SİİRT UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

SLOW AND FAST SUBSYSTEMS FOR COMPLEX BIOCHEMICAL REACTIONS

MASTER'S THESIS

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THESIS ACCEPTANCE AND APPROVAL

This thesis entitled "SLOW AND FAST SUBSYSTEMS FOR COMPLEX BIOCHEMICAL REACTIONS" submitted by AWDER SARDAR ABDALRAHMAN in partial fulfillment of the requirements for the degree of Master in Mathematics Department, Siirt University by,

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THESIS NOTIFICATION

I hereby declare that this thesis is my unique authorial work, which I have worked out by my own. Every information bases, references and literature used or excerpted through explanation of this work are correctly cited and listed in complete reference to the owing cause.

Awder Sardar Abdalrahman

SİİRT-2018

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LIST OF SYMBOLS

Symbols		**Explanation**
S	:	Susceptible
I	:	Infectious
R	:	Recovered
N	:	Total Population
M_0	:	Slow manifold
E	:	Enzyme
S	:	Substrate
P	:	Product
QSSA	:	Quasi stead state approximation
QE	:	Quasi equilibrium approximation

ÖZET

YÜKSEK LİSANS

(KOMPLEKS BİYOKİMYASAL TEPKİMELER İÇİN YAVAŞ VE HIZLI ALT SİSTEMLER)

Awder Sardar ABDALRAHMAN

Siirt Üniversitesi Fen Bilimleri Enstitüsü
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2018, 37 Sayfa

Bu çalışmada, bazı enzim inhibitörleri için matematiksel modellemeyi tanımlıyoruz ve bazı model azaltım tekniklerine dayalı değişken ve parametrenin sayısını en aza indiriyoruz. Bu tezi altı bölüme ayırdık. Bu tezin ilk bölümünde, matematiksel modelleme ve modelleme süreci ile ilgili bir giriş verilmektedir. İkinci bölüm, basit enzim reaksiyonlarının bir örneği ile biyokimyasal reaksiyonlar için model azaltma için iki yöntem sunmaktadır. Yöntemler, yarı kararlı durum yaklaşımı (QSSA) ve quasi equilibrium approximation (QEA) 'dur. Daha sonra, bölüm 3'te, rekabetçi inhibisyon modeli tanımlandık ve önerilen yöntemlerin, değişken ve parametrenin sayısını en aza indirgemek için uyguladık. Bundan sonra, rekabetsiz inhibisyon ve karışık inhibisyon gibi bazı daha karmaşık modeller tanımladık, önerilen teknikler temelinde eleman sayısını düşürdük ve bu modeller için sırasıyla dördüncü ve beşinci analitik çözümler hesapladık. Son olarak, sonuç ve öneriler bölüm altıda verilmektedir.

Anahtar Kelimeler: Matematiksel Modelleme, Kimyasal Kinetik, Quasi Kararlı Durum Yaklaşımı, Quasi Denge Yaklaşımı, Yavaş ve Hızlı Alt Sistemler, Yavaş Manifold

ABSTRACT

MS. THESIS

SLOW AND FAST SUBSYSTEMS FOR COMPLEX BIOCHEMICAL REACTIONS

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The Graduate School of Natural and Applied Science of Siirt University
The Degree of Master of Science
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Supervisor: Assoc. Prof.Dr. Ali AKGÜL

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In this study, we define mathematical modelling for some enzyme inhibitors and we minimize the number of variables and parameter based on some techniques of model reductions. We divided this thesis into the six sections. The first section of this thesis gives an introduction regarding to mathematical modeling and modeling process. The second section presents two methods of model reduction for biochemical reactions with an example of simple enzyme reactions. Methods are quasi-steady state approximations and quasi-equilibrium approximations. Then, in section three we define competitive inhibition model and we applied the proposed methods to minimize the number of variables and paraments. After that, we have defined some more complex models such as uncompetitive inhibition and mixed inhibition, we reduce the number of elements based on the suggested techniques and we calculate some analytical solution for such models in sections four and five, respectively. Finally, conclusions and recommendations are given in section six.

Keywords: Mathematical Modeling, Chemical Kinetics, Quasi Steady State Approximation, Quasi Equilibrium Approximation, Slow and Fast Subsystems, Slow Manifold.

1. INTRODUCTION

A mathematical model is a mathematical object established on a real situation and produced in the hope that its mathematical behavior resembles the real behavior. Mathematical modeling is the scientific art of creating, analyzing, validating, and construing mathematical models. Otherwise stated, mathematical modeling is a branch of applied mathematics that dealing with describing and/or predicting real-world system behavior. There are some examples of real-world systems: an object moving in a gravitational field; stock market fluctuations; predator-prey interactions; cell signaling pathways. To define a mathematical model, we should define three different things: variables, parameters and functional forms. (Baker, 2011; Ingalls, 2012; Kot, 2001; Sontag, 2014)

1.1. The Modeling Process

There are a variety of steps that can be used to convert an idea into a theoretical model and then into a quantitative model. It is clear that a theoretical model presents our idea in a model diagram that involving arrows and boxes. Mathematical equations are also used to define the rate of each process. In Figure 1.1, it can be seen that a series of steps are required to define modelling process. An example of modelling process is the SIR epidemic model (Lawson, et al., 2008).

A model diagram can be given:

$$S \xrightarrow{Transmission(\beta)} I \xrightarrow{Recovery(\alpha)} R, \qquad (1)$$

where S is susceptible population, I infectious population and R is recovered population Susceptible (S): Individuals susceptible to the disease. α and β are model parameters.

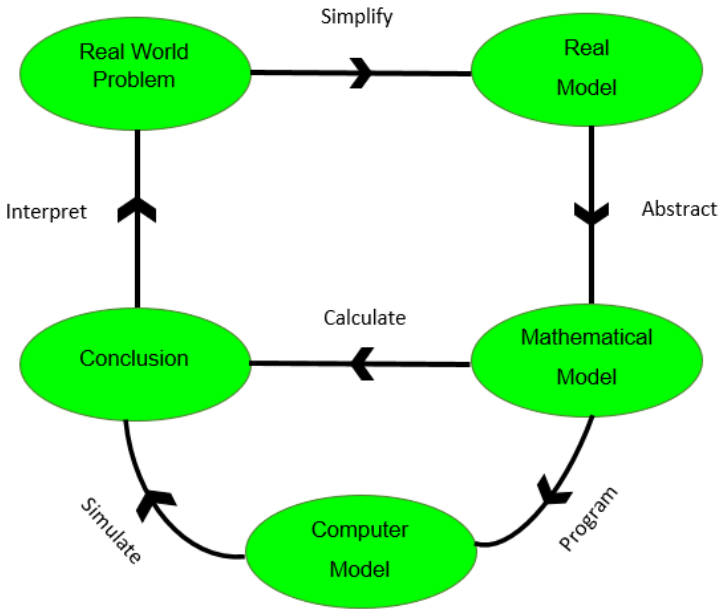

Figure 1.1. Mathematical modeling steps.

1.2. Elementary Reactions

The elementary reaction for chemical kinetics is given by the stoichiometric equation: (Gorban, et al., 2011)

$$\alpha_{p1}A_1 + \alpha_{p2}A_2 + \cdots + \alpha_{pn}A_n \rightarrow \beta_{p1}A_1 + \beta_{p2}A_2 + \cdots + \beta_{pn}A_n\,, \qquad (2)$$

where the stoichiometric coefficients $\alpha_{pi} \geq 0$ and $\beta_{pi} \geq 0$ are non-negative integers and ρ is the reaction number.

1.3. Stoichiometric Vector

For each reaction, the stoichiometric vector is defined from the stoichiometric coefficients $\alpha_{pi} \geq 0$ and $\beta_{pi} \geq 0$ with coordinates (Gorban, et al., 2008).

$$\gamma_p = \beta_{pi} - \alpha_{pi}. \tag{3}$$

1.4. Reaction Rate and Mass Action Law

Consider an irreversible reaction

$$\alpha_{p1}A_1 + \alpha_{p2}A_2 + \cdots + \alpha_{pn}A_n \xrightarrow{k_p} \beta_{p1}A_1 + \beta_{p2}A_2 + \cdots + \beta_{pn}A_n.$$

And let $C_i = [A_i]$, for $i = 1,2,\ldots,n$ are concentration species. Then we can use mass action law to define the reaction rate v_p which is explained in below:

$$v_p = k_p \prod_{i=1}^{n} C_i^{\alpha_{pi}} \tag{4}$$

where k_p is the reaction rate constant (kinetic constant) (Khoshnaw, 2015).

