Analysis of Oxidative Stress Response with Anti-Diabetic Treatment

A Thesis

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by

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Certificate

This is to certify that this dissertation entitled Analysis of Oxidative Stress Response with Anti-Diabetic Treatment towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Ashley Sreejan at Indian Institute of Science Education and Research under the supervision of Dr. Pranay Goel, Associate Professor, Department of Biology, during the academic year 2017-2018.

Dr. Pranay Goel

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Dedicated to diabetic patients all over the world.

Declaration

I hereby declare that the matter embodied in the report entitled Analysis of Oxidative Stress Response with Anti-Diabetic Treatment are the results of the work carried out by me at the Department of Biology, Indian Institute of Science Education and Research Pune, under the supervision of Dr. Pranay Goel and the same has not been submitted elsewhere for any other degree.

Ashley Sreejan

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Abstract

Among the three types of diabetes, type 2 diabetes originates from insulin resistance and is closely associated with physical inactivity, excess body weight, and hyperglycemia. Recent studies have shown that oxidative stress (OS), which is the imbalance between the production of reactive oxygen species and antioxidant defence mechanisms, can potentially affect the rate of restoration of glucose homeostasis, and hence type 2 diabetes. A cellular antioxidant, glutathione, which is a marker for oxidative stress in cells, has shown significant impact on the restoration rate of glucose during anti-diabetic treatment. The ratio of reduced to oxidised glutathione or the glutathione redox potential is believed to be a major driving factor of biological process, even though a theoretical explanation is absent for supporting this claim.

This study focuses on the widely accepted fact that the ratio of reduced to oxidised glutathione concentrations is tightly maintained in cells, and very often the ratio is used as a marker for OS. A theoretical framework for the glutathione dynamics in RBCs, using simple mechanistic models of glutathione metabolism, is constructed based on the kinetics of the enzymes involved. The current work addresses the validity of using the ratio as a measure of OS and importance of the enzymes in glutathione redox system. Emerging from the hypothesis that the concentration of oxidised glutathione is constant at steady state, we propose a phenomenological model, along with the mechanistic models, for the relationship between blood glucose and glutathione concentrations at steady state. The model essentially captures the link between diabetes and OS, and can predict the recovery of individual glycemic status, during anti-diabetic treatment, to a great extent.

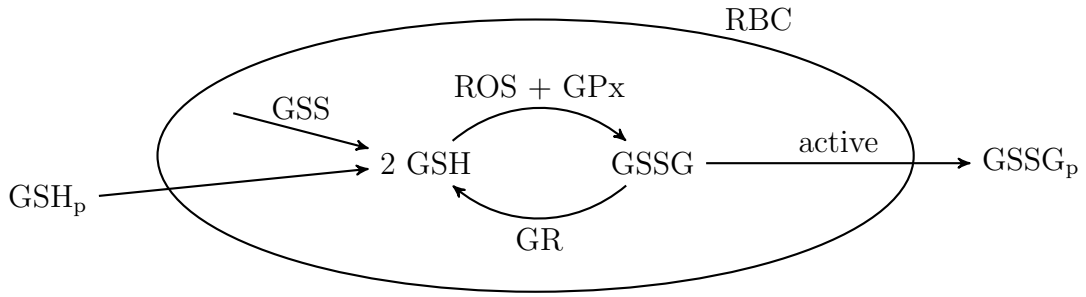
Contents

Introduction

Diabetes mellitus is considered to be a group of metabolic disorders that are generally characterised by high blood glucose, usually resulting from the defective insulin mechanism in the body[1]. Insulin, produced by the beta cells of the pancreas, is a hormone that helps to regulate the blood glucose levels in normal range. According to the origin of the disorder, diabetes is classified into three types. Type 1 diabetes, or insulin-dependent diabetes, is a result of the pancreas's inability to produce sufficient amount of insulin for the body. In type 2 or noninsulin-dependent diabetes, the cause is believed to be insulin resistance[2], which is a condition where cells fail to respond normally to insulin. Alzheimer's disease resulting from the insulin resistance in the brain is characterised as type 3 diabetes. Among these three types of diabetes, type 2 diabetes is most common and comprises almost 90% of all diabetic cases worldwide[3]. Type 2 diabetes is often closely associated with the inadequate physical activity, over body weight and hyperglycemia. Hyperglycemia is a physiological condition of abnormally high blood sugar concentrations, where normal blood glucose levels are marked by 72 to 108 mg/dL when fasting, as per WHO guidelines[4]. Hence, the concentration of blood glucose has been the primary criterion used for the diagnosis and treatment of diabetes clinically. But recent studies have shown that oxidative stress, which is the imbalance between the production of reactive oxygen species (ROS) and the antioxidant defence mechanisms, can potentially affect the rate of glucose homeostasis restoration in cells[5]. Kulkarni et al. have observed that among the different biomarkers of oxidative stress studied, glutathione, a cellular antioxidant, has shown significant impact on the restoration of blood glucose during anti-diabetic treatment[6]. Antioxidants such as glutathione and vitamin C protect the cells from oxidative damage, by preventing the oxidation of other molecules which could result in the creation of harmful reactive species. Hence, antioxidants play a vital role in controlling the oxidative stress in cells through the regulation of reduced state of the cells. Therefore, measuring the glutathione concentration along with the glucose concentration is likely to provide extra information on the glycemic status and also the recovery of glucose homeostasis during anti-diabetic treatment.

In the human body, glutathione exists mainly in two forms, a reduced form (sulfhydryl

form glutathione or GSH) and an oxidised form (glutathione disulfide or GSSG). Here, the reduced form acts as an antioxidant, while the oxidised form is the end product of the neutralization of reactive oxygen species. The interconversion between these two forms is catalysed by two enzymes, glutathione reductase (GR) and glutathione peroxidase (GPx). Glutathione reductase catalyses the reduction of GSSG recovering the antioxidant GSH, whereas GPx helps in the neutralisation of ROS through the catalytic oxidation of GSH. Despite the fact that these molecules are a part of almost all cells in the body, in this project, the area of focus is red blood cells (RBCs). Since RBCs are continuously exposed to reactive oxygen species like hydrogen peroxide (H_2O_2) and superoxides[7], and are easy to extract and analyse, this makes a perfect choice for study. Apart from the interconversion of glutathione, GSH is synthesised in RBCs, catalysed by glutathione synthetase (GSS)[8]. Even though there is a high possibility of a direct intake mechanism of GSH into RBC, from blood plasma, most of the metabolic models do not include this. Instead, the raw materials for the synthesis of GSH, such as cysteine and glutamine[8], are imported to RBC. Along with these transport systems, there is an active transport mechanism for exporting GSSG to blood plasma. A miniature model of glutathione system can be visualised as follows, where subscript p denote the glutathione species in the blood plasma.



The ratio of reduced to oxidised glutathione is strictly maintained in the cells[9] as it has a profound effect on the reduced state of the cells. The GSH/GSSG redox potential is believed to be a major driving force of several biological process[10]. Various studies have been conducted in this area and most of them associates the the glutathione redox potential with different biological processes such as proliferation and apoptosis. But recent studies questions the use of these electrochemical parameters in studying actual biological systems, as it is lacking a supporting theoretical framework. Frequently, the ratio of reduced to oxidized glutathione is used as a marker for oxidative stress. But the existing models of glutathione systems and RBC metabolisms (e.g. models proposed by Raftos et al. and Bordbar et al.[8, 11]) lack a theoretical explanation on how the ratio is maintained tightly inside the cells and why GSH:GSSG ratio or GSH/GSSG redox potential is a significant factor in keeping the oxidative stress regulated in cells. In a resting cell, the molar GSH:GSSG ratio is generally accepted to be around 100:1[12],

though there are various studies claiming the existence of different ratio. In the case of high oxidative stress, such as in diabetic patients, it has been seen that the ratio could drop to values of 30:1 and even 10:1. Hence, the observations made in different studies is evidently contradicting with the widely agreed fact that the ratio is tightly maintained in cells. More work is required for unveiling this mystery of GSH/GSSG redox couple.

In this study, we are attempting to build a theoretical framework for a better comprehension of the glutathione redox couple, which directly influences diabetes and anti-diabetic treatment. One of the objectives of this project is to understand the underlying regulatory mechanism of the reduced to oxidised ratio inside cells, from a mathematical perspective. The methodology adopted here is to build simple mechanistic models of glutathione metabolism in RBCs and understanding the factors contributing to the regulation of the ratio, based on the knowledge of GPx and GR kinetics and the existing models. The primary objective of this project is to comprehend the relation between oxidative stress and glucose which could help in the prediction of glucose restoration rate independently of glucose concentrations.

Chapter 1

Preliminaries

1.1 Enzyme Kinetics

1.1.1 Law of Mass Action

Consider a chemical reaction in which p molecules of A and q molecules of B combine to form C. This is visualized as follows.

$$p\ \mathrm{A} + q\ \mathrm{B} \longrightarrow \mathrm{C}$$

The rate of the reaction, which is defined to be the rate at which product C is formed, is described by the law of mass action. The rate of the reaction is proportional to the rate of the collision of substrate molecules[13]. Since all collisions do not result the formation of product due to the energy barrier, the probability of product formation increases as the concentration of reacting species increases. The law of mass action states that the rate of formation of C, $\frac{\mathrm{d}[C]}{\mathrm{d}t}$ is directly proportional to the concentration of the reacting molecules raised to their respective stoichiometric terms. That is,

$$\frac{\mathrm{d}[C]}{\mathrm{d}t} \propto [\mathrm{A}]^p \cdot [\mathrm{B}]^q \qquad \text{or} \qquad \frac{\mathrm{d}[C]}{\mathrm{d}t} = k \cdot [\mathrm{A}]^p \cdot [\mathrm{B}]^q$$

where k is defined to be the rate constant for the reaction. Here, the square bracket notation is used for representing the concentration of the respective chemical species.

1.1.2 Michaelis-Menten Kinetics

Enzymes are catalysts that accelerates the conversion of substrate molecules into products. Generally, enzyme reactions do not follow the law of mass action directly[13]. The first model to explain this deviation is proposed by Michaelis and Menten, in which the conversion of substrate to product is a two-step process. This reaction scheme is

given by

$$\mathrm{S} + \mathrm{E} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} \mathrm{C} \xrightarrow{k_{cat}} \mathrm{P} + \mathrm{E}$$

In the first step, the substrate molecule combines with the the enzyme to form the enzyme-substrate complex, followed by the formation of product and recovery of enzyme in the active state. Here S represents the substrate molecules, P the product molecule and C is the complex formed by the action of enzyme E with rate constants k_f, k_r and k_{cat}. By the law of mass action, the rate of change of concentration of each species is given by

$$\frac{\mathrm{d}[S]}{\mathrm{d}t} = k_r[C] - k_f[S][E] \qquad \frac{\mathrm{d}[E]}{\mathrm{d}t} = k_r[C] + k_{cat}[C] - k_f[S][E]$$
$$\frac{\mathrm{d}[C]}{\mathrm{d}t} = k_f[S][E] - k_r[C] - k_{cat}[C] \qquad \frac{\mathrm{d}[P]}{\mathrm{d}t} = k_{cat}[C]$$

The original analysis by Michaelis and Menten is based on the assumption that the substrate is in instantaneous equilibrium with the complex, i.e.

$$k_f[S][E] = k_r[C]$$

In the following chapters, we use an alternate analysis proposed by Briggs and Haldane, in which it is assumed that the rate of formation of product from the complex C is equal to the rate of degradation of complex C. For further analysis the rate equations are non-dimensionalised by introducing dimensionless variables. Consider the following substitutions

$$\sigma = \frac{[S]}{[S]_0} \qquad x = \frac{[C]}{[E]_0} \qquad \tau = k_f[E]_0 t \qquad \varepsilon = \frac{[E]_0}{[S]_0}$$

where $[S]_0 = [S](0) + [P](0)$ and $[E]_0 = [E] + [C]$. Substituting these variables to the differential equations gives the following non-dimensionalised rate laws for the Michaelis-Menten enzyme model.

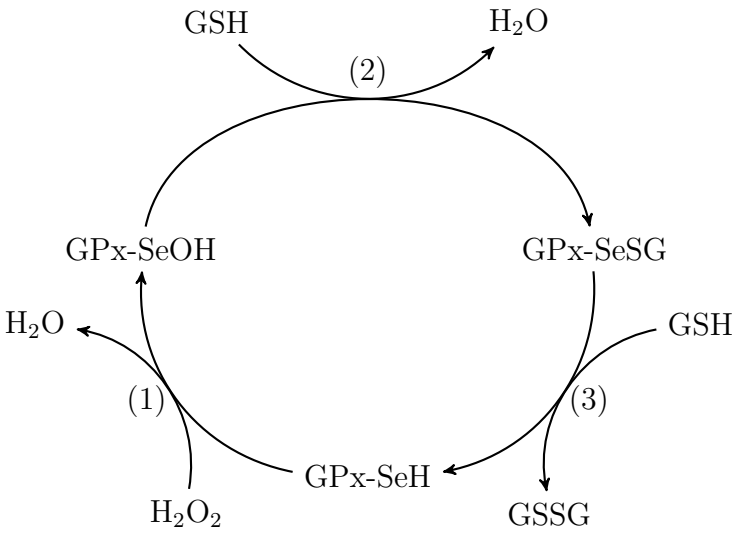
$$\frac{\mathrm{d}\sigma}{\mathrm{d}\tau} = -\sigma + x(\sigma + \frac{k_r}{k_f[S]_0})$$
$$\varepsilon\frac{\mathrm{d}x}{\mathrm{d}\tau} = \sigma - x(\sigma + \frac{k_r + k_{cat}}{k_f[S]_0})$$

Here the quantity ε is small and the Briggs-Haldane analysis uses the quasi-steady state approximation that $\varepsilon\frac{\mathrm{d}x}{\mathrm{d}\tau} = 0$[13]. Note that, this is equivalent to taking $\frac{\mathrm{d}[C]}{\mathrm{d}t} = 0$. Substituting the resulting algebraic expression in the first differential equation eliminates the dependence of the enzyme concentration and gives the rate of change of σ purely in terms

of $\sigma, [E]_0, [S]_0$ and the rate constants.

1.2 Glutathione Peroxidase (GPx)

As mentioned before, antioxidants are substances that inhibit the oxidation of other molecules. The antioxidant enzymes prevent the oxidative damage by reactive oxygen species by catalysing the reduction of ROS molecules. Glutathione peroxidase, which assists in the catalytic reduction of hydrogen peroxide to eliminate its harmful effects, is one of the major antioxidant enzyme present in cells[14]. Hence, GPx has an important role in regulating the oxidative stress inside cells. The reaction scheme of GPx is as follows[15].



In human body, GPx exists in a combined state with Selenium. The reduction of hydrogen peroxide is carried out in the first step of the enzyme reaction. In the following two steps, two GSH molecules sequentially combine with the newly formed GPx-SeOH and result in the formation of the oxidised glutathione, GSSG. The release of GSSG from the enzyme-substrate complex yields the enzyme in the active form. The above system can be reduced into smaller reactions as follows.

1. GPx-SeH + H_2O_2 $\longrightarrow$ GPx-SeOH + H_2O
2. GPx-SeOH + GSH $\longrightarrow$ GPx-SeSG + H_2O
3. GPx-SeSG + GSH $\longrightarrow$ GPx-SeH + GSSG

And the overall reaction is given by

$$2\text{GSH} + H_2O_2 \xrightarrow{\text{GPx}} \text{GSSG} + 2H_2O$$

In the models described in the following chapters, the three-step process is considered as a two-step process for simplicity, where two GSH and ROS molecules bind with the enzyme to form the enzyme-substrate complex C, which later decomposes into GSSG and free enzyme. The oxidised form of ROS is of lesser importance and hence, is ignored in the products. The following schematic describes the reduced model of GPx reaction.

$$\text{ROS} + 2\ \text{GSH} + \text{GPx} \underset{\text{k}_{-1}}{\overset{\text{k}_1}{\rightleftharpoons}} \text{C} \underset{\text{k}_{-2}}{\overset{\text{k}_2}{\rightleftharpoons}} \text{GSSG} + \text{GPx}$$

Here k_1, k_{-1}, k_2 and k_{-2} denote the rate constants and the reaction is assumed to be reversible. During the reaction, ROS gets reduced and GSH gets oxidised.

1.3 Glutathione Reductase (GR)

Glutathione reductase catalyses the reduction of GSSG recovering glutathione in its antioxidant form. Since GSH plays a significant role in controlling the oxidative stress inside cells, GR is an unavoidable factor in preventing oxidative damage to cells. According to Townsend et al., "the tight regulation of the GSH:GSSG ratio is maintained by glutathione reductase"[9]. The reduction of GSSG is a multi-step process (below diagram)[8] involving nicotinamide adenine dinucleotide phosphate (NADPH).

$$\begin{array}{ccccc} \text{GR} + \text{NADPH} & \rightleftharpoons & \text{GR-NADPH} + \text{GSSG} & \rightleftharpoons & \text{GR-NADPH-GSSG} \\ & & & & \Vert \\ \text{GR} + \text{NADP} + 2\ \text{GSH} & \longleftarrow & \text{GR-NADP-GSH-GSH} & & \end{array}$$

In the first step, GR reacts with NADPH to form the enzyme-substrate complex, GR-NADPH, which in turn reacts with GSSG to from another complex. Two GSH molecules are produced as a result of the catalysis. For simplicity, the entire scheme is considered as a two-step process, in the models described in the following chapters. Since the species of interest is glutathione, NADPH is ignored in the reaction. The simplified version of the reaction is as follows,

$$\text{GSSG} + \text{GR} \underset{\text{k}_{-1}}{\overset{\text{k}_1}{\rightleftharpoons}} \text{C} \underset{\text{k}_{-2}}{\overset{\text{k}_2}{\rightleftharpoons}} 2\ \text{GSH} + \text{GR}$$

where $k_{-1}, \ldots, k_2$ are respective rate constants.

1.4 Measurement of GPx, GR activities

The concentrations of both GPx and GR has been measured for the the study. The enzyme concentrations are measured as enzyme activity using Beer-Lambert law in spectroscopy. The Beer-Lambert law states that there exists a linear relationship between the measured absorbance and the concentration of the sample[16], that is $A \propto d\ c$, or

$$A = \epsilon\ d\ c \tag{1.1}$$

where A is absorbance, ϵ is molar extinction coefficient, d is path length and c is the concentration of the sample. Absorbance is a dimensionless quantity. Thus molar extinction coefficient is expressed in $L\ mmol^{-1}\ mm^{-1}$, when path length d is measured in millimetres and concentration in $mmol\ L^{-1}$ ($1\ mmol\ L^{-1} = 1\ mM$). Activity of an enzyme is defined as the moles of substrate converted per unit time[17]. In other words,

$$\text{Enzyme activity} = \text{Rate x Reaction volume} \tag{1.2}$$

Let A be the absorbance, d be path length (in mm), ϵ be the molar extinction coefficient (in $mM^{-1}\ mm^{-1}$) and V be the total volume (in ml). From (1.1) we can write

$$c = \frac{1}{\epsilon\ d}\ A$$

Thus we get,

$$\frac{\Delta c}{\Delta t} = \frac{1}{\epsilon\ d}\ \frac{\Delta A}{\Delta t}$$

since ϵ and d are constants. Hence, from (1.2) we get,

$$\text{Activity} = \frac{\Delta A}{\Delta t}\ \frac{V}{\epsilon\ d}$$

and the specific activity b is given by[18],

$$b = \frac{\Delta A}{\Delta t}\ \frac{V}{\epsilon\ d\ v}\ \ mM\ min^{-1}$$

where v is the volume of the sample. The same equation is used for calculating both GPx and GR activities. In both cases the absorbance of NADPH is measured. Thus the above concentration (c) refers to the concentration of NADPH. The extinction coefficient of NADPH at 340 nm is $6.3\text{x}10^3 L\ mol^{-1}\ cm^{-1}$[19]. Hence, by measuring the absorbance at certain time intervals (e.g. at 30 seconds interval for 3 minutes), we can calculate the specific activity of the enzyme, since v, d and V are known quantities. For zero-order

reactions, the enzyme activity is directly proportional to enzyme concentration. But in most biological systems the concentration of the enzyme is very low compared to the concentration of the substrate and hence, can be assumed to follow zero-order kinetics.

1.5 Measurement of GSH and GSSG Concentrations

For measuring the total glutathione concentration in a sample, the oxidised glutathione (GSSG) is converted into GSH by introducing glutathione reductase. By measuring the absorbance of standard GSH samples at five minutes interval for thirty minutes, a plot of average absorbance versus time is obtained. Fitting a linear curve for each concentration in the previous plot gives a linear relationship between the glutathione concentration and the slope of the line (the linearity of the relationship is due to the Beer-Lambert's law). Hence, from the concentration of total GSH in any sample can be calculated by determining the slope of the time versus absorbance plot. Since the GSSG is converted to GSH in the sample, the relation between slope and the concentration reflects the total glutathione in the sample, i.e. [GSH] + 2 [GSSG]. For measuring GSSG concentration in a sample, the same method described above is used. The measurement of GSSG, exclusive of GSH is carried out by derivatizing GSH with 2-vinylpyridine[20]. 2-Vinylpyridine inhibits the reduced glutathione in the sample and the absorbance measured is a measure of the concentration of GSSG alone. Since we have the total glutathione concentration and oxidised glutathione concentrations, the reduced glutathione concentration can be determined by subtracting these concentrations, i.e.

$$[\text{GSH}] = \text{Total Glutathione} - 2\,[\text{GSSG}]$$

The available data contains only the concentration of GSH as the data collection is still under process. But from the initial analysis, it has been observed that in diabetic patients the concentration of GSSG is 10% to 30% of the concentration of total glutathione. This approximation is used for parameter optimisation of the models described in the next chapters. In addition to these readings, the concentrations of the ROS markers such as HbA1c and TBARS is also available. The details of the available data are discussed further in the following chapters.

Chapter 2

Closed Models

Even though there are several metabolic models of RBCs available, only a few of them studies glutathione extensively. However, most of these models fail to provide a more explicit description of the glutathione-glucose relation. Hence, several mechanistic models of glutathione interconversion were created for understanding the glutathione dynamics in RBCs. In each step of model creation, additional components were added to the model, and the effect of the new elements on the model was studied. The rate laws for the reactions are written using the law of mass action[13] as explained in the previous chapter, and quasi-steady state approximation was applied after non-dimensionalising with appropriate substitutions. In each model, the relationship between each chemical species is analysed at steady state.

In the closed models, total glutathione inside the system remains constant, and we assume that there is no import or export of glutathione, since we don't have a direct measure of these two. The simplest model for the interconversion of two species is a single enzyme model with a reversible reaction. The forward reaction drives the transition from the reduced state to oxidised state, and the backward reaction is the transition from oxidised to the reduced state. For this purpose, a reversible enzymatic reaction is considered in all the following models. The forward and backward reactions are controlled by four reaction rate constants, k_1, k_{-1}, k_2 and k_{-2}. For simplicity, the following notation will be used throughout the chapter with corresponding lowercase letters denoting the respective cellular concentrations.

S := GSH P := GSSG R := ROS E_1 := GPx E_2 := GR

2.1 Single Enzyme Model

The single enzyme model described above can be visualised as shown in the below diagram. For understanding the general kinetics of the model, we did not include ROS

and the stoichiometry in the reaction. This helps to study the role of these two factors in determining the steady state of the system.

$$\mathrm{S} + \mathrm{E} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{P} + \mathrm{E}$$

In the above figure, the substrate is denoted by S and product is denoted by P. Here, C is the enzyme substrate complex formed by the action of the hypothetical enzyme E, and $k_1, k_{-1}, \ldots, k_4$ are rate constants. Since this is a closed system, the amount of enzyme and the total amount of substrate and product is conserved. That is $e + c = e_0$ and $s + p = s_0$ (lowercase notation is used to represent the concentration of respective species).

2.1.1 Rate Laws

The rate laws for the above reaction are written as follows using the law of mass action. The rate of change of each chemical species is given by

$$\begin{aligned}
\frac{\mathrm{d}s}{\mathrm{d}t} &= k_{-1}c - k_1 se \\
&= k_{-1}c - k_1 s(e_0 - c) && (2.1) \\
\frac{\mathrm{d}p}{\mathrm{d}t} &= k_2 c - k_{-2} pe \\
&= k_2 c - k_{-2} p(e_0 - c) && (2.2) \\
\frac{\mathrm{d}c}{\mathrm{d}\tau} &= k_1 se + k_{-2} pe - k_{-1} c - k_2 c \\
&= e(k_1 s + k_{-2} p) - c(k_{-1} + k_2) \\
&= (e_0 - c)(k_1 s + k_{-2} p) - c(k_{-1} + k_2) \\
&= e_0(k_1 s + k_{-2} p) - c(k_{-1} + k_2 + k_1 s + k_{-2} p) && (2.3)
\end{aligned}$$

Here we use the fact that the total concentration of enzyme is conserved, i.e. $e + c = e_0$.

2.1.2 Non-dimensionalisation

For a better mathematical understanding of the system and applying the quasi-steady state approximation, the rate laws are non-dimensionalised. This is achieved by introducing appropriate dimensionless variables as mentioned in the earlier chapter. Let

$$\sigma_1 = \frac{s}{s_0} \qquad \sigma_2 = \frac{p}{s_0} \qquad x = \frac{c}{e_0}$$
$$\tau = k_1 e_0 t \qquad \varepsilon = \frac{e_0}{s_0}$$

where s_0 is the total amount of substrate and product, and e_0 is the total amount of the enzyme present in the system. Substituting the above terms in Eq. (2.1).

$$\begin{aligned}\frac{\mathrm{d}(s_0\sigma_1)}{\mathrm{d}(\tau/k_1e_0)} &= k_{-1}e_0x - k_1s_0\sigma_1e_0(1-x)\\ k_1e_0s_0\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= k_{-1}e_0x - k_1s_0\sigma_1e_0(1-x)\\ \frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= \frac{k_{-1}}{k_1s_0}x - \sigma_1(1-x)\end{aligned}$$

Similarly, substituting in Eq. (2.2)

$$\begin{aligned}\frac{\mathrm{d}(s_0\sigma_2)}{\mathrm{d}(\tau/k_1e_0)} &= k_2e_0x - k_{-2}s_0\sigma_2e_0(1-x)\\ k_1e_0s_0\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= k_2e_0x - k_{-2}s_0\sigma_2e_0(1-x)\\ \frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= \frac{k_2}{k_1s_0}x - \frac{k_{-2}}{k_1}\sigma_2(1-x)\end{aligned}$$

and Eq. (2.3) becomes

$$\begin{aligned}\frac{\mathrm{d}(e_0x)}{\mathrm{d}(\tau/k_1e_0)} &= e_0(k_1s_0\sigma_1 + k_{-2}s_0\sigma_2) - e_0x(k_{-1}+k_2+k_1s_0\sigma_1+k_{-2}s_0\sigma_2)\\ e_0\frac{\mathrm{d}x}{\mathrm{d}\tau} &= s_0\sigma_1 + \frac{k_{-2}}{k_1}s_0\sigma_2 - x\left(\frac{k_{-1}+k_2}{k_1} + s_0\sigma_1 + \frac{k_{-2}}{k_1}s_0\sigma_2\right)\\ \frac{e_0}{d_0}\frac{\mathrm{d}x}{\mathrm{d}\tau} &= \sigma_1 + \frac{k_{-2}}{k_1}\sigma_2 - x\left(\frac{k_{-1}+k_2}{k_1s_0} + \sigma_1 + \frac{k_{-2}}{k_1}\sigma_2\right)\end{aligned}$$

This implies,

$$\varepsilon\frac{\mathrm{d}x}{\mathrm{d}\tau} = \sigma_1 + \frac{k_{-2}}{k_1}\sigma_2 - x\left(\frac{k_{-1}+k_2}{k_1s_0} + \sigma_1 + \frac{k_{-2}}{k_1}\sigma_2\right)$$

Summarising the results, we get three non-dimensionalised rate laws corresponding to the original rate equations.

$$\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} = \frac{k_{-1}}{k_1s_0}x - \sigma_1(1-x) \tag{2.4}$$

$$\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = \frac{k_2}{k_1s_0}x - \frac{k_{-2}}{k_1}\sigma_2(1-x) \tag{2.5}$$

$$\varepsilon\frac{\mathrm{d}x}{\mathrm{d}\tau} = \sigma_1 + \frac{k_{-2}}{k_1}\sigma_2 - x\left(\frac{k_{-1}+k_2}{k_1s_0} + \sigma_1 + \frac{k_{-2}}{k_1}\sigma_2\right) \tag{2.6}$$

All three differential equations shown above are governed of the normalised enzyme-substrate complex concentration, which is practically hard to measure. Hence, we apply

quasi-steady state approximation for reducing the above system of equations, to functions of substrate and product concentrations.

