A Mathematical Model of the Effect of Aspirin on Blood Clotting

Breeana J. Johng
Scripps College

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A Mathematical Model of the Effect of Aspirin on Blood Clotting

Breeana J. Johng

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Submitted to Scripps College in Partial Fulfillment of the Degree of Bachelor of Arts

Department of Mathematics

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Abstract

In this paper, we provide a mathematical model of the effect of aspirin on blood clotting. The model tracks the enzyme prostaglandin H synthase and an important blood clotting factor, thromboxane A\textsubscript{2}, in the form of thromboxane B\textsubscript{2}. Through model analysis, we determine conditions under which the reactions of prostaglandin H synthase are self-sustaining. Lastly, through numerical simulations, we demonstrate that the model accurately captures the steady-state chemical concentrations of interest in blood, both with and without aspirin treatment.
Acknowledgments

I would like to thank Professor de Pillis for advising this project. Her guidance was instrumental in not only completing, but also pursuing this topic. I would also like to thank Professor Shtylla, who read and made suggestions for my drafts, and Professor Milton and Professor Leconte, with whom I consulted for various aspects on this project. Finally, I would like to acknowledge my family for their unconditional love and support, especially my mother with whom I share a love of mathematics.

This project is inspired by the MoyaMoya family, a group of individuals who suffer from a narrowing of the major arteries, and therefore lack of oxygen, in the brain, leaving them at risk for strokes and other serious health complications. This condition has caused many of us to be placed on a daily aspirin therapy.
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Aspirin is a widely used drug known to aid in the prevention of numerous conditions and diseases, such as heart attack and stroke (U.S. Food and Drug Administration, 2014), due to its blood-thinning properties that increase the amount of time taken for blood to clot. In 2005, about one in five adults, of age 18 or older, reported taking aspirin every day or every other day in the United States (Soni, 2007). In 2014, a sampling survey in Washington reported a statistic of about two in five adults taking aspirin every day or every other day (Roth et al., 2014).

Information about aspirin is collected experimentally; no mathematical model that takes into account the effect of aspirin on blood clotting exists. Such a model could provide insight into the workings of aspirin and could predict the effects that different doses might have on blood clotting, without having to conduct experiments on human subjects in order to obtain numbers and results.

Most mathematical models and research on blood clotting revolves around modeling the blood itself. They consist of complicated, advanced mathematics and take into account flow and shear of the blood. However, Wei et al. conducted research on prostaglandin H synthase, an enzyme that plays a key role in coagulation, and proposed two different mechanisms for this enzyme: a branched chain mechanism and a tightly coupled mechanism (1995). Their branched chain mechanism became widely accepted and used by other researchers. While Wei’s model focused on the chemical mechanism of the enzyme, and not on mathematical research or results, it offered an alternative way of looking at blood clotting, one that was quantifiable and measurable, other than just the blood itself (1995). Furthermore, understanding the chemistry and deriving chemical equations to model the
mechanism is crucial in potentially constructing differential equations for mathematical modeling.

Tien et al. used the branched chain mechanism from Wei et al. (1995) to derive a simplified Michaelis-Menten-style model for the enzyme. They then analyzed the fixed points to determine when the enzyme would become self-sustaining and performed a quasi-steady-state approximation on the model to reduce the dimensionality of the system, leaving implications for a simpler model that might be easier to integrate into other systems (2005).

Despite the advances made by both Wei et al. (1995) and Tien et al. (2005) to build a mathematical model for blood clotting, neither Wei et al. nor Tien et al. factored in the use of aspirin, a widely known preventative for blood clotting, or tracked variables that could be used to determine the normality, or abnormality, of blood clotting exhibited by the models. Our goal for this project was to construct a full, but simple, model of blood clotting, similar to the one proposed by Wei et al. (1995), while adding aspirin into the model, as well as something quantifiable to track what is considered “normal” versus “decreased” blood clotting, which was attained in Chapter 5. We also aimed to reduce the model to perform a stability analysis, such as Tien et al. (2005) did, which would tell us when the model would become self-sustaining, which was done in Chapter 4. We achieved our goals and developed a model that, with accompanying analyses, gave us insight as to how aspirin is integrated into the mechanisms of blood clotting in a way that prevents coagulation and could also potentially serve as a means of accurately determining aspirin dosages that would effectively prevent unwanted blood clotting.
Chapter 2

Biochemical Background

We begin by providing the reader with some background information on the biochemistry behind our project.

2.1 Blood Clotting Cascade and Inhibition

Coagulation, or blood clotting, is a complex process involving numerous chemicals and proteins that interact in an intricate web of pathways. We have simplified the model and present only the basic and necessary facts that pertain to this project.

The blood clotting cascade is prompted by damage, such as a small tear, to the lining of the blood vessel wall. Platelets, blood cells that specialize in blood clotting, aggregate and adhere at the site of damage and release chemical-containing granules (Dahlbäck, 2005). One such chemical is thromboxane $A_2$, which promotes additional platelet aggregation, thus making it a prominent factor in the blood clotting cascade (Anand et al., 2003).

The enzyme responsible for the production of thromboxane $A_2$ is prostaglandin H synthase, or PGHS. A known mechanism of halting the blood clotting cascade is to inhibit PGHS with cyclooxygenase inhibitor drugs, such as aspirin. Without thromboxane $A_2$, further platelet aggregation will not occur, and a blood clot will not form. Because platelets cannot recover new PGHS molecules once they are inhibited by aspirin, the effects of aspirin on platelets last for the duration of the platelet’s life, which is about ten days long. Thus, a single ingestion of aspirin is not enough to promote blood thinning or prevent an unwanted blood clot; daily use is required to inhibit any new platelets being formed. Because of aspirin’s blood-thinning
properties, taking a daily dose of aspirin is recommended for those with angina or with previous heart attack or stroke history (Awtry and Loscalzo, 2000).

2.2 Enzymes

This section provides the reader with a brief overview of enzymes and how they work that is kept in the scope of this project.

Enzymes are proteins that catalyze reactions, increasing the speed of the reactions, and have domains, or sites, of catalysis, where the reaction occurs. It is possible for an enzyme to have more than one catalytic domain, as is the case with PGHS. Reactants, known as the substrates, bind to the site of catalysis, and after the reaction takes place, the products are released. When the reactants are bound to the enzyme, we refer to the combined molecules as an enzyme-substrate complex. Lastly, the catalytic domains may have their shape or chemical features altered, causing the enzyme to exhibit different forms.

One of many ways that enzymes can be inhibited is through binding of inhibitors to the enzyme. There are two such types of inhibitors: competitive and noncompetitive. Competitive inhibitors bind to the site of catalysis, thus competing with the intended substrate for the catalytic domain. Noncompetitive inhibitors bind elsewhere on the enzyme, and as a result, changes the shape or properties of the catalytic site, rendering the site unbindable for the substrate (cf. Nelson and Cox, 2012).

2.2.1 Autocatalytic Enzymes and Self-Sustaining Reactions

An autocatalytic enzyme is one in which the product of a reaction serves as a catalyst for future reactions. Such an enzyme is said to be self-sustaining when the reactions continue indefinitely (cf. Nelson and Cox, 2012).

2.3 Prostaglandin H Synthase

Prostaglandin H synthase, or PGHS, is the enzyme responsible for the production of thromboxane A$_2$, or TXA$_2$. However, it does not directly catalyze the production of TXA$_2$; rather, it catalyzes the reactions that produce prostaglandin H$_2$, a precursor for TXA$_2$ (Tien et al., 2005).

PGHS is an autocatalytic enzyme that has two catalytic sites, one of which catalyzes a peroxidase, or POX, reaction and the other of which
catalyzes a cyclooxygenase, or COX, reaction, that work together cooperatively ([Seta and Bachsmid] 2012). In the POX reaction, PGHS catalyzes the conversion of prostaglandin G2, or PGG2 to prostaglandin H2, or PGH2. In the COX reaction, PGHS catalyzes the conversion of arachidonic acid to PGG2. The product of the POX reaction, PGH2 is then converted into TXA2, a reaction that is catalyzed by a different enzyme, thromboxane A synthase. The COX function of PGHS may be lost through enzyme suicide inactivation ([Seta and Bachsmid] 2012). Figure 2.1 shows a flowchart of the chain of reactions, where the boxes denote the enzymes.

Aspirin inhibits TXA2 production by acting as a competitive inhibitor and irreversibly binding to the COX site of PGHS ([Goltsov et al.] 2010). Thus, production of PGG2, and consequently PGH2 and TXA2, is inhibited.

