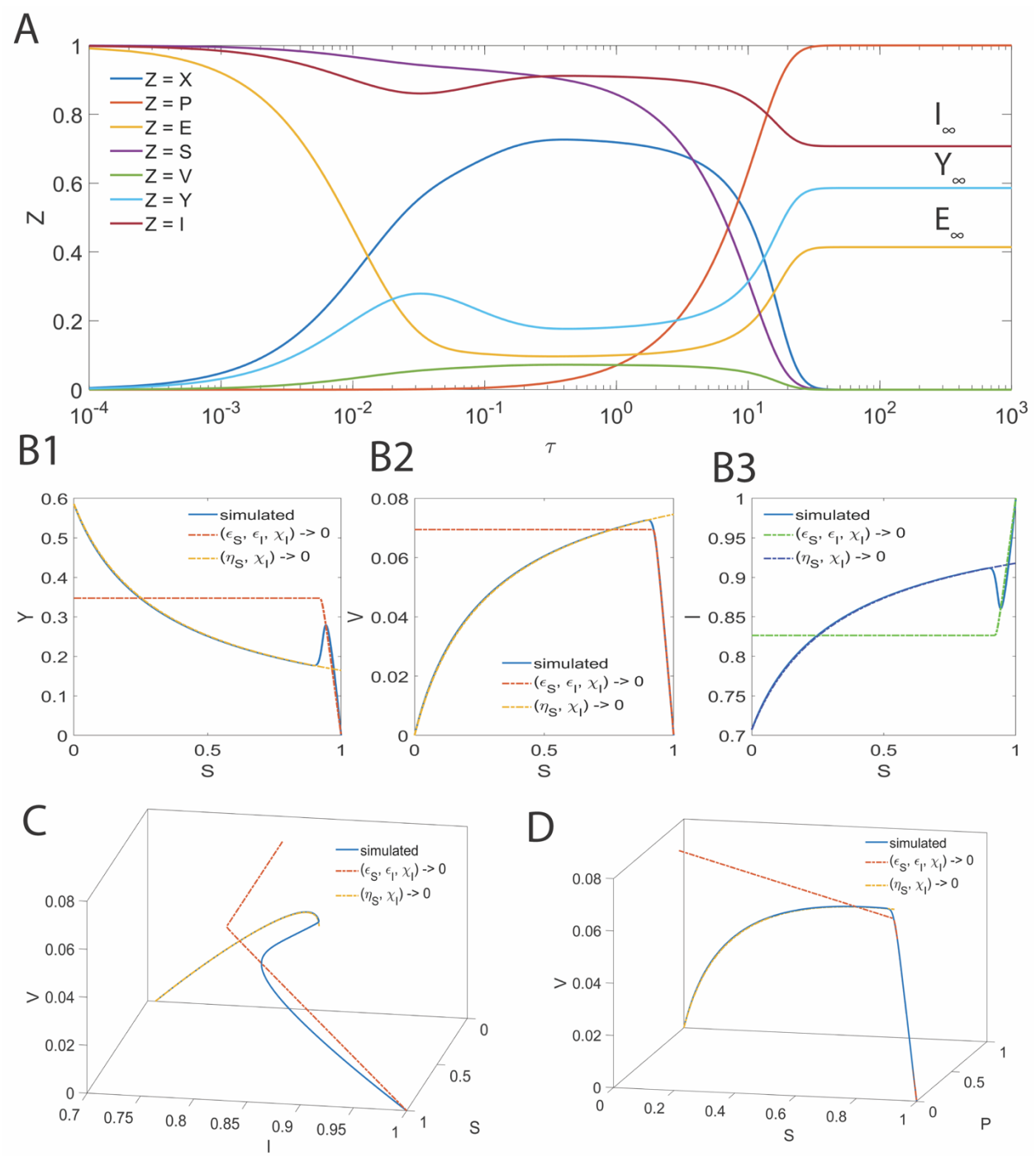

FIGURE 12. Pre- and post-steady state approximations of partially competitive inhibition scheme. Here the settings are $\eta_S = 0.02, \varepsilon_S = 0.1,\ \kappa_S = 0.1,\ \chi_I = 0.03, \varepsilon_I = 0.06, \kappa_I = 0.5$. Common initial conditions for the numerical simulation of **Eqs. 34.1-3** are $(S, I, E, X, Y, P) = (1,1,1,0,0,0)$ at τ = 0. Post-steady state approximations were generated under the conditions that $(\eta_S, \chi_I) \to 0$. Whereas, the pre-steady state approximations were computed under the conditions that $(\varepsilon_S, \chi_I, \varepsilon_I) \to 0$. **A.** Sample simulation trajectories of (S, I, E, X, Y, P). Clearly, (I, E, Y) ends at the equilibrium states $(I_\infty, E_\infty, Y_\infty)$ upon complete depletion of the substrate. **B1-3**, **C** and **D**. Phase-

space plots of trajectories in A along with the approximations in the pre- and post-steady state regimes. **B1**. (Y, S) space trajectory and approximations are computed using **Eqs. 44.4** and **48.6**. **B2**. (V, S) space trajectory with approximations using **Eqs. 44.2**. **B3**. (S, I) space trajectory and approximations using the mass conservation relationship $I = 1 - \varepsilon_I Y$. **C**. (V, S, I) space approximations. **D**. (V, P, S) space approximations.

TABLE 1. Summary of variables and parameters used in the theory section

Parameters / Variables	Definition	Remarks
e, s, i, p, q, x, y, v, u	Concentration of enzyme, substrate, inhibitor, product of substrate, product of inhibitor, enzyme-substrate complex, enzyme-inhibitor complex, velocity of substrate-product formation, velocity of inhibitor-product formation.	mol/lit, M
e_0, s_0, i_0	Initial enzyme, substrate and inhibitor concentrations.	M