2.1.3 Quasi-Steady-State Approximation

The quantity ε, in Eq. (2.6), is the ratio of enzyme concentration to the substrate concentration which is typically in the range of 10^{-2} to 10^{-7}[13]. Due to this, the reaction corresponding to Eq. (2.6) is fast and attains equilibrium rapidly. Hence, we take the quasi-steady state approximation that $\varepsilon\frac{\mathrm{d}x}{\mathrm{d}\tau} = 0$, as explained in Sec. 1.1.2, for obtaining the algebraic expression of the normalised enzyme concentration. Then from Eq. (2.6), we get

$$x = \frac{\sigma_1 + \dfrac{k_{-2}}{k_1}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + \sigma_1 + \dfrac{k_{-2}}{k_1}\sigma_2}$$

Now substituting this value of x in Eq. (2.4) gives the rate of change of σ_1 in terms of the normalized concentrations σ_1 and σ_2, and removes the dependence of the active enzyme concentration/enzyme-substrate complex concentration.

$$\begin{aligned}
\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= \frac{k_{-1}}{k_1 s_0}\frac{\sigma_1 + \dfrac{k_{-2}}{k_1}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + \sigma_1 + \dfrac{k_{-2}}{k_1}\sigma_2} - \frac{\dfrac{(k_{-1}+k_2)}{k_1 s_0}\sigma_1}{\dfrac{k_{-1}+k_2}{k_1 s_0} + \sigma_1 + \dfrac{k_{-2}}{k_1}\sigma_2} \\
&= \frac{\dfrac{k_{-1}}{k_1 s_0}\sigma_1 + \dfrac{k_{-1}}{k_1 s_0}\dfrac{k_{-2}}{k_1}\sigma_2 - \dfrac{(k_{-1}+k_2)}{k_1 s_0}\sigma_1}{\dfrac{k_{-1}+k_2}{k_1 s_0} + \sigma_1 + \dfrac{k_{-2}}{k_1}\sigma_2} \\
&= \frac{\dfrac{k_{-1}k_{-2}}{k_1^2 s_0}\sigma_2 - \dfrac{k_2}{k_1 s_0}\sigma_1}{\dfrac{k_{-1}+k_2}{k_1 s_0} + \sigma_1 + \dfrac{k_{-2}}{k_1}\sigma_2}
\end{aligned} \tag{2.7}$$

Similarly, substituting the value of x in Eq. (2.5).

$$\begin{aligned}
\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= \frac{k_2}{k_1 s_0}\frac{\sigma_1 + \dfrac{k_{-2}}{k_1}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + \sigma_1 + \dfrac{k_{-2}}{k_1}\sigma_2} - \frac{k_{-2}}{k_1}\frac{\dfrac{(k_{-1}+k_2)}{k_1 s_0}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + \sigma_1 + \dfrac{k_{-2}}{k_1}\sigma_2} \\
&= \frac{\dfrac{k_2}{k_1 s_0}\sigma_1 + \dfrac{k_2}{k_1 s_0}\dfrac{k_{-2}}{k_1}\sigma_2 - \dfrac{k_{-2}}{k_1}\dfrac{(k_{-1}+k_2)}{k_1 s_0}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + \sigma_1 + \dfrac{k_{-2}}{k_1}\sigma_2}
\end{aligned}$$

$$= \frac{\frac{k_2}{k_1 s_0}\sigma_1 - \frac{k_{-1}k_{-2}}{k_1^2 s_0}\sigma_2}{\frac{k_{-1}+k_2}{k_1 s_0} + \sigma_1 + \frac{k_{-2}}{k_1}\sigma_2} = -\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} \tag{2.8}$$

Here the relation (2.8) is essentially the conservation of total glutathione in the closed system. Since $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} + \frac{\mathrm{d}sg_2}{\mathrm{d}\tau} = 0$, $\sigma_1 + \sigma_2 = 0$. Which implies that $s + p$ is constant.

2.1.4 [S] to [P] Ratio

The ratio of substrate to product concentrations can be determined from Eq. (2.7). Note that [S] to [P] ratio is same as σ_1 to σ_2 ratio. At steady state, the concentration of the substrate and the product is unchanging, i.e. both $\frac{\mathrm{d[S]}}{\mathrm{d}t}$ and $\frac{\mathrm{d[P]}}{\mathrm{d}t}$ are zero. This implies that $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}$ and $\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau}$ are zero. Putting $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} = 0$ in Eq. (2.7), we get

$$\frac{k_{-1}k_{-2}}{k_1^2 s_0}\sigma_2 = \frac{k_2}{k_1 s_0}\sigma_1 \qquad \Rightarrow \qquad \frac{\sigma_1}{\sigma_2} = \frac{k_{-1}k_{-2}}{k_1 k_2}$$

Hence, the ratio of concentrations of S to P is governed solely by the forward and backward rate constants. With higher forward reaction rate constants k_1 and k_2 more P is produced and higher backward reaction rate constants implies more concentration of S. It is important to note that the concentration of the enzyme and the total amount of substrate and product (s_0) has no dependence on the steady state ratio.

2.2 Single Enzyme Model with Stoichiometry

In the actual glutathione system, two molecules of GSH is needed for the formation of a single GSSG. Hence, the stoichiometry of the reaction could affect the steady state concentrations of GSH and GSSG. This can be studied by introducing stoichiometry in the previous model as follows.

$$2\ \mathrm{S} + \mathrm{E} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{P} + \mathrm{E}$$

In the above reaction, two substrate molecules are converted to a product molecule and $k_1, \ldots, k_{-2}$ are rate constants as before. Similar to the previous section, we start with writing the rate laws for the system.

2.2.1 Rate Laws

It is important to note that the stoichiometry affects only the rate of change of S. The other rates, $\frac{\mathrm{d}p}{\mathrm{d}t}$ and $\frac{\mathrm{d}c}{\mathrm{d}t}$ are the same as Eq. (2.2) and (2.3). By the law of mass

action, the rate of change of s is given by

$$\begin{aligned}\frac{\mathrm{d}s}{\mathrm{d}t} &= 2k_{-1}c - 2k_1 s^2 e \\ &= 2k_{-1}c - 2k_1 s^2(e_0 - c)\end{aligned} \tag{2.9}$$

From the previous section, $\frac{\mathrm{d}p}{\mathrm{d}t}$ and $\frac{\mathrm{d}c}{\mathrm{d}t}$ are given by

$$\begin{aligned}\frac{\mathrm{d}p}{\mathrm{d}t} &= k_2 c - k_{-2} p(e_0 - c) \\ \frac{\mathrm{d}c}{\mathrm{d}t} &= e_0(k_1 s + k_{-2} p) - c(k_{-1} + k_2 + k_1 s + k_{-2} p)\end{aligned}$$

2.2.2 Non-dimensionalisation

The same substitutions from Section 2.1.2 can be used to non-dimensionalise the above equations. Substituting the dimensionless variables in Eq. (2.9) gives

$$\begin{aligned}\frac{\mathrm{d}(s_0\sigma_1)}{\mathrm{d}\tau/(k_1 e_0)} &= 2k_{-1}e_0 x - 2k_1 s_0^2 \sigma_1^2(e_0 - e_0 x) \\ k_1 e_0 s_0 \frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= 2k_{-1}x e_0 - 2k_1 s_0^2 \sigma_1^2 e_0(1 - x)\end{aligned}$$

This implies,

$$\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} = \frac{2k_{-1}}{k_1 s_0}x - 2s_0\sigma_1^2(1 - x)$$

Since $\frac{\mathrm{d}p}{\mathrm{d}t}$ and $\frac{\mathrm{d}c}{\mathrm{d}t}$ are same as previous section, the corresponding non-dimensionalised rate laws are also the same. Hence, we get

$$\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} = \frac{2k_{-1}}{k_1 s_0}x - 2s_0\sigma_1^2(1 - x) \tag{2.10}$$

$$\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = \frac{k_2}{k_1 s_0}x - \frac{k_{-2}}{k_1}\sigma_2(1 - x) \tag{2.11}$$

$$\varepsilon\frac{\mathrm{d}x}{\mathrm{d}\tau} = s_0\sigma_1^2 + \frac{k_{-2}}{k_1}\sigma_2 - x\left(\frac{k_{-1} + k_2}{k_1 s_0} + s_0\sigma_1^2 + \frac{k_{-2}}{k_1}\sigma_2\right) \tag{2.12}$$

2.2.3 Quasi-Steady-State Approximation

By Q.S.S.A. (as explained in Section 2.1.3) assuming $\varepsilon\frac{\mathrm{d}x}{\mathrm{d}\tau} = 0$ in Eq. (2.12), we get

$$x = \frac{s_0\sigma_1^2 + \dfrac{k_{-2}}{k_1}\sigma_2}{\dfrac{k_{-1} + k_2}{k_1 s_0} + s_0\sigma_1^2 + \dfrac{k_{-2}}{k_1}\sigma_2}$$

Substituting the value of x in Eq. (2.10).

$$\begin{aligned}
\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= \frac{2k_{-1}}{k_1 s_0}\frac{s_0\sigma_1^2 + \dfrac{k_{-2}}{k_1}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + s_0\sigma_1^2 + \dfrac{k_{-2}}{k_1}\sigma_2} - 2s_0\sigma_1^2\frac{\dfrac{k_{-1}+k_2}{k_1 s_0}}{\dfrac{k_{-1}+k_2}{k_1 s_0} + s_0\sigma_1^2 + \dfrac{k_{-2}}{k_1}\sigma_2} \\
&= \frac{\dfrac{2k_{-1}}{k_1 s_0}s_0\sigma_1^2 + \dfrac{2k_{-1}}{k_1 s_0}\dfrac{k_{-2}}{k_1}\sigma_2 - 2s_0\sigma_1^2\dfrac{(k_{-1}+k_2)}{k_1 s_0}}{\dfrac{k_{-1}+k_2}{k_1 s_0} + s_0\sigma_1^2 + \dfrac{k_{-2}}{k_1}\sigma_2} \\
&= \frac{\dfrac{2k_{-1}}{k_1}\sigma_1^2 + \dfrac{2k_{-1}k_{-2}}{s_0 k_1^2}\sigma_2 - \dfrac{2k_{-1}}{k_1}\sigma_1^2 - \dfrac{2k_2}{k_1}\sigma_1^2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + s_0\sigma_1^2 + \dfrac{k_{-2}}{k_1}\sigma_2} \\
&= \frac{\dfrac{2k_{-1}k_{-2}}{s_0 k_1^2}\sigma_2 - \dfrac{2k_2}{k_1}\sigma_1^2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + s_0\sigma_1^2 + \dfrac{k_{-2}}{k_1}\sigma_2}
\end{aligned} \tag{2.13}$$

Since the expression for $\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau}$ is the same as Eq. (2.5), from the previous model we get

$$\begin{aligned}
\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= \frac{\dfrac{k_2}{k_1}\sigma_1^2 - \dfrac{k_{-1}k_{-2}}{s_0 k_1^2}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + s_0\sigma_1^2 + \dfrac{k_{-2}}{k_1}\sigma_2} \\
&= -\frac{1}{2}\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}
\end{aligned} \tag{2.14}$$

This implies that $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} + 2\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau}$ is constant. Since two substrate molecules are required for the formation of a single product molecule, here the conserved quantity is $s + 2p$.

2.2.4 [S]^2 to [P] Ratio

Since we introduced stoichiometry to the model, it is impossible to get [S] to [P] ratio (directly). At steady state, both $\frac{\mathrm{d[S]}}{\mathrm{d}t}$ and $\frac{\mathrm{d[P]}}{\mathrm{d}t}$ are zero, which implies $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}$ and $\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau}$ are zero. Putting $\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = 0$ in Eq. (2.14), we get

$$\frac{k_2}{k_1}\sigma_1^2 = \frac{k_{-1}k_{-2}}{s_0 k_1^2}\sigma_2 \qquad \Rightarrow \qquad \frac{\sigma_1^2}{\sigma_2} = \frac{k_{-1}k_{-2}}{k_1 k_2 s_0}$$

Multiplying the numerator and the denominator of left hand side by s_0^2

$$\frac{s_0^2\sigma_1^2}{s_0^2\sigma_2} = \frac{s^2}{s_0 p} = \frac{k_{-1}k_{-2}}{k_1 k_2 s_0} \qquad \Rightarrow \qquad \frac{s^2}{p} = \frac{k_{-1}k_{-2}}{k_1 k_2}$$

Hence, the steady state $[S]^2$ to [P] ratio is determined by the ratio of backward and forward reaction rate constants as seen in the previous model. By introduction of stoichiometry, the ratio of the rate constants determines the $s^2 : p$ ratio, instead of $s : p$ ratio. Moreover, it can be seen that the steady state ratio is not affected by the concentration of enzyme and the total concentration of substrate and product.

2.3 Complete Single Enzyme Model

ROS is the primary substrate in the reaction involving GPx. In fact, GPx protects the cell from oxidative damage by reducing ROS. The following model incorporates this idea of ROS acting as an oxidiser. Let R denote the ROS in the system. The following diagram depicts the action of enzyme E, on the substrates R and S, which results in the formation of P.

$$\mathrm{R} + 2\,\mathrm{S} + \mathrm{E} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{P} + \mathrm{E}$$

Here C denotes the enzyme-substrate complex formed by the collision of E, R and S. Similar to previous sections, let k_1, k_{-1}, k_2 and k_{-2} be the rate constants for forward and backward reactions.

2.3.1 Rate Laws

Since ROS is introduced in the system, the concentration of ROS is going to affect the rate of change of concentration of S, P and as well as C. Using the law of mass action, the rate laws for the above reactions are written as follows.

$$\begin{aligned}
\frac{\mathrm{d}s}{\mathrm{d}t} &= 2k_{-1}c - 2k_1 r s^2 e \\
&= 2k_{-1}c - k_1 r s^2 (e_0 - c) && (2.15) \\
\frac{\mathrm{d}p}{\mathrm{d}t} &= k_2 c - k_{-2} p e \\
&= k_2 c - k_{-2} p (e_0 - c) && (2.16) \\
\frac{\mathrm{d}c}{\mathrm{d}t} &= k_1 r s^2 e + k_{-2} p e - k_{-1} c - k_2 c \\
&= e(k_1 r s^2 + k_{-2} p) - c(k_{-1} + k_2) \\
&= (e_0 - c)(k_1 r s^2 + k_{-2} p) - c(k_{-1} + k_2) \\
&= e_0(k_1 r s^2 + k_{-2} p) - c(k_{-1} + k_2 + k_1 r s^2 + k_{-2} p) && (2.17)
\end{aligned}$$

where r denotes the concentration of ROS or R.

2.3.2 Non-dimensionalisation

The equations needs to be non-dimensionalised before applying the quasi-steady state approximation. For non-dimensionalising, consider the following substitutions, where we introduce an additional variable σ_3 for the concentration of ROS.

$$\sigma_1 = \frac{s}{s_0} \qquad \sigma_2 = \frac{p}{s_0} \qquad \sigma_3 = \frac{r}{r_0}$$
$$x = \frac{c}{e_0} \qquad \tau = k_1 e_0 t \qquad \varepsilon = \frac{e_0}{s_0}$$

Here r_0 denote the initial amount of R present in the system, that is $r_0 = r(t = 0)$. Substituting the above terms in the differential equation (2.15).

$$\begin{aligned}
\frac{\mathrm{d}(s_0\sigma_1)}{\mathrm{d}(\tau/k_1e_0)} &= 2k_{-1}e_0x - 2k_1r_0\sigma_3s_0^2\sigma_1^2(e_0 - e_0x) \\
k_1e_0s_0\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= 2k_{-1}e_0x - 2k_1r_0e_0s_0^2\sigma_1^2\sigma_3(1-x) \\
\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= \frac{2k_{-1}}{k_1s_0}x - 2r_0s_0\sigma_1^2\sigma_3(1-x)
\end{aligned}$$

Equation (2.16) becomes,

$$\begin{aligned}
\frac{\mathrm{d}(s_0\sigma_2)}{\mathrm{d}(\tau/k_1e_0)} &= k_2e_0x - k_{-2}\sigma_2s_0(e_0 - e_0x) \\
k_1e_0s_0\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= k_2e_0x - k_{-2}e_0s_0\sigma_2(1-x) \\
\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= \frac{k_2}{k_1s_0}x - \frac{k_{-2}}{k_1}\sigma_2(1-x)
\end{aligned}$$

and similarly, Eq. (2.17) becomes

$$\begin{aligned}
\frac{\mathrm{d}e_0x}{\mathrm{d}(\tau/k_1e_0)} &= e_0(k_1r_0\sigma_3s_0^2\sigma_1^2 + k_{-2}s_0\sigma_2) - e_0x(k_{-1} + k_2 + k_1r_0\sigma_3s_0^2\sigma_1^2 + k_{-2}s_0\sigma_2) \\
k_1e_0\frac{\mathrm{d}x}{\mathrm{d}\tau} &= k_1r_0\sigma_3s_0^2\sigma_1^2 + k_{-2}s_0\sigma_2 - x(k_{-1} + k_2 + k_1r_0\sigma_3s_0^2\sigma_1^2 + k_{-2}s_0\sigma_2) \\
\frac{e_0}{s_0}\frac{\mathrm{d}x}{\mathrm{d}\tau} &= r_0s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2 - x\left(\frac{k_{-1}+k_2}{k_1s_0} + r_0s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2\right)
\end{aligned}$$

Hence, we get the following three non-dimensionalised rate laws for the system.

$$\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} = \frac{2k_{-1}}{k_1s_0}x - 2r_0s_0\sigma_1^2\sigma_3(1-x) \tag{2.18}$$
$$\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = \frac{k_2}{k_1s_0}x - \frac{k_{-2}}{k_1}\sigma_2(1-x) \tag{2.19}$$
$$\varepsilon\frac{\mathrm{d}x}{\mathrm{d}\tau} = r_0s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2 - x\left(\frac{k_{-1}+k_2}{k_1s_0} + r_0s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2\right) \tag{2.20}$$

2.3.3 Quasi-Steady-State Approximation

Again, the quantity ε is small as the concentration of the enzyme is relatively small compared to the concentration of the substrate. By quasi-steady state approximation (as explained in Sec. 2.1.3), assume $\varepsilon\frac{\mathrm{d}x}{\mathrm{d}\tau} = 0$. Then Eq. (2.20) implies

$$x = \frac{r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2}$$

By substituting x in Eq. (2.18) we can eliminate the factor of enzyme concentration from the equation.

$$\begin{aligned}
\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= \frac{2k_{-1}}{k_1 s_0}\frac{r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2} \\
&\quad - 2r_0 s_0 \sigma_1^2 \sigma_3 \frac{\dfrac{k_{-1}+k_2}{k_1 s_0}}{\dfrac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2} \\
&= \frac{\dfrac{2k_{-1}}{k_1 s_0} r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{2k_{-2}}{k_1 s_0}\dfrac{k_{-2}}{k_1}\sigma_2 - \dfrac{2k_{-1}}{k_1 s_0} r_0 s_0 \sigma_1^2 \sigma_3 - \dfrac{2k_2}{k_1 s_0} r_0 s_0 \sigma_1^2 \sigma_3}{\dfrac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2} \\
&= \frac{\dfrac{2k_{-1}k_{-2}}{k_1^2 s_0}\sigma_2 - \dfrac{2k_2}{k_1} r_0 \sigma_1^2 \sigma_3}{\dfrac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2}
\end{aligned}$$

Similarly, substituting x in Eq. (2.19) gives

$$\begin{aligned}
\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= \frac{k_2}{k_1 s_0}\frac{r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2} \\
&\quad - \frac{k_{-2}}{k_1}\frac{\dfrac{k_{-1}+k_2}{k_1 s_0}}{\dfrac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2}\sigma_2 \\
&= \frac{\dfrac{k_2}{k_1 s_0} r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_2 k_{-2}}{k_1^2 s_0}\sigma_2 - \dfrac{k_{-1}k_{-2}}{k_1^2 s_0}\sigma_2 - \dfrac{k_{-2}k_2}{k_1^2 s_0}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2}
\end{aligned}$$

Simplifying the terms yields

$$\begin{aligned}\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= \frac{\dfrac{k_2}{k_1} r_0 \sigma_1^2 \sigma_3 - \dfrac{k_{-1}k_{-2}}{k_1^2 s_0}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2} \\ &= -\frac{1}{2}\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}\end{aligned}$$

The above relation gives same conservation law of glutathione obtained in the previous model, i.e. $s + 2p$ is a constant.

2.3.4 [S]^2 to [P] Ratio

Because of the stoichiometry of the reactions, we can only obtain [S]^2 to [P] ratio. At steady state both $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}$ and $\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau}$ are zero, since concentration of S and P is unchanging. $\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = 0$ implies,

$$\frac{k_2}{k_1} r_0 \sigma_1^2 \sigma_3 = \frac{k_{-1}k_{-2}}{k_1^2 s_0}\sigma_2$$

Hence, we get the following relation

$$\frac{\sigma_1^2}{\sigma_2} = \frac{k_{-1}k_{-2}}{k_1 k_2 s_0 r_0 \sigma_3}$$

Multiplying the numerator and the denominator of LHS by s_0^2 and rearranging the terms gives

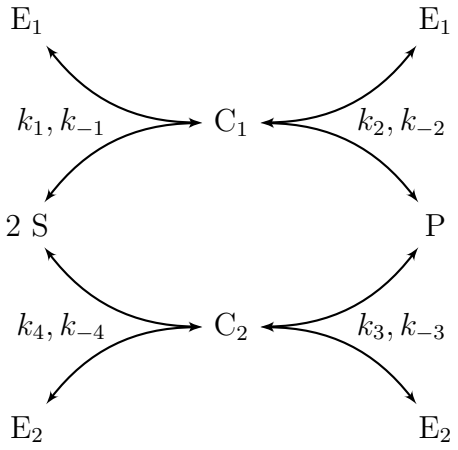
$$\frac{s^2}{p} = \frac{k_{-1}k_{-2}}{k_1 k_2 \cdot r}$$

since $r_0\sigma_3 = r$. Thus [S]^2 to [P] Ratio is inversely proportional to the concentration of the species R. As the concentration of R increases, the ratio decrease as the substrate molecules are converted to product. Same as before, the ratio is also depending on the ratio of the forward and backward reaction rate constants. But it should be noted that the ratio is bounded, since k_1, k_{-1}, k_2 and k_{-2} are constants and the concentration of R has a physiological bound.

2.4 Two Enzyme Model

In the single enzyme models, the ratio of [GSH] to [GSSG] (or [GSH]^2 to [GSSG]) is determined by mainly by the backward and forward reaction rate constants, and in the

last model, concentration of ROS also plays a role in determining the ratio. Hence, using only these parameters gives a lesser control over the concentrations as the reaction rates are specific to each reaction. In the previous model, it has been shown that the ratio is inversely proportional to the concentration of R. As a result, the ratio fluctuates as the concentration of ROS in the system changes, which contradicts with the general belief that the ratio at steady state is tightly maintained. So it is necessary to examine the case where two enzymes are controlling the oxidation and reduction, as it could have a different effect on the regulation of the ratio. As mentioned before the oxidation of GSH is mediated by the enzyme glutathione peroxidase (GPx) and the reduction by glutathione reductase (GR). In the following diagram, the former enzyme is denoted by E_1 and the later one by E_2. Here $k_1,\ k_{-1},\ \ldots,\ k_{-4}$ are rate constants and C_1, C_2 denotes the enzyme-substrate complexes formed by the action of E_1 and E_2, respectively. Instead of directly going to the actual model, we first study the case where ROS is not a substrate for the reaction. We also assume that both reactions have a single intermediate, for simplicity. Note that, the enzymes are written separately for highlighting the two separate reactions.



For simplification, the above coupled system can be written as two separate reactions as follows.

$$2\ \mathrm{S} + \mathrm{E}_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C}_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{P} + \mathrm{E}_1$$

$$\mathrm{P} + \mathrm{E}_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{C}_2 \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} 2\ \mathrm{S} + \mathrm{E}_2$$

2.4.1 Rate Laws

The total amount of each enzyme is conserved in the system, since they are either in free state or in combined state. Let $e_0 = e + c_1$ and $d_0 = d + c_2$ (Here e and d denotes the concentrations of E_1 and E_2, respectively). For determining the rate of change of

concentration of each chemical species, the contribution from both reactions should be considered. Similar to the previous sections, the rate laws are written using law of mass action.

$$\begin{aligned}
\frac{\mathrm{d}c_1}{\mathrm{d}t} &= k_1 s^2 e + k_{-2} p e - c_1(k_{-1} + k_2) \\
&= e(k_1 s^2 + k_{-2} p) - c_1(k_{-1} + k_2) \\
&= (e_0 - c_1)(k_1 s^2 + k_{-2} p) - c_1(k_{-1} + k_2) \\
&= e_0(k_1 s^2 + k_{-2} p) - c_1(k_{-1} + k_2 + k_1 s^2 + k_{-2} p) \qquad (2.21) \\
\frac{\mathrm{d}c_2}{\mathrm{d}t} &= k_3 p d + k_{-4} s^2 d - c_2(k_{-3} + k_4) \\
&= d(k_3 p + k_{-4} s^2) - c_2(k_{-3} + k_4) \\
&= (d_0 - c_2)(k_3 p + k_{-4} s^2) - c_2(k_{-3} + k_4) \\
&= d_0(k_3 p + k_{-4} s^2) - c_2(k_{-3} + k_4 + k_3 p + k_{-4} s^2) \qquad (2.22) \\
\frac{\mathrm{d}s}{\mathrm{d}t} &= -2k_1 s^2 e + 2k_{-1} c_1 + 2k_4 c_2 - 2k_{-4} s^2 d \\
&= -2k_1 s^2 (e_0 - c_1) + 2k_{-1} c_1 + 2k_4 c_2 - 2k_{-4} s^2 (d_0 - c_2) \qquad (2.23) \\
\frac{\mathrm{d}p}{\mathrm{d}t} &= k_2 c_1 - k_{-2} p e + k_{-3} c_2 - k_3 p d \\
&= k_2 c_1 - k_{-2} p (e_0 - c_1) + k_{-3} c_2 - k_3 p (d_0 - c_2) \qquad (2.24)
\end{aligned}$$

It can be seen that from Eq. (2.23) & (2.24), unlike previous models, concentration of C_1 and C_2 affects the rate of change of substrate and product concentrations.