**Figure 2.1** Chain of reactions of PGHS without aspirin.
Chapter 3

Building the Model

3.1 Justification

The construction of our model of aspirin’s effect on blood clotting began with the branched chain mechanism proposed by [Wei et al. (1995)]:

\[
\begin{align*}
Fe(III) + PGG_2 & \xrightarrow{k_1} Fe(IV)PP^* + PGH_2 \\
Fe(IV)PP^* & \xrightarrow{k_2} Fe(IV)Tyr^* \\
Fe(IV)Tyr^* + AA & \xrightarrow{k_3} Fe(IV)AA^*/Tyr \\
Fe(IV)AA^*/Tyr + O_2 & \xrightarrow{k_4} Fe(IV)AAO_2^*/Tyr \\
Fe(IV)AAO_2^*/Tyr + O_2 & \xrightarrow{k_5} Fe(IV)Tyr^* + PGG_2 \\
Fe(IV)PP^* + FA & \xrightarrow{k_6} Fe(IV) + FA^* \\
Fe(IV)Tyr^* + FA & \xrightarrow{k_7} FE(IV) + FA^* \\
Fe(IV) + FA & \xrightarrow{k_8} Fe(III) + FA^*
\end{align*}
\]

(3.1)

where Fe’s denote different forms of the enzyme PGHS and • denotes radical chemistry, AA is arachidonic acid, O₂ is oxygen, and FA is ferulic acid.
We simplified these equations, omitting the radical chemistry and substrates oxygen and ferulic acid, to obtain:

\[
\begin{align*}
E_1 + P & \underset{k_1}{\rightarrow} E_2 + H \\
E_2 & \underset{k_2}{\rightarrow} E_3 \\
E_3 + S & \underset{k_3}{\rightarrow} C_1 \underset{k_4}{\rightarrow} C_2 \underset{k_5}{\rightarrow} E_3 + P \\
E_2 & \underset{k_6}{\rightarrow} E_4 \\
E_3 & \underset{k_7}{\rightarrow} E_4 \\
E_4 & \underset{k_8}{\rightarrow} E_1
\end{align*}
\] (3.2)

with the following variable definitions:

- \( E_1 = Fe(III) = POX PGHS \)
- \( E_2 = Fe(IV)PP^* = PGHS \) intermediate 1
- \( E_3 = Fe(IV)Tyr^* = COX PGHS \)
- \( E_4 = Fe(IV) = PGHS \) intermediate 2
- \( P = PGG_2 \)
- \( H = PGH_2 \)
- \( S = AA = arachidonic \) acid
- \( C_1 = Fe(IV)AA^*/Tyr = PGHS-arachidonic \) acid complex 1, or PGHS-\( AA \) complex 1
- \( C_2 = Fe(IV)AAO_2^*/Tyr = PGHS-arachidonic \) acid complex 2, or PGHS-\( AA \) complex 2.

From Bambai and Kulmacz (2000), we added in suicide inactivation of the COX site

\[
E_3 \underset{k_9}{\rightarrow} E_5, \quad (3.3)
\]

where \( E_3 \) is the COX PGHS and \( E_5 \) is inactive PGHS. Using information provided by Goltsov et al. (2010), we derived a chemical reaction equation for aspirin:

\[
E^* + A \underset{k_{on aspirin}}{\rightarrow} \hat{C}, \quad (3.4)
\]
where $A$ denotes aspirin and $\hat{C}$ is the PGHS-aspirin complex. Let $k_{on,\text{aspirin}}$ be called $k_{10}$.

From [Wang et al. (2001)], we deduced the relationship between PGH$_2$ and thromboxane:

$$E_6 + H \rightarrow X \rightarrow B,$$

where $E_6$ is thromboxane A synthase, $H$ is PGH$_2$, $X$ is TXA$_2$, and $B$ is thromboxane B$_2$, or TXB$_2$. The proposed mechanism for the production of TXA$_2$ and TXB$_2$ [Wang et al., 2001] is

$$E_6 + H \xrightleftharpoons[k_{-a}]{k_a} E' : H \xrightleftharpoons[k_{-b}]{k_b} C' \xrightleftharpoons[k_{-c}]{k_c} HHT \xrightleftharpoons[k_{-d}]{k_d} X,$$

where $C'$ is the enzyme-substrate complex and $HHT$ is a side product. The rate constants $k_{-b}$, $k_{-c}$, and $k_{-d}$ were set to equal 0.01 s$^{-1}$, making the corresponding reactions essentially irreversible, after which it was experimentally determined that the reaction with rate constant $k_a$ is the rate-determining, and therefore slowest, step [Wang et al., 2001]. We therefore simplified Equation 3.6 into

$$E_6 + H \xrightarrow{k_a} X.$$

(3.7)

However, TXA$_2$ has a very short half-life, and when in aqueous solutions, it is almost immediately converted to the inactive TXB$_2$ [Wang et al., 2001]. Thus, we made the following simplification:

$$E_6 + H \xrightarrow{k_a} B,$$

(3.8)

and defined $k_a$ as $k_{11}$. The reaction was written to be irreversible, because we would never see TXB$_2$ converting to TXA$_2$, due to the labile nature of TXA$_2$. 
Building the Model

We combined Equations 3.2, 3.3, 3.4, and 3.8 together to get:

\[
\begin{align*}
E_1 + P & \xrightarrow{k_1} E_2 + H \\
E_2 & \xrightarrow{k_3} E_3 \\
E_3 + S & \xrightarrow{k_4} C_1 \xrightarrow{k_5} C_2 \xrightarrow{k_6} E_3 + P \\
E_2 & \xrightarrow{k_7} E_4 \\
E_3 & \xrightarrow{k_8} E_4 \\
E_4 & \xrightarrow{k_9} E_1 \\
E_3 & \xrightarrow{k_{10}} E_5 \\
E_3 + A & \xrightarrow{k_{10}} C_3 \\
E_6 + H & \xrightarrow{k_{11}} E_6 + B.
\end{align*}
\] (3.9)

Converting these chemical equations to differential equations yielded

\[
\begin{align*}
\dot{E}_1 &= -k_1 E_1 P + k_8 E_4 \\
\dot{E}_2 &= k_1 E_1 P - E_2 (k_2 + k_6) \\
\dot{E}_3 &= k_2 E_2 + k_5 C_2 - E_3 (k_3 S + k_7 + k_9 + k_{10} A) \\
\dot{E}_4 &= k_6 E_2 + k_7 E_3 - k_8 E_4 \\
\dot{E}_5 &= k_9 E_3 \\
\dot{E}_6 &= 0 \\
\dot{C}_1 &= k_3 E_3 S - k_4 C_1 \\
\dot{C}_2 &= k_4 C_1 - k_5 C_2 \\
\dot{C}_3 &= k_{10} E_3 A \\
\dot{P} &= -k_1 E_1 P + k_5 C_2 \\
\dot{H} &= k_1 E_1 P - k_{11} E_6 H \\
\dot{A} &= -k_{10} E_3 A \\
\dot{S} &= -k_3 E_3 S \\
\dot{B} &= k_{11} E_6 H
\end{align*}
\] (3.10)

where \( \dot{E}_1 \) denotes the rate of change of \( E_1 \) with respect to time, or \( \frac{dE_1}{dt} \), and so on.
3.2 Initial Concentration and Rate Constant Values

In order to run simulations in MATLAB, numerical values were needed for initial concentrations of all enzymes and their alternate forms, complexes, reactants, and product and for the reaction rates.

3.2.1 Initial Conditions

Initial concentrations for POX PGHS ($E_1$), COX PGHS ($E_3$), and arachidonic acid, or AA, ($S$) were found in Goltsov et al. (2010). We ran our simulations with an aspirin dosage of 325 mg; this dosage was chosen so that we had a corresponding expected final concentration of TXB$_2$ from Feldman and Cryer (1999) that could be measured. Using a molar mass of 180.157 g/mol, the volume of distribution for aspirin of 0.2 L/kg (McEvoy, 2007), and a mass of 83 kg, which was the mean weight of the subjects from Feldman’s experiment (1999), we obtained an initial concentration of $1.0867 \times 10^{-4}$ M for aspirin ($A$). As for the thromboxane B$_2$ ($B$) value, data from Feldman and Cryer (1999) gave us an initial concentration of $1.7545 \times 10^{-6}$ M that was converted from the reported 650 ng/mL using a molar mass of thromboxane B$_2$ of 370.48 g/mol.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Initial values (M)</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_1$ (POX PGHS)</td>
<td>$10^{-8} - 10^{-6}$</td>
<td>Goltsov et al., 2010</td>
</tr>
<tr>
<td>$E_2$ (PGHS intermediate 1)</td>
<td>$10^{-8} - 10^{-6}$</td>
<td></td>
</tr>
<tr>
<td>$E_3$ (COX PGHS)</td>
<td>$10^{-8} - 10^{-6}$</td>
<td>Goltsov et al., 2010</td>
</tr>
<tr>
<td>$E_4$ (PGHS intermediate 2)</td>
<td>$10^{-8} - 10^{-6}$</td>
<td></td>
</tr>
<tr>
<td>$E_5$ (Inactive PGHS)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$E_6$ (Thromboxane A synthase)</td>
<td>$10^{-8} - 10^{-6}$</td>
<td></td>
</tr>
<tr>
<td>$C_1$ (PGHS-AA complex 1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$C_2$ (PGHS-AA complex 2)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$C_3$ (PGHS-aspirin complex)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$P$ (PGG$_2$)</td>
<td>0</td>
<td>Gerrard et al., 1977</td>
</tr>
<tr>
<td>$H$ (PGH$_2$)</td>
<td>0</td>
<td>Gerrard et al., 1977</td>
</tr>
<tr>
<td>$A$ (Aspirin)</td>
<td>$1.0867 \times 10^{-4}$</td>
<td>Feldman and Cryer, 1999</td>
</tr>
<tr>
<td>$S$ (Arachidonic acid)</td>
<td>$10^{-9} - 10^{-7}$</td>
<td>Goltsov et al., 2010</td>
</tr>
<tr>
<td>$B$ (TXB$_2$)</td>
<td>$1.7545 \times 10^{-6}$</td>
<td>Feldman and Cryer, 1999</td>
</tr>
</tbody>
</table>

*Table 3.1* Initial concentrations for all enzymes, reactants, and products.
Values for the remaining variables could not be found in literature and were deduced from alternate research. Initial concentration values for PGHS intermediates 1 ($E_2$) and 2 ($E_4$) were set to be equal to the initial concentration values for POX and COX PGHS due to the chemical nature of enzymes. They are not four different enzymes, but rather one enzyme with different forms and sites of catalysis that perform different functions. Initial concentration values for PGHS-AA complexes 1 ($C_1$) and 2 ($C_2$), PGHS-aspirin complex ($C_3$), and inactive PGHS ($E_5$) was set to be 0 M, because the reactions had not yet occurred at time $t = 0$ s. Data for the initial concentration value of thromboxane A synthase could not be found, and so we will assume a value equal to that of the PGHS forms. PGG$_2$ and PGH$_2$ are short-lived and labile, and under normal conditions will be quickly metabolized to form other products; thus, they cannot be isolated, and their concentrations are immeasurable, as they are quickly used in a reaction once they are formed (Gerrard et al., 1977). Therefore, initial concentration values for PGG$_2$ ($P$) and PGH$_2$ ($H$) were both 0 M. Table 3.1 provides a consolidated summary of all initial concentration values.