E, S, I, P, Q, X, Y, V, U	$S = \frac{s}{s_0}, E = \frac{e}{e_0}, P = \frac{p}{s_0}, Y = \frac{y}{e_0}, X = \frac{x}{e_0}, I = \frac{i}{i_0}, Q = \frac{q}{i_0}$ are the normalized concentrations of substrate, enzyme, product of substrate, enzyme-inhibitor complex, enzyme-substrate complex, inhibitor and product of inhibitor, velocity of product of substrate formation, and velocity of product of inhibitor formation.	dimensionless
E_C, S_C, I_C, P_C, Q_C, X_C, Y_C, V_C, U_C	Steady state values.	dimensionless
k_2, k_3	Unimolecular product formation rate constants.	1/second
τ	$= k_2 t$	dimensionless
v_{max}, u_{max}	$v_{max} = k_2\, e_0$, $u_{max} = k_3\, e_0$	M/second
k_1, k_i	Bimolecular rate constants associated with the binding of substrate and inhibitor respectively with the same enzyme.	1 / (M second)
k_{-1}, k_{-i}	Dissociation rate constants associated with the enzyme-substrate and enzyme-inhibitor complexes.	1/second
K_{RS}, K_{RI}	$K_{RS} = k_2 / k_1$, $K_{RI} = k_3 / k_i$	M
K_{DS}, K_{DI}	$K_{DS} = k_{-1} / k_1$, $K_{DI} = k_{-i} / k_i$	M
K_{MS}, K_{MI}	$K_{MS} = K_{RS} + K_{DS}$, $K_{MI} = K_{RI} + K_{DI}$	M
η_S, η_I	$\eta_S = k_2 / k_1\, s_0$, $\eta_I = k_3 / k_i\, i_0$	dimensionless
ε_S, ε_I, ε_{IS}	$\varepsilon_S = e_0 / s_0$, $\varepsilon_I = e_0 / i_0$, $\varepsilon_{IS} = \varepsilon_I / \varepsilon_S = s_0 / i_0$	dimensionless
κ_S, κ_I	$\kappa_S = k_{-1} / k_1 s_0$, $\kappa_I = k_{-i} / k_i\, i_0$	dimensionless
χ_I	$= k_2 / k_i i_0$ defined for the partially competitive inhibition scheme.	dimensionless
ρ	$= k_3 / k_2 = u_{max} / v_{max}$	dimensionless
σ	$= k_1 / k_i$	dimensionless
μ_S, μ_I	$\mu_S = \eta_S + \kappa_S$, $\mu_I = \eta_I + \kappa_I$	dimensionless
Υ	$= k_{-1} / k_{-i}$	dimensionless
δ	$= \tilde{\mu}_I \varepsilon_S / \rho \tilde{\mu}_S \varepsilon_I = (v_{max}/\tilde{\mu}_S)/(u_{max}/\tilde{\mu}_I)$	dimensionless
$\tilde{\mu}_I$	$= \varepsilon_I + \kappa_I + \eta_I = \varepsilon_S + \mu_S$	dimensionless