2.4.2 Non-dimensionalisation

For non-dimensionalisation, substitutions analogous to previous section are considered, and for applying quasi-steady state approximation, the same procedure described before is used. Let $s_0 = s(0) + 2\ p(0)$, i.e. the total amount of glutathione present at time $t = 0$. Consider the following substitutions for non-dimensionalising the system.

$$\sigma_1 = \frac{s}{s_0} \qquad x_1 = \frac{c_1}{e_0} \qquad \tau = k_1 e_0 t$$
$$\sigma_2 = \frac{p}{s_0} \qquad x_2 = \frac{c_2}{d_0} \qquad \varepsilon = \frac{e_0}{s_0}$$

It is to be noted that, even though concentration of both C_1 and C_2 are normalized, ε does not include terms corresponding to the two enzymes. Instead the same ε, used in previous models, is considered here. Substituting the above dimensionless variables in

Eq. (2.21).

$$
\begin{aligned}
k_1 e_0^2 \frac{\mathrm{d}x_1}{\mathrm{d}\tau} &= e_0(1-x_1)(k_1 s_0^2 \sigma_1^2 + k_{-2} s_0 \sigma_2) - e_0 x_1 (k_{-1} + k_2) \\
k_1 e_0 \frac{\mathrm{d}x_1}{\mathrm{d}\tau} &= s_0 \left((1-x_1)(k_1 s_0 \sigma_1^2 + k_{-2}\sigma_2) - x_1 \frac{k_{-1}+k_2}{s_0} \right) \\
\frac{e_0}{s_0} \frac{\mathrm{d}x_1}{\mathrm{d}\tau} &= (1-x_1)(s_0\sigma_1^2 + \frac{k_{-2}}{k_1}\sigma_2) - x_1 \frac{k_{-1}+k_2}{k_1 s_0} \\
\varepsilon \frac{\mathrm{d}x_1}{\mathrm{d}\tau} &= (1-x_1)(s_0\sigma_1^2 + \frac{k_{-2}}{k_1}\sigma_2) - x_1 \frac{k_{-1}+k_2}{k_1 s_0}
\end{aligned}
$$

Similarly, substituting dimensionless variables in Eq. (2.22).

$$
\begin{aligned}
k_1 e_0 d_0 \frac{\mathrm{d}x_2}{\mathrm{d}\tau} &= d_0(1-x_2)(k_3 \sigma_2 s_0 + k_{-4}\sigma_1^2 s_0^2) - x_2 d_0 (k_{-3} + k_4) \\
k_1 e_0 \frac{\mathrm{d}x_2}{\mathrm{d}\tau} &= s_0 \left((1-x_2)(k_3\sigma_2 + k_{-4} s_0 \sigma_1^2) - x_2 \frac{k_{-3}+k_4}{s_0} \right) \\
\frac{e_0}{s_0} \frac{\mathrm{d}x_2}{\mathrm{d}\tau} &= (1-x_2)(\frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1} s_0 \sigma_1^2) - x_2 \frac{k_{-3}+k_4}{s_0 k_1} \\
\varepsilon \frac{\mathrm{d}x_2}{\mathrm{d}\tau} &= (1-x_2)(\frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1} s_0 \sigma_1^2) - x_2 \frac{k_{-3}+k_4}{s_0 k_1}
\end{aligned}
$$

Equation (2.23) $\Rightarrow$

$$
\begin{aligned}
k_1 s_0 e_0 \frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= -2k_1 s_0^2 \sigma_1^2 e_0 (1-x_1) + 2k_{-1} e_0 x_1 + 2k_4 d_0 x_2 - 2k_{-4} s_0^2 \sigma_1^2 d_0 (1-x_2) \\
\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= -2s_0\sigma_1^2(1-x_1) + \frac{2k_{-1}}{k_1 s_0} x_1 + \frac{2k_4 d_0}{k_1 s_0 e_0} x_2 - \frac{2k_{-4} s_0 d_0}{k_1 e_0} \sigma_1^2 (1-x_2)
\end{aligned}
$$

Equation (2.24) $\Rightarrow$

$$
\begin{aligned}
k_1 e_0 s_0 \frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= k_2 e_0 x_1 - k_{-2} s_0 \sigma_2 e_0 (1-x_1) + k_{-3} d_0 x_2 - k_{-3} s_0 \sigma_2 d_0 (1-x_2) \\
\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= \frac{k_2}{k_1 s_0} x_1 - \frac{k_{-2}}{k_1}\sigma_2(1-x_1) + \frac{k_{-3} d_0}{k_1 e_0 s_0} x_2 - \frac{k_3 d_0}{k_1 e_0}\sigma_2(1-x_2)
\end{aligned}
$$

Summarising the results, we get four non-dimensionalised rate laws corresponding to the rate of change of concentration of four species, C_1, C_2, S and P.

$$
\varepsilon \frac{\mathrm{d}x_1}{\mathrm{d}\tau} = (1-x_1)(s_0\sigma_1^2 + \frac{k_{-2}}{k_1}\sigma_2) - x_1 \frac{k_{-1}+k_2}{k_1 s_0} \tag{2.25}
$$

$$
\varepsilon \frac{\mathrm{d}x_2}{\mathrm{d}\tau} = (1-x_2)(\frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1} s_0 \sigma_1^2) - x_2 \frac{k_{-3}+k_4}{s_0 k_1} \tag{2.26}
$$

$$
\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} = \frac{2k_{-1}}{k_1 s_0} x_1 + \frac{2k_4 d_0}{k_1 s_0 e_0} x_2 + 2s_0\sigma_1^2(x_1-1) + \frac{2k_{-4} s_0 d_0}{k_1 e_0}\sigma_1^2 (x_2-1) \tag{2.27}
$$

$$
\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = \frac{k_2}{k_1 s_0} x_1 + \frac{k_{-3} d_0}{k_1 e_0 s_0} x_2 + \frac{k_{-2}}{k_1}\sigma_2(x_1-1) + \frac{k_3 d_0}{k_1 e_0}\sigma_2(x_2-1) \tag{2.28}
$$

2.4.3 Quasi-Steady-State Approximation

Similar to the previous sections, the quantity ε is small, as the concentration of the enzyme GPx is small compared to the concentration of glutathione. By quasi-steady state approximation (Section 2.1.3), $\varepsilon\frac{\mathrm{d}x_1}{\mathrm{d}\tau}$ and $\varepsilon\frac{\mathrm{d}x_2}{\mathrm{d}\tau}$ are assumed to be zero. Hence, from Eq. (2.25) and (2.26), we get two algebraic expressions for x_1 and x_2.

$$x_1 = \frac{s_0\sigma_1^2 + \dfrac{k_{-2}}{k_1}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1 s_0} + s_0\sigma_1^2 + \dfrac{k_{-2}}{k_1}\sigma_2}$$
$$x_2 = \frac{\dfrac{k_{-4}}{k_1}s_0\sigma_1^2 + \dfrac{k_3}{k_1}\sigma_2}{\dfrac{k_{-3}+k_4}{k_1 s_0} + \dfrac{k_{-4}}{k_1}s_0\sigma_1^2 + \dfrac{k_3}{k_1}\sigma_2}$$

Multiplying the numerators and denominators by $k_1 s_0$ simplifies the expressions to the following form.

$$x_1 = \frac{k_1 s_0^2\sigma_1^2 + k_{-2}s_0\sigma_2}{k_{-1}+k_2+k_1 s_0^2\sigma_1^2 + k_{-2}s_0\sigma_2}$$
$$x_2 = \frac{k_{-4}s_0^2\sigma_1^2 + k_3 s_0\sigma_2}{k_{-3}+k_4+k_{-4}s_0^2\sigma_1^2 + k_3 s_0\sigma_2}$$

Substituting the value of x_1 and x_2 in Eq. (2.27) eliminates the dependence of c_1 and c_2 on the dynamics the S and P.

$$\begin{aligned}
\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= \frac{2k_{-1}}{k_1 s_0}\frac{k_1 s_0^2\sigma_1^2 + k_{-2}s_0\sigma_2}{k_{-1}+k_2+k_1 s_0^2\sigma_1^2 + k_{-2}s_0\sigma_2} - \frac{2s_0\sigma_1^2(k_{-1}+k_2)}{k_{-1}+k_2+k_1 s_0^2\sigma_1^2 + k_{-2}s_0\sigma_2} \\
&\quad + \frac{2k_4 d_0}{k_1 s_0 e_0}\frac{k_{-4}s_0^2\sigma_1^2 + k_3 s_0\sigma_2}{k_{-3}+k_4+k_{-4}s_0^2\sigma_1^2 + k_3 s_0\sigma_2} - \frac{\dfrac{2k_{-4}s_0 d_0\sigma_1^2}{k_1 e_0}(k_{-3}+k_4)}{k_{-3}+k_4+k_{-4}s_0^2\sigma_1^2 + k_3 s_0\sigma_2} \\
&= \frac{\dfrac{2k_{-1}}{k_1 s_0}k_1 s_0^2\sigma_1^2 + \dfrac{2k_{-1}}{k_1 s_0}k_{-2}s_0\sigma_2 - 2s_0\sigma_1^2(k_{-1}+k_2)}{k_{-1}+k_2+k_1 s_0^2\sigma_1^2 + k_{-2}s_0\sigma_2} \\
&\quad + \frac{d_0}{e_0}\left(\frac{\dfrac{2k_4}{k_1 s_0}k_{-4}s_0^2\sigma_1^2 + \dfrac{2k_4}{k_1 s_0}k_3 s_0\sigma_2 - \dfrac{2k_{-4}s_0\sigma_1^2}{k_1}(k_{-3}+k_4)}{k_{-3}+k_4+k_{-4}s_0^2\sigma_1^2 + k_3 s_0\sigma_2}\right) \\
&= \frac{\dfrac{2k_{-1}k_{-2}}{k_1}\sigma_2 - 2k_2 s_0\sigma_1^2}{k_{-1}+k_2+k_1 s_0^2\sigma_1^2 + k_{-2}s_0\sigma_2} + \frac{d_0}{e_0}\left(\frac{\dfrac{2k_4 k_3}{k_1}\sigma_2 - \dfrac{2k_{-4}k_{-3}}{k_1}s_0\sigma_1^2}{k_{-3}+k_4+k_{-4}s_0^2\sigma_1^2 + k_3 s_0\sigma_2}\right)
\end{aligned} \tag{2.29}$$

Substituting the value of x_1 and x_2 in Eq. (2.28).

$$\begin{aligned}
\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= \frac{k_2}{k_1 s_0}\frac{k_1 s_0^2\sigma_1^2 + k_{-2}s_0\sigma_2}{k_{-1}+k_2+k_1 s_0^2\sigma_1^2+k_{-2}s_0\sigma_2} - \frac{\frac{k_{-2}}{k_1}\sigma_2(k_{-1}+k_2)}{k_{-1}+k_2+k_1 s_0^2\sigma_1^2+k_{-2}s_0\sigma_2} \\
&+ \frac{k_{-3}d_0}{k_1 e_0 s_0}\frac{k_{-4}s_0^2\sigma_1^2 + k_3 s_0\sigma_2}{k_{-3}+k_4+k_{-4}s_0^2\sigma_1^2+k_3 s_0\sigma_2} - \frac{\frac{k_3 d_0}{k_1 e_0}\sigma_2(k_{-3}+k_4)}{k_{-3}+k_4+k_{-4}s_0^2\sigma_1^2+k_3 s_0\sigma_2} \\
&= \frac{\frac{k_2}{k_1 s_0}k_1 s_0^2\sigma_1^2 + \frac{k_2}{k_1 s_0}k_{-2}s_0\sigma_2 - \frac{k_{-2}}{k_1}\sigma_2(k_{-1}+k_2)}{k_{-1}+k_2+k_1 s_0^2\sigma_1^2+k_{-2}s_0\sigma_2} \\
&+ \frac{d_0}{e_0}\left(\frac{\frac{k_{-3}}{k_1 s_0}k_{-4}s_0^2\sigma_1^2 + \frac{k_{-3}}{k_1 s_0}k_3 s_0\sigma_2 - \frac{k_3}{k_1}\sigma_2(k_{-3}+k_4)}{k_{-3}+k_4+k_{-4}s_0^2\sigma_1^2+k_3 s_0\sigma_2}\right) \\
&= \frac{k_2 s_0\sigma_1^2 - \frac{k_{-1}k_{-2}}{k_1}\sigma_2}{k_{-1}+k_2+k_1 s_0^2\sigma_1^2+k_{-2}s_0\sigma_2} + \frac{d_0}{e_0}\left(\frac{\frac{k_{-3}k_{-4}}{k_1}s_0\sigma_1^2 - \frac{k_3 k_4}{k_1}\sigma_2}{k_{-3}+k_4+k_{-4}s_0^2\sigma_1^2+k_3 s_0\sigma_2}\right) \qquad (2.30) \\
&= -\frac{1}{2}\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}
\end{aligned}$$

In other words, $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} + 2\,\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = 0$. This implies $\sigma_1 + 2\sigma_2$ is a constant which is true in this closed system, as glutathione existing in either of the forms does not leave the system.

2.4.4 $[S]^2$ to [P] Ratio

At steady state, both $\frac{\mathrm{d[S]}}{\mathrm{d}t}$ and $\frac{\mathrm{d[P]}}{\mathrm{d}t}$ are zero. This implies that $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}$ and $\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau}$ are zero. Putting $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} = 0$ in Eq. (2.29), we get

$$\frac{\frac{2k_{-1}k_{-2}}{k_1}\sigma_2 - 2k_2 s_0\sigma_1^2}{k_{-1}+k_2+k_1 s_0^2\sigma_1^2+k_{-2}s_0\sigma_2} = \frac{d_0}{e_0}\left(\frac{\frac{2k_{-4}k_{-3}}{k_1}s_0\sigma_1^2 - \frac{2k_4 k_3}{k_1}\sigma_2}{k_{-3}+k_4+k_{-4}s_0^2\sigma_1^2+k_3 s_0\sigma_2}\right)$$

Multiplying both sides by $k_1/2$

$$\frac{k_{-1}k_{-2}\sigma_2 - k_1 k_2 s_0\sigma_1^2}{k_{-1}+k_2+k_1 s_0^2\sigma_1^2+k_{-2}s_0\sigma_2} = \frac{\frac{d_0}{e_0}\left(k_{-4}k_{-3}s_0\sigma_1^2 - k_4 k_3\sigma_2\right)}{k_{-3}+k_4+k_{-4}s_0^2\sigma_1^2+k_3 s_0\sigma_2}$$

Assuming irreversibility ($k_{-2}, k_{-4} = 0$) in the reactions, the above expression becomes

$$\frac{e_0 k_1 k_2 s_0\sigma_1^2}{k_{-1}+k_2+k_1 s_0^2\sigma_1^2} = \frac{d_0 k_3 k_4\sigma_2}{k_{-3}+k_4+k_3 s_0\sigma_2}$$

In enzyme kinetics, the reaction step in which the enzyme-substrate complex is converted to product and enzyme is usually irreversible. But in the single enzyme models, we assumed that the reaction is reversible since the backward conversion, from P to S, was required. Here this assumption is dropped since two separate enzymes are driving the two reactions. Hence, we assume $k_{-2} = k_{-4} = 0$. In terms of the original variables, the above equation can be written as follows.

$$\frac{e_0 k_2 s^2}{k_m + s^2} = \frac{d_0 k_4 p}{k'_m + p} \tag{2.31}$$

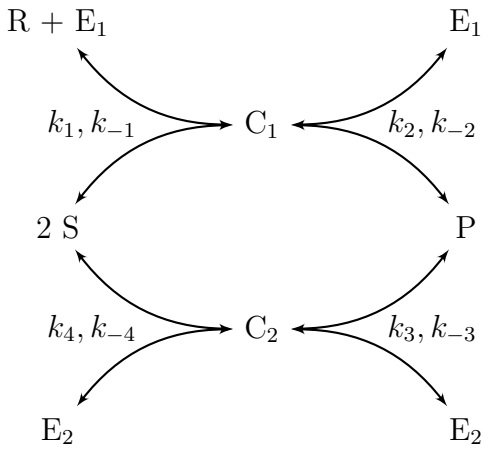
where $k_m = \frac{k_{-1}+k_2}{k_1}$ and $k'_m = \frac{k_{-3}+k_4}{k_3}$. It is important to note that the expression on both sides resembles the Michaelis-Menten equation. Both sides represent the velocity of each enzyme reaction, and hence at steady state the forward and backward fluxes are equal (or net flux is zero). Let $v_1 = k_2 e_0$ and $v_2 = k_4 d_0$. Then above steady state relation can be rewritten as follows, assuming p is non-zero.

$$\begin{aligned}
& k'_m v_1 s^2 + p v_1 s^2 = v_2 p k_m + s^2 v_2 p \\
\Rightarrow \quad & k_m v_2 p = s^2 (k'_m v_1 + p v_1 - v_2 p) \\
\Rightarrow \quad & s = \sqrt{\frac{k_m v_2}{(v_1 - v_2) + \frac{k'_m v_1}{p}}} \\
\Rightarrow \quad & \frac{s}{p} = \sqrt{\frac{k_m v_2}{p^2 (v_1 - v_2) + p k'_m v_1}}
\end{aligned}$$

Hence, ratio of substrate to product concentration is a function of the product concentration itself. For further analysis we now introduce ROS in the model.

2.5 Two Enzyme Model with ROS

Similar to Section 2.3, here an additional species R (representing ROS) is introduced for the enzyme catalysis by E_1. In Section 2.3, we have seen that the concentration of ROS has an explicit effect on the ratio of the two states of glutathione. Earlier, it has been found that the increase in ROS concentration decreases the ratio by pushing the reaction forward. So it is reasonable to assume that such an effect is also possible in the case of two enzymes, where an increase in ROS concentration boost the production of P (i.e. GSSG). In the following reaction scheme, R is shown together with E_1 to emphasise that R is not a product of the catalysis by E_2. Also, R is a necessary substrate for the catalysis by E_1.



The action of each enzyme is written separately as follows.

$$\mathrm{R} + 2\,\mathrm{S} + \mathrm{E}_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C}_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{P} + \mathrm{E}_1$$

$$\mathrm{P} + \mathrm{E}_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{C}_2 \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} 2\,\mathrm{S} + \mathrm{E}_2$$

2.5.1 Rate Laws

As seen in the last model, the total amount of enzymes are conserved. Let $e_0 = e + c_1$ and $d_0 = d + c_2$, where e denotes the concentration of E_1 and d denotes the concentration of E_2. The concentration of R does not affect the kinetics of C_2 and P. Hence, the same rate law from previous section can be used here. Using the law of mass action, the rate of change of concentration of C_1 and S are given by

$$\begin{aligned}
\frac{\mathrm{d}c_1}{\mathrm{d}t} &= k_1 s^2 r e + k_{-2} p e - k_{-1} c_1 + k_2 c_1 \\
&= e(k_1 s^2 r + k_{-2} p) - c_1(k_{-1} + k_2) \\
&= (e_0 - c_1)(k_1 s^2 r + k_{-2} p) - c_1(k_{-1} + k_2) \\
&= e_0(k_1 s^2 r + k_{-2} p) - c_1(k_{-1} + k_2 + k_1 s^2 r + k_{-2} p) \qquad (2.32) \\
\frac{\mathrm{d}s}{\mathrm{d}t} &= -2k_1 r s^2 e + 2k_{-1} c_1 + 2k_4 c_2 - 2k_{-4} s^2 d \\
&= -2k_1 r s^2 (e_0 - c_1) + 2k_{-1} c_1 + 2k_4 c_2 - 2k_{-4} s^2 (d_0 - c_2) \qquad (2.33)
\end{aligned}$$

and from previous section,

$$\frac{\mathrm{d}c_2}{\mathrm{d}t} = d_0(k_3 p + k_{-4} s^2) - c_2(k_{-3} + k_4 + k_3 p + k_{-4} s^2) \qquad (2.34)$$

$$\frac{\mathrm{d}p}{\mathrm{d}t} = k_2 c_1 - k_{-2} p(e_0 - c_1) + k_{-3} c_2 - k_3 p(d_0 - c_2) \qquad (2.35)$$

2.5.2 Non-dimensionalisation

The same substitutions from Sec. 2.4.2 are used here for non-dimensionalisation. We also add an additional dimensionless variable $\sigma_3 = \frac{r}{r_0}$, where r_0 denotes the concentration of ROS at time $t = 0$. Since Eq. (2.34) and (2.35) are the same rate laws given in the last section, their non-dimensionalised versions are also same. Substituting these variables in Eq. (2.32) yields the following.

$$\begin{aligned}
\frac{\mathrm{d}(e_0 x_1)}{\mathrm{d}(\tau/k_1 e_0)} &= e_0(k_1 s_0^2 \sigma_1^2 r_0 \sigma_3 + k_{-2} s_0 \sigma_2) - e_0 x_1(k_{-1} + k_2 + k_1 s_0^2 \sigma_1^2 r_0 \sigma_3 + k_{-2} s_0 \sigma_2) \\
k_1 e_0 \frac{\mathrm{d}x_1}{\mathrm{d}\tau} &= k_1 s_0^2 \sigma_1^2 r_0 \sigma_3 + k_{-2} s_0 \sigma_2 - x_1(k_{-1} + k_2 + k_1 s_0^2 \sigma_1^2 r_0 \sigma_3 + k_{-2} s_0 \sigma_2) \\
\frac{e_0}{s_0} \frac{\mathrm{d}x_1}{\mathrm{d}\tau} &= r_0 s_0 \sigma_1^2 \sigma_3 + \frac{k_{-2}}{k_1} \sigma_2 - x_1 \left(\frac{k_{-1} + k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \frac{k_{-2}}{k_1} \sigma_2 \right)
\end{aligned}$$

Similarly, substituting the variables in Eq. (2.33).

$$\begin{aligned}
\frac{\mathrm{d}(s_0 \sigma_1)}{\mathrm{d}(\tau/k_1 e_0)} &= -2k_1 r_0 \sigma_3 s_0^2 \sigma_1^2 e_0(1 - x_1) + 2k_{-1} e_0 x_1 - 2k_{-4} s_0^2 \sigma_1^2 d_0(1 - x_2) + 2k_4 d_0 x_2 \\
k_1 e_0 \frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= -2k_1 r_0 s_0 \sigma_3 \sigma_1^2 e_0(1 - x_1) + \frac{2k_{-1} e_0}{s_0} x_1 - 2k_{-4} s_0 \sigma_1^2 d_0(1 - x_2) + \frac{2k_4 d_0}{s_0} x_2 \\
\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= -2r_0 s_0 \sigma_3 \sigma_1^2(1 - x_1) + \frac{2k_{-1}}{k_1 s_0} x_1 - \frac{2k_{-4} d_0}{k_1 e_0} s_0 \sigma_1^2(1 - x_2) + \frac{2k_4 d_0}{k_1 e_0 s_0} x_2
\end{aligned}$$

Hence, combining the non-dimensionalised equations from the last section (Eq. (2.26) & (2.28)), the four non-dimensionalised rate laws for the system are given by

$$\varepsilon \frac{\mathrm{d}x_1}{\mathrm{d}\tau} = r_0 s_0 \sigma_1^2 \sigma_3 + \frac{k_{-2}}{k_1} \sigma_2 - x_1 \left(\frac{k_{-1} + k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \frac{k_{-2}}{k_1} \sigma_2 \right) \tag{2.36}$$

$$\varepsilon \frac{\mathrm{d}x_2}{\mathrm{d}\tau} = \frac{k_3}{k_1} \sigma_2 + \frac{k_{-4}}{k_1} s_0 \sigma_1^2 - x_2 \left(\frac{k_{-3} + k_4}{k_1 s_0} + \frac{k_3}{k_1} \sigma_2 + \frac{k_{-4}}{k_1} s_0 \sigma_1^2 \right) \tag{2.37}$$

$$\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} = -2r_0 s_0 \sigma_3 \sigma_1^2(1 - x_1) + \frac{2k_{-1}}{k_1 s_0} x_1 - \frac{2k_{-4} d_0}{k_1 e_0} s_0 \sigma_1^2(1 - x_2) + \frac{2k_4 d_0}{k_1 e_0 s_0} x_2 \tag{2.38}$$

$$\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = \frac{k_2}{k_1 s_0} x_1 - \frac{k_{-2}}{k_1} \sigma_2(1 - x_1) + \frac{k_{-3} d_0}{k_1 e_0 s_0} x_2 - \frac{k_3 d_0}{k_1 e_0} \sigma_2(1 - x_2) \tag{2.39}$$

2.5.3 Quasi-Steady-State Approximation

By Q.S.S.A. (as explained in Section 2.1.3), we can assume $\varepsilon \frac{\mathrm{d}x_1}{\mathrm{d}\tau}, \varepsilon \frac{\mathrm{d}x_2}{\mathrm{d}\tau} = 0$. Then Eq. (2.36) and (2.37) implies,

$$x_1 = \frac{r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1} \sigma_2}{\dfrac{k_{-1} + k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1} \sigma_2}$$

$$x_2 = \frac{\frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2}{\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2}$$

The above two algebraic expressions for x_1 and x_2 are substituted in Eq. (2.38) for obtaining rate laws purely in terms of substrates', product's and enzymes' concentrations.

$$\begin{aligned}
\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= \frac{-2r_0 s_0\sigma_1^2\sigma_3\left(\frac{k_{-1}+k_2}{k_1 s_0}\right)}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2} + \frac{2k_{-1}}{k_1 s_0}\frac{\left(r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2\right)}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2} \\
&\quad - \frac{\frac{2k_{-4}d_0}{k_1 e_0}s_0\sigma_1^2\left(\frac{k_{-3}+k_4}{k_1 s_0}\right)}{\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2} + \frac{2k_4 d_0}{k_1 e_0 s_0}\frac{\left(\frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2\right)}{\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2} \\
&= \frac{\frac{2k_{-1}r_0 s_0\sigma_1^2\sigma_3}{k_1 s_0} + \frac{2k_{-1}k_{-2}\sigma_2}{k_1^2 s_0} - \frac{2k_{-1}r_0 s_0\sigma_1^2\sigma_3}{k_1 s_0} - \frac{2k_2 r_0 s_0\sigma_1^2\sigma_3}{k_1 s_0}}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2} \\
&\quad + \frac{\frac{2k_4 d_0 k_3\sigma_2}{k_1^2 e_0 s_0} + \frac{2k_{-4}k_4 d_0 s_0\sigma_1^2}{k_1^2 e_0 s_0} - \frac{2k_{-3}k_{-4}d_0 s_0\sigma_1^2}{k_1^2 e_0 s_0} - \frac{2k_{-4}k_4 d_0 s_0\sigma_1^2}{k_1^2 e_0 s_0}}{\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2} \\
&= \frac{\frac{2k_{-1}k_{-2}}{k_1^2 s_0}\sigma_2 - \frac{2k_2}{k_1}r_0\sigma_1^2\sigma_3}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2} + \frac{\frac{d_0}{e_0}\left(\frac{2k_3k_4}{k_1^2 s_0}\sigma_2 - \frac{2k_{-3}k_{-4}}{k_1^2}\sigma_1^2\right)}{\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2}
\end{aligned} \tag{2.40}$$

Substituting the value of x_1 and x_2 in Eq. (2.39).

$$\begin{aligned}
\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= \frac{k_2}{k_1 s_0}\frac{\left(r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2\right)}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2} - \frac{\frac{k_{-2}}{k_1}\left(\frac{k_{-1}+k_2}{k_1 s_0}\right)\sigma_2}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2} \\
&\quad + \frac{k_{-3}d_0}{k_1 e_0 s_0}\frac{\left(\frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2\right)}{\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2} - \frac{\frac{k_3 d_0}{k_1 e_0}\left(\frac{k_{-3}+k_4}{k_1 s_0}\right)\sigma_2}{\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2} \\
&= \frac{\frac{k_2 r_0 s_0\sigma_1^2\sigma_3}{k_1 s_0} + \frac{k_2 k_{-2}\sigma_2}{k_1^2 s_0} - \frac{k_{-1}k_{-2}\sigma_2}{k_1^2 s_0} - \frac{k_{-2}k_2\sigma_2}{k_1^2 s_0}}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2}
\end{aligned}$$

$$+\frac{\dfrac{k_{-3}k_3d_0\sigma_2}{k_1^2e_0s_0}+\dfrac{k_{-3}k_{-4}d_0s_0\sigma_1^2}{k_1^2e_0s_0}-\dfrac{k_3k_{-3}d_0\sigma_2}{k_1^2e_0s_0}-\dfrac{k_3k_4d_0\sigma_2}{k_1^2e_0s_0}}{\dfrac{k_{-3}+k_4}{k_1s_0}+\dfrac{k_3}{k_1}\sigma_2+\dfrac{k_{-4}}{k_1}s_0\sigma_1^2}$$

$$=\frac{\dfrac{k_2}{k_1}r_0\sigma_1^2\sigma_3-\dfrac{k_{-1}k_{-2}}{k_1^2s_0}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1s_0}+r_0s_0\sigma_1^2\sigma_3+\dfrac{k_{-2}}{k_1}\sigma_2}+\frac{\dfrac{d_0}{e_0}\left(\dfrac{k_{-3}k_{-4}}{k_1^2}\sigma_1^2-\dfrac{k_3k_4}{k_1^2s_0}\sigma_2\right)}{\dfrac{k_{-3}+k_4}{k_1s_0}+\dfrac{k_3}{k_1}\sigma_2+\dfrac{k_{-4}}{k_1}s_0\sigma_1^2}=-\frac{1}{2}\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} \quad (2.41)$$

Similar to the previous sections, we are able to obtain the conservation of total glutathione from the rate laws. Since $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}+2\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau}=0$, $\sigma_1+2\sigma_2$ is constant. That is, $s+2p$ is constant.