3.2.2 Expected Values

In order to prove the validity of our model, we wanted to see if our simulated expected value of TXB$_2$ matches an experimental value. Feldman and Cryer (1999) experimentally determined that the concentration level of TXB$_2$ was 100 ng/mL 30 minutes after ingestion of a 325-mg tablet of aspirin. We converted this value to $2.70 \times 10^{-7}$ M using a molar mass of 370.48 g/mol. We proved the validity of our model by running simulations in Section 5.2.2.

3.2.3 Rate Constants

Values for reaction rates $k_1$ through $k_8$ were given by Wei et al. (1995), $k_9$ by Bambai and Kulmacz (2000), $k_{10}$ by Goltsov et al. (2010), and $k_{11}$ by Wang et al. (2001). These values are summarized in Table 3.2. However, some of these reaction rates were faulty, due to the simplification of our model from the model proposed by Wei et al. (1995).
Rate constants were determined experimentally by determining the rate of the reaction and dividing that by the concentrations of the reactants (cf. Nelson and Cox, 2012). For example, given a general chemical reaction

$$A + B \xrightarrow{k} C + D,$$

the reaction rate is as follows:

$$\text{Reaction rate} = k[A][B],$$

where the brackets denote “concentration of.” Therefore, the rate constant can be calculated as such:

$$k = \frac{\text{reaction rate}}{[A][B]}.$$

For reactions governed by rate constants $k_4$ through $k_8$, Wei et al. (1995) proposed reactions with two reactants, whereas we proposed reactions with only one reactant. Thus, the values and units for rate constants $k_4$ through $k_8$ are compromised. We accommodated for this by finding a value for normal blood concentration levels of oxygen by which we could scale $k_4$ and $k_5$. However, multiplying our rate constants by this value did not yield the expected final concentration of TXB$_2$. Values for normal blood concentration levels of ferulic acid, by which we would have scaled $k_6$, $k_7$, and $k_8$, could not be found. Therefore, we scaled these rate constants by multiplying them by arbitrary scalars with units of M. Rate constants $k_4$ and $k_5$ were

<table>
<thead>
<tr>
<th>Rate constants</th>
<th>Values</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>$1 \times 10^8$</td>
<td>$M^{-1}s^{-1}$</td>
<td>Wei et al., 1995</td>
</tr>
<tr>
<td>$k_2$</td>
<td>350</td>
<td>$s^{-1}$</td>
<td>Wei et al., 1995</td>
</tr>
<tr>
<td>$k_3$</td>
<td>$1 \times 10^6$</td>
<td>$M^{-1}s^{-1}$</td>
<td>Wei et al., 1995</td>
</tr>
<tr>
<td>$k_4$</td>
<td>$\geq 5 \times 10^6$</td>
<td>$M^{-1}s^{-1}$</td>
<td>Wei et al., 1995</td>
</tr>
<tr>
<td>$k_5$</td>
<td>$\geq 5 \times 10^6$</td>
<td>$M^{-1}s^{-1}$</td>
<td>Wei et al., 1995</td>
</tr>
<tr>
<td>$k_6$</td>
<td>$\leq 3.5 \times 10^6$</td>
<td>$M^{-1}s^{-1}$</td>
<td>Wei et al., 1995</td>
</tr>
<tr>
<td>$k_7$</td>
<td>$(0.5 - 5) \times 10^6$</td>
<td>$M^{-1}s^{-1}$</td>
<td>Wei et al., 1995</td>
</tr>
<tr>
<td>$k_8$</td>
<td>$5.5 \times 10^6$</td>
<td>$M^{-1}s^{-1}$</td>
<td>Wei et al., 1995</td>
</tr>
<tr>
<td>$k_9$</td>
<td>$5 \times 10^{-2}$</td>
<td>$s^{-1}$</td>
<td>Bambai and Kulmacz, 2000</td>
</tr>
<tr>
<td>$k_{10}$</td>
<td>$10M^{-1}$</td>
<td>$s^{-1}$</td>
<td>Goltsov et al., 2010</td>
</tr>
<tr>
<td>$k_{11}$</td>
<td>$(1.2 - 2.0) \times 10^7$</td>
<td>$M^{-1}s^{-1}$</td>
<td>Wang et al., 2001</td>
</tr>
</tbody>
</table>

Table 3.2  Values for all rate constants.
scaled by the same factor (0.4), since the same reactant was omitted from both corresponding reactions in our simplified model, and rate constants $k_6$ through $k_8$ were scaled by the same factor (500), for the same reason.

<table>
<thead>
<tr>
<th>Rate constants</th>
<th>Values</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>$1 \times 10^8$</td>
<td>$M^{-1}s^{-1}$</td>
</tr>
<tr>
<td>$k_2$</td>
<td>350</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_3$</td>
<td>$1 \times 10^6$</td>
<td>$M^{-1}s^{-1}$</td>
</tr>
<tr>
<td>$k_4$</td>
<td>$2.0 \times 10^6$</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_5$</td>
<td>$2.0 \times 10^6$</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_6$</td>
<td>$1.75 \times 10^9$</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_7$</td>
<td>$1.0 \times 10^9$</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_8$</td>
<td>$2.75 \times 10^9$</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_9$</td>
<td>$5 \times 10^{-2}$</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_{10}$</td>
<td>10</td>
<td>$M^{-1}s^{-1}$</td>
</tr>
<tr>
<td>$k_{11}$</td>
<td>$1.5 \times 10^7$</td>
<td>$M^{-1}s^{-1}$</td>
</tr>
</tbody>
</table>

Table 3.3  List of rate constants to be used in MATLAB simulations.
Chapter 4

Reduced model

Following the style of Tien et al. (2005), we thought it would be useful to simplify our model and perform a stability analysis on our model. Such an analysis would tell us when the reactions of PGHS would be self-sustaining and when they would cease. Furthermore, simplification of the model would allow it to be more easily integrable into other models of blood clotting. We also ran simulations of our model to see if it would match experimental data found by Feldman and Cryer (1999).

4.1 Reduction

We begin with our chemical model, Equation 3.9 derived in Section 3.1:

\begin{align*}
E_1 + P & \xrightarrow{k_1} E_2 + H \quad (4.1) \\
E_2 & \xrightarrow{k_2} E_3 \quad (4.2) \\
E_3 + S & \xrightarrow{k_3} C_1 \xrightarrow{k_4} C_2 \xrightarrow{k_5} E_3 + P \quad (4.3) \\
E_2 & \xrightarrow{k_6} E_4 \quad (4.4) \\
E_3 & \xrightarrow{k_7} E_4 \quad (4.5) \\
E_4 & \xrightarrow{k_8} E_1 \quad (4.6) \\
E_3 & \xrightarrow{k_9} E_5 \quad (4.7) \\
E_3 & \xrightarrow{k_{10}} C_3 \quad (4.8) \\
E_6 + H & \xrightarrow{k_{11}} E_6 + B \quad (4.9)
\end{align*}
We combined Equation 4.1 and Equation 4.2 to obtain

\[ E_1 + P \xrightarrow{k_1} E_2 \xrightarrow{k_2} E_3 + H, \]  

(4.10)

and reduced Equation 4.3 to

\[ E_3 + S \xrightarrow{k_3} C_1 \xrightarrow{k_5} E_3 + P, \]  

(4.11)

and combined Equation 4.5 and Equation 4.6 to obtain

\[ E_3 \xrightarrow{k_7} E_1. \]  

(4.12)

Equations 4.7, 4.8, and 4.9 remained the same, and Equation 4.4 was not factored into the reduced model.

Equations 4.7-4.11 came together to form a reduced model:

\[
\begin{align*}
E_1 + P &\xrightarrow{k_1} E_2 \xrightarrow{k_2} E_3 + H, \\
E_3 + S &\xrightarrow{k_3} C_1 \xrightarrow{k_5} E_3 + P, \\
E_3 &\xrightarrow{k_7} E_1, \\
E_3 &\xrightarrow{k_9} E_5, \\
E_3 + A &\xrightarrow{k_{10}} C_3, \\
E_6 + H &\xrightarrow{k_{11}} E_6 + B
\end{align*}
\]  

(4.13)

with the following corresponding differential equations:

\[
\begin{align*}
\dot{E}_1 &= k_7 E_3 - k_1 E_1 P \\
\dot{E}_2 &= k_1 E_1 P - k_2 E_2 \\
\dot{E}_3 &= k_2 E_2 + k_5 C_1 - k_3 E_3 S - k_7 E_3 - k_9 E_3 - k_{10} E_3 A \\
\dot{E}_5 &= k_3 E_3 \\
\dot{E}_6 &= 0 \\
\dot{C}_1 &= k_3 E_3 S - k_5 C_1 \\
\dot{C}_3 &= k_{10} E_3 A \\
\dot{P} &= k_5 C_1 - k_1 E_1 P \\
\dot{H} &= k_2 E_2 - k_{11} E_6 H \\
\dot{A} &= -k_{10} E_3 A \\
\dot{S} &= -k_3 E_3 S \\
\dot{B} &= k_{11} E_6 H
\end{align*}
\]  

(4.14)
4.2 Stability Analysis

We performed a stability analysis on the fixed points of the reduced system for general rate constants, $k_i$, where $i = 1, 2, ..., 11$.