1.5. Kinetic Equations

If the elementary reactions are given with their stoichiometric equations and reaction rate constants then the kinetic equations (differential equations) for the concentration vector are

$$\frac{dc}{dt} = \sum_{p} \gamma_p v_p, \tag{5}$$

where p is the number of reactions, γ_p is stoichiometric vector and v_p is reaction rate, C is the concentration vector. (Khoshnaw, et al., 2017).

Example 1.5.1

We consider the simplest model of spread of an infectious disease. In this model, people are categorized as either susceptible S or infective I. It can be seen that susceptible people are healthy whereas infective people are sick. We assume that the model population is closed. The chemical reaction of the model takes the following: (Murray, 2001).

$$S + I \xrightarrow{\alpha} 2I, \tag{6}$$

where α is kinetic constant (parameter). Then the stoichiometric vector becomes

$$\gamma = \begin{pmatrix} -1 \\ 1 \end{pmatrix}$$

and we have one reaction rate $v = \alpha SI$.

Then the kinetic equations based on the mass action law are given as follows:

$$\begin{aligned} \frac{dS}{dt} &= -\alpha SI, \\ \frac{dI}{dt} &= \alpha SI. \end{aligned} \tag{7}$$

With initial model populations $S(0) = S_0$ and $I(0) = I_0$. Adding differential equations (7), we obtain $\frac{dS}{dt} + \frac{dI}{dt} = 0$, then we have the following model relation

$$S + I = N. \tag{8}$$

The equation (8) is called conservation law for the model, where N is the total population (constant). For solving the system analytically, we can start from

$$\frac{dS}{dt} = -\alpha SI$$

Then, the equation becomes a separable differential equation.

$$\frac{dS}{S(N-S)} = -\alpha dt.$$

This is separable differential equation. The equation can be solved analytically. Then, the model analytical solutions are

$$S(t) = \frac{N}{1 + \frac{N - S_0}{S_0} e^{\alpha N t}} \qquad \text{and} \qquad I(t) = \frac{N}{1 + \frac{S_0}{N - S_0} e^{-\alpha N t}} .$$

Interestingly, we can conclude that $S(t) \to 0$ and $I(t) \to N$ as $t \to \infty$.

2. METHODS OF MODEL REDUCTION

Identifying the most important model reduction technique for biochemical reactions is often a difficult task. This is sometimes required some scientific classifications such as citation indexes and impact factors. This was simply asked Google about some model reduction words. This was asked on 17*th* February 2018. We obtained some important results: (Fenichel, 1979; Segel, et al., 1989; Jones, 1995; Vora, et al., 2001; Khoshnaw, 2015)

– We asked about "quasi–steady state" we obtained 479, 000 and regarding "pseudo–steady state" we had 114, 000 links; all together 593, 000.
– For well-known technique "quasi–equilibrium" we obtained 289, 000 links;

– but for "geometric singular perturbation" we have 34, 000 links, while for "singular perturbation" we have 305, 000 links.

Model reduction is a transformation process on the original system to another system in which the new model contains a smaller number of elements (variables and parameters). We have a variety of techniques of model reduction for systems biology. Methods of model reduction here are very important in systems biology for minimizing chemical reaction parameters and species. In this study, some essential techniques of model reductions are reviewed and applied for some enzymatic reactions. Techniques are simply given in the following sections.

2.1. Quasi-Steady State Approximation (QSSA)

The concept of the quasi–steady state was suggested as a model reduction technique. Bodeustein in 1913 proposed the classical method of quasi–steady state approximation. Then, there are more explanations about the method that suggested by Briggs and Haldane in 1925. It was about the simplest enzyme reaction $E + S \leftrightharpoons ES \rightarrow E + P$. Briggs and Haldane assumed that the total enzyme concentration is very small in compared to the substrate concentration $[S]$. (Volk, et al., 1977; Schnell, et al., 2002; Pedersen, et al., 2008; Li, et al., 2008; Li, 2013; Khoshnaw, 2015)

Then the method became as important technique of model reductions and model analysis for biochemical reactions. In order to define the method, we simply divide a set of variables $C(t)$ for two sets. The first set is called slow species (basics) $C^s(t)$. The second set is called fast species (fast intermediate) $C^f(t)$. A new variable $C^{\sim f}(t) = \frac{1}{\varepsilon} C^f(t)$ can be intoduced where ε is a small parameter. Then, the differential equations of a biochemical reaction model can be divided into two subsystems:

$$\frac{dC^s}{dt} = W^s\ (C^s(t), C^{\sim f}(t), K), \tag{9}$$

$$\frac{d\check{C}^f}{dt} = \frac{1}{\epsilon} W^f\ (C^s(t), C^{\sim f}(t), K). \tag{10}$$

The first subsystem (9) is called the slow, while the second one (10) is called the fast subsystem. We can analyze fast subsystem (10) and the standard singular perturbation method can be also applied. We can also calculate a slow manifold of the system from the algebraic equations $\mathrm{W}^f(C^s(t), C^{\sim f}(t), K) = 0$ when $\varepsilon \to 0$.

2.1.1. Simple Enzymatic Reactions

We consider an irreversible enzymatic reaction. The model was suggested by Briggs and Haldane. The chemical reactions are given: (Briggs, et al., 1925; Khoshnaw, 2013)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \overset{k_2}{\to} E + P, \tag{11}$$

where E is enzyme, S is substrate, ES is enzyme-substrate complex and P is product. We have three model paramers k_1, k_{-1} and k_2. The model varible concentrations are defined by

$$E = [E], \qquad S = [S], \qquad P = [P], \qquad C = [ES].$$

Using mass action law for Eq. (11), we can define a system of ordinary differential equations:

$$\begin{aligned} \frac{dE}{dt} &= -k_1 ES + k_{-1} C + k_2 C, \\ \frac{dS}{dt} &= -k_1 ES + k_{-1} C, \\ \frac{dC}{dt} &= k_1 ES - (k_{-1} + k_2) C, \\ \frac{dP}{dt} &= k_2 C. \end{aligned} \tag{12}$$

With the initial concentrations

$$E(0) = e_0, \;\; S(0) = s_0, \text{and } C(0) = P(0) = 0. \tag{13}$$

We have two independent conservation laws for system (12), they are given below:

$$E + C = e_0, \;\; S + P + C = s_0. \tag{14}$$

We subsitute Eq. (14) into Eq. (12), then the system of equations becomes

$$\begin{aligned} \frac{dS}{dt} &= -k_1 S(e_0 - C) + k_{-1} C, \\ \frac{dC}{dt} &= k_1 S(e_0 - C) - (k_{-1} + k_2) C. \end{aligned} \tag{15}$$

Briggs and Haldane assumed that the total of enzymes is much smaller than the total of substrates ($i.e.\;\; e_0 \ll s_0$). To monimze the number of parameters , a simple scaling is used . We intoduce the following new variables:

$$\tau = k_1 e_0 t, \;\; u(\tau) = \frac{S(t)}{s_0}, \text{and } \; v(\tau) = \frac{C(t)}{e_0}.$$

Then, the system of ODEs (15) becomes

$$\begin{aligned} \frac{du}{d\tau} &= -u(1 - v) + \alpha v, \qquad u(0) = 1, \\ \epsilon \frac{dv}{d\tau} &= u(1 - v) - \beta v, \qquad v(0) = 0, \end{aligned} \tag{16}$$

where

$$\epsilon = \frac{e_0}{s_0}, \quad \alpha = \frac{k_{-1}}{s_0 k_1}, \qquad \text{and} \qquad \beta = \frac{k_{-1} + k_2}{s_0 k_1}.$$

The system (15) is called slow and fast subsystems.