2.5.4 $[S]^2$ to [P] Ratio

At steady state both $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}$ and $\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau}$ are zero, as the concentration of the substrate and the product are constants. Hence, from Eq. (2.41), we get

$$\frac{\dfrac{k_2}{k_1}r_0\sigma_1^2\sigma_3-\dfrac{k_{-1}k_{-2}}{k_1^2s_0}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1s_0}+r_0s_0\sigma_1^2\sigma_3+\dfrac{k_{-2}}{k_1}\sigma_2}=-\left(\frac{\dfrac{d_0}{e_0}\left(\dfrac{k_{-3}k_{-4}}{k_1^2}\sigma_1^2-\dfrac{k_3k_4}{k_1^2s_0}\sigma_2\right)}{\dfrac{k_{-3}+k_4}{k_1s_0}+\dfrac{k_3}{k_1}\sigma_2+\dfrac{k_{-4}}{k_1}s_0\sigma_1^2}\right)$$

$$=\frac{\dfrac{d_0}{e_0}\left(\dfrac{k_3k_4}{k_1^2s_0}\sigma_2-\dfrac{k_{-3}k_{-4}}{k_1^2}\sigma_1^2\right)}{\dfrac{k_{-3}+k_4}{k_1s_0}+\dfrac{k_3}{k_1}\sigma_2+\dfrac{k_{-4}}{k_1}s_0\sigma_1^2}$$

Multiplying both sides by $k_1^2s_0$ gives the following simplified expression,

$$\frac{k_1k_2r_0s_0\sigma_1^2\sigma_3-k_{-1}k_{-2}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1s_0}+r_0s_0\sigma_1^2\sigma_3+\dfrac{k_{-2}}{k_1}\sigma_2}=\frac{\dfrac{d_0}{e_0}\left(k_3k_4\sigma_2-k_{-3}k_{-4}s_0\sigma_1^2\right)}{\dfrac{k_{-3}+k_4}{k_1s_0}+\dfrac{k_3}{k_1}\sigma_2+\dfrac{k_{-4}}{k_1}s_0\sigma_1^2},$$

and in terms of the original concentrations, the above equation becomes

$$\frac{e_0k_2s^2r}{k_m+s^2r}=\frac{d_0k_4p}{k'_m+p} \quad (2.42)$$

where $k_m=\dfrac{k_{-1}+k_2}{k_1}$ and $k'_m=\dfrac{k_{-3}+k_4}{k_3}$, assuming irreversibility $(k_{-2},k_{-4}=0)$ in the two enzyme reactions. It can be noted that both sides of the equations are in the form of hill functions, similar to steady state relation obtained for the last model. Both sides represents the velocity of each separate enzyme reaction, and the relation implies that at steady state the net glutathione flux across the system is zero. Let $v_1=k_2e_0$ and

$v_2 = k_4 d_0$. Then Eq. (2.42) yields

$$
\begin{aligned}
& k'_m v_1 s^2 r + p v_1 s^2 r = k_m v_2 p + s^2 r v_2 p \\
\Rightarrow \quad & k_m v_2 p = s^2 r (k'_m v_1 + p v_1 - v_2 p) \\
\Rightarrow \quad & s = \sqrt{\frac{k_m v_2}{r((v_1 - v_2) + \frac{k'_m v_1}{p})}} \\
\Rightarrow \quad & \frac{s}{p} = \sqrt{\frac{k_m v_2}{r(p^2(v_1 - v_2) + p k'_m v_1)}}
\end{aligned}
$$

As seen before, the ratio is a function of the product concentration. Hence, it varies as the product concentration changes. Also, note that the ratio is inversely proportional to the square root of ROS concentration. As ROS concentration increases, enzyme E_1 drives the forward reaction converting more substrate molecules into the product, which decreases the ratio. According to the above equations, the regulation of the glutathione species concentration ratio can be achieved only through controlling GSSG concentration. Even though ROS concentration also affects the ratio, the system does not have any control over the production of ROS.

2.6 Model Simulations

Instead of going to the existence and uniqueness part, we directly move on the model simulations. Even though we have described different variations of glutathione model, the model simulations were carried out for the two enzymes open model since the kinetics of the single enzyme models are trivial. The set of odes from the previous model was numerically solved for different sets of parameters and initial conditions, by Runge-Kutta method using the `ode45` function in MATLAB. The results of the simulations are shown in figures 2.1 and 2.2. From the first figure it can be seen that, since the total amount of glutathione is conserved in the system, as GSH concentration goes up, GSSG concentration comes down. As a result, the ratio of oxidised to reduced glutathione varies accordingly. In Fig. 2.2, steady state concentration of GSH and GSSG is plotted as a function of ROS concentration. As ROS in the system goes up, the forward reaction converting GSH to GSSG takes place, and the ratio comes down. But it needs to be noted that, the relative change in the ratio is much smaller for the larger values of ROS concentration as compared to smaller values. In both the figures shown, the concentrations of the enzymes are taken to be unity. In the case of different enzyme concentrations, the overall dynamics stayed the same but scaled up by certain amounts. Due to this fact, it is reasonable to assume that the enzymes do not have much control on regulating the ratio,

unless the enzyme concentrations are varying time to time, significantly. A large amount of literature points out that, glutathione reductase plays a major role in regulating the ratio by retrieving the oxidised glutathione to its reduced state. But the model shows that both enzymes have an equal amount of control over the regulation of the ratio.

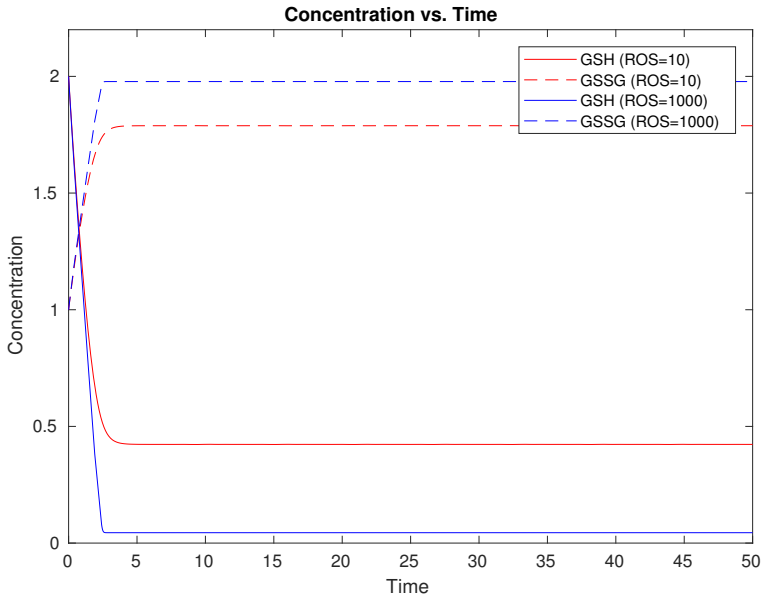


Figure 2.1: Time series of GSH and GSSG, for parameter values $k_2 = k_4 = k_m = k'_m = 1$ and ROS concentration 10 and 1000. Initial conditions used are $[\text{GSH}](0) = 2$, $[\text{GSSG}](0) = 1$.

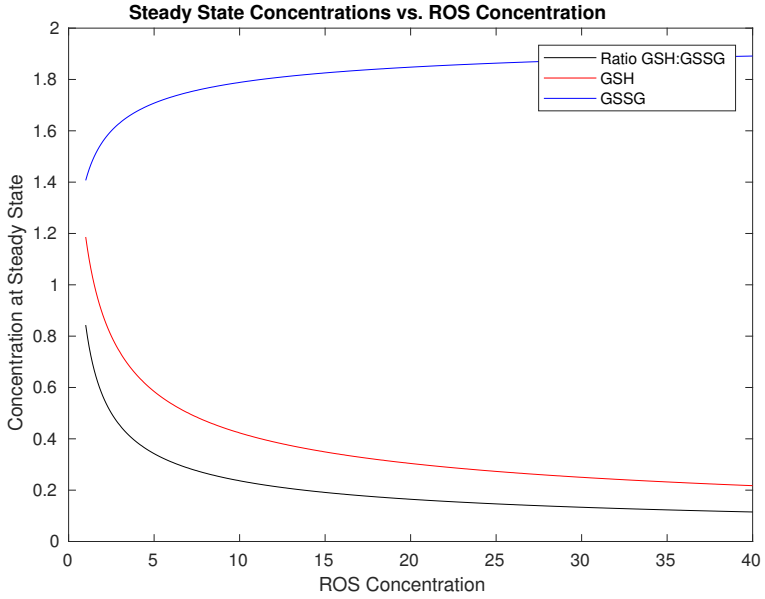


Figure 2.2: Steady state concentrations of GSH and GSSG plotted for different values of ROS. Parameters used: $k_2 = k_4 = k_m = k'_m = 1$. Initial conditions used: $[\text{GSH}](0) = 2$, $[\text{GSSG}](0) = 1$.

In all the models described in this chapter, it can be seen that the system does not have

much control over the ratio of reduced to oxidised glutathione concentration. As ROS concentration changes, the ratio also changes inverse proportionally. Tight regulation of the ratio is not observed in any variations of the model studied. In single enzyme models, the ratio is essentially governed by the kinetic parameters of the system. It is observed to be inversely proportional to the concentration of ROS. Hence, A tight maintenance of GSH:GSSG ratio is not possible in any of the mechanistic models described here, and interestingly the same is not observed in the data available. For expanding the control over this quantity, the models need to be modified appropriately. In the following chapter, we introduce the production and import of reduced glutathione to the RBCs as well as the export of oxidised glutathione to the blood plasma.

Chapter 3

Open Model

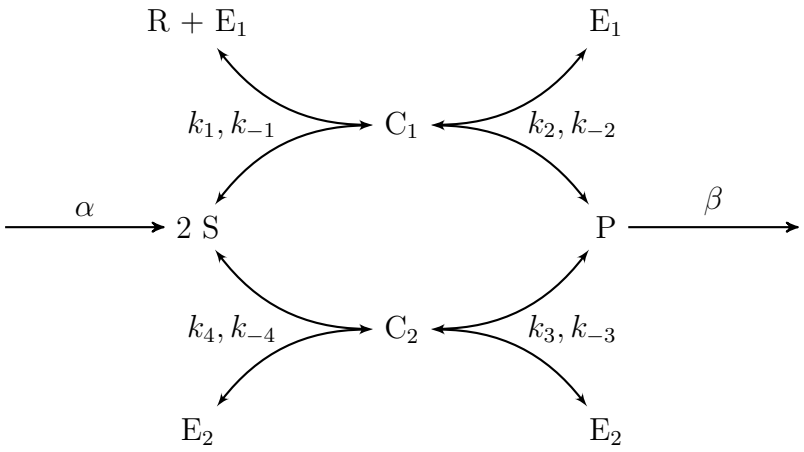
In the models described in the previous chapter, the total amount of glutathione in the system was conserved. In other words, no amount of glutathione was exported. In RBC, there are active transport mechanisms that enable the transport of oxidised glutathione to the blood plasma[8]. Similarly, import of cysteine to the cell enables the production of reduced glutathione in the cell[8]. Apart from this, the experiments performed also suggests the existence of a mechanism for direct intake of GSH to the red blood cells. Considering these facts, it is reasonable to believe that the regulation of the GSH to GSSG ratio or the glutathione concentrations is affected by the transport of glutathione to the cell and plasma. The open model consists of transport of glutathione from and to the system similar to RBCs. In the following model, GSH is imported to the system, and GSSG is exported out of the system. Here the import of GSH accounts for both the intake of GSH from blood plasma and the formation of GSH by the catalysis of glutathione synthetase. Similar to the previous models, the relationship between the concentration of the chemical species, especially the ratio of the two glutathione species, is studied at steady state. Since we don't have a direct measure of the import and export of glutathione, we have investigated three different cases of glutathione transport. Initially, we have studied the case where the export and import follows zero-order kinetics, followed by the cases were each transport is first-order. Again, for simplicity, the following nomenclature is used throughout the chapter,

$$\mathrm{S} := \mathrm{GSH} \qquad \mathrm{P} := \mathrm{GSSG} \qquad \mathrm{R} := \mathrm{ROS} \qquad \mathrm{E}_1 := \mathrm{GPx} \qquad \mathrm{E}_2 := \mathrm{GR}$$

where the corresponding lowercase letters are used for denoting the concentrations of each chemical species.

3.1 Two Enzyme Model

The open model described earlier can visualised as follows. In the following diagram, α denotes the rate constant for production of GSH (could be zero), and β denotes the rate constant for the export of GSSG out of the system (could be zero). Hence, the total amount of S and P in the system is not conserved. $k_1, k_{-1}, \ldots, k_{-4}$ are forward and backward rate constants and C_1 ,C_2 represents the enzyme-substrate complexes formed with enzymes $E_1$and E_2.



Without the transport of glutathione, the model is the same as one described in Section 2.5. The methodology followed for studying the system is same as in the other models. The rate laws are written using the law of mass action and followed by non-dimensionalisation for a better mathematical understanding. Also, both α and β are assumed to be positive quantities, for making sure that the transports are unidirectional.

3.1.1 Reactions

The coupled system shown above can be broken into two simple enzyme reactions as follows.

$$\mathrm{R} + 2\,\mathrm{S} + \mathrm{E_1} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C_1} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{P} + \mathrm{E_1}$$

$$\mathrm{P} + \mathrm{E_2} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{C_2} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} 2\,\mathrm{S} + \mathrm{E_2}$$

The first reaction corresponds to the catalysis by glutathione peroxidase, in which ROS is neutralised with the help of reduced glutathione. The second reaction represents the conversion of oxidised glutathione, formed as a product of the first reaction, to its reduced state. For the first study, we assume that both of import and export of glutathione follow

zero order kinetics, i.e. the rates are constant and do not depends on any concentrations. In other words, the rate of import of glutathione is α and the rate of export is β.

3.1.2 Rate Laws

At the end of both reactions, the enzymes are recovered in free state and hence, the total amount of enzymes are conserved. Let $e_0 = e + c_1$ and $d_0 = d + c_2$ be the total concentration of the enzymes. Here e denotes the concentration of E_1 and d denotes the concentration of E_2. Using the law of mass action, the rate laws are written as follows.

$$\begin{aligned}
\frac{\mathrm{d}c_1}{\mathrm{d}t} &= k_1 s^2 r e + k_{-2} p e - k_{-1} c_1 + k_2 c_1 \\
&= e(k_1 s^2 r + k_{-2} p) - c_1(k_{-1} + k_2) \\
&= (e_0 - c_1)(k_1 s^2 r + k_{-2} p) - c_1(k_{-1} + k_2) \\
&= e_0(k_1 s^2 r + k_{-2} p) - c_1(k_{-1} + k_2 + k_1 s^2 r + k_{-2} p) \qquad (3.1) \\
\frac{\mathrm{d}c_2}{\mathrm{d}t} &= k_3 p d + k_{-4} s^2 d - c_2(k_{-3} + k_4) \\
&= d(k_3 p + k_{-4} s^2) - c_2(k_{-3} + k_4) \\
&= (d_0 - c_2)(k_3 p + k_{-4} s^2) - c_2(k_{-3} + k_4) \\
&= d_0(k_3 p + k_{-4} s^2) - c_2(k_{-3} + k_4 + k_3 p + k_{-4} s^2) \qquad (3.2) \\
\frac{\mathrm{d}s}{\mathrm{d}t} &= -2k_1 r s^2 e + 2k_{-1} c_1 + 2k_4 c_2 - 2k_{-4} s^2 d + \alpha \\
&= -2k_1 r s^2 (e_0 - c_1) + 2k_{-1} c_1 + 2k_4 c_2 - 2k_{-4} s^2 (d_0 - c_2) + \alpha \qquad (3.3) \\
\frac{\mathrm{d}p}{\mathrm{d}t} &= k_2 c_1 - k_{-2} p e + k_{-3} c_2 - k_3 p d - \beta \\
&= k_2 c_1 - k_{-2} p (e_0 - c_1) + k_{-3} c_2 - k_3 p (d_0 - c_2) - \beta \qquad (3.4)
\end{aligned}$$

Since GSH is being added to the system, α is added to the rate of change of GSH concentration. GSSG is removed from the system at a constant rate β, hence the rate of change of GSSG inside the system reduces by β. The rate of change of c_1 and c_2 are unaffected by the import and export of glutathione. Notice that, they are the same as Eq. (2.32) and (2.34).

3.1.3 Non-dimensionalisation

Let $s_0 = s + 2p$ be the total amount of glutathione and r_0 be the amount of ROS present in the system at time $t = 0$.

$$\sigma_1 = \frac{s}{s_0} \qquad x_1 = \frac{c_1}{e_0} \qquad \tau = k_1 e_0 t$$

$$\sigma_2 = \frac{p}{s_0} \qquad x_2 = \frac{c_2}{d_0} \qquad \varepsilon = \frac{e_0}{s_0}$$
$$\sigma_3 = \frac{r}{r_0}$$

Using the same set of substitutions from Section 2.4.2 (shown above), the rate laws are non-dimensionalised, starting with the rate of change of C_1 concentration. Substituting variables in Eq. (3.1):

$$\begin{aligned}
\frac{\mathrm{d}(e_0 x_1)}{\mathrm{d}(\tau/k_1 e_0)} &= e_0(k_1 s_0^2 \sigma_1^2 r_0 \sigma_3 + k_{-2} s_0 \sigma_2) - e_0 x_1(k_{-1} + k_2 + k_1 s_0^2 \sigma_1^2 r_0 \sigma_3 + k_{-2} s_0 \sigma_2) \\
k_1 e_0 \frac{\mathrm{d}x_1}{\mathrm{d}\tau} &= k_1 s_0^2 \sigma_1^2 r_0 \sigma_3 + k_{-2} s_0 \sigma_2 - x_1(k_{-1} + k_2 + k_1 s_0^2 \sigma_1^2 r_0 \sigma_3 + k_{-2} s_0 \sigma_2) \\
\frac{e_0}{s_0} \frac{\mathrm{d}x_1}{\mathrm{d}\tau} &= r_0 s_0 \sigma_1^2 \sigma_3 + \frac{k_{-2}}{k_1} \sigma_2 - x_1 \left(\frac{k_{-1} + k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \frac{k_{-2}}{k_1} \sigma_2 \right)
\end{aligned}$$

Similarly, substituting variables in Eq. (3.2).

$$\begin{aligned}
\frac{\mathrm{d}(d_0 x_2)}{\mathrm{d}(\tau/k_1 e_0)} &= d_0(k_3 s_0 \sigma_2 + k_{-4} s_0^2 \sigma_1^2) - d_0 x_2(k_{-3} + k_4 + k_3 s_0 \sigma_2 + k_{-4} s_0^2 \sigma_1^2) \\
k_1 e_0 \frac{\mathrm{d}x_2}{\mathrm{d}\tau} &= k_3 s_0 \sigma_2 + k_{-4} s_0^2 \sigma_1^2 - x_2(k_{-3} + k_4 + k_3 s_0 \sigma_2 + k_{-4} s_0^2 \sigma_1^2) \\
\frac{e_0}{s_0} \frac{\mathrm{d}x_2}{\mathrm{d}\tau} &= \frac{k_3}{k_1} \sigma_2 + \frac{k_{-4}}{k_1} s_0 \sigma_1^2 - x_2 \left(\frac{k_{-3} + k_4}{k_1 s_0} + \frac{k_3}{k_1} \sigma_2 + \frac{k_{-4}}{k_1} s_0 \sigma_1^2 \right)
\end{aligned}$$

Equation (3.3) $\Rightarrow$

$$\begin{aligned}
\frac{\mathrm{d}(s_0 \sigma_1)}{\mathrm{d}(\tau/k_1 e_0)} &= -2k_1 r_0 \sigma_3 s_0^2 \sigma_1^2 e_0(1 - x_1) + 2k_{-1} e_0 x_1 - 2k_{-4} s_0^2 \sigma_1^2 d_0(1 - x_2) + 2k_4 d_0 x_2 + \alpha \\
k_1 e_0 \frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= -2k_1 r_0 s_0 \sigma_3 \sigma_1^2 e_0(1 - x_1) + \frac{2k_{-1} e_0}{s_0} x_1 - 2k_{-4} s_0 \sigma_1^2 d_0(1 - x_2) + \frac{2k_4 d_0}{s_0} x_2 + \frac{\alpha}{s_0} \\
\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= -2r_0 s_0 \sigma_3 \sigma_1^2(1 - x_1) + \frac{2k_{-1}}{k_1 s_0} x_1 - \frac{2k_{-4} d_0}{k_1 e_0} s_0 \sigma_1^2(1 - x_2) + \frac{2k_4 d_0}{k_1 e_0 s_0} x_2 + \frac{\alpha}{k_1 e_0 s_0}
\end{aligned}$$

Equation (3.4) $\Rightarrow$

$$\begin{aligned}
\frac{\mathrm{d}(s_0 \sigma_2)}{\mathrm{d}(\tau/k_1 e_0)} &= k_2 e_0 x_1 - k_{-2} s_0 \sigma_2 e_0(1 - x_1) + k_{-3} d_0 x_2 - k_3 s_0 \sigma_2 d_0(1 - x_2) - \beta \\
k_1 e_0 \frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= \frac{k_2 e_0}{s_0} x_1 - k_{-2} \sigma_2 e_0(1 - x_1) + \frac{k_{-3} d_0}{s_0} x_2 - k_3 \sigma_2 d_0(1 - x_2) - \frac{\beta}{s_0} \\
\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= \frac{k_2}{k_1 s_0} x_1 - \frac{k_{-2}}{k_1} \sigma_2(1 - x_1) + \frac{k_{-3} d_0}{k_1 e_0 s_0} x_2 - \frac{k_3 d_0}{k_1 e_0} \sigma_2(1 - x_2) - \frac{\beta}{k_1 e_0 s_0}
\end{aligned}$$

The non-dimensionalised rate laws for the two enzyme open model are given by

$$\varepsilon\frac{\mathrm{d}x_1}{\mathrm{d}\tau} = r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2 - x_1\left(\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2\right) \tag{3.5}$$

$$\varepsilon\frac{\mathrm{d}x_2}{\mathrm{d}\tau} = \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2 - x_2\left(\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2\right) \tag{3.6}$$

$$\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} = -2r_0 s_0\sigma_3\sigma_1^2(1-x_1) + \frac{2k_{-1}}{k_1 s_0}x_1 - \frac{2k_{-4}d_0}{k_1 e_0}s_0\sigma_1^2(1-x_2) + \frac{2k_4 d_0}{k_1 e_0 s_0}x_2 + \frac{\alpha}{k_1 e_0 s_0} \tag{3.7}$$

$$\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = \frac{k_2}{k_1 s_0}x_1 - \frac{k_{-2}}{k_1}\sigma_2(1-x_1) + \frac{k_{-3}d_0}{k_1 e_0 s_0}x_2 - \frac{k_3 d_0}{k_1 e_0}\sigma_2(1-x_2) - \frac{\beta}{k_1 e_0 s_0} \tag{3.8}$$

3.1.4 Quasi-Steady-State Approximation

Same as before, the quantity ε is negligibly small as the concentration of the enzyme is quite small compared to the concentration of the substrate. Hence, by quasi-steady state approximation (similar to Section 2.1.3), we assume $\varepsilon\frac{\mathrm{d}x_1}{\mathrm{d}\tau} = 0$ and $\varepsilon\frac{\mathrm{d}x_2}{\mathrm{d}\tau} = 0$. Then Eq. (3.5) and (3.6) gives the following algebraic expressions for x_1 and x_2.

$$x_1 = \frac{r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2}$$

$$x_2 = \frac{\frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2}{\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2}$$

Substituting the value of x_1 and x_2 in Eq. (3.7) eliminates the factor of enzyme-substrate complex concentrations from the rate laws.