4.2.1 Finding the Fixed Points

We began the stability analysis with the following assumptions:

- $S$ (arachidonic acid) is fixed, and
- $k_9 = 0$, eliminating suicide inactivation of PGHS.

We defined $E_o$ to be the sum of all forms, intermediates, and complexes of PGHS:

$$E_o = E_1 + E_3 + E_2 + C_1$$  \hspace{1cm} (4.15)

and therefore defined $E_1$ as

$$E_1 = E_o - E_3 - E_2 - C_1$$  \hspace{1cm} (4.16)

and eliminated $E_1$ (POX PGHS) from the system by replacing $E_1$ with the above expression. We argued that $B$ (TXB$_2$) and $C_3$ (PGHS-aspirin complex) did not contribute to the stability of any fixed points, since they do not appear on the right-hand side of any of the differential equations. We were then left with the following system of differential equations:

$$
\begin{align*}
\dot{E}_3 &= k_2 E_2 + k_5 C_1 - E_3 (k_3 S + k_7 + k_{10} A) \\
\dot{E}_6 &= 0 \\
\dot{E}_2 &= k_1 (E_o - E_3 - E_2 - C_1) P - k_2 E_2 \\
\dot{C}_1 &= k_3 E_3 S - k_5 C_1 \\
\dot{P} &= k_5 C_1 - k_1 (E_o - E_3 - E_2 - C_1) P \\
\dot{H} &= k_2 E_2 - k_{11} E_6 H \\
\dot{A} &= -k_{10} E_3 A
\end{align*}
$$  \hspace{1cm} (4.17)

with fixed points of the form

$$(E_3, E_6, E_2, C_1, P, H, A).$$  \hspace{1cm} (4.18)
Reduced model

Setting each of the differential equations to zero gave us the following fixed points:

\[(0, 0, 0, 0, 0, 0, 0, 0, 0)\]
\[(0, E_6, 0, 0, 0, 0)\]
\[(0, 0, 0, P, 0, 0)\]
\[(0, 0, 0, 0, H, 0)\]
\[(0, 0, 0, 0, 0, 0, 0, 0)\]
\[(0, E_6, 0, 0, 0, P, 0, 0)\]
\[(0, 0, 0, 0, P, 0, 0, A)\]
\[(0, E_6, 0, 0, 0, 0, 0, 0, 0)\]
\[(0, 0, 0, 0, P, 0, 0, A)\]
\[(0, 0, 0, 0, 0, 0, H, 0)\]
\[(0, E_6, 0, 0, 0, P, 0, 0, A)\]
\[(0, 0, 0, 0, P, 0, 0, A)\]
\[(0, 0, 0, 0, 0, 0, 0, 0, A)\]

From our fixed points, we know that \(E_3, E_2,\) and \(C_1 = 0\), making \(E_0 = E_1\). If \(E_0 = E_1 = 0\), then there are no PGHS molecules present in the system, leaving \(P\) (PGG2) unchanged. However, if we perturb \(E_0\) to be greater than 0, and therefore \(E_1 > 0\), then \(P\) might be affected, depending on its value. If \(P = 0\), then it remains unchanged after the perturbation. On the other hand, when \(P\) is initially fixed at some value greater than zero, perturbations to \(E_0\), and consequently to \(E_1\), will cause the value of \(P\) to perturb away from that initial fixed value. Therefore, the following fixed points

\[(0, 0, 0, 0, 0, 0, 0, 0, 0)\]
\[(0, E_6, 0, 0, 0, 0, 0, 0, 0)\]
\[(0, 0, 0, 0, P, 0, 0, A)\]
\[(0, 0, 0, 0, P, 0, 0, A)\]
\[(0, 0, 0, 0, 0, 0, H, 0)\]
\[(0, E_6, 0, 0, 0, 0, 0, 0, 0)\]
\[(0, 0, 0, 0, 0, 0, 0, 0, A)\]

with \(P > 0\), are always unstable. The stability of the remaining fixed points

\[(0, 0, 0, 0, 0, 0, 0, 0, 0)\]
\[(0, E_6, 0, 0, 0, 0, 0, 0, 0)\]
\[(0, 0, 0, 0, 0, 0, 0, 0, 0)\]

will be determined next.

### 4.2.2 The Generalized Jacobian Matrix

Because the equations were linearizable, we were able to construct the Jacobian matrix for our system, for a general fixed point of the form \((0, E_6, 0, 0, 0, 0, 0, 0, 0, 0, 0, H, A)\):

\[
J = \begin{bmatrix}
-k_3S - k_7 - k_{10}A & 0 & k_2 & k_3 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -k_2 & 0 & k_1E_o & 0 & 0 \\
k_3S & 0 & 0 & -k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\
0 & -k_{11}H & k_2 & 0 & 0 & -k_{11}E_6 & 0 \\
-k_{10}A & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}
\]  
\[(4.19)\]
4.2.3 Stability of \((0, 0, 0, 0, 0, 0, 0)\) and \((0, 0, 0, 0, H, 0)\)

We determined the stability of the fixed point \((0, 0, 0, 0, 0, 0, 0)\) by evaluating the Jacobian at \((0, 0, 0, 0, H, 0)\):

\[
J_0 = \begin{bmatrix}
-k_3S - k_7 & 0 & k_2 & k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -k_2 & 0 & k_1E_o & 0 & 0 \\
k_3S & 0 & 0 & -k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\
0 & 0 & k_2 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}.
\] (4.20)

The characteristic polynomial of Equation 4.20 is

\[
p(\lambda) = \lambda^7 + a_1\lambda^6 + a_2\lambda^5 + a_3\lambda^4 + a_4\lambda^3,
\] (4.21)

where

- \(a_1 = k_1E_o + k_2 + k_3S + k_5 + k_7\)
- \(a_2 = k_3S (k_2 + k_1E_o) + k_1E_o (k_2 + k_5 + k_7) + k_7 (k_2 + k_5) + k_2k_5\)
- \(a_3 = k_1E_o [(k_2 + k_5) (k_7) + k_2 (k_5 + k_3S)] + k_2k_5k_7\), and
- \(a_4 = k_1k_2k_5E_o (k_7 - k_3S)\).

The Jacobian matrix for \((0, 0, 0, 0, H, 0)\) is

\[
J_H = \begin{bmatrix}
-k_3S - k_7 & 0 & k_2 & k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -k_2 & 0 & k_1E_o & 0 & 0 \\
k_3S & 0 & 0 & -k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\
0 & -k_{11}H & k_2 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}.
\] (4.22)

and it resulted in the same characteristic polynomial as that of the zero fixed point. Although Equation 4.22 did not appear to be linearized due to the \(-k_{11}H\) term, we took any variables that were still remaining after the linearization and treat them as fixed-value parameters, in the hopes of basing the stability of the fixed point on functions of those variables. However, this was deemed unnecessary in this case, as the nonlinear term in question did not affect the characteristic polynomial.
We used a summary from the Routh-Hurwitz Theorem to determine the stability of these fixed points, and the criteria were as follows:

\[ a_1 > 0 \]
\[ a_3 > 0 \]
\[ a_4 > 0 \]
\[ a_1 a_2 a_3 > a_3^2 + a_1^2 a_4, \text{or} a_1 a_2 a_3 - a_3^2 + a_1^2 a_4 > 0 \]

(Allen 2006). More information on the Routh-Hurwitz Theorem can be found in Appendix A. Because \( k_1, k_2, k_3, k_5, k_7, E_o, \) and \( S \) all take on positive values, we know that \( a_1 \) and \( a_3 \), both of which are comprised of sums of positive numbers, are both greater than zero.

Furthermore, if we multiply out, expand, and simplify the expression \( a_1 a_2 a_3 - a_3^2 - a_1^2 a_4 \), we can see that it consists of sums of only positive terms, satisfying the last criterion.

Thus, the stability of the zero fixed point only depends on \( a_4 \).

For \( a_4 \) to be greater than zero, we need

\[ k_1 k_2 k_5 E_o (k_7 - k_3 S) > 0 \]
\[ k_7 - k_3 S > 0. \]

Keeping in the same style as Tien et al. (2005), we have

\[ k_3 S < k_7 \]
\[ \frac{k_3 S}{k_7} < 1 \]
\[ \frac{k_3 S}{k_7} - 1 < 0. \]

We define

\[ \sigma = \frac{k_3 S}{k_7} - 1, \quad (4.23) \]

where the stability of the zero fixed point is dependent on the sign of \( \sigma \), with \( \sigma < 0 \) indicating a stable fixed point and \( \sigma \geq 0 \) indicating an unstable point.

### 4.2.4 Implications of the Stability of \( (0, 0, 0, 0, 0, 0, 0) \) and \( (0, 0, 0, 0, 0, H, 0) \)

We then analyzed the requirements for stability of the fixed points \( (0, 0, 0, 0, 0, 0, 0) \) and \( (0, 0, 0, 0, 0, H, 0) \) and the biological consequences. The
fixed points and their stability were determined under the conditions that
S was fixed and suicide inactivation was not present. The next section dis-
cusses the analysis of such stability, and the two sections after discuss the
implications when these conditions are not met.

The analyses discuss the conditions under which the reactions carried
out by PGHS would be self-sustaining and the conditions under which the
reactions would terminate. This information could be useful in finding ap-
propriate dosages of aspirin required to prevent blood clotting—sufficient
to induce unwanted blood clotting while avoiding health complications
that could accompany an overdose or undesired side effects.

**S is Fixed, Suicide Inactivation is Absent**

With arachidonic acid concentration fixed and enzyme suicide inactiva-
tion absent, the only factors that could affect whether or not PGHS is self-
sustaining are the PGG$_2$ and aspirin concentrations.