It is clear that $\alpha < \beta$ because $k_{-1}, k_2 > 0$, and $\alpha = \beta$ when $k_2 = 0$. The system (16) clearly has the form of the systems (9) and (10). We can apply geometric singular perturbation technique; the differential equations can be minimized when $\varepsilon = 0$. Then, the system becomes

$$\frac{du}{d\tau} = -u(1 - v) + \alpha v, \tag{17}$$

$$0 = u(1 - v) - \beta v. \tag{18}$$

The equation (18) can be simply solved for *v*,

$$v = \frac{u}{\beta + u}. \tag{19}$$

Thus, the analytical solutions are necessarily close to the slow manifold M_0. The slow manifold M_0 can be given:

$$M_0 = \left\{(u, v): u \in [0,1], v = \frac{u}{\beta + u}\right\}. \tag{20}$$

Finally, the reduced differential equation is obtained and it is close to the manifold M_0,

$$\frac{du}{d\tau} = \frac{(\alpha - \beta)u}{\beta + u}. \tag{21}$$

It can be concluded that the equation (21) becomes Michaelis-Menten equation. The slow manifold here is normally hyperbolic and stable. The function *F* (*u*, *v*) be the left side of equation (18), this means

$$F(u, v) = u(1 - v) - \beta v \rightarrow \frac{\partial F(u, v)}{\partial v} = -(\beta + u) < 0.$$

2.2. Quasi-Equilibrium Approximation (QEA)

It is not quite clear who proposed the quasi-equilibrium approximation. The idea of QEA has been proposed as a model reduction method for minimizing the number of variables and parameters. The idea of QEA is that the fast reactions become equilibrium very quickly. In other words, the set of fast reactions will reach equilibrium very quickly compared to set of slow reactions. We consider a system of differential equations of chemical reaction as follows: (Volk, et al., 1977; Schnell, et al., 2002; Khoshnaw, 2015)

$$\frac{dC}{dt} = \sum_{s,slow} R^s(c,k,t)\gamma^s + \frac{1}{\epsilon}\sum_{f,fast} R^f(c,k,t)\gamma^f, \tag{22}$$

where ε is a small parameter ($0 < \varepsilon \ll 1$), reaction rates are R^s and R^f. The stoichiometric vectors are γ^s and γ^f. Then, the fast subsystem takes the following form

$$\frac{dC}{dt} = \frac{1}{\epsilon}\sum_{f,fast} R^f(c,k,t)\gamma^f. \tag{23}$$

The QEA is used to separate variables into slow and fast. In order to do that it is required to study the linear conservation laws for the original model (22) and subsystem (23). In general, we have some conservation laws for system (22), and they are denoted by linear functions $h^1(C)$, $h^2(C)$, ..., $h^k(C)$. Interestingly, there are two main cases regarding to linear conservation space. Firstly, if all conservation laws of (23) are also given by the system (22). Then, we do not have any fast–slow separation for variables. Therefore, the system (23) gives the dynamics of fast variables. Secondly, if the fast subsystem (23) has some more linearly independent conservation laws such as $h^{k+1}(C)$, $h^{k+2}(C)$, ..., $h^{k+p}(C)$. Then, such equations are not obtained by the system (22). More interestingly, we can calculate the quasi–equilibrium manifold using the following equations

$$\sum_{f,fast} R^f(c,k,t)\gamma^f = 0, \tag{24}$$

$$h^i(C) = b_i\,, 1 \le i \le k+p. \tag{25}$$

2.2.1 Revisable Enzymatic Reactions

We consider the simple revisable enzymatic reactions as follows: (Khoshnaw, 2013)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E + P, \tag{26}$$

where E, S, ES and P are variables of the model. They are called enzyme, substrate, enzyme–substrate complex, and product. We have four model parameters and they are k_1, k_{-1}, k_2 and k_{-2}. We assume the model concentrations variables as $E = [E], S = [S], P = [P], C = [ES]$. The model differential equations are:

$$\begin{aligned} \frac{dS}{dt} &= -k_1 ES + k_{-1} C, \\ \frac{dE}{dt} &= -k_1 ES + (k_{-1} + k_2) C - k_{-2} PE, \\ \frac{dC}{dt} &= k_1 ES - (k_{-1} + k_2) C + k_{-2} PE, \\ \frac{dP}{dt} &= k_2 C - k_{-2} PE. \end{aligned} \tag{27}$$

with the initial conditions

$$E(0) = e_0, S(0) = s_0 \text{ and } C(0) = P(0) = 0\,. \tag{28}$$

For applying QEA of the chemical reactions (26), it can be supposed that the first reaction

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES$$

goes equilibrium very quickly. This means that

$k_1 = \frac{k^+}{\epsilon}$ and $k_{-1} = \frac{k^-}{\epsilon}$ where $k^+ = \frac{k_1 e_0}{s_0}$ and $k^- = \frac{k_{-1} e_0}{s_0}$.

In other words, k_1 and k_{-1} become are large parameters in comparison with k_2 and k_{-2}. Then, the system (27) are classified into fast and slow reaction rates

$$\begin{aligned}
\frac{dS}{dt} &= \frac{1}{\epsilon} g^f(S,E,C,t),\\
\frac{dE}{dt} &= \frac{1}{\epsilon} g^f(S,C,E,t) + g^s(E,C,P,t),\\
\frac{dC}{dt} &= -\frac{1}{\epsilon} g^f(S,C,E,t) + g^s(E,C,P,t),\\
\frac{dP}{dt} &= g^s(E,C,P,t),
\end{aligned} \tag{29}$$

where $g^f(S,E,C,t) = -k^+ES + k^-C$ and $g^s(E,C,P,t) = k_2C - k_{-2}PE$.

When $\varepsilon \to 0$, we can use the quasi–equilibrium approximation. Thus, such fast reaction has two slow variables and they are called the stoichiometric conservation laws. They are denoted by $b_1(S,C) = S + C$ and $b_2(E,C) = E + C$. We can use the equation $g^f(S,E,C,t) = 0$ to calculate slow manifold. The slow manifold becomes

$$M_0 = \left\{(S,E,C) \in R^3 : S = \frac{k^-C}{k^+E}\right\}. \tag{30}$$

We assume that the slow variables b_1 and b_2 are fixed, then we have the following equations:

$$\begin{aligned}
k^+ES - k^-C &= 0,\\
S + C &= b_1\,,\\
E + C &= b_2.
\end{aligned} \tag{31}$$

Then, from equation (31) we have the following quadratic equation

$$k^+C^2 - (k^+b_1 + k^+b_2 + k^-)C + k^+b_1b_2 = 0. \tag{32}$$

It can be solved analytically for *C*

$$C(b_1,b_2) = \frac{1}{2}\left[\left(b_1 + b_2 + \frac{k^-}{k^+}\right) \pm \sqrt{\left(b_1 + b_2 + \frac{k^-}{k^+}\right)^2 - 4b_1\,b_2}\right].$$

A sign "–" should be selected in order to have positive concentrations. If $b_1 \to 0$ and $b_1 \to 0$ then $C \to 0$. Moreover, the variables S and E are also given

$$S(b_1, b_2) = b_1 - \frac{1}{2}\left[\left(b_1 + b_2 + \frac{k^-}{k^+}\right) - \sqrt{\left(b_1 + b_2 + \frac{k^-}{k^+}\right)^2 - 4b_1\, b_2}\ \right],$$

$$E(b_1, b_2) = b_2 - \frac{1}{2}\left[\left(b_1 + b_2 + \frac{k^-}{k^+}\right) - \sqrt{\left(b_1 + b_2 + \frac{k^-}{k^+}\right)^2 - 4b_1\, b_2}\ \right].$$

3. COMPETITIVE INHIBITION

Enzyme inhibitors are occurred as molecules. They are involved with catalysis and enzymatic reactions. It is clear that studying of enzyme inhibitors provided a good information about enzyme mechanisms and helped us to define some metabolic pathways. Reversible and irreversible inhibitors are two main important types of enzyme inhibitors. Reversible inhibitors are also classified into three types: competitive inhibitors, uncompetitive inhibitors and mixed inhibitors (Mohan, et al., 2015).

A competitive inhibitor is the first common type of reversible inhibition, see Fig. 3.1. A competitive inhibitor and substrate are competed for the active site of an enzyme. The active side is occupied by inhibitor (I). It prevents binding of the substrate to the enzyme (Klonowski, 1983).

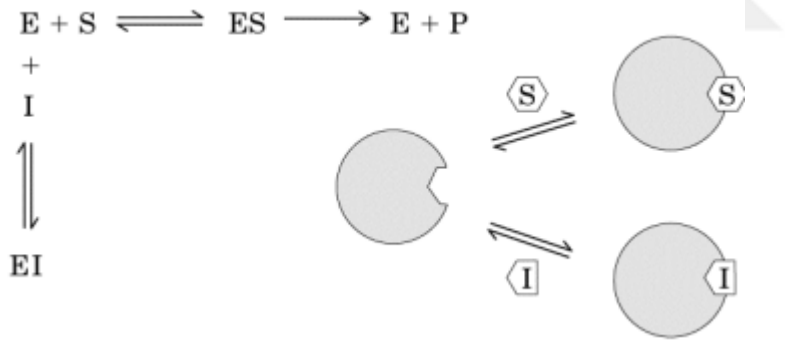

Figure 3.1. Competitive Inhibition.