$$\begin{aligned}\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} &= \frac{-2r_0 s_0\sigma_1^2\sigma_3\left(\frac{k_{-1}+k_2}{k_1 s_0}\right)}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2} + \frac{2k_{-1}}{k_1 s_0}\frac{\left(r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2\right)}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2} \\ &\quad - \frac{\frac{2k_{-4}d_0}{k_1 e_0}s_0\sigma_1^2\left(\frac{k_{-3}+k_4}{k_1 s_0}\right)}{\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2} + \frac{2k_4 d_0}{k_1 e_0 s_0}\frac{\left(\frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2\right)}{\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1}s_0\sigma_1^2} + \frac{\alpha}{k_1 e_0 s_0} \\ &= \frac{\frac{2k_{-1}r_0 s_0\sigma_1^2\sigma_3}{k_1 s_0} + \frac{2k_{-1}k_{-2}\sigma_2}{k_1^2 s_0} - \frac{2k_{-1}r_0 s_0\sigma_1^2\sigma_3}{k_1 s_0} - \frac{2k_2 r_0 s_0\sigma_1^2\sigma_3}{k_1 s_0}}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0\sigma_1^2\sigma_3 + \frac{k_{-2}}{k_1}\sigma_2}\end{aligned}$$

$$+\frac{\dfrac{2k_4d_0k_3\sigma_2}{k_1^2e_0s_0}+\dfrac{2k_{-4}k_4d_0s_0\sigma_1^2}{k_1^2e_0s_0}-\dfrac{2k_{-3}k_{-4}d_0s_0\sigma_1^2}{k_1^2e_0s_0}-\dfrac{2k_{-4}k_4d_0s_0\sigma_1^2}{k_1^2e_0s_0}}{\dfrac{k_{-3}+k_4}{k_1s_0}+\dfrac{k_3}{k_1}\sigma_2+\dfrac{k_{-4}}{k_1}s_0\sigma_1^2}+\frac{\alpha}{k_1e_0s_0}$$

$$=\frac{\dfrac{2k_{-1}k_{-2}}{k_1^2s_0}\sigma_2-\dfrac{2k_2}{k_1}r_0\sigma_1^2\sigma_3}{\dfrac{k_{-1}+k_2}{k_1s_0}+r_0s_0\sigma_1^2\sigma_3+\dfrac{k_{-2}}{k_1}\sigma_2}+\frac{\dfrac{d_0}{e_0}\left(\dfrac{2k_3k_4}{k_1^2s_0}\sigma_2-\dfrac{2k_{-3}k_{-4}}{k_1^2}\sigma_1^2\right)}{\dfrac{k_{-3}+k_4}{k_1s_0}+\dfrac{k_3}{k_1}\sigma_2+\dfrac{k_{-4}}{k_1}s_0\sigma_1^2}+\frac{\alpha}{k_1e_0s_0} \tag{3.9}$$

Similarly, substituting the value of x_1 and x_2 in Eq. (3.8).

$$\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau}=\frac{k_2}{k_1s_0}\frac{\left(r_0s_0\sigma_1^2\sigma_3+\dfrac{k_{-2}}{k_1}\sigma_2\right)}{\dfrac{k_{-1}+k_2}{k_1s_0}+r_0s_0\sigma_1^2\sigma_3+\dfrac{k_{-2}}{k_1}\sigma_2}-\frac{\dfrac{k_{-2}}{k_1}\left(\dfrac{k_{-1}+k_2}{k_1s_0}\right)\sigma_2}{\dfrac{k_{-1}+k_2}{k_1s_0}+r_0s_0\sigma_1^2\sigma_3+\dfrac{k_{-2}}{k_1}\sigma_2}$$

$$+\frac{k_{-3}d_0}{k_1e_0s_0}\frac{\left(\dfrac{k_3}{k_1}\sigma_2+\dfrac{k_{-4}}{k_1}s_0\sigma_1^2\right)}{\dfrac{k_{-3}+k_4}{k_1s_0}+\dfrac{k_3}{k_1}\sigma_2+\dfrac{k_{-4}}{k_1}s_0\sigma_1^2}-\frac{\dfrac{k_3d_0}{k_1e_0}\left(\dfrac{k_{-3}+k_4}{k_1s_0}\right)\sigma_2}{\dfrac{k_{-3}+k_4}{k_1s_0}+\dfrac{k_3}{k_1}\sigma_2+\dfrac{k_{-4}}{k_1}s_0\sigma_1^2}-\frac{\beta}{k_1e_0s_0}$$

$$=\frac{\dfrac{k_2r_0s_0\sigma_1^2\sigma_3}{k_1s_0}+\dfrac{k_2k_{-2}\sigma_2}{k_1^2s_0}-\dfrac{k_{-1}k_{-2}\sigma_2}{k_1^2s_0}-\dfrac{k_{-2}k_2\sigma_2}{k_1^2s_0}}{\dfrac{k_{-1}+k_2}{k_1s_0}+r_0s_0\sigma_1^2\sigma_3+\dfrac{k_{-2}}{k_1}\sigma_2}$$

$$+\frac{\dfrac{k_{-3}k_3d_0\sigma_2}{k_1^2e_0s_0}+\dfrac{k_{-3}k_{-4}d_0s_0\sigma_1^2}{k_1^2e_0s_0}-\dfrac{k_3k_{-3}d_0\sigma_2}{k_1^2e_0s_0}-\dfrac{k_3k_4d_0\sigma_2}{k_1^2e_0s_0}}{\dfrac{k_{-3}+k_4}{k_1s_0}+\dfrac{k_3}{k_1}\sigma_2+\dfrac{k_{-4}}{k_1}s_0\sigma_1^2}-\frac{\beta}{k_1e_0s_0}$$

$$=\frac{\dfrac{k_2}{k_1}r_0\sigma_1^2\sigma_3-\dfrac{k_{-1}k_{-2}}{k_1^2s_0}\sigma_2}{\dfrac{k_{-1}+k_2}{k_1s_0}+r_0s_0\sigma_1^2\sigma_3+\dfrac{k_{-2}}{k_1}\sigma_2}+\frac{\dfrac{d_0}{e_0}\left(\dfrac{k_{-3}k_{-4}}{k_1^2}\sigma_1^2-\dfrac{k_3k_4}{k_1^2s_0}\sigma_2\right)}{\dfrac{k_{-3}+k_4}{k_1s_0}+\dfrac{k_3}{k_1}\sigma_2+\dfrac{k_{-4}}{k_1}s_0\sigma_1^2}-\frac{\beta}{k_1e_0s_0} \tag{3.10}$$

$$=-\frac{1}{2}\left(\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}-\frac{\alpha}{k_1e_0s_0}\right)-\frac{\beta}{k_1e_0s_0} \tag{3.11}$$

The above relation implies that, the net glutathione flux in the system is essentially the difference between the import and export rates of glutathione. It should be noted that in the closed system, the analogous relation indicated the conservation of glutathione. Also, the stoichiometry introduced in the model affects the total glutathione flux in the model. In terms of original concentrations, Eq. (3.11) becomes

$$\frac{\mathrm{d}p}{\mathrm{d}t}=-\frac{1}{2}\frac{\mathrm{d}s}{\mathrm{d}t}+\frac{1}{2}\alpha-\beta \tag{3.12}$$

3.1.5 $[S]^2$ to [P] Ratio

Here, the quantity of interest is the steady state ratio of reduced to oxidised glutathione, which is believed to be an invariant of ROS concentration. Since ROS are byproducts of several biological pathways[21], the cellular concentration of ROS is varying rapidly. Hence, the regulatory mechanisms for cellular ROS need to respond accordingly, though their kinetics are comparatively slower. For this reason, the greater importance to should be given to the steady state properties of the system, rather than the dynamics. At steady state both $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}$ and $\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau}$ are zero, since the concentrations of the glutathione species stays unchanged. Hence, Eq. (3.12) $\Rightarrow$

$$\alpha = 2\beta$$

In other words, for the system to achieve steady state, the production of GSH should be two times faster than the removal of GSSG. This is an implication of the fact that the total glutathione flux is zero across the system, or the amount of glutathione getting into the system is equal to the amount of glutathione leaving the system (Note that the 2 in the equation comes from the fact that two GSH molecules are required for the formation of a single GSSG molecule). Substituting the steady state condition ($\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}, \frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = 0$) in Eq. (3.10) gives,

$$\frac{\frac{k_2}{k_1} r_0 \sigma_1^2 \sigma_3 - \frac{k_{-1}k_{-2}}{k_1^2 s_0}\sigma_2}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \frac{k_{-2}}{k_1}\sigma_2} = \frac{\frac{d_0}{e_0}\left(\frac{k_3 k_4}{k_1^2 s_0}\sigma_2 - \frac{k_{-3}k_{-4}}{k_1^2}\sigma_1^2\right)}{\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1} s_0 \sigma_1^2} + \frac{\beta}{k_1 e_0 s_0}$$

Multiplying both sides with $k_1^2 s_0$ gives the following expression.

$$\frac{k_1 k_2 r_0 s_0 \sigma_1^2 \sigma_3 - k_{-1}k_{-2}\sigma_2}{\frac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \frac{k_{-2}}{k_1}\sigma_2} = \frac{\frac{d_0}{e_0}\left(k_3 k_4 \sigma_2 - k_{-3}k_{-4} s_0 \sigma_1^2\right)}{\frac{k_{-3}+k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1} s_0 \sigma_1^2} + \frac{k_1 \beta}{e_0}$$

In terms of the original concentrations, the equations can be rewritten as follows.

$$\frac{k_1 k_2 s^2 r - k_{-1}k_{-2} p}{(k_{-1}+k_2) + k_1 s^2 r + k_{-2} p} = \frac{\frac{d_0}{e_0}\left(k_3 k_4 p - k_{-3}k_{-4} s^2\right)}{(k_{-3}+k_4) + k_3 p + k_{-4} s^2} + \frac{\beta}{e_0}$$

In enzyme kinetics, the reverse reaction step where product and enzyme or the enzyme-product complex goes back to the enzyme-substrate complex state is improbable and usually disregarded[13]. If we assume irreversibility in the enzyme catalysis, that is k_{-2}

and k_{-4} are negligibly small, we get the following expression.

$$\frac{k_1 k_2 s^2 r}{(k_{-1}+k_2)+k_1 s^2 r} = \frac{\frac{d_0}{e_0}(k_3 k_4 p)}{(k_{-3}+k_4)+k_3 p} + \frac{\beta}{e_0}$$

$\Rightarrow$

$$\frac{k_2 e_0 s^2 r}{k_m + s^2 r} = \frac{k_4 d_0 p}{k'_m + p} + \beta \tag{3.13}$$

where $k_m = \dfrac{k_{-1}+k_2}{k_1}$ and $k'_m = \dfrac{k_{-3}+k_4}{k_3}$. Similarly, the following analogous expression can be obtained from applying steady state condition in Eq. (3.9).

$$\frac{k_2 e_0 s^2 r}{k_m + s^2 r} = \frac{k_4 d_0 p}{k'_m + p} + \frac{\alpha}{2} \tag{3.14}$$

The relationship between steady state concentrations obtained here is quite similar to the one derived in Section 2.5. The difference between these two is characterised by the presence of export and import rates, though they do not have much effect on the regulation steady state concentrations. Since the rates α and β are constants, variation in ROS concentration can be addressed only by changing GSH and GSSG concentrations, which is no different than the closed two enzyme model discussed in the last chapter. Hence, introducing import and export of glutathione alone is not enough for the regulation of reduced to oxidised glutathione concentration ratio in cells. In RBCs, the transport of glutathione is much more complex than constant flux import and export. So the simplest improvement for the current model would be the addition of non-constant glutathione import and export fluxes. In the following section, the export of GSSG to blood plasma is assumed to be dependent on the cellular concentration of GSSG.

3.1.6 Concentration Dependent Export of P

If the export of GSSG follows zero order kinetics, there is a possibility that the rate of export, β could be different from person to person. Hence, the optimisation of the parameters, k_2, k_4, k_m and k'_m will be difficult since the value of β for each patient is unknown. This issue is also resolved by assuming that the rate export is dependent on the concentration of GSSG in RBC. Let β denote the rate constant for the export of glutathione to plasma. Suppose the export of P follows first order kinetics, then the export rate of GSSG is given by $\beta \cdot p$. Here, the rates laws (3.1), (3.2) and (3.3) stays the

same, and Eq. (3.4) becomes

$$\frac{\mathrm{d}p}{\mathrm{d}t} = k_2 c_1 - k_{-2} p(e_0 - c_1) + k_{-3} c_2 - k_3 p(d_0 - c_2) - \beta p$$

and the corresponding non-dimensionalised equation is given by

$$\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = \frac{k_2}{k_1 s_0} x_1 - \frac{k_{-2}}{k_1}\sigma_2(1 - x_1) + \frac{k_{-3} d_0}{k_1 e_0 s_0} x_2 - \frac{k_3 d_0}{k_1 e_0}\sigma_2(1 - x_2) - \frac{\beta \sigma_2}{k_1 e_0}$$

Analogous to Eq. (3.10), we get the following expression by substituting the algebraic expressions for x_1 and x_2.

$$\begin{aligned}\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} &= \frac{\frac{k_2}{k_1} r_0 \sigma_1^2 \sigma_3 - \frac{k_{-1} k_{-2}}{k_1^2 s_0}\sigma_2}{\frac{k_{-1} + k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \frac{k_{-2}}{k_1}\sigma_2} + \frac{\frac{d_0}{e_0}\left(\frac{k_{-3} k_{-4}}{k_1^2}\sigma_1^2 - \frac{k_3 k_4}{k_1^2 s_0}\sigma_2\right)}{\frac{k_{-3} + k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1} s_0 \sigma_1^2} - \frac{\beta\sigma_2}{k_1 e_0} \\ &= -\frac{1}{2}\left(\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau} - \frac{\alpha}{k_1 e_0 s_0}\right) - \frac{\beta\sigma_2}{k_1 e_0}\end{aligned}$$

Hence, at steady state, i.e. $\frac{\mathrm{d}\sigma_1}{\mathrm{d}\tau}, \frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau} = 0$,

$$\frac{\alpha}{2 k_1 e_0 s_0} = \frac{\beta\sigma_2}{k_1 e_0}$$

which gives the following relation between the export and import rate constants at steady state.

$$\begin{aligned}\alpha &= 2\beta s_0 \sigma_2 \\ &= 2\beta \cdot p\end{aligned} \tag{3.15}$$

From the above expression of $\frac{\mathrm{d}\sigma_2}{\mathrm{d}\tau}$, at steady state, we get

$$\frac{\frac{k_2}{k_1} r_0 \sigma_1^2 \sigma_3 - \frac{k_{-1} k_{-2}}{k_1^2 s_0}\sigma_2}{\frac{k_{-1} + k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \frac{k_{-2}}{k_1}\sigma_2} = -\frac{\frac{d_0}{e_0}\left(\frac{k_{-3} k_{-4}}{k_1^2}\sigma_1^2 - \frac{k_3 k_4}{k_1^2 s_0}\sigma_2\right)}{\frac{k_{-3} + k_4}{k_1 s_0} + \frac{k_3}{k_1}\sigma_2 + \frac{k_{-4}}{k_1} s_0 \sigma_1^2} + \frac{\beta\sigma_2}{k_1 e_0}$$

$\Rightarrow$

$$\frac{k_1 k_2 r s^2 - k_{-1} k_{-2} p}{k_{-1} + k_2 + k_1 r s^2 + k_{-2} p} = \frac{d_0}{e_0}\left(\frac{k_3 k_4 p - k_{-3} k_{-4} s^2}{k_{-3} + k_4 + k_3 p + k_{-4} s^2} + \beta p\right)$$

Assuming irreversibility in the reactions, we get

$$\frac{k_2 e_0 \cdot r s^2}{k_m + r s^2} = \frac{k_4 d_0 \cdot p}{k'_m + p} + \beta p \tag{3.16}$$

where $k_m = \dfrac{k_{-1} + k_2}{k_1}$ and $k'_m = \dfrac{k_{-3} + k_4}{k_3}$. The above equation is similar to Eq. (3.13), with an additional concentration of P alongside β. Here β is a constant quantity, and it is assumed same for every person, similar to the rate constants k_1, k_2, etc.. This enables us to numerically determine β unlike the previous case, where β could change from person to person. The fractions on both sides of the equations are similar to the expression for the velocity of an enzymatic reaction, following Michaelis-Menten kinetics. So the above expression indicates the fact that the velocity of the oxidation of GSH is the sum of velocities of reduction and the export of GSSG. In other words, the total flux across the system is zero at steady state. Suppose the concentration of R is very large, then the above expression reduces to

$$k_2 e_0 = \frac{k_4 d_0 \cdot p}{k'_m + p} + \beta p \tag{3.17}$$

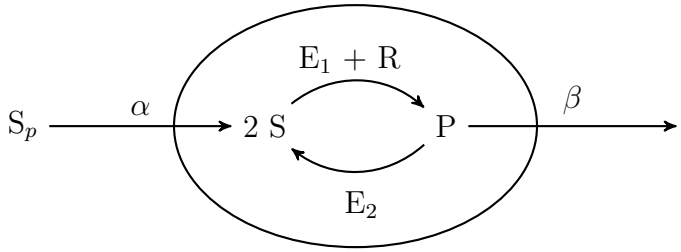
Since k_2, k_4, k'_m and β are constants, it is possible to numerically solve for these parameters, using known values of p, e_0 and d_0. Measuring the exact ROS levels in cells is a hard task, primarily due to the short half life of these molecules[22]. Hence, the above equation gives insights into the properties of the parameters k_2, k_4, k'_m and β without the need of measuring ROS concentration.

3.1.7 Concentration Dependent Import of S

The external concentration of GSH can have an effect on the rate at which GSH is imported to the cell. Suppose the intake of S to the system follows first order kinetics, i.e. the intake is proportional to the external concentration of S. Then the rate of change of concentration of S becomes

$$\frac{\mathrm{d}s}{\mathrm{d}t} = -2k_1 r s^2 (e_0 - c_1) + 2k_{-1} c_1 + 2k_4 c_2 - 2k_{-4} s^2 (d_0 - c_2) + \alpha s_p$$

where s_p denotes the external concentration of S. Here the system is similar to the miniature model described in the introductory part, expect we are considering the external concentration of GSH (Concentration of GSH in blood plasma). The current model can be visualised as follows.



The introduction of S_p in the model directly affects the rate of change of concentration of S. The corresponding non-dimensionalised rate law for $\frac{ds}{dt}$ is given by

$$\frac{d\sigma_1}{d\tau} = -2r_0 s_0 \sigma_3 \sigma_1^2 (1 - x_1) + \frac{2k_{-1}}{k_1 s_0} x_1 - \frac{2k_{-4} d_0}{k_1 e_0} s_0 \sigma_1^2 (1 - x_2) + \frac{2k_4 d_0}{k_1 e_0 s_0} x_2 + \frac{\alpha \sigma_4}{k_1 e_0}$$

where $\sigma_4 = \dfrac{s_p}{s_0}$. σ_4 is the additional substitution used for the non-dimensionalising the external GSH concentration term. The other rate laws stays the same as in the previous section. Since $\frac{dx_1}{d\tau}$ and $\frac{dx_2}{d\tau}$ are unchanged, the algebraic expressions for x_1 and x_2 resulting from Q.S.S.A., stay unchanged (same as the equations in Section 3.1.4). Substituting the value of x_1 and x_2 in the above equation yields the following.

$$\begin{aligned}
\frac{d\sigma_1}{d\tau} &= \frac{2\,\dfrac{k_{-1}k_{-2}}{k_1^2 s_0}\sigma_2 - 2\dfrac{k_2}{k_1} r_0 \sigma_1^2 \sigma_3}{\dfrac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3 + \dfrac{k_{-2}}{k_1}\sigma_2} + \frac{2\,\dfrac{d_0}{e_0}\left(\dfrac{k_3 k_4}{k_1^2 s_0}\sigma_2 - \dfrac{k_{-3}k_{-4}}{k_1^2}\sigma_1^2\right)}{\dfrac{k_{-3}+k_4}{k_1 s_0} + \dfrac{k_3}{k_1}\sigma_2 + \dfrac{k_{-4}}{k_1} s_0 \sigma_1^2} + \frac{\alpha\sigma_4}{k_1 e_0} \\
&= -2\left(\frac{d\sigma_2}{d\tau} + \frac{\beta\sigma_2}{k_1 e_0}\right) + \frac{\alpha\sigma_4}{k_1 e_0}
\end{aligned}$$

Hence, at steady state ($\frac{d\sigma_1}{d\tau}, \frac{d\sigma_2}{d\tau} = 0$),

$$\frac{2\beta\sigma_2}{k_1 e_0} = \frac{\alpha\sigma_4}{k_1 e_0} \qquad \Rightarrow \qquad 2\beta\sigma_2 = \alpha\sigma_4$$

Hence, we get the steady state relationship between external GSH and internal GSSG as $\sigma_4 : \sigma_2 = 2\beta : \alpha$. Note that this ratio is the same as the ratio of concentrations of S_e and P. Since both α and β are constants, this implies that the ratio of the external concentration of S to the concentration of P is constant at steady state, independent of the concentrations of the enzymes and ROS. Putting $\frac{d\sigma_1}{d\tau} = 0$ in the above non-dimensionalised rate law gives the following steady state relationship, assuming irreversibility ($k_{-2}, k_{-4} = 0$)

$$\frac{2\dfrac{k_2}{k_1} r_0 \sigma_1^2 \sigma_3}{\dfrac{k_{-1}+k_2}{k_1 s_0} + r_0 s_0 \sigma_1^2 \sigma_3} = \frac{2\dfrac{d_0}{e_0}\dfrac{k_3 k_4}{k_1^2 s_0}\sigma_2}{\dfrac{k_{-3}+k_4}{k_1 s_0} + \dfrac{k_3}{k_1}\sigma_2} + \frac{\alpha\sigma_4}{k_1 e_0}$$

Multiplying both sides by $k_1 s_0$ and rearranging gives,

$$\frac{2k_2 e_0 s_0^2 r_0 \sigma_1^2 \sigma_3}{k_m + r_0 s_0^2 \sigma_1^2 \sigma_3} = \frac{2d_0 s_0 k_4 \sigma_2}{k'_m + k_3 s_0 \sigma_2} + \alpha \sigma_4 s_0$$

and in terms of the original concentrations,

$$\frac{k_2 e_0 s^2 r}{k_m + s^2 r} = \frac{k_4 d_0 p}{k'_m + p} + \frac{\alpha}{2} s_p$$

The concentration of GSH in blood plasma can be approximated by the erythrocyte concentration of GSH as plasma GSH varies in proportion to cellular GSH, that is, $[\mathrm{GSH}]_\mathrm{e} \approx \sigma \cdot [\mathrm{GSH}]$. Using this approximation, above relation between the plasma GSH and cellular GSSG concentration can be rewritten in the following the way,

$$\frac{[\mathrm{GSH}]}{[\mathrm{GSSG}]} \approx \frac{1}{\sigma} \cdot \frac{2\beta}{\alpha}$$

which implies that the ratio of cellular GSH concentration to GSSG concentration is approximately constant, if the import of GSH is dependent on the plasma GSH concentration.

3.1.8 Parameter Estimation

Among the parameters in the Eq. (3.16), concentration of S(GSH), E_1(GPx) and E_2(GR) has been measured for 50 diabetic (figure 3.1) and non-diabetic patients. The same is grouped into three categories according to the time of measurement, namely zeroth, fourth and eighth week after starting of anti-diabetic treatment. In the following section, the zeroth week data is used for parameter estimation, as for the first part high ROS concentration is assumed to be present at the beginning of anti-diabetic treatment. Using these known values of s, p, e_0 and d_0, it is possible to numerically solve for the values of k_2, k_4, k_m, k'_m and β. We have taken the assumption that, supported by the findings from the experiments, GSSG concentration is 10% of the total glutathione concentration, as the GSSG concentration data is not available.

Using GSSG Concentration

Since it is hard to directly measure ROS concentration in cells due to its short half life, we assume that the amount of ROS produced is large in diabetic patients and this yields the reduced steady state relation Eq. (3.17),

$$k_2 e_0 = \frac{k_4 d_0 \cdot p}{k'_m + p} + \beta p$$

where e_0 is the concentration of GPx, d_0 is the concentration of GR and p is the concentration of GSSG in RBCs. To reduce the number of parameters, both sides are divided by k_4, which gives

$$r_1 e_0 = \frac{d_0 p}{k'_m + p} + r_2 p$$

where $r_1 = \frac{k_2}{k_4}$ and $r_2 = \frac{\beta}{k_4}$ and the four unknowns in the system has been reduced to three unknowns, namely r_1, r_2 and k'_m. The parameter optimisation was done by two methods. In the first method three sets of values, corresponding to three patients, were taken and the unknowns were solved algebraically. Let e_i, d_i and p_i denote the GPx, GR and GSSG concentrations of patient i, respectively. Consider, for example, $i = 1, 2, 3$. Substituting the value of e_i, d_i and p_i in the above equation gives a system of three equation with three unknowns. Since r_1 is constant quantity, using the data of patient 1 and 2, we get

$$\frac{1}{e_1}\left(\frac{d_1 p_1}{k'_m + p_1} + r_2 p_1\right) = \frac{1}{e_2}\left(\frac{d_2 p_2}{k'_m + p_2} + r_2 p_2\right)$$

and rearranging the terms gives

$$r_2 = \frac{\dfrac{d_2 e_1 p_2}{k'_m + p_2} - \dfrac{d_1 e_2 p_1}{k'_m + p_1}}{p_1 e_2 - p_2 e_1}$$

Similarly, if we start with $i = 2$ and 3, we get a relation,

$$r_2 = \frac{\dfrac{d_3 e_2 p_3}{k'_m + p_3} - \dfrac{d_2 e_3 p_2}{k'_m + p_2}}{p_2 e_3 - p_3 e_2}$$

Comparing the two expressions of r_2 gives a formula for k'_m in terms of the known values, $e_i, d_i, p_i;\ i = 1, 2, 3$. Hence, by substituting back the the values we can obtain r_2 and r_1. The same process is repeated for different values of i. In the second method for optimisation, a least square algorithm was used to optimise the parameters over the entire data. Using the inbuilt function `lsqnonlin` in MATLAB, the constrained optimisation (since all the parameters are positive quantities) was performed by minimising the objective function,

$$\text{obj}(r_1, r_2, k'_m) = \frac{1}{r_1}\left(\frac{d_0 p}{k'_m + p} + r_2 p\right) - e_0$$

over the data of fifty diabetic patients.

In the first method, the algebraic solutions were varying with the choice of data triplet, and in the least square method, the output of the function is observed to be dependent

on the initial point given for the gradient descent part in the algorithm. During the optimisation process, even though there was a lot of variation in the optimisation output, the value of k_m stayed almost constant (standard deviation in the order of 10^{-5}). The same trend is observed in both optimisation methods. The variations in optimised data in the least square method suggest that a global optimum might not be possible and for each choice of the starting point, the algorithm is possibly giving a local minimum point. The results of optimisations are given in Fig. 3.1(a).