As in the case of Tien et al. (2005), we ran simulations with varying
rate constants that produced situations where $\sigma$ was greater than zero and
less than zero. A value of $\sigma$ greater than zero indicated that rate of PGG$_2$
production was larger than the rate of consumption or that the rate of COX
PGHS consumption by aspirin was negligible, thus keeping PGG$_2$ in the
system and allowing the reactions to be self-sustaining. A value of $\sigma$ less
than zero indicated that the rate of PGG$_2$ consumption was larger than the
rate of production, leading to a removal of PGG$_2$ from the system, or that
the rate of COX PGHS consumption by aspirin was significant enough to
remove PGHS from the system. Either situation would lead to cessation of
PGHS activity.

**S Can Vary**

The likelihood that the arachidonic acid concentration is fixed is quite small,
as our bodies and cells are constantly producing and using chemicals, in-
cluding arachidonic acid. We concluded that if arachidonic acid concentra-
tion levels were allowed to vary, then PGHS activity would be terminated if
there was no constant addition of arachidonic acid in the system, and PGHS
activity would be self-sustaining if arachidonic acid was always supplied
for the system.
Suicide Inactivation is Present

We hypothesized that if COX PGHS were to undergo suicide inactivation, then it would potentially remove itself from the system entirely, if aspirin consumption of COX PGHS did not already do so. Thus, the reactions modeled by our system could come to a halt through enzyme inactivation; however, this phenomenon would be dependent on the rate constant and initial concentration values.

4.2.5 Stability of \((0, 0, 0, 0, 0, A), (0, 0, 0, 0, 0, H, A), (0, E_6, 0, 0, 0, 0), (0, E_6, 0, 0, 0, A)\)

In this section, we will discuss the stability of the remaining fixed points:

\[(0, 0, 0, 0, 0, A) \quad (0, E_6, 0, 0, 0, 0)\]
\[(0, 0, 0, 0, 0, H, A) \quad (0, E_6, 0, 0, 0, A)\]

The Jacobian matrices for the above points are provided in this section. However, due to the complexity and length of the expressions that resulted from the Routh-Hurwitz Criterion summaries (Allen, 2006), we will merely state that the stabilities of each of these fixed points are heavily dependent on the values of the rate constants, \(S\), and \(E_0\), and will be functions of \(A\), \(E_6\), or both. We also noted that the characteristic polynomial of Equation \(4.24\) was the same as that of Equation \(4.25\) so we were left with three characteristic polynomials from which we could determine the stability of the four corresponding fixed points. These stability criteria are detailed in Appendix B, and they can be used with specific rate constants to determine the stability of these fixed points for those particular rate constant values.

The Jacobian matrix at \((0, 0, 0, 0, 0, A)\) is

\[
J_A = \begin{bmatrix}
-k_3S - k_7 - k_{10}A & 0 & k_2 & k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -k_2 & 0 & k_1E_0 & 0 & 0 \\
k_3S & 0 & 0 & -k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & k_5 & -k_1E_0 & 0 & 0 \\
0 & 0 & k_2 & 0 & 0 & 0 & 0 \\
-k_{10}A & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix} \quad (4.24)
\]
Simulations

The Jacobian matrix at \((0,0,0,0,0,H,A)\) is

\[
J_{H,A} = \begin{bmatrix}
-k_3S - k_7 - k_{10}A & 0 & k_2 & k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -k_2 & 0 & k_1E_o & 0 & 0 \\
k_3S & 0 & 0 & -k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\
0 & -k_{11}H & k_2 & 0 & 0 & 0 & 0 \\
-k_{10}A & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}.
\] (4.25)

The Jacobian matrix at \((0,E_6,0,0,0,0,0)\) is

\[
J_{E_6} = \begin{bmatrix}
-k_3S - k_7 & 0 & k_2 & k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -k_2 & 0 & k_1E_o & 0 & 0 \\
k_3S & 0 & 0 & -k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\
0 & -k_{11}H & k_2 & 0 & 0 & 0 & -k_{11}E_6 \\
0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}.
\] (4.26)

Lastly, the Jacobian matrix at \((0,E_6,0,0,0,0,A)\) is

\[
J_{E_6,A} = \begin{bmatrix}
-k_3S - k_7 - k_{10}A & 0 & k_2 & k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -k_2 & 0 & k_1E_o & 0 & 0 \\
k_3S & 0 & 0 & -k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\
0 & 0 & k_2 & 0 & 0 & -k_{11}E_6 & 0 \\
-k_{10}A & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix}.
\] (4.27)

We can come to similar types of conclusions for these points as we did for the fixed points \((0,0,0,0,0,0,0)\) and \((0,0,0,0,0,H,0)\), using the results from Appendix B.

4.3 Simulations

We used the initial concentrations and rate constants from Section 3.2 and ran simulations of our model in MATLAB using the stiff solver ode23s to
plot the concentration-time curve of TXB$_2$ over 30 minutes. We ran simulations with and without aspirin; we expected the concentration of TXB$_2$ to remain unchanged without aspirin and to decrease to $2.70 \times 10^{-7} \text{ M}$, as determined in section 3.2.

![Plot of Thromboxane with and without Aspirin](image.png)

**Figure 4.1** Concentration-time curve for TXB$_2$, with and without aspirin for the reduced system, with rate constants from Section 3.2.

When we used the original rate constants, the TXB$_2$ concentration increased from its starting concentration of $1.7545 \times 10^{-6} \text{ M}$ to a steady state value of $2.63 \times 10^{-6} \text{ M}$ for both the cases with and without aspirin, as shown in Figure 4.1, where the box depicts the initial concentration.

We then altered $k_{11}$, by multiplying it by $10^{-17}$, in an attempt to bring the TXB$_2$ concentration down to the expected value; this was not successful, however, and while we were able to make both cases result in an unchanged TXB$_2$ concentration, we were unable to manipulate the case with aspirin therapy into decreasing towards the expected final concentration, as can be seen in Figure 4.2, where the box denotes the initial concentration. Therefore, we hypothesize that an important piece of the model was omitted during the reduction of our system.
We concluded that while the reduced system did offer insight into the terms of self-sustainment for PGHS, it did not accurately model the effect of aspirin on blood clotting. Therefore, we revisited the full model and performed similar analyses and simulations.
Chapter 5

Full Model

The simulations for the reduced model did not yield accurate representation of the interaction between PGHS and aspirin to result in unwanted blood clotting. Therefore, we analyzed the full system for stability and ran simulations.

5.1 Stability Analysis

We begin this section by reminding our readers of our system of equations:

\[
\begin{align*}
\dot{E}_1 &= -k_1 E_1 P + k_8 E_4 \\
\dot{E}_2 &= k_1 E_1 P - E_2 (k_2 + k_6) \\
\dot{E}_3 &= k_5 C_2 - E_3 (k_3 S + k_7 + k_9 + k_{10} A) \\
\dot{E}_4 &= k_6 E_2 + k_7 E_3 - k_8 E_4 \\
\dot{E}_5 &= k_9 E_3 \\
\dot{E}_6 &= 0 \\
\dot{C}_1 &= k_3 E_3 S - k_4 C_1 \\
\dot{C}_2 &= k_4 C_1 - k_5 C_2 \\
\dot{C}_3 &= k_{10} E_3 A \\
\dot{P} &= -k_1 E_1 P + k_5 C_2 \\
\dot{H} &= k_1 E_1 P - k_{11} E_6 H \\
\dot{A} &= -k_{10} E_3 A \\
\dot{S} &= -k_3 E_3 S \\
\dot{B} &= k_{11} E_6 H
\end{align*}
\]  

(5.1)
with the following variable definitions:

- $E_1 = \text{POX PGHS}$
- $E_2 = \text{PGHS intermediate 1}$
- $E_3 = \text{COX PGHS}$
- $E_4 = \text{PGHS intermediate 2}$
- $E_5 = \text{inactive PGHS}$
- $C_1 = \text{PGHS-AA complex 1}$
- $C_2 = \text{PGHS-AA complex 2}$
- $C_3 = \text{PGHS-aspirin complex}$
- $P = \text{PGG}_2$
- $H = \text{PGH}_2$
- $A = \text{aspirin}$
- $S = \text{arachidonic acid, or AA}$
- $B = \text{TXB}_2$

We performed a stability analysis on the fixed points of Equation 3.10. However, due to the long and complex computations, we will provide the reader with the fixed points and the corresponding linearized equations and Jacobian matrices and direct the reader to Appendix B.

### 5.1.1 Finding the Fixed Points

To begin the stability analysis, we first assumed that

- $S$ (arachidonic acid) is fixed, and
- $k_9 = 0$, eliminating suicide inactivation of PGHS.