The kinetic reactions of competitive inhibition are given:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E + P,$$
$$E + I \underset{k_5}{\overset{k_4}{\rightleftarrows}} EI \tag{33}$$

where I is inhibitor, ES and EI are complex intermediate species. The model has five parameters and they are k_1 , k_2 , k_3 , k_4 and k_5. Model variables are $E = [E],\ S = [S],\ P = [P],\ C_1 = [ES],\ C_2 = [EI]$. The model equations can be expressed as follows:

$$\begin{aligned}
\frac{dS}{dt} &= -k_1SE + k_2C_1, \\
\frac{dE}{dt} &= -k_1SE + k_2C_1 + k_3C_1 - k_4EI + k_5C_2, \\
\frac{dI}{dt} &= -k_4EI + k_5C_2, \\
\frac{dC_1}{dt} &= k_1SE - k_2C_1 - k_3C_1, \\
\frac{dp}{dt} &= k_3C_1, \\
\frac{dC_2}{dt} &= k_4EI - k_5C_2.
\end{aligned} \tag{34}$$

We have the following initial conditions

$$E(0) = e_0\ , S(0) = s_0, I(0) = i_0 \text{ and } C_1(0) = C_2(0) = P(0) = 0\ . \tag{35}$$

The model has the following conservation equations:

$$C_2 + I = i_0\ ,\quad C_1 + S + P = s_0 \text{ and } E + C_1 + C_2 = e_0. \tag{36}$$

By substituting the conservation laws into system (34), the kinetic equations take the form:

$$\begin{aligned}
\frac{dS}{dt} &= -k_1S(e_0 - i_0 + I - C_1) + k_2C_1, \\
\frac{dI}{dt} &= -k_4I(e_0 - i_0 + I - C_1) + k_5(i_0 - I),
\end{aligned} \tag{37}$$

$$\frac{dC_1}{dt} = k_1 S(e_0 - i_0 + I - C_1) - (k_2 + k_3)C_1.$$

By introducing the following new variables:

$$\tau = k_1 e_0 t\,, \qquad u(\tau) = \frac{I(t)}{i_0}, \qquad v(\tau) = \frac{S(t)}{s_0}, \qquad \text{and} \qquad w(\tau) = \frac{C_1(t)}{e_0}.$$

Therefore, the system (37) takes the form:

$$\frac{dv}{d\tau} = -v\,((1-w) + \alpha_1(u-1)) + \alpha_2 w, \tag{38}$$

$$\frac{du}{d\tau} = -u(\alpha_3(1-w) - \alpha_4(u-1)) + \alpha_5(1-u), \tag{39}$$

$$\epsilon\frac{dw}{d\tau} = v((1-w) + \alpha_1(u-1)) - \alpha_6 w, \tag{40}$$

with initial conditions $u(0) = 1$, and $v_1(0) = v_2(0) = v_3(0) = 0$,

where

$$\epsilon = \frac{e_0}{s_0}, \qquad \alpha_1 = \frac{i_0}{e_0}, \qquad \alpha_2 = \frac{k_2}{s_0 k_1}, \qquad \alpha_3 = \frac{-k_4}{k_1}, \qquad \alpha_4 = \frac{i_0 k_4}{e_0 k_1},$$
$$\alpha_5 = \frac{k_5}{e_0 k_1} \qquad \text{and} \qquad \alpha_6 = \frac{k_2 + k_3}{s_0 k_1}.$$

It is clear that the above thee equations (38), (39) and (40) have the form of equations (9) and (10). We can sue QSSA when $\varepsilon \to 0$, then equations (38)- (40) take the form

$$\frac{dv}{d\tau} = -v\,((1-w) + \alpha_1(u-1)) + \alpha_2 w, \tag{41}$$

$$\frac{du}{d\tau} = -u(\alpha_3(1-w) - \alpha_4(u-1)) + \alpha_5(1-u), \tag{42}$$

$$0 = v((1-w) + \alpha_1(u-1)) - \alpha_6 w, \tag{43}$$

Equation (43) can be solved for w in terms of u and v

$$w = \frac{v(1+\alpha_1(u-1)}{v+\alpha_6}\ . \tag{44}$$

Thus, the approximate solution for equations (38), (39) and (40) and the manifold M_0 are relatively close. The slow manifold M_0 is given

$$M_0 = \left\{(u,v)\colon u\ and\ v \in [0,1], w = \frac{v(1+\alpha_1(u-1)}{v+\alpha_6}\right\} \tag{45}$$

By substituting equation (44) into equations (41) and (42), the following differential equation close to the manifold M_0 are obtained.

$$\frac{dv}{d\tau} = \frac{v[(v+\alpha_2)\big(1+\alpha_1(u-1)\big) - (1-\alpha_1(u-1))(v+\alpha_6)]}{v+\alpha_6} \tag{46}$$

$$\begin{aligned}\frac{du}{d\tau} = {} & \frac{-u\alpha_3[(v+\alpha_6) - v(1+\alpha_1(u-1)\ )\ ]}{v+\alpha_6} \\ & + \frac{(u-1))(v+\alpha_6)(u\alpha_4-\alpha_5)}{v+\alpha_6}\end{aligned} \tag{47}$$

Using the technique of **QEA** for chemical reactions (33), we assume that the first reaction

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES,$$

becomes quasi–equilibrium. It means that the parameters can be given

$$k_1 = \frac{k^+}{\epsilon}\ \text{and}\ k_2 = \frac{k^-}{\epsilon}\ \text{where}\ k^+ = \frac{k_1 e_0}{s_0}\ \text{and}\ k^- = \frac{k_2 e_0}{s_0}.$$

In other words, k_1 and k_2 are large constants in comparison with k_4 and k_5. Thus, the equations (34) take the form of equation (22)

$$\begin{aligned}\frac{dS}{dt} &= \frac{-1}{\epsilon} g^f(S,E,C_1), \\ \frac{dE}{dt} &= \frac{-1}{\epsilon} g^f(S,E,C_1) + g^{s_1}(\ C_1) + g^{s_2}(E,I,C_2),\end{aligned} \tag{48}$$

$$\frac{dI}{dt} = g^{s_2}(E, I, C_2),$$

$$\frac{dC_1}{dt} = \frac{1}{\epsilon} g^f(S, E, C_1) - g^{s_1}(C_1),$$

$$\frac{dP}{dt} = g^{s_1}(C_1),$$

$$\frac{dC_2}{dt} = -g^{s_2}(E, I, C_2),$$

where $g^f(S, E, C_1) = k^+SE - k^-C_1$, $g^{s_1}(C_1) = k_3C_1$ and $g^{s_2}(E, I, C_2) = -k_4EI + k_5C_2$.

When $\varepsilon \to 0$, we can apply the quasi–equilibrium approximation. Therefore, the model has two slow variables. They are given $b_1(S, C_1) = S + C_1$ and $b_2(E, C_1) = E + C_1$. Slow manifold can be expressed from fast reaction rate equation $g^f(S, E, C_1) = 0$. The model manifold is given below:

$$M_0 = \left\{(S, E, C_1) \in R^3 : S = \frac{k^-C_1}{k^+E}\right\}. \tag{49}$$

We assume that the slow variables b_1 and b_2 are fixed,

$$\begin{aligned} k^+SE - k^-C_1 &= 0, \\ S + C_1 &= b_1, \\ E + C_1 &= b_2. \end{aligned} \tag{50}$$

Then, the following quadratic equation for C_1 is obtained:

$$k^+{C_1}^2 - (k^+b_1 + k^+b_2 + k^-)C_1 + k^+b_1b_2 = 0. \tag{51}$$

We can solve equation (51) for $C_1$and we have

$$C_1(b_1, b_2) = \frac{1}{2}\left[\left(b_1 + b_2 + \frac{k^-}{k^+}\right) \pm \sqrt{\left(b_1 + b_2 + \frac{k^-}{k^+}\right)^2 - 4b_1\, b_2}\ \right].$$

We select a sign "–" in order to have positive concentrations. If $b_1 \rightarrow 0$ and $b_1 \rightarrow 0$ then $C_1 \rightarrow 0$. Moreover, the solution for other variables take the form

$$S(b_1, b_2) = b_1 - \frac{1}{2}\left[\left(b_1 + b_2 + \frac{k^-}{k^+}\right) - \sqrt{\left(b_1 + b_2 + \frac{k^-}{k^+}\right)^2 - 4b_1\, b_2}\ \right],$$

$$E(b_1, b_2) = b_2 - \frac{1}{2}\left[\left(b_1 + b_2 + \frac{k^-}{k^+}\right) - \sqrt{\left(b_1 + b_2 + \frac{k^-}{k^+}\right)^2 - 4b_1\, b_2}\ \right].$$

4. UNCOMPETITIVE INHIBITION

Uncompetitive inhibitors are also another common type of reversible inhibition. An uncompetitive inhibitor binds at a site different from the substrate active site and binds only to the ES complex. This type of reaction requires that one or more substrates bind to E before the inhibitor can bind; see Fig. 4.1. (Mohan, et al., 2015).