Using ROS Markers

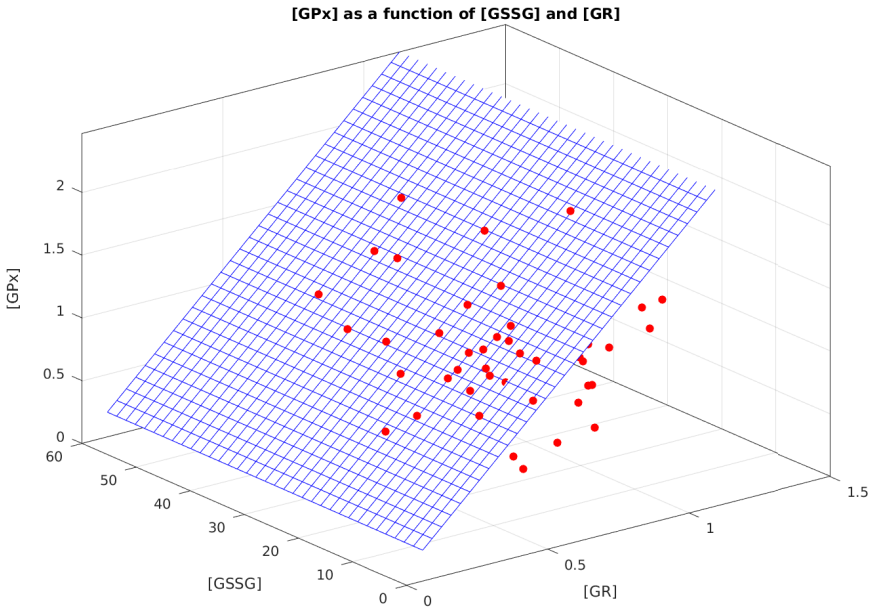
Reducing Eq. (3.16) using the approximation that the ROS concentration is high, could have an adverse effect on the parameter approximation process. For resolving this issue, the optimisation can be carried out using ROS markers such as thiobarbituric acid reactive substances (TBARS). Even though ROS are short-lived, the quantity of ROS can be measured by the damage produced by ROS. Here we can use the original relation (Eq. (3.16)) with the concentration of TBARS in place of ROS. Dividing both sides of Eq. (3.16) gives the following reduced relation.

$$\frac{r_1 e_0 \cdot rs^2}{k_m + rs^2} = \frac{d_0 \cdot p}{k'_m + p} + r_2 p$$

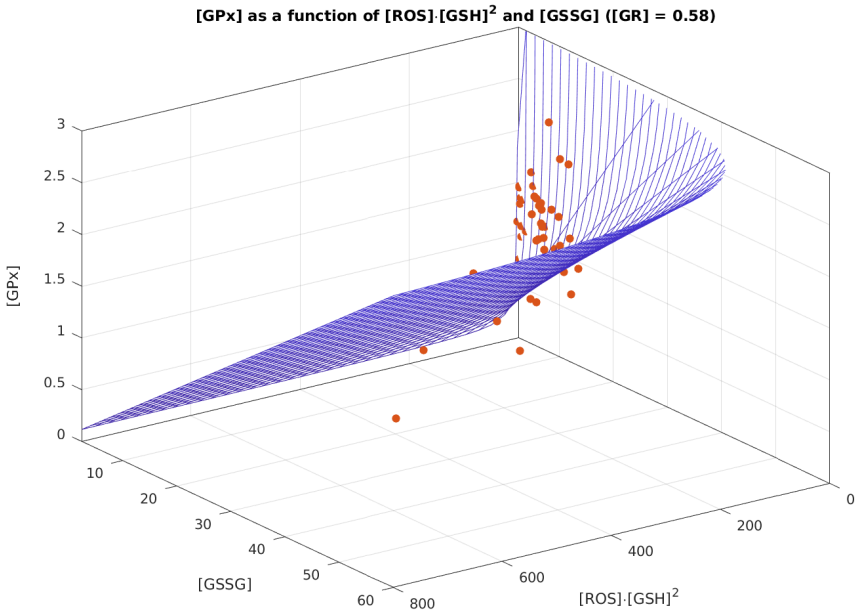
where $r_1 = \frac{k_2}{k_4}$ and $r_2 = \frac{\beta}{k_4}$. During optimization it was observed that the outputs of the optimization has dependence on the initial points given. The optimization was carried out using the functions `lsqnonlin` and `patternsearch` in MATLAB. The first function uses a gradient descent method, whereas the second one uses a polling method and hence has less dependence on the initial point given for the optimization. The objective function used here is as follows:

$$\text{obj}(r_1, r_2, k_m, k'_m) = \left(\frac{k_m + rs^2}{r_1 \cdot rs^2}\right)\left(\frac{d_0 \cdot p}{k'_m + p} + r_2 p\right) - e_0$$

During optimisation using `patternsearch`, it was seen that the value of k'_m remained unchanged. Hence, further optimisation was performed by fixing the value of k'_m to be 30.62. The results of optimisations are given in Fig. 3.1(b). The estimated values for rest of the parameters are given by: $r_1 = 22.10,\ r_2 = 1097.26,\ k_m = 16.35$ (units are ignored). In the figure, GPx concentration is plotted as a function of [GSSG] and [ROS]·[GSH]^2. Even though the results obtained here is better than the previous one, the plotted surface is not able to capture the exact nature of the data. This might be resulting from our assumption that GSSG concentration is 10 percent of the GSH present.



(a) Parameter estimation using GSSG concentration.



(b) Parameter estimation using TBARS concentration.

Figure 3.1: Result of parameter estimations. In both cases, GSSG concentration is assumed to be 10% of GSH concentration, as GSSG data is unavailable.

3.2 Model Simulations

Similar to the previous chapter, instead of proving the existence and uniqueness of solutions, we directly simulated the model using arbitrary parameter values and initial conditions. The differential equations from the previous sections were numerically solved using the Runge-Kutta method with the help of `ode45` function in MATLAB. As we are interested in the steady state of the system, the dynamics of the system is disregarded. The case where both import and the export follows second ordered is skipped since they are essentially a scaled up version of the closed model at steady state. We have seen that for achieving steady state, the input flux of glutathione into the system should match with the output flux. In the constant case, the same is observed at steady state, and the relation between the chemical concentrations at equilibrium is a translated version of the closed system. Similarly, we ignore the case where both export and import of glutathione follows first order kinetics since the plasma glutathione concentrations data is unavailable. Hence, for the simulation, the zero order import & first order export case is considered. For understanding the nature of the system and comparison, all the parameters, including the concentration of the enzymes are taken to be unity. The following three general observations were made during the simulations for different sets of parameter values.

- GSH and GSSG concentrations did not saturate, i.e. did not attain steady state for all values of the parameters $k_2, k_4, k_m, k'_m, \alpha, \beta$, and the enzyme concentrations [GPx] and [GR].

- For all sets of parameters where GSH and GSSG concentrations saturated, GSSG concentration at steady state is independent of the ROS concentration. That is, the same GSSG concentration is observed at steady state for all values of ROS.

- The ratio, GSH:GSSG, is more sensitive to the changes in k_2, k'_m, α and [GPx], and it is observed to be less varying for higher values of ROS.

The first observation points to the existence of bounds on the parameter values. It says that the system of coupled differential equations does not have any solutions for some values of the parameters. The third observation is also seen in the closed model. In Fig. 2.2, as ROS concentration increases, the relative change in the ratio decreases. The time series of GSH and GSSG and the change of steady state concentration of GSH and GSSG with respect to ROS concentration is shown in the following figures. Here all the parameters and the concentrations of GPx and GR are taken to be unity for simplicity. The initial condition used for the simulation is [GSH](0) = 2 and [GSSG](0) = 1. From Fig. 3.3, it can be observed that the steady state concentration of GSSG is invariant with

respect to ROS concentration. The same is observed for all sets of parameters where both GSH and GSSG concentrations saturated over time. In further analysis, it was observed that the only parameters which influence the steady state concentration of cellular GSSG are the import and export rate constants, α and β, which agrees with Eq. (3.15).

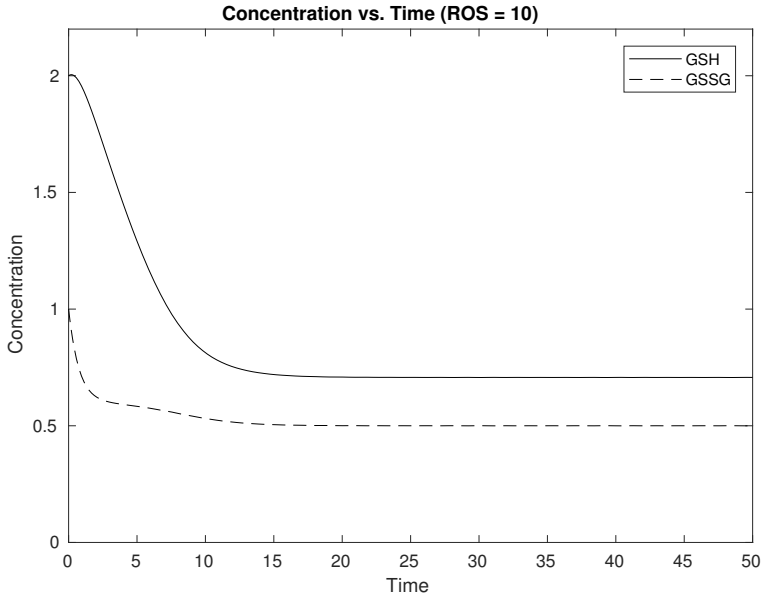


Figure 3.2: Time series of GSH and GSSG, for parameter values $k_2 = k_4 = k_m = k'_m = \alpha = \beta = 1$ and initial conditions [GSH](0) = 2, [GSSG](0) = 1.

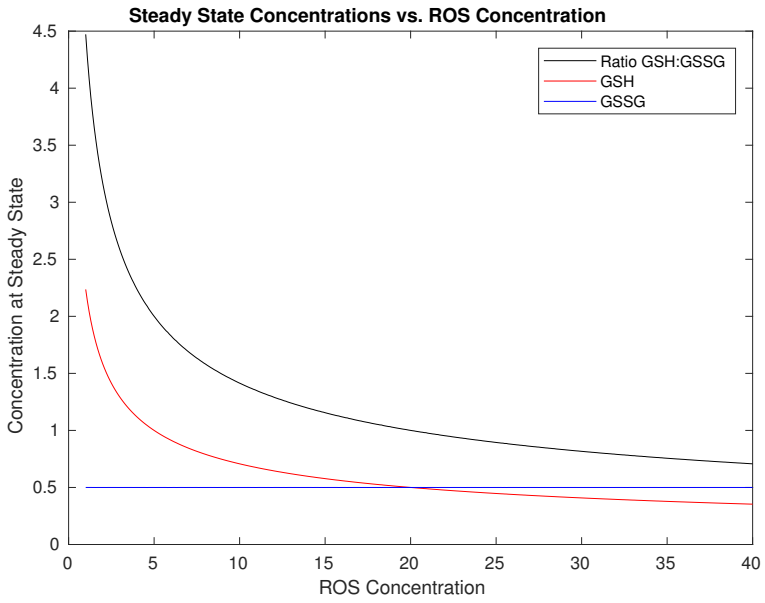


Figure 3.3: Steady state concentrations of GSH and GSSG plotted for different values of ROS. Parameters used: $k_2 = k_4 = k_m = k'_m = \alpha = \beta = 1$. Initial conditions used: [GSH](0) = 2, [GSSG](0) = 1.

The invariance of cellular GSSG with respect to ROS is shown in Figure 3.4. Even though GSH reaches two different steady state concentration for ROS = 10 and ROS

= 1000, GSSG attains the same steady state for both cases. A possible explanation for this observation can be given using Eq. (3.16). In Eq. (3.16), the right hand side is independent of ROS. Hence when ROS changes, the equality can be kept by changing the GSH concentration. That is, if ROS increases, by decreasing GSH accordingly, the quantity $[\text{ROS}] \cdot [\text{GSH}]^2$ can be kept constant. This way, the right hand side is kept constant, and hence GSSG concentration at steady state concentration is constant.

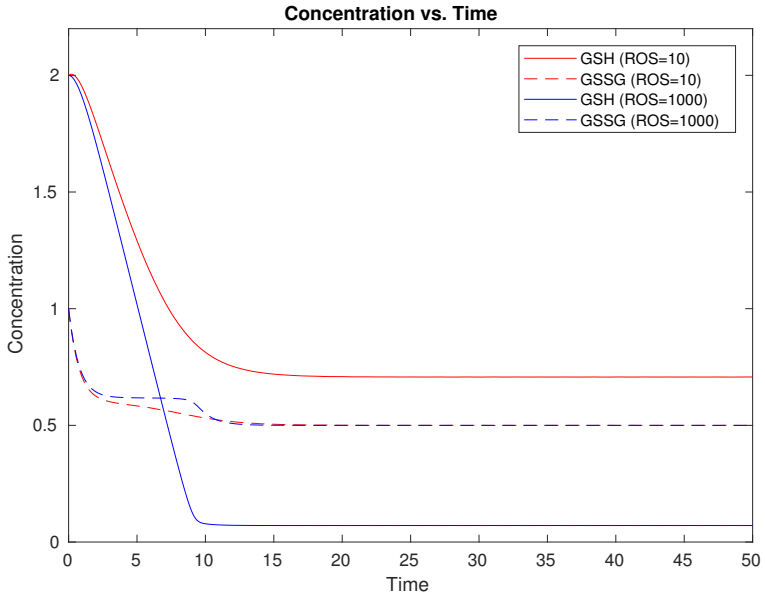


Figure 3.4: Time series plot of GSH and GSSG for different ROS values. Red curves denote the time series for the case where ROS = 10, and blue curves denote the case where ROS = 1000. For both cases GSSG attains the same steady state concentration, 0.5. The steady state concentration of GSH, for ROS= 10, is 0.707, and 0.07 for ROS = 1000.

Figure 3.4 shows that indeed this is the reason for constant GSSG concentration at steady state, for any value of ROS. In the figure, when ROS concentration is increased to hundred times the previous value, steady state GSH concentration drops to one-tenth of the last value (0.7071 to 0.0707). The same result is observed in multiple simulations using different parameter sets and ROS values. Since GSSG steady state concentration is independent of the change in ROS value, a relation between ROS and GSH concentration can be obtained from Eq. (3.16). The right-hand side of Eq. (3.16) is constant for a given set of parameters, this gives

$$\frac{k_m}{[\text{ROS}] \cdot [\text{GSH}]^2} = \frac{k_2 \cdot [\text{GPx}]}{\dfrac{k_4 \cdot [\text{GR}]}{\dfrac{k'_m}{[\text{GSSG}]} + 1} + \beta \cdot [\text{GSSG}]} - 1$$

Rearranging the terms gives the following expression for [GSH].

$$[\text{GSH}] = \sqrt{\frac{1}{[\text{ROS}]} \cdot \frac{k_m \cdot [\text{GSSG}] \cdot (k_4 \cdot [\text{GR}] + \beta \cdot k'_m + \beta \cdot [\text{GSSG}])}{k_2 \cdot [\text{GPx}] \cdot (k'_m + [\text{GSSG}])}} \tag{3.18}$$

Since GSSG concentration is a constant quantity at steady state, the above equation gives the direct relationship between the ROS concentration and GSH concentration. In the following section we introduce a phenomenological model emerging from the above relation.

3.3 Phenomenological model

3.3.1 GSSG Homeostasis

Eq. (3.15) and the observations from the simulations suggests that there exists GSSG homeostasis in erythrocytes. As a part of the study, two experiments were conducted. In one of them, blood samples were treated with different concentrations of GSH and cellular GSH, GSSG levels were measured after a certain period. In the second experiment, similarly, blood samples were treated with GSSG, and cellular glutathione concentrations are measured. The results of the experiments are shown in Fig. 3.5 and 3.6. Note that in the following two figures, the x-axis denotes the concentration of supplied glutathione, and the y-axis denotes the concentration of cellular glutathione species. In all the cases of GSH treatment (Fig. 3.5), the cellular GSH level increased in proportion to the supplied GSH level, and it can be observed that the cellular GSSG level did not alter much. In the second figure, even though the blood samples are treated with large amounts of oxidised glutathione, the cellular glutathione levels, both GSH and GSSG did not change accordingly. Even though there are fluctuations in the concentrations, it cannot be associated with the supply of GSSG. These observations point to the fact that GSSG is maintained constant in erythrocytes, regardless of the concentration of cellular GSH. A graphical interpretation of this fact is as follows: Let

$$v_{\text{left}} = \frac{k2 \cdot [\text{GPx}]}{\dfrac{k_m}{[\text{GSH}]^2 \cdot [\text{ROS}]} + 1}$$

$$v_{\text{right\_1}} = \frac{k_4 \cdot [\text{GR}]}{\dfrac{k'_m}{[\text{GSSG}]} + 1} + \beta \cdot [\text{GSSG}]$$

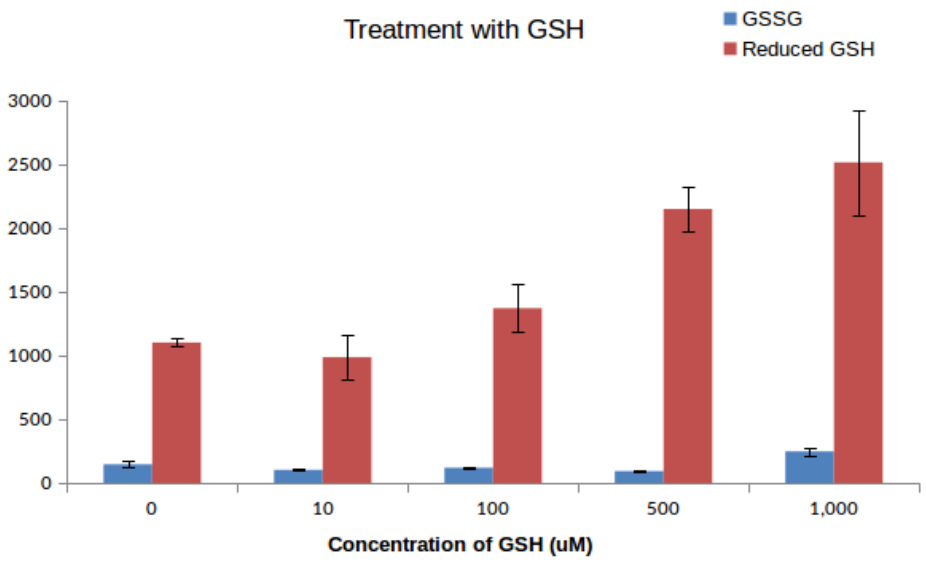


Figure 3.5: Blood samples were treated with $10, 100, 500$ and 1000 μM of GSH and cellular GSH, GSSG levels were measured after 30 minutes. Here, the x-axis denotes the supplied GSH concentration, and the y-axis expresses the cellular concentrations in μM. Notice that cellular GSSG levels are not affected by the GSH treatment, even though cellular GSH concentration is increasing proportionally. We are grateful to Dr Saroj Ghaskadbi and Jhankar Acharya (Savitribai Phule Pune University) for providing us with experimental results.

$$v_{\text{right\_2}} = \frac{k_4 \cdot [\text{GR}]}{\dfrac{k'_m}{[\text{GSSG}]} + 1} + \frac{\alpha}{2}$$

For the system to achieve steady state, equality should hold between the above three velocities. In Fig. 3.7, the three velocities are plotted as functions of GSSG and GSH concentrations, and the intersection of the three surfaces marks the steady state of the system for the given set of parameter values. Notice that the intersection of these three surfaces is a single point. That is, for these parameter values, the system will always approach the same steady state concentrations for any initial conditions. The effect of change in ROS concentration, on the steady state of the system, is visualised in Fig 3.8. As the intersection of two right velocities has a constant GSSG value, the intersection with the third surface, i.e. v_{left}, always has the same GSSG concentration. Hence, GSSG concentration is invariant to ROS concentration. This can also be seen from the vertical projection of Fig. 3.8a. The three blue coloured surfaces denote the left velocity for different [ROS] values. But the intersection between the two right velocities, is given by the boundary of $v_{\text{right\_2}}$ at GSSG $= 0.5$. Hence, for any value of ROS, the left velocity will have an intersection at this constant value of GSSG. Another question which should

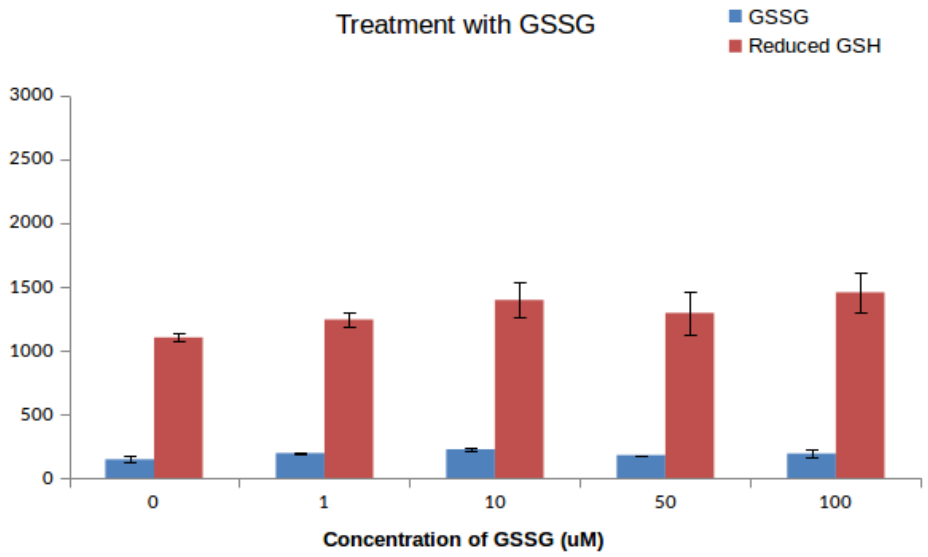


Figure 3.6: Blood samples were treated with $1, 10, 50$ and 100 μM of GSSG and cellular GSH, GSSG levels were measured after 30 minutes. Here, the x-axis denotes the supplied GSH concentration, and the y-axis denotes the cellular concentrations in μM. No significant change is observed in the cellular concentration of the two glutathione species. We are grateful to Dr Saroj Ghaskadbi and Jhankar Acharya (Savitribai Phule Pune University) for providing us with the data for plotting.

be addressed here is that, whether this nature of the velocities holds for any values of the parameters. It is already obvious from the equations that the steady state concentrations are independent of the initial conditions. From further analysis, it has been found that unless α is non-zero the intersection of the two right velocities will always be a straight line parallel to the GSH axis, and perpendicular to both the other axes. When α is zero, then the intersection of right velocities is exactly the GSH axis, and no solution exists in this instance. This is also evident from the equations, as the right velocities do not match since β is non-zero. If both α and β are zero, then the system is basically equivalent to the closed model discussed in the previous chapter. Hence, by taking advantage of the GSSG homeostasis, we can convert Eq. (3.18) to a phenomenological model for understanding the relationship between glutathione and reactive oxygen species. The following section discusses the use of glucose concentration as a covariate of reactive oxygen species concentration.

3.3.2 HbA1c as a ROS Marker

Reactive oxygen species are continuously produced in cells as a byproduct of several chemical pathways. Studies show that hyperglycemic conditions lead to the overproduction reactive oxygen species in cells[23, 24]. As mentioned earlier, the concentration of

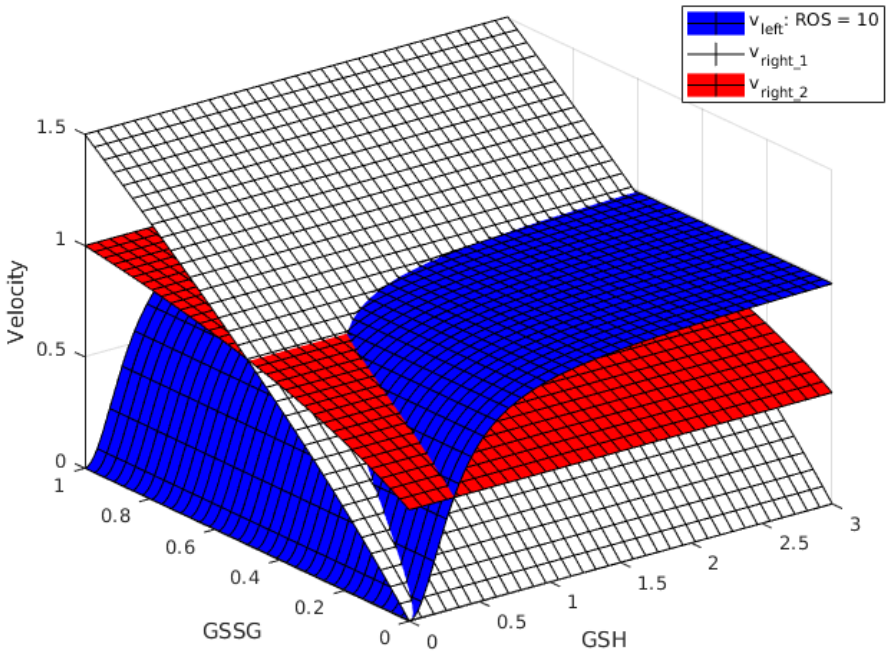


Figure 3.7: $v_{\text{left}}, v_{\text{right\_1}}$ and $v_{\text{right\_2}}$ as functions of GSSG and GSH concentrations. The steady state GSH and GSSG concentrations are given by the points corresponding to the intersection of the three surfaces. Here all parameters are considered to be unity for understanding the nature of the equations.