$\tilde{\mu}_S$	$= \varepsilon_S + \kappa_S + \eta_S = \varepsilon_S + \mu_I$	dimensionless
$\tilde{\kappa}_I$	$= \varepsilon_I + \kappa_I$	dimensionless
α_S	$= 1 + \varepsilon_S + \kappa_S + \eta_S$	dimensionless
α_I	$= 1 + \varepsilon_I + \kappa_I + \eta_I$	dimensionless
β_I	$= 1 + \varepsilon_I + \kappa_I$	dimensionless
ϕ_S	$= \eta_S \varepsilon_S$	dimensionless
ϕ_I	$= \eta_I \varepsilon_I$	dimensionless
f_S, f_S	$f_S = v_{max}/\tilde{\mu}_S$, $f_I = u_{max}/\tilde{\mu}_I$ are the reaction acceleration factors associated with the conversion of substrate and inhibitor into their products.	dimensionless

TABLE 2. Phase-space approximations of fully competitive inhibition scheme

Conditions	Approximations	Remarks
$(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I) \to 0$	$V \cong \frac{\varepsilon_S S}{\left(S + \tilde{\mu}_S\left(1 + \frac{S^{1/\delta}}{\tilde{\mu}_I}\right)\right)}$; $U \cong \frac{\rho \varepsilon_I I}{I + \tilde{\mu}_I\left(1 + \frac{I^{\delta}}{\tilde{\mu}_S}\right)}$ In (V, P, S) space $S \in (0,1)$ and $P = 1 - V - S$. In (U, Q, I) space $I \in (0,1)$ and $Q = 1 - U/\rho - I$.	Post steady state regime of (V, S) and (U, I) spaces. **Eqs. 8.2**
$(\eta_S, \eta_I, Q) \to 0$	$V \cong \frac{\varepsilon_S S}{2\varepsilon_I \mu_S (S + \mu_S)}\left((S + \mu_S)\alpha_I + (S + 2\mu_S)\varepsilon_I + \sqrt{\mu_I{}^2 S^2 + 2\alpha_I \mu_S \mu_I S - 4\left(-\frac{\alpha_I{}^2}{4} + \varepsilon_I\right)\mu_S{}^2} + S\right)$ In (V, P, S) space $S \in (0,1)$ and $P = 1 - V - S$.	Post-steady state regime of the (V, S) space. **Eq. 6.12**
$(\eta_S, \eta_I, P) \to 0$	$U \cong \frac{I \rho \varepsilon_I}{2\varepsilon_S \mu_I (I + \mu_I)}\left(-(I + \mu_I)\alpha_S + (I + 2\mu_I)\varepsilon_S - \sqrt{\mu_S{}^2 I^2 + 2\alpha_S \mu_S \mu_I I - 4\mu_I{}^2\left(-\frac{\alpha_S{}^2}{4} + \varepsilon_S\right)} + I\right)$ In (U, Q, I) space $I \in (0,1)$ and $Q = 1 - U/\rho - I$.	Post-steady state regime of the (U, I) space. **Eq. 6.16**
$(\varepsilon_S, \eta_I, \varepsilon_I) \to 0$	$V \cong \frac{b\varepsilon_S \eta_S{}^2}{a}\left(1 - \frac{1}{R}\text{LambertW}\left(R exp\left(R + \frac{a^2(S-1)}{b\varepsilon_S \eta_S{}^2}\right)\right)\right)$ In (V, P, S) space $S \in (0,1)$ and $P = 1 - V - S$. $a = \frac{\alpha_S}{\eta_S}\left(1 - \frac{1}{\alpha_S \alpha_I}\right)$; $b = \frac{1}{\eta_S{}^3}\left(1 - \frac{1}{\alpha_I}\right)$; $R = a - 1$	Pre-steady state regime of the (V, S) space. **Eq. 23.4**
$(\varepsilon_I, \eta_S, \varepsilon_S) \to 0$	$U \cong R\left(1 - \text{LambertW}\left(-exp\left(\frac{(g - \rho)}{\rho} + \frac{g^2(I-1)}{h\rho\varepsilon_I \eta_I{}^2}\right)\right)\right)$ $g = \frac{\rho\alpha_I}{\eta_I}\left(1 - \frac{1}{\alpha_I \alpha_S}\right)$; $h = \frac{\rho^2}{\eta_I{}^3}\left(1 - \frac{1}{\alpha_S}\right)$; $R = \frac{b\varepsilon_I \eta_I{}^2}{g}$ In (U, Q, I) space $I \in (0,1)$ and $Q = 1 - U/\rho - I$	Pre-steady state regime of the (U, I) space. **Eq. 29.3**