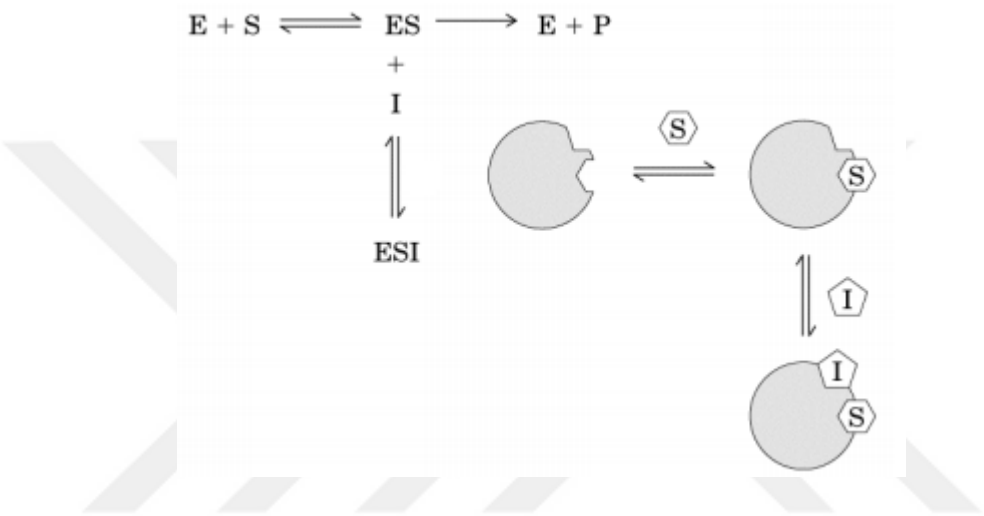

Figure 4.1. Uncompetitive Inhibition

The kinetic reactions of uncompetitive inhibition are given:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E + P,$$
$$ES + I \underset{k_5}{\overset{k_4}{\rightleftarrows}} ESI, \tag{52}$$

where I is inhibition; ES and ESI are intermediate components. Model parameters are k_1 , k_2 , k_3 , k_4 and k_5. The model variables are $E = [E]$, $P = [P]$, $S = [S], I = [I]$, $C_1 = [ES]$, $C_2 = [ESI]$. The model differential equations are given based on mass action law

$$\frac{dE}{dt} = -k_1 ES + k_2 c_1 + k_3 C_1,$$

$$\frac{dS}{dt} = -k_1 ES + k_2 C_1,$$

$$\frac{dC_1}{dt} = k_1 ES - k_2 C_1 - k_3 C_1 - k_4 C_1 I + k_5 C_2, \tag{53}$$

$$\frac{dp}{dt} = k_3 C_1,$$

$$\frac{dI}{dt} = -k_4 C_1 I + k_5 C_2,$$

$$\frac{dC_2}{dt} = k_4 C_1 I - k_5 C_2,$$

with the initial conditions

$$E(0) = e_0, S(0) = s_0 \text{ and } I(0) = i_0, C_1(0) = C_2(0) = P(0) = 0. \tag{54}$$

The following three independent stoichiometric conservation laws have obtained from the system (53):

$$I + C_2 = i_0\,, \quad C_2 + C_1 + E = e_0\,, \quad C_1 + P + S + c_2 = s_0. \tag{55}$$

By substituting the conservation laws into system (53), the kinetic equations take the form

$$\frac{dS}{dt} = -k_1 S(e_0 - i_0 + I - C_1) + k_2 C_1,$$

$$\frac{dC_1}{dt} = k_1 S(e_0 - i_0 + I - C_1) - (k_2 + k_3) C_1, \tag{56}$$

$$\frac{dI}{dt} = -k_4 C_1 I + k_5 (i_0 - I).$$

The following new variables are introduced:

$$\tau = k_1 e_0 t\,, \qquad u(\tau) = \frac{I(t)}{i_0}, \qquad v(\tau) = \frac{S(t)}{s_0}, \qquad \text{and} \qquad w(\tau) = \frac{C_1(t)}{e_0},$$

Then, system (56) can be expressed as fast and slow subsystems:

$$\frac{dv}{d\tau} = -v((1-w) - \alpha_1(1-v)) + \alpha_2 w, \tag{57}$$

$$\epsilon \frac{dw}{d\tau} = v((1-w) - \alpha_1(1-u)) - \alpha_3 w, \tag{58}$$

$$\frac{du}{d\tau} = -\alpha_4 w u + \alpha_5(1-u), \tag{59}$$

where

$$\epsilon = \frac{e_0}{s_0}, \qquad \alpha_1 = \frac{i_0}{e_0}, \qquad \alpha_2 = \frac{k_2}{s_0 k_1}, \qquad \alpha_3 = \frac{k_2 + k_3}{s_0 k_1}, \qquad \alpha_4 = \frac{k_4}{k_1}, \qquad \alpha_5 = \frac{k_5}{e_0 k_1}\,.$$

With initial conditions $u(0) = v(0) = 1\,, w(0) = 0$.

When $\varepsilon \to 0$, equations (57)-(59) can be written as follows

$$\frac{dv}{d\tau} = -v((1-w) - \alpha_1(1-v)) + \alpha_2 w, \tag{60}$$

$$0 = v((1-w) - \alpha_1(1-u)) - \alpha_3 w, \tag{61}$$

$$\frac{du}{d\tau} = -\alpha_4 w u + \alpha_5(1-u). \tag{62}$$

We can solve equation (61) for w in terms of v and u analytically. It can be given:

$$w = \frac{v(1 - \alpha_1(u-1)}{v + \alpha_3}\,. \tag{63}$$

Furthermore, the approximate solutions and slow manifold are relatively close to each other. The manifold M_0 is given below:

$$M_0 = \left\{(u, v, w): u \text{ and } v \in [0,1], w = \frac{v(1-\alpha_1(u-1)}{v+\alpha_3}\right\}. \tag{64}$$

Therefore, we have nonlinear differential equations and they are close to M_0

$$\frac{du}{d\tau} = \frac{-\alpha_4 uv + (1-u)(\alpha_4\alpha_1 uv + \alpha_5(v+\alpha_3))}{v+\alpha_3}, \tag{65}$$

$$\frac{dv}{d\tau} = \frac{(v+\alpha_2)v(1-\alpha_1(1-u) + v(\alpha_1(1-v)-1)(v+\alpha_3)}{v+\alpha_3}. \tag{66}$$

For **QEA** of the chemical reactions (52), we suppose that the second reaction

$$ES + I \underset{k_5}{\overset{k_4}{\rightleftarrows}} ESI,$$

goes equilibrium very quickly:

$$k_4 = \frac{k^+}{\epsilon} \text{ and } k_5 = \frac{k^-}{\epsilon} \text{ where } k^+ = \frac{k_4 e_0}{s_0} \text{ and } k^- = \frac{k_5 e_0}{s_0} .$$

This means k_4 and k_5 are large constants in compassion with k_1 and k_2. Thus, equations (53) has the form of equation (22)

$$\frac{dE}{dt} = g^{s_1}(S, E, C_1) + g^{s_2}(C_1),$$

$$\frac{dS}{dt} = g^{s_1}(S, E, C_1),$$

$$\frac{dC_1}{dt} = -g^{s_1}(S, E, C_1) - g^{s_2}(C_1) + \frac{1}{\epsilon} g^f(C_1, \mathrm{I}, C_2), \tag{67}$$

$$\frac{dP}{dt} = g^{s_2}(C_1),$$

$$\frac{dI}{dt} = \frac{1}{\epsilon} g^f(C_1, \mathrm{I}, C_2),$$

$$\frac{dC_2}{dt} = \frac{-1}{\epsilon} g^f(C_1, \mathrm{I}, C_2).$$

where $g^f(C_1, \mathrm{I}, C_2) = -k^+ C_1 I + k^- C_2$, $g^{s_1}(S, E, C_1) = -k_1 ES + k_2 C_1$ and, $g^{s_2}(C_1) = k_3 C_1$.