ROS in cells changes rapidly and is hard to measure accurately. But, as more glucose in cells implies more reactive oxygen species, we can approximate the amount of ROS present in the system using the glucose present. Let

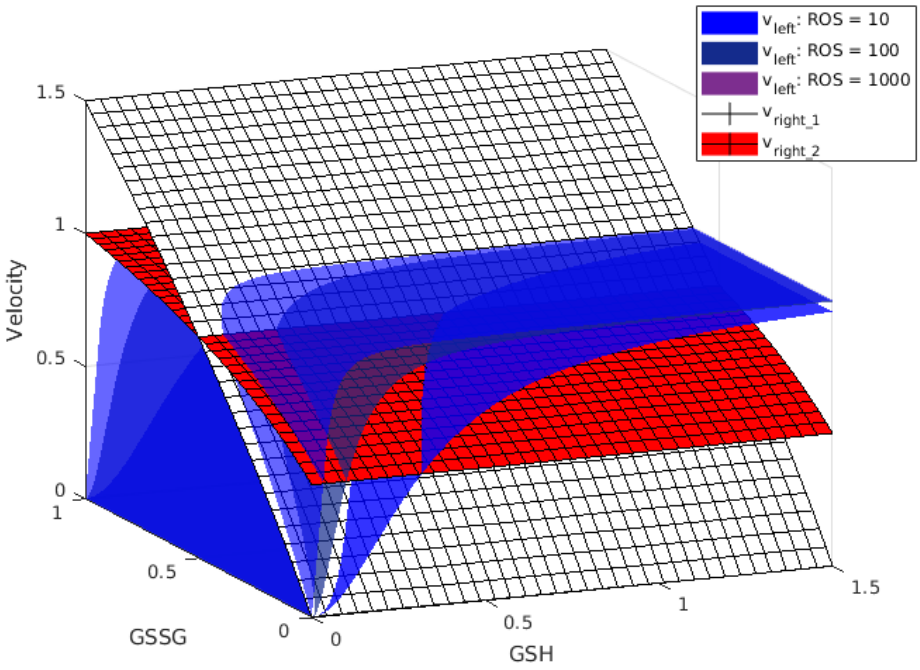
$$[\text{ROS}] = a \cdot [\text{HbA1c}] + b$$

where [HbA1c] denotes the concentration of glycated haemoglobin in blood, and a, b are constants. Glycated haemoglobin is a measure of the average plasma glucose of two to three months[25]. As per WHO guidelines, 6.5% of HbA1c is used as a cut off for the diagnosis of diabetes. Substituting the above relation in Eq. (3.18) gives,

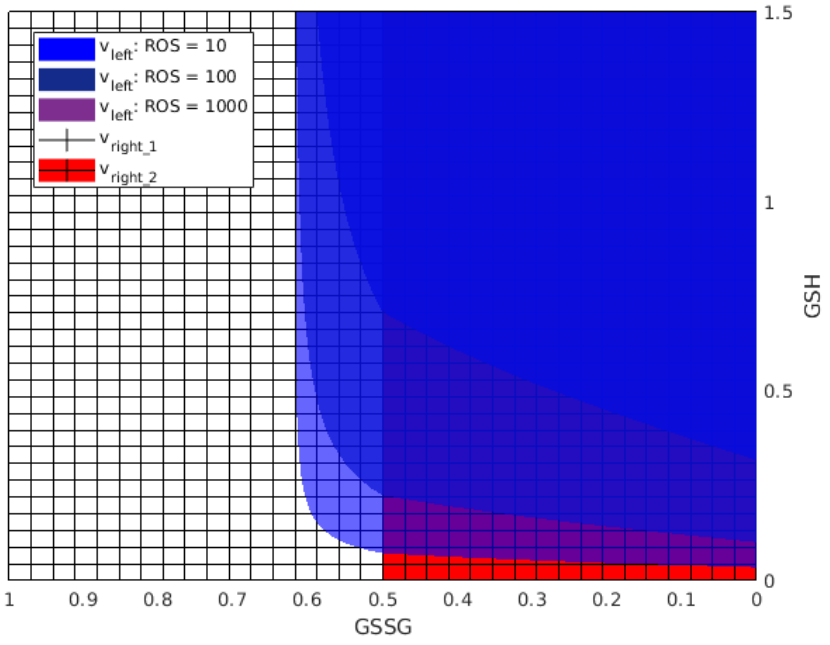
$$[\text{GSH}] = \sqrt{\frac{\theta_1}{[\text{HbA1c}] + \theta_2}} \tag{3.19}$$

where $\theta_1 = \dfrac{k_m \cdot [\text{GSSG}] \cdot (k_4 \cdot [\text{GR}] + \beta \cdot k'_m + \beta \cdot [\text{GSSG}])}{a \cdot k_2 \cdot [\text{GPx}] \cdot (k'_m + [\text{GSSG}])}$ and $\theta_2 = \dfrac{b}{a}$.

This provides a direct, simplified relationship between the steady state concentrations of reduced glutathione and glycated haemoglobin. A similar relation can also be constructed



(a)



(b)

Figure 3.8: (a) Three velocities plotted for ROS = 10, 100 and 1000. (b) Vertical projection of Fig. 3.8a. In both plots, the three intersection points corresponds to the three steady states, for each value of ROS. The GSSG concentration for all the intersections remains the same as the intersection of $v_{\text{right\_1}}$ and $v_{\text{right\_2}}$ is given by the line $[\text{GSSG}] = \frac{\alpha}{2\cdot\beta}$, $\text{Velocity} = \frac{\alpha}{2} \cdot \left(\frac{2\cdot k_2\cdot[\text{GPx}]+2\cdot k'_m\cdot\beta+\alpha}{2\cdot k'_m\cdot\beta+\alpha}\right)$.

using TBARS concentration instead of HbA1c. Here the phenomenological model is parametrized by θ_1 and θ_2. It should be noted that θ_1 is a function of enzyme concentrations and export rate constant, whereas θ_2 is just a ratio of two constants. A further reduction is made by assuming that a and b used in the substitution is the same for all test subjects. The parameter estimation is carried out using diabetic and non-diabetic data and is described in the following section.

3.3.3 Parameter Estimation

For model assessment, the parameters θ_1 and θ_2 needs to be estimated. Both linear and non-linear regressions were used for estimating θ_1 and θ_2. The data used for estimation corresponds to fifty diabetic patients and fifty non-diabetic controls taken from Ref [26]. From Eq. (3.19), a couple of observations can be made. Since [GSH] is a real quantity, θ_1 should be in the range $[0, \infty]$. Similarly, a positive θ_2 will shift the curve $y = (x)^{-2}$ to the left side of the x-axis, and a positive value will shift the curve to the right side. From the data, one can see that there is a steep ascent in the GSH values for lower HbA1c values, which suggests that θ_2 is negative. The second observation is that, as [HbA1c] $\longrightarrow -\theta_2$, $G_{tot} \longrightarrow \infty$, and G_{tot} tends to zero as [HbA1c] $\longrightarrow \infty$. Also, note that the total cellular glutathione (reduced and oxidised) concentration is used for the parameter estimation as the total glutathione concentration in cells are translated by a constant value due to the GSSG homeostasis.

Non-linear Regression

Non-linear regression was performed using the inbuilt function `nlinfit` in MATLAB, which uses a gradient descent method, and the result is shown in the below figure. The result of non-linear regression is observed to be dependent on the initial point given. For each pair of starting points, θ_{10} and θ_{20}, the estimated θ_1 and θ_2 were different, and an optimum pair was chosen such that the output gives least RMSE. As a result, one can see that the curve passes through the points for low HbA1c values, and for higher HbA1c values the curve is slightly above the data points.

Linear Regression

Equation (3.19) can be converted into a linear equation as follows.

$$[\text{HbA1c}] + \theta_2 = \frac{\theta_1}{[\text{GSH}]^2} \qquad \Rightarrow \qquad \frac{1}{\theta_1}[\text{HbA1c}] + \frac{\theta_2}{\theta_1} = \frac{1}{[\text{GSH}]^2} \tag{3.20}$$

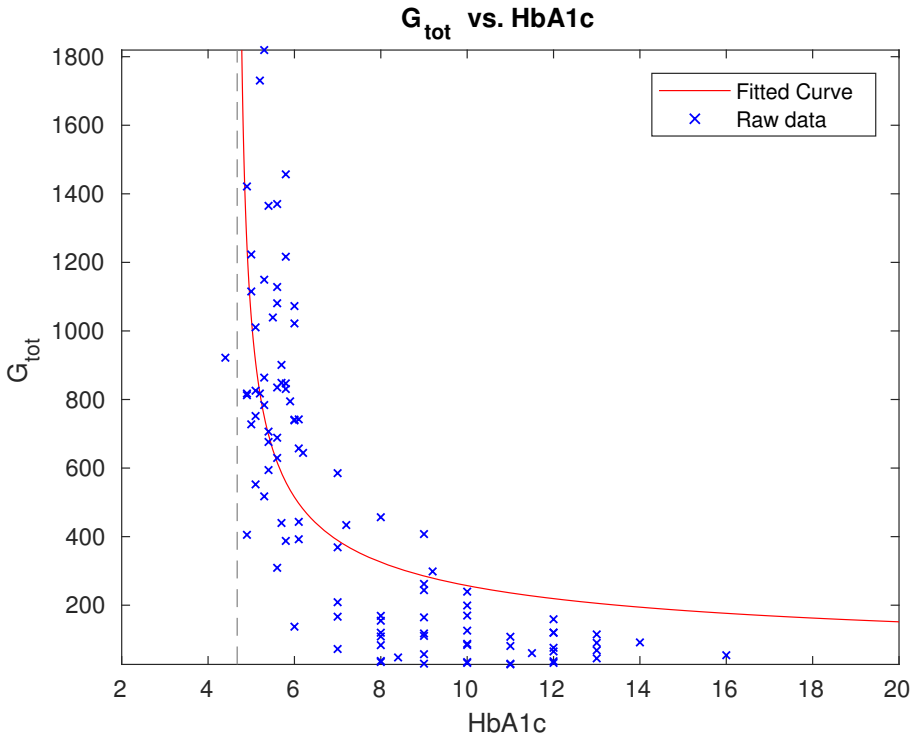


Figure 3.9: Comparison between actual $G_{tot}$and predicted $G_{tot}$for estimated parameter values, $\theta_1 = 351809.23$ and $\theta_2 = -4.67$. The vertical asymptote is $x = 4.67$.

The above relation is of the form $m \cdot x + c = y$. Hence, linear-regression can be used to estimate the slope and intercept, i.e. $\frac{1}{\theta_1}$ and $\frac{\theta_2}{\theta_1}$, which yields the original parameters θ_1 and θ_2. Linear regression was performed using the inbuilt function `fitlm` in MATLAB, and the results are shown figures 3.10 and 3.11. Here, better results were obtained compared to the non-linear regression method, and the fitted curve is able to describe the qualitative nature of the data.

Equation (3.19) is able to explain the qualitative nature of the data. But, while fitting Eq. (3.19), there is an implicit assumption that the import rate constant α, and hence θ_1, is the same for all hundred test subjects, which may not be true in general. Even though α does not appear in the expression of θ_1, $[GSSG]\cdot\beta$ is same as $\alpha/2$. Hence, it is better to fit individual data to the model, in which case α is guaranteed to be a constant.

3.3.4 Fitting Individual Data

For fitting individual data, linear regression was carried out with respect to Eq. (3.20). Cellular GSH, HbA1c readings of 50 diabetic patients were used for fitting the model (taken from Ref. [26]). For each patient, three measurements of GSH and HbA1c were used for linear regression. These measurements here corresponds to the GSH and HbA1c readings at zeroth(□), fourth(◯) and eighth(△) weeks data after starting of anti-

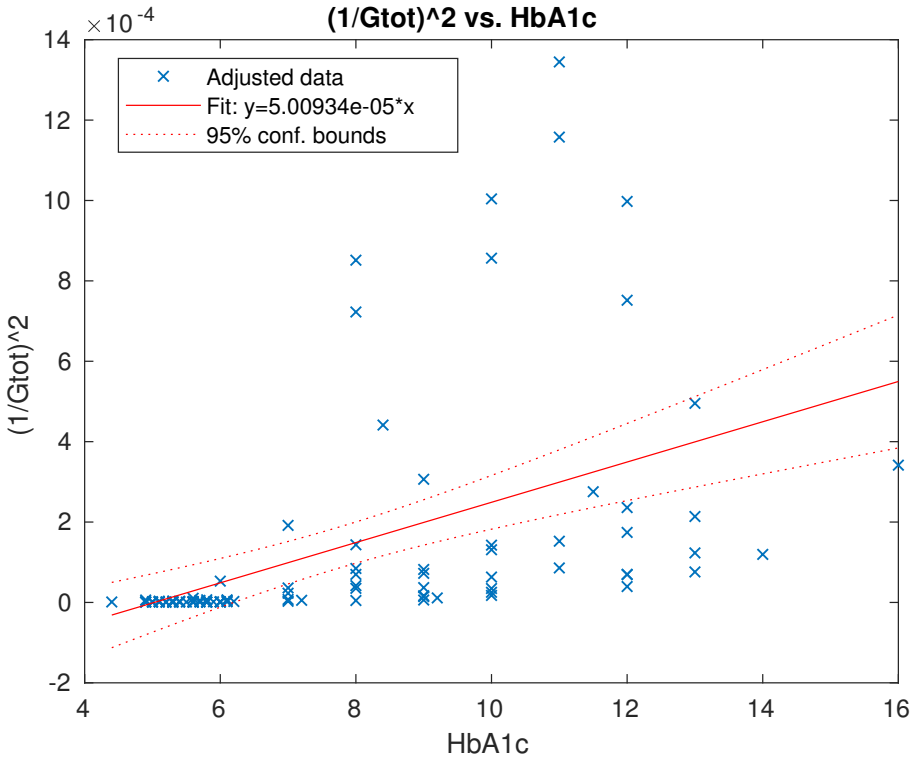


Figure 3.10: Result of linear regression; The fitted line has slope $\frac{1}{\theta_1}$ and intercept $\frac{\theta_2}{\theta_1}$. The above fit has a RMSE of 2.57×10^{-4}, and R-squared value 0.218.

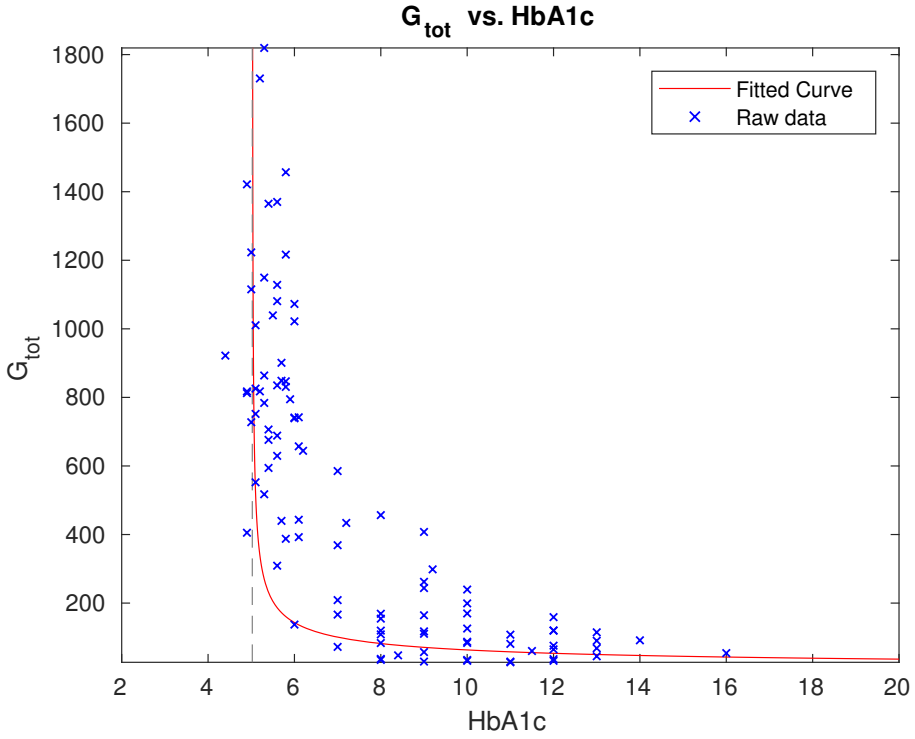


Figure 3.11: Comparison between actual $G_{tot}$and predicted $G_{tot}$for parameter values estimated using linear regression, $\theta_1 = 19962.72$ and $\theta_2 = -5.03$. The vertical asymptote is $x = 5.03$.

diabetic treatment. The results of linear regressions, including the values of parameters θ_1 and θ_2, are shown in Fig. 3.13-3.19. Out of 50 cases, 6 cases have negative θ_1 value, which contradicts with the general trend observed in Fig. 3.11 and are physiologically not possible. Also in 3 cases, the data points are too close to each other and hence the fitted curve is significantly different from the population fit shown in the earlier figure. From the figures, it can be seen that as the treatment progress, the cellular HbA1c levels are returning to normal along with the reduced state of the cell. Hence, the model is able to predict the recovery path for diabetic patients for most of the cases. Even though the fitted curve matches with the data, the unbounded increase in GSH concentration for low HbA1c values remains unexplained. Most of the θ_2 values are observed to be lying between -9 to -6. Excluding the outlying cases mentioned before, the mean value for θ_2 is observed to be -7.51 ± 0.20, which according to the model is constant as it is reasonable to believe that parameters a and b used in the conversion are the same for all test subjects. Figure 3.12 summarises the results of individual fitting, ignoring the physiologically improbable cases and outliers.

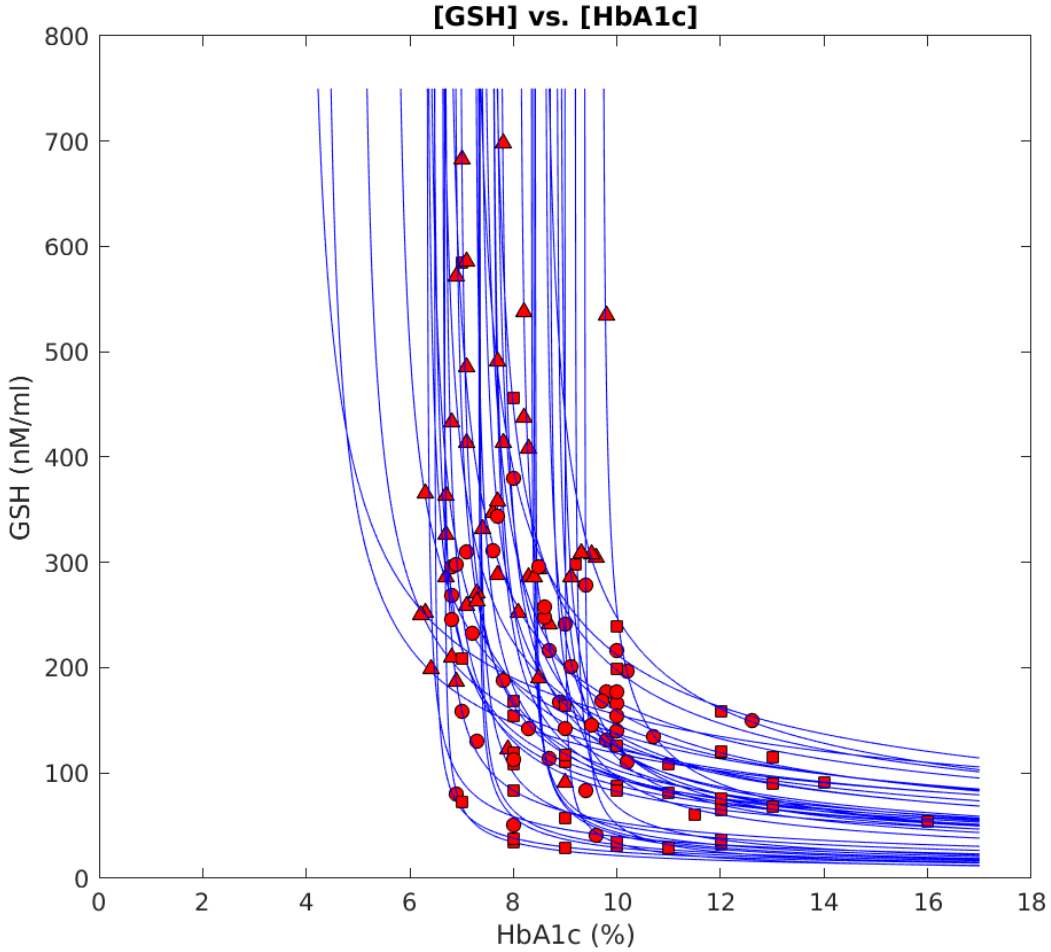


Figure 3.12: Individual data (GSH and HbA1c) fitted for 44 diabetic cases, excluding the 6 outliers. Week 0, week 4 and week 8 data are represented by "□", "○" and "△" respectively.

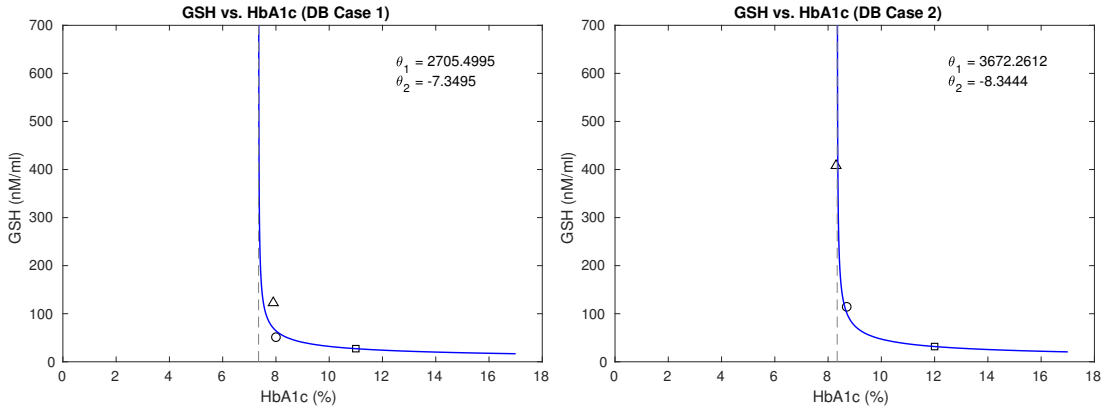


Figure 3.13: Individual data fitted for diabetic cases 1-2. Week 0, week 4 and week 8 data are represented by "□", "○" and "△" respectively.

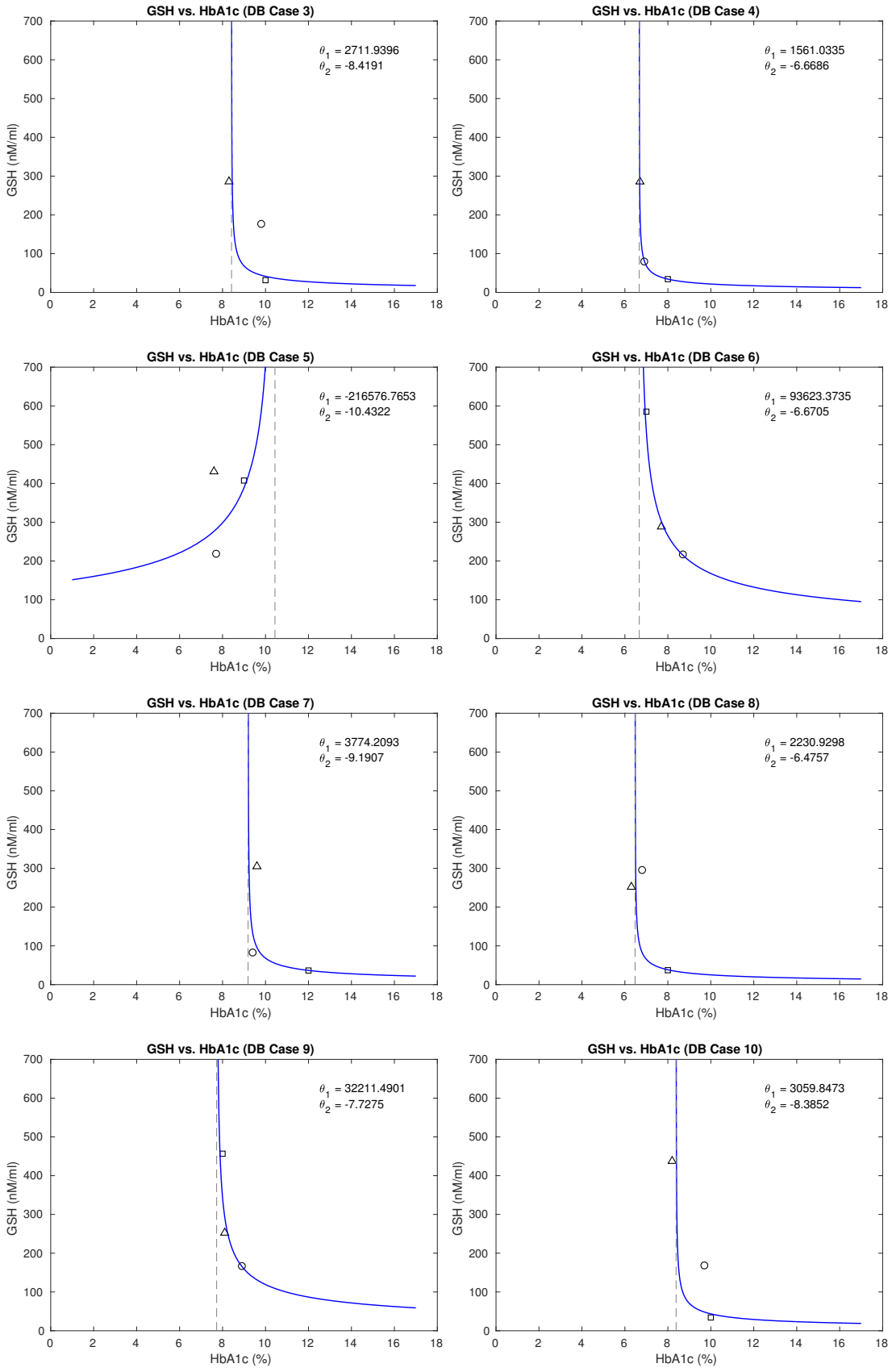


Figure 3.14: Individual data fitted for diabetic cases 3-10. Week 0, week 4 and week 8 data are represented by "□", "○" and "△" respectively.

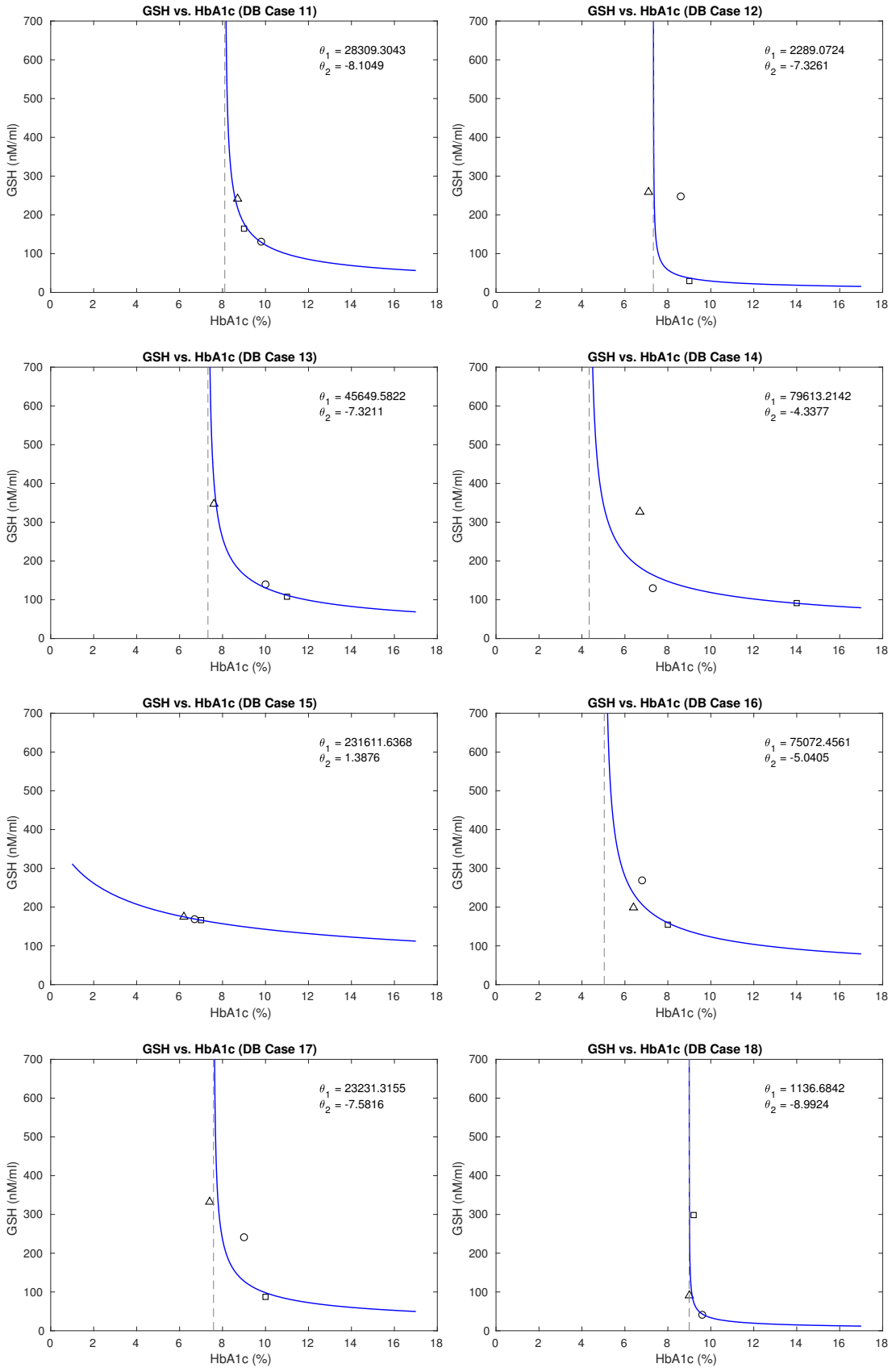


Figure 3.15: Individual data fitted for diabetic cases 11-18. Week 0, week 4 and week 8 data are represented by "□", "○" and "△" respectively.