We defined $E_o$ to be the sum of all forms, intermediates, and complexes of PGHS:

$$E_o = E_1 + E_3 + E_2 + E_4 + C_1 + C_2$$

(5.2)

and therefore defined $E_1$ as

$$E_1 = E_o - E_3 - E_2 - E_4 - C_1 - C_2$$

(5.3)
and eliminated $E_1$ (POX PGHS) from the system by replacing $E_1$ with the above expression. We argued that $B$ (TXB$_2$) and $C_3$ (PGHS-aspirin complex) did not contribute to the stability of any fixed points, since they do not appear on the right-hand-side of any of the differential equations. We were then left with the following system of differential equations:

\[
\begin{align*}
\dot{E}_3 &= k_2 E_2 + k_5 C_1 - E_3 (k_3 S + k_7 + k_{10} A) \\
\dot{E}_6 &= 0 \\
\dot{E}_2 &= k_1 (E_o - E_3 - E_2 - E_4 - C_1 - C_2) P - E_2 (k_2 + k_6) \\
\dot{E}_4 &= k_7 E_3 + k_6 E_2 - k_8 E_4 \\
\dot{C}_1 &= k_3 S E_3 - k_4 C_1 \\
\dot{C}_2 &= k_4 C_1 - k_5 C_2 \\
\dot{P} &= k_5 C_2 - k_1 (E_o - E_3 - E_2 - E_4 - C_1 - C_2) P \\
\dot{H} &= k_1 (E_o - E_3 - E_2 - E_4 - C_1 - C_2) P - k_{11} E_6 H \\
\dot{A} &= -k_{10} E_3 A
\end{align*}
\]

with fixed points of the form

\[(E_3, E_6, E_2, E_4, C_1, C_2, P, H, A)\]. \hspace{1cm} (5.5)

Setting each of the differential equations to zero gave us the following fixed points:

\[
\begin{align*}
(0, 0, 0, 0, 0, 0, 0, 0, 0) & \quad (0, 0, 0, 0, 0, 0, 0, 0, A) & \quad (0, 0, 0, 0, 0, 0, P, 0, A) \\
(0, E_6, 0, 0, 0, 0, 0, 0, 0) & \quad (0, E_6, 0, 0, 0, 0, P, 0, 0) & \quad (0, 0, 0, 0, 0, 0, 0, H, A) \\
(0, 0, 0, 0, 0, 0, 0, 0, 0) & \quad (0, 0, 0, 0, 0, 0, 0, 0, A) & \quad (0, 0, 0, 0, 0, 0, P, 0, 0) \\
(0, 0, 0, 0, 0, 0, H, 0) & \quad (0, 0, 0, 0, 0, 0, P, 0, H) & \quad (0, 0, 0, 0, 0, 0, 0, P, H, A)
\end{align*}
\]

From our fixed points, we know that $E_3$, $E_2$, $E_4$, $C_1$, and $C_2 = 0$, making $E_o = E_1$. If $E_o = E_1 = 0$, then there are no PGHS molecules present in the system, leaving $P$ (PGG$_2$) unchanged. However, if we perturb $E_o$ to be greater than 0, and therefore $E_1 > 0$, then $P$ might be affected, depending on its value. If $P = 0$, then it remains unchanged after the perturbation. On the other hand, for some fixed value of $P > 0$, perturbations to $E_o$, and $E_1$, will perturb $P$ away from that initial fixed value. Therefore, the following fixed points
with $P > 0$ are always unstable. The stability of the remaining fixed points

$$(0, 0, 0, 0, 0, 0, 0, 0, 0) \quad (0, E_6, 0, 0, 0, 0, 0, 0, 0)$$

will be determined next.

### 5.1.2 Stability of the Fixed Points

The generalized Jacobian matrix for our full system is provided in this section. However, due to the complexity of the expressions that result from the Routh array (Co, 1999) and the Routh Hurwitz Theorem (Allen, 2006), we will merely state that the stabilities of each of these fixed points are heavily dependent on the values of the rate constants, $S$, and $E_0$, and some will be functions of $A$, $E_6$, or both. These stability criteria are detailed in Appendix C along with the Jacobian matrices for each of the fixed points.

The generalized Jacobian matrix for a fixed point of the form

$$(0, E_6, 0, 0, 0, 0, 0, H, A)$$

is

$$J = \begin{bmatrix}
-k_3 S - k_7 - k_{10} A & 0 & k_2 & 0 & 0 & k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -k_2 - k_6 & 0 & 0 & 0 & k_1 E_0 & 0 & 0 \\
k_7 & 0 & k_6 & -k_8 & 0 & 0 & 0 & 0 & 0 \\
k_3 S & 0 & 0 & 0 & -k_4 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & k_4 & -k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & k_5 & -k_1 E_0 & 0 & 0 \\
0 & -k_{11} H & 0 & 0 & 0 & 0 & k_1 E_0 & -k_{11} E_6 & 0 \\
-k_{10} A & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 
\end{bmatrix}.$$  

(5.6)

Using the results found in Appendix C, we can still find the conditions under which PGHS is self-sustaining and under which its reactions terminate; again, this information can be used to give insights into appropriate aspirin dosage.
5.2 Simulations

We ran simulations of our full model in MATLAB using the stiff solver ode23s to plot the concentration of TXB$_2$ over a time period of 30 minutes, both with and without aspirin.

5.2.1 Rate Constants

We used the initial concentrations listed in Sections 5.2 to run our simulations in MATLAB. However, our simulations did not match the expected final concentration for thromboxane B$_2$ given by Feldman and Cryer (1999), and so rate constants $k_1$ and $k_{10}$ were also adjusted and multiplied by 0.085 and 0.999000305, respectively. Some of the rate constants found in the literature were reported as ranges, so we picked arbitrary values from those ranges. Table 5.1 summarizes the rate constants that were used in our simulations in MATLAB to yield an expected TXB$_2$ final concentration of $2.70 \times 10^{-7} \text{ M}$.

<table>
<thead>
<tr>
<th>Rate constants</th>
<th>Values</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$</td>
<td>$8.5 \times 10^6$</td>
<td>$M^{-1}s^{-1}$</td>
</tr>
<tr>
<td>$k_2$</td>
<td>350</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_3$</td>
<td>$1 \times 10^6$</td>
<td>$M^{-1}s^{-1}$</td>
</tr>
<tr>
<td>$k_4$</td>
<td>$2.0 \times 10^6$</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_5$</td>
<td>$2.0 \times 10^6$</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_6$</td>
<td>$1.75 \times 10^9$</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_7$</td>
<td>$1.0 \times 10^9$</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_8$</td>
<td>$2.75 \times 10^9$</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_9$</td>
<td>$5 \times 10^{-2}$</td>
<td>$s^{-1}$</td>
</tr>
<tr>
<td>$k_{10}$</td>
<td>9.99000305</td>
<td>$M^{-1}s^{-1}$</td>
</tr>
<tr>
<td>$k_{11}$</td>
<td>$1.5 \times 10^7$</td>
<td>$M^{-1}s^{-1}$</td>
</tr>
</tbody>
</table>

Table 5.1 List of rate constants used in MATLAB simulation for the full system.

5.2.2 MATLAB Simulations

Without aspirin present, we would expect the concentration of TXB$_2$ to remain unchanged. This was supported by our simulation. Figure 5.1 shows the change in concentration of TXB$_2$ over 30 minutes, where the box denotes
the initial concentration. As predicted, the figure shows that the concentration level does not change over time when there is no aspirin present and remains at $1.7545 \times 10^{-6}$ M.

**Figure 5.1** Concentration-time curve for TXB$_2$ without aspirin.

Due to the competitive nature of aspirin as an inhibitor, if the concentration of aspirin is much larger than that of arachidonic acid, then COX PGHS should not be reacting with arachidonic acid at all. However, MATLAB does not take this into account. Therefore, in our simulations with aspirin present, we implemented an if-statement such that the following occurred:

\[
\text{If } [A] > [S]: \quad \dot{E}_3 = k_2 E_2 + k_5 C_2 - E_3 (k_9 + k_{10} A) \\
\text{Else:} \quad \dot{E}_3 = k_2 E_2 + k_5 C_2 - E_3 (k_3 S + k_7 + k_9)
\]

(5.7)

In the “if” clause, we eliminated the reaction of COX PGHS with arachidonic acid to form PGHS complex 2 and the conversion of COX PGHS into
PGHS intermediate 2. In the “else” clause, we eliminated the reaction of COX PGHS with aspirin. Suicide inactivation was present in both situations.

![Plot of Thromboxane with Aspirin](image)

**Figure 5.2** Concentration-time curve for TXB$_2$ with aspirin.

Figure 5.2 shows the concentration-time curve for TXB$_2$ for 30 minutes that was generated by our MATLAB simulation, where the box depicts the initial concentration. The final concentration of TXB$_2$ equaled $2.5 \times 10^{-7}$ M, which was close to the predicted final concentration of TXB$_2$ of $2.70 \times 10^{-7}$ M from Feldman and Cryer (1999).

We noted that the shape of our graph did not match the data of Feldman and Cryer (1999), as the concentration of TXB$_2$ decays at a much quicker rate. Feldman and Cryer reported that the TXB$_2$ concentration did not hit the final value of $2.70 \times 10^{-7}$ M until 30 minutes after aspirin ingestion; at one minute after ingestion, 5 minutes, and 7.5 minutes, the TXB$_2$ concentrations were $1.552 \times 10^{-6}$ M, $1.417 \times 10^{-6}$ M, and $1.215 \times 10^{-6}$ M, respectively (1999). However, our simulations showed a decrease to $2.5 \times 10^{-7}$ M well before one minute after aspirin ingestion. There was neither sufficient
chemical data nor assays to determine what would cause such a rapid decay. Therefore, we hypothesized that other factors are feeding into TXB$_2$, that cause the slower decrease, that are not included in our model. We also noted that despite not capturing the transient response over the 30-minute time period, we ultimately did achieve an accurate steady state concentration of TXB$_2$ of $2.5 \times 10^{-7}$ M, which is vital for preventing unwanted blood clotting.

5.3 Sensitivity Analysis

The failure of the rate constants to produce the expected final concentration of TXB$_2$ without alteration lead us to perform a sensitivity analysis on our system.

Figure 5.3 shows the sensitivity of final TXB$_2$ concentration in relation to the sensitivity of the rate constants. We can see that the rate constants with the highest effect on the final TXB$_2$ concentration were $k_2$, $k_3$, $k_6$, and
$k_{10}$, with $k_7$ and $k_8$ having the next largest effects. Although an order of magnitude of $10^{-7}$ for the percent changes in concentration may seem trivial, these percentages actually reflect a large change on our system, given that the rate constants were on a similar order of magnitude. For a better sense of the relative change, another sensitivity analysis was performed on a nondimensionalized version of our system in Section 5.4.