When $\varepsilon \to 0$, we can apply the quasi–equilibrium approximation. As a result, the model has two slow variables $b_1(C_1, I) = C_1 - I$ and $b_2(I, C_2) = I + C_2$. The slow manifold is calculated from nonlinear equation $g^f(C_1, \mathrm{I}, C_2) = 0$. This is given by

$$M_0 = \left\{(C_1, \mathrm{I}, C_2) \in R^2 : I = \frac{k^- C_2}{k^+ C_1}\right\}. \tag{68}$$

After fixing the slow variables b_1 and b_2, we have the following equations

$$\begin{aligned} -k^+ C_1 I + k^- C_2 &= 0, \\ C_1 - I &= b_1, \\ I + C_2 &= b_2. \end{aligned} \tag{69}$$

The following quadratic equation for C_2 is obtained:

$$-k^+ {C_2}^2 + (k^+ b_1 + 2k^+ b_2 + k^-) C_2 - k^+ (b_1 + b_2) b_2 = 0. \tag{70}$$

The equation (70) can be solved analytically for C_2. We obtain

$$C_2(b_1, b_2) = \frac{1}{2}\left[-\left(b_1 + 2\, b_2 + \frac{k^-}{k^+}\right) \pm \sqrt{\left(b_1 + 2\, b_2 + \frac{k^-}{k^+}\right)^2 - 4(b_1 + b_2) b_2} \right].$$

We select a sign "–" in order have positive concentrations of I, C_1 and C_2. If $b_1 \to 0$ and $b_1 \to 0$ then $C_2 \to 0$. Moreover, the variables I and C_1 can be also given:

$$I(b_1, b_2) = b_2 - \frac{1}{2}\left[-\left(b_1 + 2\, b_2 + \frac{k^-}{k^+}\right) - \sqrt{\left(b_1 + 2\, b_2 + \frac{k^-}{k^+}\right)^2 - 4(b_1 + b_2) b_2} \right],$$

$$C_1(b_1, b_2) = b_1 + b_2 - \frac{1}{2}\left[-\left(b_1 + 2\, b_2 + \frac{k^-}{k^+}\right) - \sqrt{\left(b_1 + 2\, b_2 + \frac{k^-}{k^+}\right)^2 - 4(b_1 + b_2)b_2}\ \right].$$

5. MIXED INHIBITION

The third type of reversible inhibition is mixed inhibitors. This kind of inhibitors is different compared with the other types of inhibitors because it binds at a site distinct from the substrate active site, but it binds to either E or ES; see Fig. 5.1. (Mohan, et al., 2015).

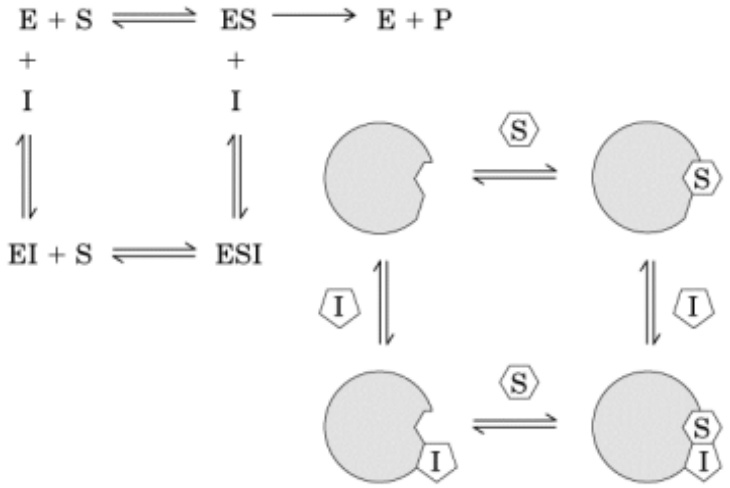

Figure 5.1. Mixed Inhibition.

The kinetic reactions of mixed inhibition are given:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E + P,$$

$$E + I \underset{k_5}{\overset{k_4}{\rightleftarrows}} EI + S\,,$$

$$ES + I \underset{k_7}{\overset{k_6}{\rightleftarrows}} ESI\,, \tag{71}$$

$$EI + S \underset{k_9}{\overset{k_8}{\rightleftarrows}} ESI\,,$$

where *S*, *E*, *P* and *I* are substrate, enzyme, product and inhibition, respectively; *ES*, *EI* and *ESI* are complex intermediate species. Whereas, k_i for $i = 1,2,3,\dots,9$ are parametrs. The model variables are expressed by $E = [E]$, $S = [S]$, $P = [P]$, $I = [I]$, $C_1 = [ES]$, $C_2 = [EI]$ and $C_3 = [ESI]$. The corresponding system of ODFs of the reactions (71) can be written as follows:

$$\begin{aligned}
\frac{dE}{dt} &= -k_1ES + k_2C_1 + k_3C_1 - k_4EI + k_5C_2S,\\
\frac{dS}{dt} &= -k_1ES + k_2C_1 + k_4EI - k_5C_2S - k_8C_2S + k_9c_3,\\
\frac{dC_1}{dt} &= k_1ES - k_2C_1 - k_3C_1 - k_6C_1I + k_7C_3,\\
\frac{dp}{dt} &= k_3C_1,\\
\frac{dI}{dt} &= -k_4EI + k_5C_2S - k_6C_1I + k_7C_3,\\
\frac{dC_2}{dt} &= k_4EI - k_5C_2S - k_8C_2S + k_9C_3,\\
\frac{dC_3}{dt} &= k_6C_1I - k_7C_3 + k_8C_2S - k_9C_3,
\end{aligned} \tag{72}$$

where the initial conditions are

$$\begin{aligned}
E(0) = e_0, S(0) = s_0, I(0) = i_0 \text{ and } C_1(0) = C_2(0) = C_3(0)\\
= P(0) = 0.
\end{aligned} \tag{73}$$

The model includes two independent conservation equations:

$$E + C_1 - I = e_0 - i_0\,,\ I + C_2 + C_3 = i_0. \tag{74}$$

Substituting linear conservation equations into the original equations, some equations can be reduced:

$$\frac{dS}{dt} = -k_1 S(e_0 - C_1 - C_2 - C_3) + k_2 C_1 + k_4(e_0 - C_1 - C_2 - C_3)(i_0 - C_2 - C_3) - k_5 C_2 S - k_8 C_2 S + k_9 C_3,$$

$$\frac{dC_1}{dt} = k_1 S(e_0 - C_1 - C_2 - C_3) - k_2 C_1 - k_3 C_1 - k_6 C_1(i_0 - C_2 - C_3) + k_7 C_3, \tag{75}$$

$$\frac{dp}{dt} = k_3 C_1,$$

$$\frac{dC_2}{dt} = k_4(e_0 - C_1 - C_2 - C_3)(i_0 - C_2 - C_3) - k_5 C_2 S - k_8 C_2 S + k_9 C_3,$$

$$\frac{dC_3}{dt} = k_6 C_1(i_0 - C_2 - C_3) - k_7 C_3 + k_8 C_2 S - k_9 C_3.$$

We introduce the following new variables:

$$\tau = k_1 e_0 t\,, u(\tau) = \frac{S(t)}{s_0}, v(\tau) = \frac{P(t)}{s_0}, w_1(\tau) = \frac{C_1(t)}{i_0}, w_2(\tau) = \frac{C_2(t)}{i_0}, \text{and } w_3(\tau) = \frac{C_3(t)}{i_0}$$