$(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I) \to 0$	$V \cong \frac{\varepsilon_S S}{S+\tilde{\mu}_S\left(1+\frac{I}{\tilde{\mu}_I}\right)}$; $U \cong \frac{\rho\varepsilon_I I}{I+\tilde{\mu}_I\left(1+\frac{S}{\tilde{\mu}_S}\right)}$ In (V, S, I) space, $S \in (0,1)$ and $I \cong S^{1/\delta}$. In (U, S, I) space, $I \in (0,1)$ and $S \cong I^{\delta}$	Post steady state regime in the (V, S, I) and (U, S, I) spaces. **Eqs. 8.1** and **6.19**
$(\eta_S, \eta_I, \varepsilon_S, \varepsilon_I) \to 0$	$V \cong \frac{\varepsilon_S(1-P)}{(1-P)+\tilde{\mu}_S\left(1+\frac{1-Q}{\tilde{\mu}_I}\right)}$; $U \cong \frac{\rho\varepsilon_I(1-Q)}{(1-Q)+\tilde{\mu}_I\left(1+\frac{1-P}{\tilde{\mu}_S}\right)}$ In (V, P, Q) space, $P \in (0,1)$ and $Q \cong 1-(1-P)^{\frac{1}{\delta}}$. In (U, P, Q) space, $Q \in (0,1)$ and $P \cong 1-(1-Q)^{\delta}$	Post steady state regime in the (V, P, Q) and (U, P, Q) spaces. **Eqs. 8.1** and **6.18**

TABLE 3. Phase-space approximations of partially competitive inhibition scheme

Conditions	Approximations	Remarks
$(\varepsilon_I, \chi_I, \phi_S) \to 0$	$V \cong \varepsilon_S \eta_S^2 \frac{c}{\sqrt{(a^2-4b)}}\left(\exp\left(\frac{\left(-a+\sqrt{(a^2-4b)}\right)\tau}{2}\right) - \exp\left(-\frac{\left(a+\sqrt{(a^2-4b)}\right)\tau}{2}\right)\right)$ $a = \frac{\alpha_S}{\eta_S}\left(1-\frac{1}{\alpha_S\beta_I}\right)$; $b = \frac{\varepsilon_S}{\eta_S}$; $c = \frac{1}{\eta_S{}^3}\left(1-\frac{1}{\beta_I}\right)$	Entire pre- and post-steady state regimes of (V, τ) space. **Eqs. 38.1**
$(\eta_S, \chi_I, \varepsilon_S, \varepsilon_I) \to 0$	$V \cong \frac{\varepsilon_S S}{S+\tilde{\mu}_S\left(1+\frac{1}{\tilde{\kappa}_I}\right)}$; $Y \cong \frac{1}{1+\tilde{\kappa}_I\left(1+\frac{S}{\tilde{\mu}_S}\right)}$ In (I, S) space, the approximation can be expressed in parametric form where $I = 1-\varepsilon_I Y$ and Y is expressed in terms of $S \in (0,1)$.	Post-steady state regime in (V, S) and (Y, S) and (I, S) spaces. **Eqs.35.8**
$\eta_S \to 0$	$Y \cong \frac{\eta_I}{2\varepsilon_I}\left(1-\frac{R}{\eta_I}\tanh\left(\operatorname{arctanh}\left(\frac{\eta_I}{R}\right)+\frac{R}{2\eta_I}\tau\right)\right)$ $R = \sqrt{\beta_I{}^2 - 4\varepsilon_I}$	Pre-steady state regime of (Y, τ) space. **Eqs. 40**
$(\varepsilon_S, \chi_I, \varepsilon_I) \to 0$	$V \cong \frac{\varepsilon_S(\beta_I-1)}{(\alpha_S\beta_I-1)}\left(1+\text{LambertW}\left(-exp\left(-1-\frac{\eta_S(\alpha_S\beta_I-1)^2}{\alpha_S{}^2\beta_I\varepsilon_S(\beta_I-1)}P\right)\right)\right)$	Pre-steady state regime of (V, P) space. **Eq. 44.1**.
$(\varepsilon_S, \chi_I, \varepsilon_I) \to 0$	$V \cong \frac{b\varepsilon_S\eta_S{}^2}{a}\left(1-\frac{1}{R}\text{LambertW}\left(Rexp\left(R+\frac{a^2(S-1)}{b\eta_S{}^2\varepsilon_S}\right)\right)\right)$ $a = \frac{\alpha_S}{\eta_S}\left(1-\frac{1}{\alpha_S\beta_I}\right)$; $b = \frac{1}{\eta_S{}^3}\left(1-\frac{1}{\beta_I}\right)$; $R = (a-1)$	Pre-steady state regime of (V, S) space. **Eq. 44.2**.