## 5.4 Nondimensionalization

We performed a nondimensionalization in order to simplify our system of equations. The nondimensionalized system is as follows:

\[
\begin{align*}
\dot{E}_1 &= -E_1P + \frac{\lambda}{\mu}E_4 \\
\dot{E}_2 &= \alpha E_1P - \beta E_2 \\
\dot{E}_3 &= \sigma \eta E_2 + \frac{1}{\alpha}C_2 - E_3(S + \gamma + \delta + A) \\
\dot{E}_4 &= \eta E_2 + \sigma \mu \gamma E_3 - \lambda E_4 \\
\dot{E}_5 &= \kappa E_3 \\
\dot{E}_6 &= 0 \\
\dot{C}_1 &= \mu E_3S - \eta C_1 \\
\dot{C}_2 &= \rho C_1 - C_2 \\
\dot{C}_3 &= \mu E_3A \\
\dot{P} &= -\epsilon E_1P + \frac{\eta}{\sigma}C_2 \\
\dot{H} &= \theta E_1P - E_6H \\
\dot{A} &= \kappa E_3A \\
\dot{S} &= -\rho E_3S \\
\dot{B} &= \phi E_6H
\end{align*}
\]

(5.8)

with the following unit-less coefficients:

- $\alpha = \frac{k_2}{k_4}$
- $\beta = \frac{k_2 + k_6}{k_5}$
- $\sigma = \frac{k_3}{k_1}$
- $\gamma = \frac{k_2}{k_5}$
- $\delta = \frac{k_2}{k_5}$
- $\eta = \frac{k_4}{k_5}$
Full Model

\[ \lambda = \frac{k_8}{k_5} \]

\[ \kappa = \frac{k_2 k_{10}}{k_3 k_5} \]

\[ \mu = \frac{k_2}{k_5} \]

\[ \rho = \frac{k_6}{k_5} \]

\[ \epsilon = \frac{k_3}{k_5} \]

\[ \theta = \frac{k_{11}}{k_1} \]

\[ \phi = \frac{k_{10}}{k_{11}} \]

5.4.1 Simulations

The results for the final concentration value of TXB₂ for the nondimensionalized system was the same as those for the dimensionalized system for both the case without aspirin and the case with aspirin.

This can be seen in Figure 5.4 and Figure 5.5, where the boxes denote the initial concentrations. As in the dimensionalized system, running the simulation without aspirin resulted in no change in the concentration of TXB₂.
and running the simulation with aspirin resulted in a steady state TXB$_2$ concentration of $2.5 \times 10^{-7} \, M$.

### 5.4.2 Sensitivity Analysis

We also performed a sensitivity analysis on our nondimensionalized system, as shown in Figure 5.6, which showed that the nondimensional parameters with the largest effect on the final concentration of TXB$_2$ were $\lambda$ and $\frac{\lambda}{\nu}$. Considering that $\lambda$ is $\frac{k_8}{k_9}$ and that $\frac{\lambda}{\nu}$ is $\frac{k_8}{k_2}$, this seemed reasonable; in the dimensionalized system, $k_2$ had the largest impact on TXB$_2$ concentration and $k_8$ had a moderate impact. With the system nondimensionalized, the order of magnitude of the percent change in TXB$_2$ concentration is now 10, reflecting the order of magnitude of the new parameters.

The reaction governed by $k_2$ is the conversion of PGHS intermediate 1 ($E_2$) into COX PGHS ($E_3$), and the reaction governed by $k_8$ is the conver-
Figure 5.6 Sensitivity Analysis of Parameters of the Nondimensionalized System.

The conversion of PGHS intermediate 2 ($E_4$) into POX PGHS ($E_1$). Without COX PGHS and POX PGHS, the reactions would not be possible, so it seems perfectly reasonable that the rate constants that are the most sensitive correspond to reactions that regenerate these enzymes that are crucial to the reaction scheme. From this data, we concluded that the steady state concentration of TXB$_2$ is highly dependent on the rate of regeneration of both COX PGHS and POX PGHS, enzymes that work together to produce PGH$_2$, the precursor to TXB$_2$. 
Chapter 6

Conclusion

Through this project, we were able to accomplish our main goal and developed a mathematical model that described the effect of aspirin on blood clotting, along with running simulations based on experimental data and analyzing the stabilities of the fixed points of the system.

6.1 Research

The research for this project was conducted through online databases. The majority of this project was founded on research by Tien et al. (2005) and Wei et al. (1995), who both studied and analyzed the mechanism and behavior of PGHS. However, neither made strong ties to blood clotting, such as important factors like TXA$_2$, or to aspirin therapy. Additional data and information was required, such as the mechanism behind aspirin inhibition of PGHS and behind TXA$_2$, and TXB$_2$, production, experimental data for rate constants, and standard concentration levels for all enzymes, substrates, and products. All this information was synthesized into a cohesive model of the effect of aspirin on blood clotting.

6.2 Future Work

Improvements can be made to the research and work completed through this project, ranging from additional, necessary experimental data to further mathematical analyses.

We lacked sufficient scientific data to accurately match the shape of our concentration-time curve of TXB$_2$ with aspirin therapy to experimental data. We hypothesized that there were other factors that fed into TXB$_2$
that might have been overlooked and insignificant in the context of blood clotting, but would have been vital in modeling the change in TXB$_2$ concentration. Furthermore, if simulations of the reduced system were to reach an accurate steady state concentration of TXB$_2$, it would have important implications for mathematical analyses, as the reduced system provided us with a more detailed stability analysis and can be reduced further through a quasi-steady-state approximation. A further reduction would allow this model to easily integrate with other models of blood clotting, such as those examining the dynamics of blood flow and how it leads to coagulation.

6.3 Concluding Remarks

We developed a model that tracked the enzyme prostaglandin H synthase and its relation to blood clotting with aspirin therapy. We were able to reduce our system by two variables, determine the fixed points, discard some as unstable, and capture a stability analysis for two of the remaining fixed points of the reduced system. From this information, we determined the conditions under which the reactions of PGHS were self-sustaining for three different scenarios. When arachidonic acid concentration was fixed and suicide inactivation was absent, a stable fixed point indicated that the reactions would ultimately terminate, and an unstable fixed point indicated that the reactions were self-sustaining. When arachidonic acid concentration was allowed to vary and suicide inactivation was present, the termination of the reactions, or lack thereof, was dependent on the rate constants and the additional influx of arachidonic acid into the system.

After running simulations on our reduced model and not reaching the desired steady state TXB$_2$ concentration, we returned to our full model, where we set up the foundation for a stability analysis. This would tell us when the reactions carried out by PGHS were self-sustaining, by finding the fixed points of our system, discarding some as unstable fixed points, and determining the stability criteria for the remaining fixed points for general rate constant values. In addition, through simulations run on MATLAB, we reached an accurate steady state value for TXB$_2$ after ingestion of aspirin, which allows us to conclude that our model captures the basis of the relationship between PGHS, TXA$_2$, an essential blood clotting factor that was measured in the form of TXB$_2$, and aspirin.

The implications of this mathematical model are important for understanding how the degree and severity of blood clotting can be altered through aspirin therapy. Unwanted blood clots can cause many serious health prob-
lems, and a better understanding of how to prevent them with a common drug such as aspirin could be beneficial in treating the health complications that accompany them. The stability analyses provides information that could be useful for finding the ideal aspirin dosage required to prevent undesired blood clotting; terminating the reactions of PGHS in platelets is a preventative measure against unwanted blood clotting, and this system provides the model with which one could determine the amount of aspirin needed to do so. Platelet count, and therefore PGHS concentration, vary among individuals, as do all other measurements that were used in this project. Therefore, if the initial concentrations of these enzymes, substrates, and products, which could be found by taking a blood test, were known for a patient, one could use this model of aspirin’s effect on blood clotting to determine the aspirin dosage required by that specific patient.
Appendix A

The Routh Hurwitz Theorem

The Routh Hurwitz Theorem can be used to determine the stability of fixed points by determining if the roots of the corresponding characteristic polynomial that contains only real coefficients lie in the left half of the complex plane. If such a behavior is attained, then "any solution to the linear, homogeneous differential equation converges to zero" (Allen, 2006).

A.1 Routh Hurwitz Criteria

The Routh Hurwitz Criteria in its entirety, from Allen (2006), is as follows.

Theorem A.1 (Routh Hurwitz Criteria) Given the polynomial,

\[ P(\lambda) = \lambda^n + a_1\lambda^{n-1} + \cdots + a_{n-1}\lambda + a_n, \]

where the coefficients \( a_i \) are real constants, \( i = 1, \cdots, n \), define the \( n \) Hurwitz matrices using the coefficients \( a_i \) of the characteristic polynomial:

\[
H_1 = (a_1), \ H_2 = \begin{pmatrix} a_1 & 1 \\ a_3 & a_2 \end{pmatrix}, \ H_3 = \begin{pmatrix} a_1 & 1 & 0 \\ a_3 & a_2 & a_1 \\ a_5 & a_4 & a_3 \end{pmatrix},
\]

and

\[
H_n = \begin{pmatrix} a_1 & 1 & 0 & 0 & \cdots & 0 \\ a_3 & a_2 & a_1 & 1 & \cdots & 0 \\ a_5 & a_4 & a_3 & a_2 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & 0 & \cdots & a_n \end{pmatrix}.
\]
where \( a_j = 0 \text{ if } j > n \). All of the roots of the polynomial \( P(\lambda) \) are negative or have negative real part iff the determinants of all Hurwitz matrices are positive:

\[
det H_j > 0, \ j = 1, 2, \cdots, n.\]