The following differential equation of dimensionless from can be obtained:

$$\frac{du}{d\tau} = -\alpha_1 u\left(\frac{1}{\alpha_1} - w_1 - w_2 - w_3\right) + \alpha_1\alpha_2 w_1 + \alpha_3\alpha_1\left(\frac{1}{\alpha_1} - w_1 - w_2 - w_3\right)(1 - w_2 - w_3) - \alpha_1\alpha_7 u - \alpha_1\alpha_8 u w_2 + \alpha_1\alpha_9 w_3, \tag{76}$$

$$\epsilon\frac{dw_1}{d\tau} = u\left(\frac{1}{\alpha_1} - w_1 - w_2 - w_3\right) - \alpha_2 w_1 - \alpha_4 w_1 - \alpha_5 w_1(1 - w_2 - w_3) + \alpha_6 w_3 \tag{77}$$

$$\frac{dv}{d\tau} = \alpha_1\alpha_4 w_1, \tag{78}$$

$$\epsilon \frac{dw_2}{d\tau} = \epsilon\alpha_1\alpha_{10}\left(\frac{1}{\alpha_1} - w_1 - w_2 - w_3\right)(1 - w_2 - w_3) - \alpha_7 u w_2 - \alpha_8 u w_2 + \alpha_9 w_3, \tag{79}$$

$$\epsilon \frac{dw_3}{d\tau} = \alpha_5 w_1(1 - w_2 - w_3) - \alpha_6 w_3 + \alpha_8 u w_2 - \alpha_9 w_3, \tag{80}$$

where

$$\epsilon = \frac{e_0}{s_0}, \quad \alpha_1 = \frac{i_0}{e_0}, \quad \alpha_2 = \frac{k_2}{s_0 k_1}, \quad \alpha_3 = \frac{i_0 k_4}{s_0 k_1}, \quad \alpha_4 = \frac{k_3}{s_0 k_1}, \quad \alpha_5 = \frac{i_0 k_6}{s_0 k_1},$$
$$\alpha_6 = \frac{k_7}{s_0 k_1}, \quad \alpha_7 = \frac{k_5}{k_1}, \quad \alpha_8 = \frac{k_8}{k_1}, \quad \alpha_9 = \frac{k_9}{s_0 k_1}, \text{and } \alpha_{10} = \frac{k_4}{k_1}.$$

With initial conditions $u(0) = 1$, $v(0) = w_1(0) = w_2(0) = w_3(0) = 0$.

The equations (76)-(80) are slow and fast subsystems. When $\varepsilon \to 0$, equations (77) - (80) become

$$\frac{du}{d\tau} = -\alpha_1 u\left(\frac{1}{\alpha_1} - w_1 - w_2 - w_3\right) + \alpha_1\alpha_2 w_1 + \alpha_3\alpha_1\left(\frac{1}{\alpha_1} - w_1 - w_2 - w_3\right)(1 - w_2 - w_3) - \alpha_1\alpha_7 u - \alpha_1\alpha_8 u w_2 + \alpha_1\alpha_9 w_3, \tag{81}$$

$$0 = u\left(\frac{1}{\alpha_1} - w_1 - w_2 - w_3\right) - \alpha_2 w_1 - \alpha_4 w_1 - \alpha_5 w_1(1 - w_2 - w_3) + \alpha_6 w_3, \tag{82}$$

$$\frac{dv}{d\tau} = \alpha_1\alpha_4 w_1, \tag{83}$$

$$0 = -\alpha_7 u w_2 - \alpha_8 u w_2 + \alpha_9 w_3, \tag{84}$$

$$0 = \alpha_5 w_1(1 - w_2 - w_3) - \alpha_6 w_3 + \alpha_8 u w_2 - \alpha_9 w_3. \tag{85}$$

We can solve equations (82), (84) and (85) for w_1, w_2 and w_3 in terms of v and u. They are given:

$$w_1 = \frac{\beta_4 h_3(u)u}{\alpha_5 - \alpha_5 h_3(u) - \alpha_5\beta_1 h_3(u)u}, \tag{86}$$

$$w_2 = h_3(u), \tag{87}$$

$$w_3 = \beta_1 h_3(u)u. \tag{88}$$

Where

$\beta_1 = \frac{\alpha_7 + \alpha_8}{\alpha_9}$, $\beta_2 = \alpha_2 + \alpha_4 + \alpha_5$, $\beta_3 = \alpha_6 + \alpha_9$, $\beta_4 = \beta_1\beta_3 - \alpha_8$,

$h_1(u) = \alpha_5\beta_4 u + \alpha_5\beta_1 u^2 + \alpha_5 u + (u - \alpha_6)\alpha_5\beta_1 u + (u - \alpha_6)\alpha_5{\beta_1}^2 u^2 + \alpha_5\beta_1\beta_4 u^2$,

$h_2(u) = \frac{u\alpha_5}{\alpha_1} + \frac{\alpha_5\beta_1 u^2}{\alpha_1} + (u + \beta_2)\beta_4 u + u\alpha_5 + (u - \alpha_6)\alpha_5\beta_1 u$, and

$$h_3(u) = \frac{h_2(u) \pm \sqrt{(h_2(u))^2 - 4h_1(u)\frac{\alpha_5 u}{\alpha_1}}}{2h_1(u)} .$$

The slow manifold M_0 can be given:

$$M_0 = \left\{(u, v, w): u \text{ and } v \in [0,1], w = \frac{v(1 - \alpha_1(u-1)}{v + \alpha_3}\right\}. \tag{89}$$

Therefore, the nonlinear kinetic equations are the manifold M_0 are relatively close to each other:

$$\begin{aligned}\frac{du}{d\tau} = &-\alpha_1 u\left(\frac{1}{\alpha_1} - h_4(u) - h_3(u) - \beta_1 h_3(u)u\right) + \alpha_1\alpha_2 h_4(u) \\ &+ \alpha_3\alpha_1\left(\frac{1}{\alpha_1} - h_4(u) - h_3(u) - \beta_1 h_3(u)u\right)(1 \\ &- h_3(u) - \beta_1 h_3(u)u) - \alpha_1\alpha_7 u - \alpha_1\alpha_8 u h_3(u) \\ &+ \alpha_1\alpha_9\beta_1 h_3(u)u,\end{aligned} \tag{90}$$

$$\frac{dv}{d\tau} = \alpha_1\alpha_4 h_4(u). \tag{91}$$

Where $h_4(u) = \frac{\beta_4 h_3(u)u}{\alpha_5 - \alpha_5 h_3(u) - \alpha_5\beta_1 h_3(u)u}$.

For applying **QEA**, we propose two different cases based on quasi-equilibrium reactions:

Case One: If possible suppose that the reversible reaction

$$EI + S \underset{k_9}{\overset{k_8}{\rightleftarrows}} ESI$$

goes quasi–equilibrium

$k_8 = \frac{k^+}{\epsilon}$ and $k_9 = \frac{k^-}{\epsilon}$ where $k^+ = \frac{k_8 e_0}{s_0}$ and $k^- = \frac{k_9 e_0}{s_0}$.

In other words, k_8 and k_9 are large kinetic constants compared to k_1, k_2, k_4, k_5, k_6, and k_7. Then, the system (72) takes the form:

$$\frac{dE}{dt} = g^{s_1}(\mathrm{E}, \mathrm{S}, C_1) + g^{s_2}(C_1) + g^{s_3}(E, I, C_2, \mathrm{S}),$$

$$\frac{dS}{dt} = g^{s_1}(\mathrm{E}, \mathrm{S}, C_1) - g^{s_3}(E, I, C_2, \mathrm{S}) + \frac{1}{\epsilon} g^{f}(C_2, S, C_3),$$

$$\frac{dC_1}{dt} = -g^{s_1}(\mathrm{E}, \mathrm{S}, C_1) - g^{s_2}(C_1) + g^{s_4}(C_1, I, C_3),$$

$$\frac{dp}{dt} = g^{s_2}(C_1), \tag{92}$$

$$\frac{dI}{dt} = g^{s_3}(E, I, C_2, \mathrm{S}) + g^{s_4}(C_1, I, C_3),$$

$$\frac{dC_2}{dt} = -g^{s_3}(E, I, C_2, \mathrm{S}) + \frac{1}{\epsilon} g^{f}(C_2, S, C_3),$$

$$\frac{dC_3}{dt} = -g^{s_4}(C_1, I, C_3) - \frac{1}{\epsilon} g^{f}(C_2, S, C_3),$$

where $g^f(C_2, S, C_3) = -k^+C_2S + k^-C_3$, $g^{s_1}(\mathrm{E}, \mathrm{S}, C_1) = -k_1ES + k_2C_1$, $g^{s_2}(C_1) = k_3C_1$, $g^{s_3}(E, I, C_2, \mathrm{S}) = -k_4EI + k_5C_2S$ and $g^{s_4}(C_1, I, C_3) = -k_6C_1I + k_7C_3$.