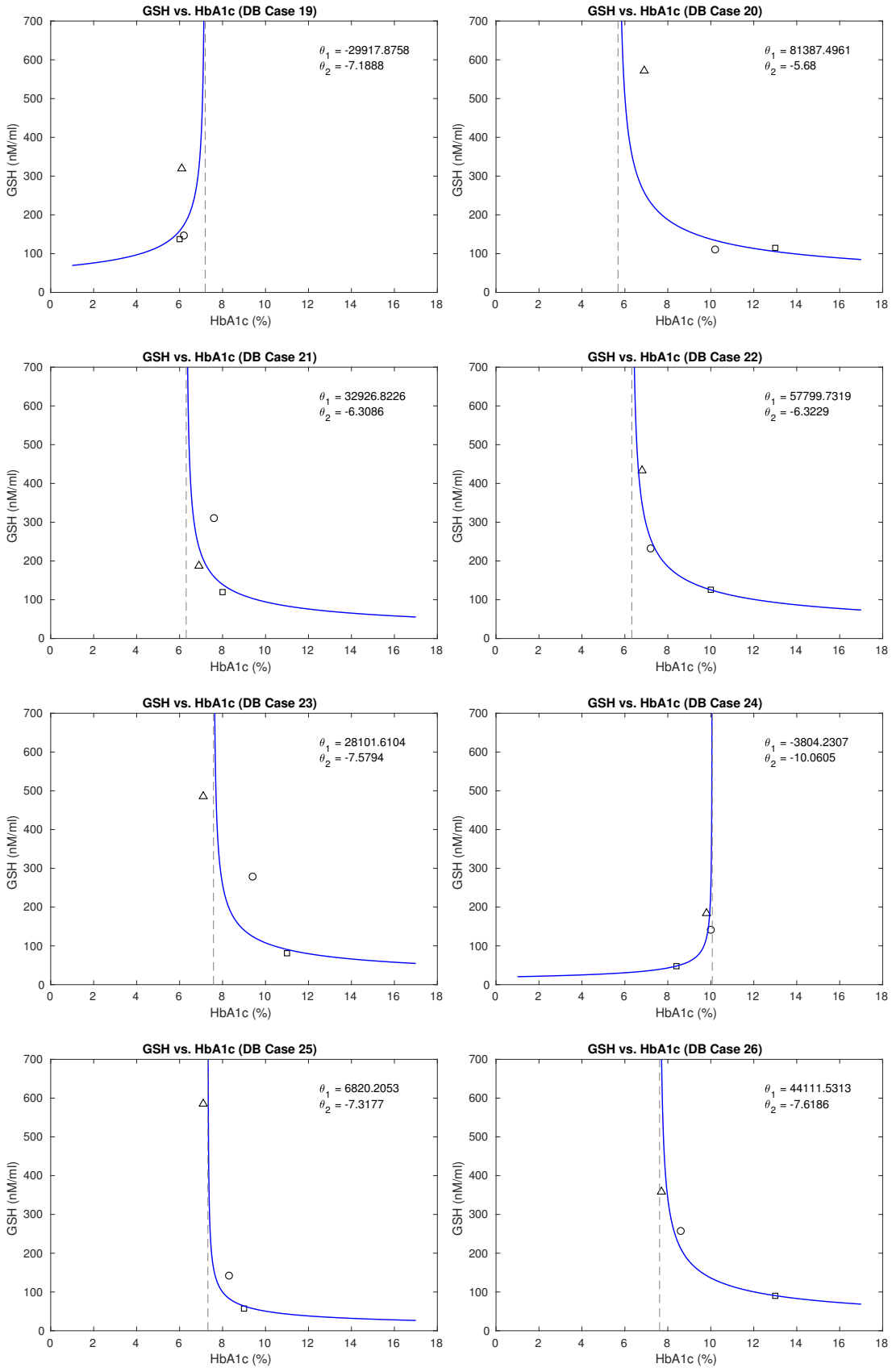


Figure 3.16: Individual data fitted for diabetic cases 19-26. Week 0, week 4 and week 8 data are represented by "□", "○" and "△" respectively.

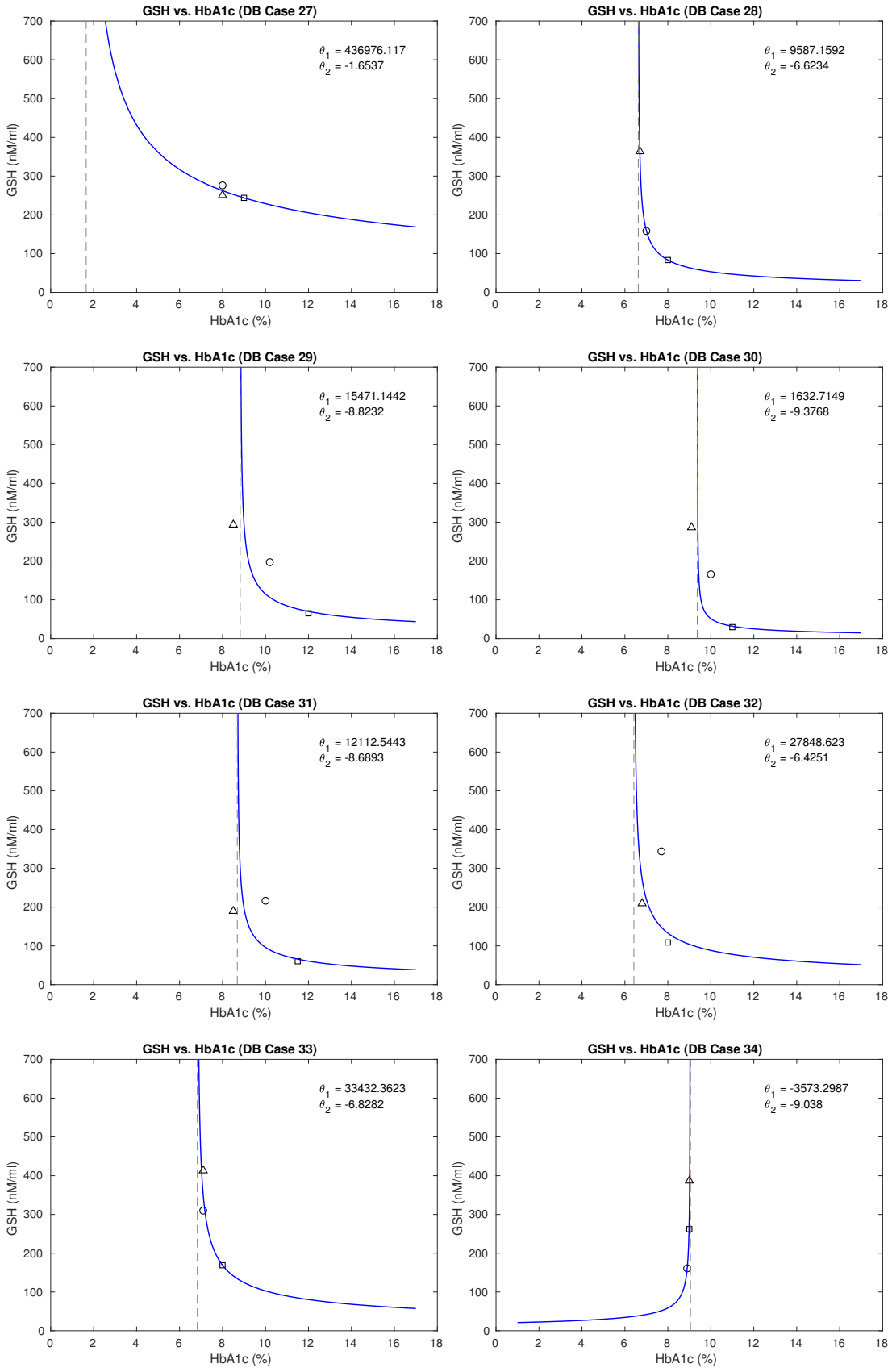


Figure 3.17: Individual data fitted for diabetic cases 27-34. Week 0, week 4 and week 8 data are represented by "□", "○" and "△" respectively.

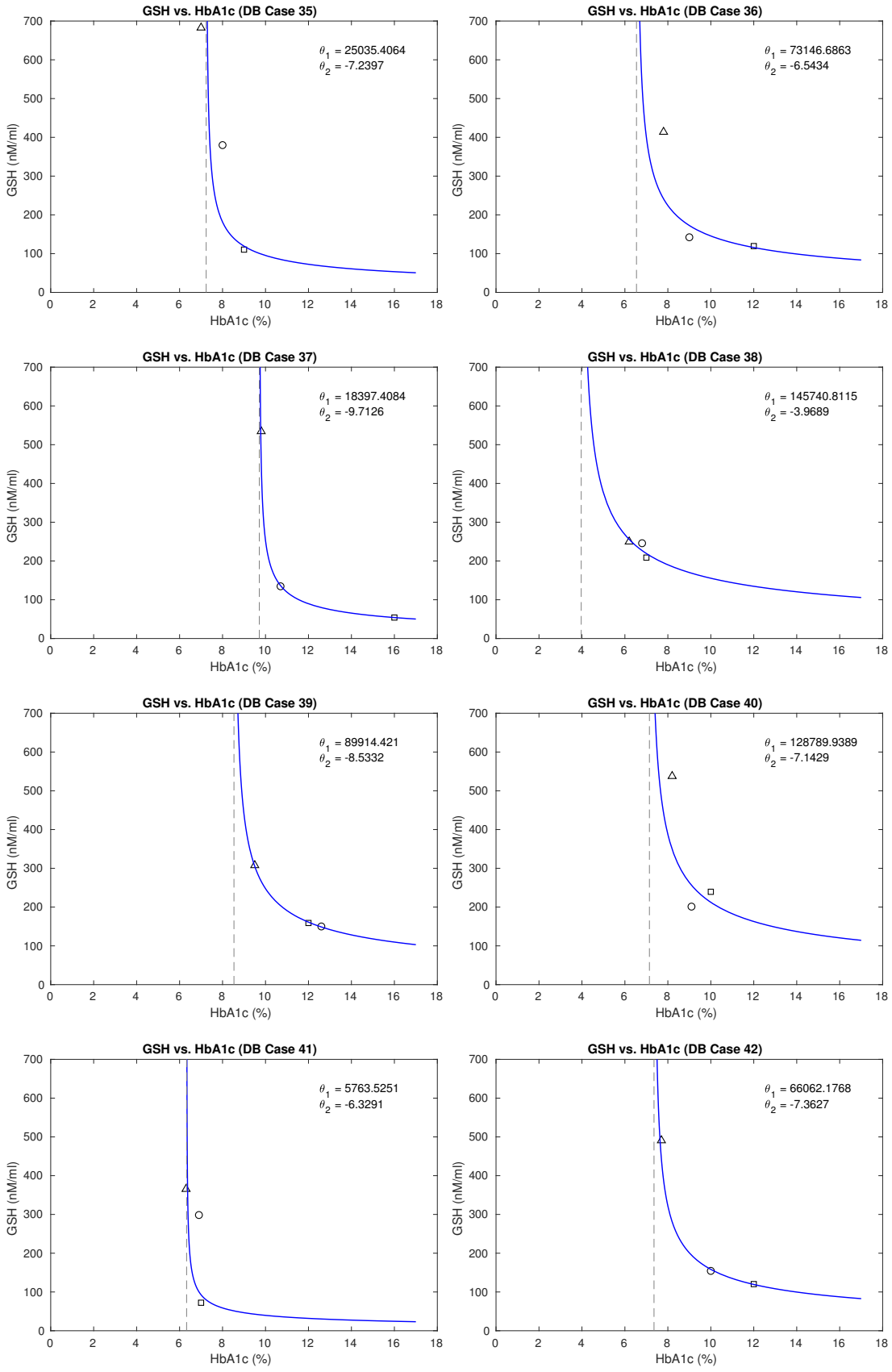


Figure 3.18: Individual data fitted for diabetic cases 35-42. Week 0, week 4 and week 8 data are represented by "□", "○" and "△" respectively.

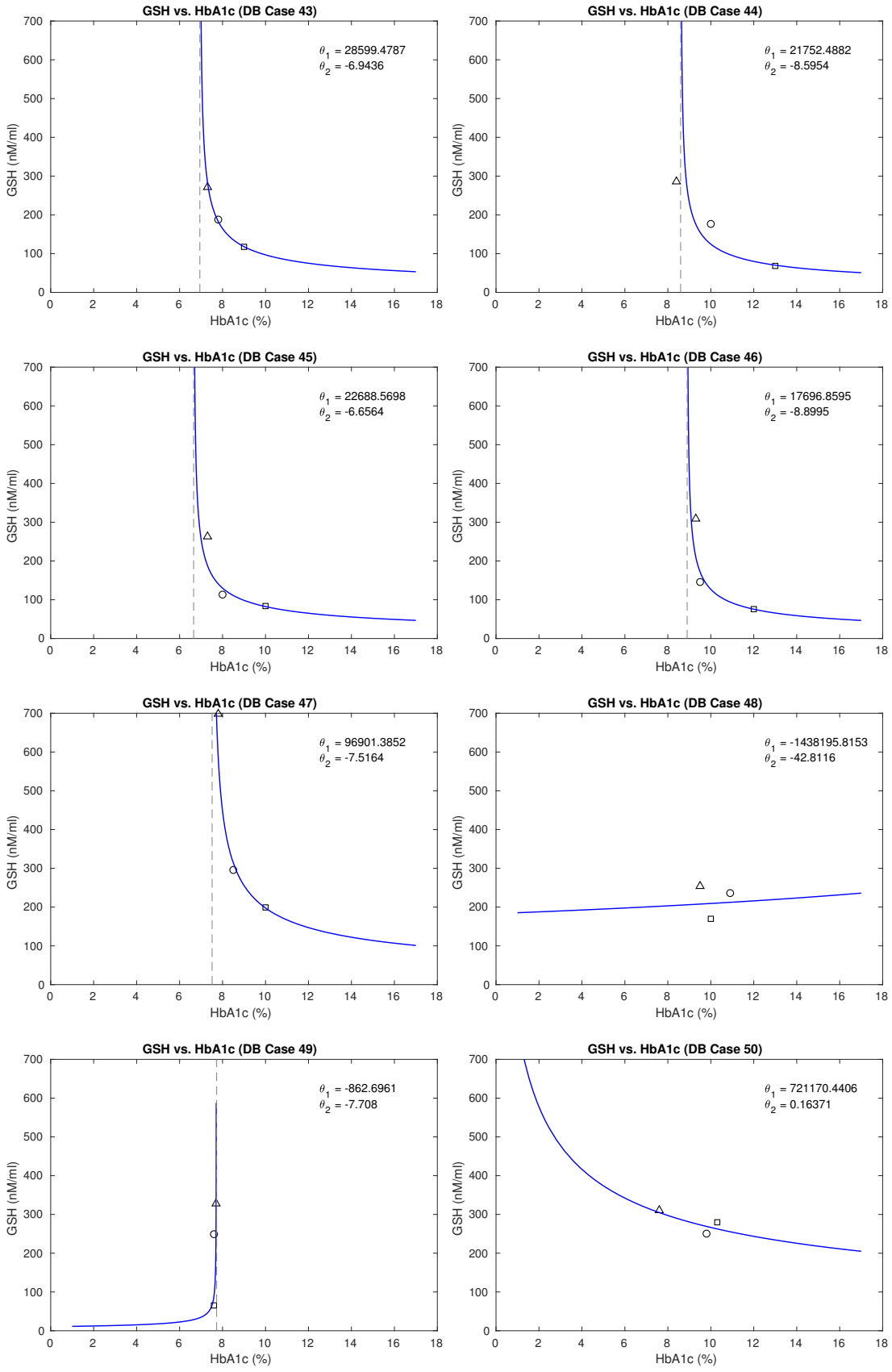


Figure 3.19: Individual data fitted for diabetic cases 43-50. Week 0, week 4 and week 8 data are represented by "□", "○" and "△" respectively.

3.4 Thermodynamics vs. Enzyme Kinetics

In this section, we discuss the importance of the reaction kinetics over the thermodynamics in the glutathione system in cells. The redox potential or the reduction potential of a chemical compound describes its tendency to get reduced by gaining electrons. In human cells, the GSH/GSSG system essentially acts as a redox couple and is generally categorised as a thiol/disulfide system. The GSH/GSSG redox potential is believed to be an essential driving force of different biological processes in cells. Various studies have been conducted on the importance of thiol/disulfide systems on the smooth working of cells, such as the work by Freedman et al.[27]. But it should be noted that this assumption ignores the kinetics of the reaction, and the enzymes hardly have any role in this process. The studies conducted by Flohe et al. point out the major drawbacks of this approach[28]. In the case of simple inorganic redox reactions, the electrochemical potential can be measured to a relatively high accuracy, which not true in the case of a complex multistep enzymatic reaction. Though a lot of studies points to the importance of redox potentials, it needs to be noted that the calculations were made under laboratory conditions which are quite far from the actual biological systems. Hence, the applicability of these electrochemical parameters needs to be questioned.

The redox potential of a chemical compound is affected by various factors, such as temperature, pH, non-equilibrium systems, the presence of multiple redox systems, etc. In the case of the enzyme reactions, the major difference that characterises this is the formation of stable intermediate or a number of intermediates. In simple inorganic reactions, the intermediate formed is unstable and has less importance compared to the enzyme-substrate complexes. Another interesting fact to notice is that the redox potential does not describe the rate of the reaction. Instead, it only discloses the direction in which it is proceeding. This is evident from the Nernst equation which is used for the estimation of redox potential, as it is usually hard to measure the exact redox potential experimentally. The Nernst equation is given by

$$\Delta E_{cell} = \Delta E^0_{cell} - \frac{\mathrm{R} \cdot \mathrm{T}}{\mathrm{z} \cdot \mathrm{F}} \log Q_r$$

where E_{cell} is the cell potential (redox potential in the case of redox cell) and E^0_{cell} is the standard cell potential which is a constant quantity. The standard cell potential are measured with reference to electrodes such as SHE (Standard Hydrogen Electrode) at standard conditions (temperature, pH and pressure/concentration). In the above equation, R is the universal gas constant, T is the temperature, F denotes the Faraday constant and z is the number of electron transferred during the reaction. The quantity of interest here, which has the major influence on the redox potential is the reaction

quotient Q_r, which is given by

$$Q_r = \frac{[C]^\gamma [D]^\delta}{[A]^\alpha [B]^\beta}$$

for a hypothetical reaction $\alpha A + \beta B \leftrightharpoons \gamma C + \delta D$. From this definition, it can be seen that even for an enzymatic reaction, only the concentration of substrates and products influences the redox potential. In the case of enzyme reactions, since enzymes are recovered in active state at the end, the reaction quotient only depends on the substrate and product concentrations. For the glutathione system, the reactions can be written as follows:

$$2\mathrm{GSH} - 2\mathrm{H}^+ - 2\mathrm{e}^- \leftrightharpoons \mathrm{GSSG}$$
$$\mathrm{H_2O_2} + 2\mathrm{H}^+ + 2\mathrm{e}^- \leftrightharpoons \mathrm{H_2O}$$

and the overall reaction is given by

$$2\mathrm{GSH} + \mathrm{H_2O_2} \leftrightharpoons \mathrm{GSSG} + 2\mathrm{H_2O}$$

Hence, the reaction quotient becomes

$$Q_r = \frac{[\mathrm{GSSG}]}{[\mathrm{GSH}]^2[\mathrm{H_2O_2}]}$$

As a result, the redox potential of the glutathione system only depends on the concentrations of the two glutathione forms and ROS. Unlike inorganic reactions, organic reactions, especially those involving enzymes, face a barrier of activation energy which is usually attained with the help of enzymes. Because of this reason, the value of redox potential cannot be used directly as a measure of reaction velocity. The experimental determination of GSH/GSSG redox potential in 1952 indicates the importance of the enzymes in glutathione system[10]. The study shows that, even when the redox potentials are matched, no reaction occurs (or reaction occur in a biologically slow time scale) without the presence of enzymes. Therefore the reduction potential alone does not carry much information regarding the redox regulation. Furthermore, enzymes play a significant role in regulating the reactions, and hence, are crucial for maintaining the reduced state of the cells.

Chapter 4

Conclusions

We have studied three existing models of glutathione redox systems and red blood cell metabolism in detail. The model proposed by Raftos et al.[8] focuses solely on the glutathione turnover in red blood cells, and is more complex compared to the other two, mainly due to the number of species involved in the glutathione system. The model accounts for both the production and removal of glutathione from RBCs, even though an import mechanism for the reduced glutathione is absent. The other two models, proposed by Jamshidi et al.[29] and Bordbar et al.[11], are elaborate models of RBC metabolic networks. Both of these models provide a broad view of the metabolic pathways in RBCs rather than focusing on each component. Even though implementations of these models are available in SBML (Systems Biology Markup Language) format, various parts of the models were missing, and we were unable to study them further. As for the glutathione turnover model, the complexity of the model prevents us from implementing it on a smaller scale. It should be noted that in all three models the enzymes and ROS is given less importance, and hence all three of them were unable to provide a clear description of the glutathione dynamics and regulatory mechanisms in RBC. Therefore, from the basics of enzyme kinetics and the knowledge from the existing models, different mechanistic models of glutathione turnover were created, and various aspects of the models were analysed under different conditions. In each step, models were modified with smaller components for obtaining a better picture of the glutathione system, and the effect of each element on the dynamics of the glutathione and the regulation of oxidative stress has been studied. The transport of glutathione across the RBCs is found to have a significant influence on the regulation of OS. The models studied can be divided into two categories, closed models and open models. In closed models, the importance of the two enzymes for the interconversion has been examined. In the case of single enzyme models, the reduced to oxidised glutathione ratio is mainly modulated by the rate constants of the forward and backward reactions. Since the rate constant is a property of the

reaction, the system has lesser control over the regulation of the ratio. Here, the ratio is observed to be varying inversely with respect to the change in ROS concentration. Therefore, the existence of two enzymes driving each conversion provides the system with relatively better control over the concentration of glutathione species, where the ratio is affected by the concentrations of the enzymes. Interestingly in two enzyme models, the steady state relationships between the concentration of chemical species split into the Michaelis-Menten equation for each enzyme, even though the system is coupled. Among the models studied, the open model of glutathione turnover, resembling the Raftos model, has significant control over the concentrations of the glutathione species arising from the glutathione transport mechanisms across RBCs.

The parameter estimations for the open model (Sec. 3.1.8 and 3.3.3) are performed using raw experimental data. Both closed and open two enzyme models were numerically solved for arbitrary parameters and initial conditions, and no strict regulation of the ratio is observed in any of the models. The parameter estimation for r_1, r_2, k_m and k'_m in the open model (Sec. 3.1.8) returned values with large deviation. The variation seen in the optimised values could be the result of the method used for optimisation, or the approximation that the GSSG concentration in RBC is 10% of the GSH concentration. Among the parameters, the value of k'_m is observed to be less varying in both the estimations using reduced equations and ROS markers. This consistency in the optimised value of k'_m could be arising from the limitations of the method used for optimisation. Reducing the Eq. (3.16) using the approximation that the ROS concentration is high, could also have an adverse effect on the approximation process. For resolving this issue, the optimisation was be carried out using ROS markers such as thiobarbituric acid reactive substances (TBARS) concentrations in place of ROS concentration, and better results are obtained. To summarise, the mechanistic model is able to capture the nature of the relationship between glutathione and glucose for the diabetic cases.

Since the steady state GSSG concentration is invariant to ROS concentration in zero order import and first order export model (Sec. 3.1.6), cellular GSH concentration gives the same information as the ratio, GSH:GSSG does. It should be noted that GSSG homeostasis is a result of our assumption that the export of GSSG to blood plasma follows first order kinetics and import of GSH follows zeroth order kinetics. The changes in ROS concentration is accounted by changing the reduced glutathione concentration, which results in a constant oxidised glutathione concentration for any parameter values. The concentration of GSSG, in this scenario, is observed to be a function of the transport rate constants α and β. Due to the construction of the model, the rate constant α can change from person to person. As a result, the steady state concentration of GSSG is also different for each person. If the GSH import were concentration dependent, then it is possible that the ratio of the two glutathione species is approximately constant, but it

appears to contradict the observations from the experiments (Fig. 3.5).

The hypothesis that there exists a cellular GSSG homeostasis gives rise to a phenomenological model for the relation between glucose and glutathione (Sec. 3.3), by approximating the reactive oxygen species concentration with the glycated haemoglobin concentration. The parameter estimation for the model was carried out using glutathione and HbA1c data obtained from Ref. [26]. Optimisations for the parameters, θ_1 and θ_2 were performed at both population and individual level. In both cases, the phenomenological model proposed is able to explain the data better than the mechanistic model, and captures the recovery of glycemic status during anti-diabetic treatment to a great extent.

To conclude, strict maintenance of the reduced to oxidised ratio of glutathione is not observed in any of the models studied and the same is not observed in any of the data analysed. A large variation in both the ratio and individual concentrations is observed even within the diabetic and control groups. The experimental results are consistent with the GSSG homeostasis hypothesis, though a theoretical explanation is still needed for explaining this. It is known that ROS follows fast kinetics and HbA1c has comparatively slower kinetics[22]. Hence, there is also a question of comparing these two quantities. Whether ROS can be averaged for the time scale of HbA1c needs to be verified. If GSSG concentration is not affected by the amount of ROS present, as suggested by the phenomenological model, the concentration of reduced glutathione is sufficient for understanding oxidative stress, and the concept of glutathione redox potential as a driving force of biological processes can be disregarded. The phenomenological model obtained from the GSSG homeostasis hypothesis is able to the explain the glycemic recovery of diabetic patients with respect to the glutathione concentration, and it is able to capture the fluctuations within the diabetic group.

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