	In (V, P, S) space, $S \in (0,1)$ and P = 1 – V – S.	
$(\varepsilon_S, \chi_I, \varepsilon_I) \to 0$	$V \cong \frac{\varepsilon_S}{1+(\varepsilon_S+\mu_S)\left(1+\frac{1}{\varepsilon_I+\kappa_I}\right)}\left(1-exp(-R\tau)\right)$ $R = \frac{\alpha_S}{\eta_S}\left(1-\frac{1}{\alpha_S\beta_I}\right)$	Pre-steady state regime of (Y, τ) space. **Eqs. 46.1**
$(\varepsilon_S, \chi_I, \varepsilon_I) \to 0$	$Y \cong \frac{b\eta_S{}^2}{a\beta_I(a-1)}\left(R - \mathrm{LambertW}\left(Rexp\left(R + \frac{a^2(S-1)}{b\eta_S{}^2\varepsilon_S}\right)\right)\right)$ $a = \frac{\alpha_S}{\eta_S}\left(1-\frac{1}{\alpha_S\beta_I}\right); b = \frac{1}{\eta_S{}^3}\left(1-\frac{1}{\beta_I}\right); R = (a-1)$	Pre-steady state regime in the (Y, S) space. **Eq. 44.4**
$(\eta_S, \chi_I) \to 0$	$V \cong \frac{\varepsilon_S S}{(S+\mu_S)}\left(\frac{1}{2\varepsilon_I\mu_S}\left(-\kappa_I S + \mu_S\left(\varepsilon_I - (1+\kappa_I)\right) + \sqrt{\kappa_I{}^2S^2 - 2(1+\varepsilon_I+\kappa_I)\mu_S\kappa_I S - 4\mu_S^2 R}\right)\right)$ $R = \left(-\frac{(1+\varepsilon_I+\kappa_I)^2}{4} + \varepsilon_I\right)$ In (V, P, S) space, $S \in (0,1)$ and P = 1 – V – S.	Post-steady state regime of (V, S) space. **Eqs. 48.4**
$(\eta_S, \chi_I) \to 0$	$Y \cong \frac{(S+\mu_S)\alpha_I - S(1+\varepsilon_I) - \sqrt{\kappa_I{}^2S^2 - 2(1+\varepsilon_I+\kappa_I)\mu_S\kappa_I S - 4\mu_S^2 R}}{2\varepsilon_I\mu_S}$ $R = \left(-\frac{(1+\varepsilon_I+\kappa_I)^2}{4} + \varepsilon_I\right)$ In (V, S, I) space, $S \in (0,1)$ and I = 1 - $\varepsilon_I Y$.	Post-steady state regime of (Y, S) space. **Eq. 48.6**