When $\varepsilon \to 0$, we can apply the quasi–equilibrium approximation. Thus, the fast reactions have two slow variables $b_1(S, C_3) = S + C_3$ and $b_2(S, C_2) = S - C_2$. We can analytically calculate the slow manifold from nonlinear equation $g^f(C_2, S, C_3) = 0$. This is given by

$$M_0 = \left\{(C_2, S, C_3) \in R^3 : S = \frac{k^-C_3}{k^+C_2}\right\}. \tag{93}$$

After fixing the slow variables b_1 and b_2, and we have the following system of equations:

$$\begin{aligned} -k^+C_2S + k^-C_3 &= 0, \\ S + C_3 &= b_1, \\ S - C_2 &= b_2. \end{aligned} \tag{94}$$

Then, the following quadratic equation for C_2 is obtained:

$$-k^+{C_2}^2 - (k^+b_2 + k^-)C_2 + k^-(b_1 - b_2) = 0. \tag{95}$$

Then, the roots of equation (95) are

$$C_2(b_1, b_2) = \frac{1}{2}\left[-\left(b_2 + \frac{k^-}{k^+}\right) \pm \sqrt{\left(b_2 + \frac{k^-}{k^+}\right)^2 + \frac{4k^-(b_1 - b_2)}{k^+}}\ \right].$$

Where the notation "–" is used for providing positive concentrations of S, C_2 and C_3. If $b_1 \to 0$ and $b_2 \to 0$ then $C_2 \to 0$. Finally, we can also calculate the solution for S and C_3:

$$S(b_1, b_2) = b_2 + \frac{1}{2}\left[-\left(b_2 + \frac{k^-}{k^+}\right) \pm \sqrt{\left(b_2 + \frac{k^-}{k^+}\right)^2 + \frac{4k^-(b_1 - b_2)}{k^+}}\ \right],$$

$$C_3(b_1, b_2) = b_1 - b_2 - \frac{1}{2}\left[-\left(b_2 + \frac{k^-}{k^+}\right) \pm \sqrt{\left(b_2 + \frac{k^-}{k^+}\right)^2 + \frac{4k^-(b_1 - b_2)}{k^+}} \right].$$

Case Two: If possible suppose that the given reactions

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES\,,$$

$$EI + S \underset{k_9}{\overset{k_8}{\rightleftarrows}} ESI,$$

are also quasi–equilibrium:

$k_1 = \frac{k^{\alpha_1}}{\epsilon}$, $k_2 = \frac{k^{\alpha_2}}{\epsilon}$, $k_8 = \frac{k^{\alpha_3}}{\epsilon}$ and $k_9 = \frac{k^{\alpha_4}}{\epsilon}$ where $k^{\alpha_1} = \frac{k_1 e_0}{s_0}$,

$k^{\alpha_2} = \frac{k_2 e_0}{s_0}$, $k^{\alpha_3} = \frac{k_8 e_0}{s_0}$ and $k^{\alpha_4} = \frac{k_9 e_0}{s_0}$.

This means $k_1, k_2, k_8,$ and k_9 are large reaction constants parameters compared to $k_4, k_5, k_6,$ and k_7. Then, the system (72) classifies into fast and slow reaction rates:

$$\frac{dE}{dt} = \frac{1}{\epsilon} g^{f_1}(\mathrm{E}, \mathrm{S}, C_1) + g^{s_1}(C_1) + g^{s_2}(E, I, C_2, \mathrm{S}),$$

$$\frac{dS}{dt} = \frac{1}{\epsilon} g^{f_1}(\mathrm{E}, \mathrm{S}, C_1) + g^{s_2}(E, I, C_2, \mathrm{S}) + \frac{1}{\epsilon} g^{f_2}(C_2, S, C_3),$$

$$\frac{dC_1}{dt} = \frac{-1}{\epsilon} g^{f_1}(\mathrm{E}, \mathrm{S}, C_1) - g^{s_1}(C_1) + g^{s_3}(C_1, I, C_3),$$

$$\frac{dp}{dt} = g^{s_1}(C_1), \tag{96}$$

$$\frac{dI}{dt} = g^{s_2}(E, I, C_2, \mathrm{S}) + g^{s_3}(C_1, I, C_3),$$

$$\frac{dC_2}{dt} = -g^{s_2}(E, I, C_2, \mathrm{S}) + \frac{1}{\epsilon} g^{f_2}(C_2, S, C_3),$$

$$\frac{dC_3}{dt} = -g^{s_3}(C_1, I, C_3) - \frac{1}{\epsilon} g^{f_2}(C_2, S, C_3).$$

Where $g^{f_1}(\mathrm{E}, \mathrm{S}, C_1) = -k^{\alpha_1} ES + k^{\alpha_2} C_1$, $g^{f_2}(C_2, S, C_3) = -k^{\alpha_3} C_2 S + k^{\alpha_4} C_3$, $g^{s_1}(C_1) = k_3 C_1$, $g^{s_2}(E, I, C_2, \mathrm{S}) = -k_4 EI + k_5 C_2 S$ and $g^{s_3}(C_1, I, C_3) = -k_6 C_1 I + k_7 C_3$.

When $\varepsilon \to 0$, we can apply the quasi–equilibrium approximation. Thus, the fast reactions have three slow variables $b_1(E, C_1) = E + C_1$, $b_2(S, C_1, C_3) = S + C_1 + C_3$ and $b_3(S, C_1, C_2) = S + C_1 - C_2$. The slow manifolds are found from nonlinear algebraic equations $g^{f_1}(\mathrm{E}, \mathrm{S}, C_1) = 0$ and $g^{f_2}(C_2, S, C_3)$=0. They are given:

$$M_0 = \left\{ (\mathrm{E}, \mathrm{S}, C_1) \in R^3 : S = \frac{k^{\alpha_2} C_1}{k^{\alpha_1} E} \right\},$$

$$M_0^* = \left\{ (C_2, S, C_3) \in R^3 : S = \frac{k^{\alpha_4} C_3}{k^{\alpha_3} C_2} \right\}. \tag{97}$$

By fixing the slow variables b_1, b_2 and b_3, we have the following system of equations:

$$-k^{\alpha_1} ES + k^{\alpha_2} C_1 = 0,$$

$$-k^{\alpha_3} C_2 S + k^{\alpha_4} C_3 = 0, \tag{98}$$

$$E + C_1 = b_1,$$

$$S + C_1 + C_3 = b_2,$$

$$S + C_1 - C_2 = b_3.$$

There are two non-linear system of equations for C_1 and C_3 by using $E = b_1 - C_1, S = b_2 - C_1 - C_3$ and $C_2 = b_2 - C_3 - b_3$:

$$-k^{\alpha_1}{C_1}^2 + (k^{\alpha_1}b_1 + k^{\alpha_1}b_2 + k^{\alpha_2})C_1 - k^{\alpha_1}C_1C_3 + k^{\alpha_1}b_1C_3 - k^{\alpha_1}b_1b_2 = 0, \quad (99)$$

$$-k^{\alpha_3}{C_3}^2 + (2k^{\alpha_3}b_2 - k^{\alpha_3}b_3 + k^{\alpha_4})C_3 + (k^{\alpha_3}b_2 - k^{\alpha_3}b_3)C_1 - k^{\alpha_3}C_1C_3 - k^{\alpha_2}{b_2}^2 + k^{\alpha_2}b_2b_3 = 0. \quad (100)$$

The non-linear equations (99) and (100) cannot be solved analytically. Thus, some numerical techniques can be applied in order to have some approximate solutions for such systems.

6. CONCLUSIONS AND RECOMMENDATIONS

Techniques of model reductions for biochemical reactions are essential tools for models in biology. Studying the dynamics behavior of nonlinear biochemical models needs some methods of model reductions. In this thesis, some models of enzymatic reactions are studied such as simple enzymatic reactions, competitive inhibition, uncompetitive inhibition and mixed inhibition. We studied two methods of model reductions. The first method is quasi steady state approximation and the other method is quasi equilibrium approximation.

The suggested model reduction approaches here significantly show an important role in many ways. Firstly, the proposed techniques are very useful tools for reducing the number of elements for such models. Because they allow us to divide the original system into slow and fast subsystems and they can be used to calculate slow manifolds. Another way is that classifying the reaction rates into slow and fast reactions based on quasi equilibrium approximation is also another important technique in model reduction because it allows us to study species concentrations participated in fast reactions. More interestingly, scaling variables is also used in this study that provides us to calculate some approximate solutions for non-linear enzymatic reaction models.

In conclusion, the results in this study will help one to study more about complex enzymatic reactions including enzyme inhibitors. More interestingly, the proposed techniques of model reductions here will be applied to a wide range of complex enzyme inhibitor models in systems biology.

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