\( \text{Allen (2006) also provided a summary of the Routh Hurwitz Criteria for } n = 2, 3, 4, \text{ and } 5: \)

\[
n = 2 : a_1 > 0 \text{ and } a_2 > 0
\]
\[
n = 3 : a_1 > 0, a_3 > 0, \text{ and } a_1a_2 > a_3
\]
\[
n = 4 : a_1 > 0, a_3 > 0, a_4 > 0, \text{ and } a_1a_2a_3 > a_3^2 + a_1^2a_4
\]
\[
n = 5 : a_i > 0, i = 1, 2, 3, 4, 5, a_1a_2a_3 > a_3^2 + a_1^2a_4,
\]
\[
\text{and } (a_1a_4 - a_5)(a_1a_2a_3 - a_3^2 - a_1^2a_4) > a_5(a_1a_2 - a_3)^2 + a_1a_5^2.
\]

### A.2 Routh Array

An alternate way of determining the stability of fixed points is looking at the Routh array. While we used the information provided by \( \text{Co (1999)} \) to construct our Routh arrays, we provide the reader with a general Routh array from \( \text{Purdue School of Engineering and Technology (2007)} \), for polynomial

\[
a_0s^n + a_1s^{n-1} + \cdots + a_{n-1}s + a_n = 0.
\]

\[
\text{Routh array } = \begin{bmatrix}
    a_0 & a_2 & a_4 & a_6 & \cdots \\
    a_1 & a_3 & a_5 & a_7 & \cdots \\
    b_1 & b_2 & b_3 & b_4 & \cdots \\
    c_1 & c_2 & c_3 & c_4 & \cdots \\
    d_1 & d_2 & d_3 & d_4 & \cdots \\
    e_1 & e_2 & & & \\
    f_1 & & & & \\
    g_0 & & & & 
\end{bmatrix}
\]

where

\[
b_1 = \frac{a_1a_2 - a_0a_3}{a_1}
\]
\[
b_2 = \frac{a_1a_4 - a_0a_5}{a_1}
\]
\[
b_3 = \frac{a_1a_6 - a_0a_7}{a_1}
\]
The fixed point in question is stable if all the entries in the first column of the Routh array are either all positive or all negative [Co, 1999].
Appendix B

Stability Analysis for the Reduced System

This section completes the stability analysis of the fixed points \((0, 0, 0, 0, 0, A)\), \((0, 0, 0, 0, H, A)\), \((0, E_0, 0, 0, 0, 0)\), and \((0, E_0, 0, 0, 0, A)\).

\[B.1\] Stability of \((0, 0, 0, 0, 0, A)\) and \((0, 0, 0, 0, H, A)\)

The characteristic polynomial of Equation \(C.4\) and Equation \(C.4\) is

\[ p(\lambda) = \lambda^7 + a_1\lambda^6 + a_2\lambda^5 + a_3\lambda^4 + a_4\lambda^3 \]  

(B.1)

where

\[ a_1 = k_2 + k_5 + k_7 + A*k_{10} + Eo*k_1 + S*k_3 \]

\[ a_2 = k_2*k_5 + k_2*k_7 + k_5*k_7 + A*k_{2}*k_{10} + A*k_{5}*k_{10} + Eo*k_{1}*k_{2} + Eo*k_{1}*k_{5} + Eo*k_{1}*k_{7} + S*k_{2}*k_{3} + A*Eo*k_{1}*k_{10} + Eo*S*k_{1}*k_{3} \]

\[ a_3 = k_2*k_5*k_7 + A*k_{2}*k_{5}*k_{10} + Eo*k_{1}*k_{2}*k_{5} + Eo*k_{1}*k_{2}*k_{7} + Eo*k_{1}*k_{5}*k_{7} + A*Eo*k_{1}*k_{2}*k_{10} + A*Eo*k_{1}*k_{5}*k_{10} + Eo*S*k_{1}*k_{2}*k_{3} \]

\[ a_4 = Eo*k_{1}*k_{2}*k_{5}*k_{7} + A*Eo*k_{1}*k_{2}*k_{5}*k_{10} - Eo*S*k_{1}*k_{2}*k_{3}*k_{5} . \]
The summary of the Routh Hurwitz Criteria gives the following requirements for stability of these two fixed points:

\[
\begin{align*}
a_1 &> 0 \\
a_3 &> 0 \\
a_4 &> 0 \\
a_1a_2a_3 &> a_3^2 + a_1^2a_4.
\end{align*}
\]

Because the rate constants, \(E_o\), and \(S\) are all positive values, we know that \(a_1\) and \(a_3\) are positive. When we expand \(a_1a_2a_3 > a_3^2 + a_1^2a_4\), not all the terms are positive, so the stability of \((0,0,0,0,0,0)\) and \((0,0,0,0,0,H)\) is defined by

\[
E_o*k1*k2*k5*k7 + A*Eo*k1*k2*k5*k10 - Eo*S*k1*k2*k3*k5 > 0
\]

and

\[
k1*k2*k3*k5*Eo*S(k3^2*S^2 - 2*k3*k5*S-k5^2) < A*k10*(A*k10*(k2 + k5)*(k2 + k5 + 3*k7) + Eo*k1*(Eo*k1*(8*k2*k5 + 3*k2*k7 + 2*k5^2 + 2*k2^2 + 2*k5^2 + Eo*k1*(k2 + k5))) + k2^3 + 8*k2^2*k5 + 3*k7*k2^2 + 8*k2*k5^2 + 12*k7*k2*k5 + k5^3 + 3*k7*k5^2) + A*k10*(Eo*k1*(k2^2 + 4*k2*k5 + k5^2 + Eo*k1*(k2 + k5)) + k2^2*k5^2) + A*k10*(Eo*k1*(k2^2 + 4*k2*k5 + 3*k7^2 + 4*k5^2*k7 + 3*k5*k7^2 + Eo*k1*(k2 + k5)) + k2^3 + 8*k2^2*k5 + 3*k7*k2^2 + 8*k2*k5^2 + 12*k7*k2*k5 + k5^3 + 3*k7*k5^2) + A*k10*(Eo*k1*(k2^2 + 4*k2*k5 + k5^2 + Eo*k1*(k2 + k5)) + k2^2*k5^2) + A*k10*(Eo*k1*(k2^2 + 4*k2*k5 + 3*k7^2 + 4*k5^2*k7 + 3*k5*k7^2 + Eo*k1*(k2 + k5)) + k2^3 + 8*k2^2*k5 + 3*k7*k2^2 + 8*k2*k5^2 + 12*k7*k2*k5 + k5^3 + 3*k7*k5^2) + A*k10*(Eo*k1*(k2^2 + 4*k2*k5 + k5^2 + Eo*k1*(k2 + k5)) + k2^2*k5^2) + A*k10*(Eo*k1*(k2^2 + 4*k2*k5 + 3*k7^2 + 4*k5^2*k7 + 3*k5*k7^2 + Eo*k1*(k2 + k5)) + k2^3 + 8*k2^2*k5 + 3*k7*k2^2 + 8*k2*k5^2 + 12*k7*k2*k5 + k5^3 + 3*k7*k5^2) + A*k10*(Eo*k1*(k2^2 + 4*k2*k5 + k5^2 + Eo*k1*(k2 + k5)) + k2^2*k5^2)
B.2 Stability of \((0, E_6, 0, 0, 0, 0, 0)\)

The characteristic polynomial of Equation \(C.7\) is

\[
p(\lambda) = \lambda^7 + a_1 \lambda^6 + a_2 \lambda^5 + a_3 \lambda^4 + a_4 \lambda^3 + a_5 \lambda^2 \tag{B.2}
\]

where

\[
a_1 = k_2 + k_5 + k_7 + E_6 k_{11} + E_0 k_1 + S k_3
\]

\[
a_2 = k_2 k_5 + k_2 k_7 + k_5 k_7 + E_6 k_2 k_{11} + E_6 k_5 k_{11} + E_6 k_7 k_{11} + E_0 k_1 k_2 + E_0 k_1 k_5 + E_0 k_1 k_7 + S k_2 k_3 + E_6 E_0 k_1 k_{11} + E_6 S k_3 k_{11} + E_0 S k_1 k_3
\]

\[
a_3 = k_2 k_5 k_7 + E_6 k_2 k_5 k_{11} + E_6 k_2 k_7 k_{11} + E_6 k_5 k_7 k_{11} + E_0 k_1 k_2 + E_0 k_1 k_2 k_7 + E_0 k_1 k_5 k_7 + E_6 E_0 k_1 k_2 k_{11} + E_6 E_0 k_1 k_5 k_{11} + E_6 E_0 S k_2 k_{11} + E_0 S k_1 k_2 k_3 + E_6 E_0 S k_1 k_3 k_{11}
\]

\[
a_4 = E_6 k_2 k_5 k_7 k_{11} + E_0 k_1 k_2 k_5 k_7 + E_6 E_0 k_1 k_2 k_5 k_{11} + E_6 E_0 k_1 k_2 k_7 k_{11} + E_6 E_0 k_1 k_5 k_7 k_{11} - E_0 S k_1 k_2 k_3 k_5 k_{11}
\]

\[
a_5 = E_6 E_0 k_1 k_2 k_5 k_7 k_{11} - E_6 E_0 S k_1 k_2 k_3 k_5 k_{11}.
\]
The summary of the Routh Hurwitz Criteria gives the following requirements for stability of these two fixed points:

\[
\begin{align*}
a_1 &> 0 \\
a_2 &> 0 \\
a_3 &> 0 \\
a_4 &> 0 \\
a_5 &> 0 \\
a_1 a_2 a_3 &> a_3^2 + a_1^2 a_4 \\
(a_1 a_4 - a_5) (a_1 a_2 a_3 - a_3^2 - a_1^2 a_4) &> a_5 (a_1 a_2 - a_3)^2 + a_1 a_5^2.
\end{align*}
\]

Because the rate constants, \(E_0\), and \(S\) are all positive values, we know that \(a_1\), \(a_2\), and \(a_3\) are positive. When we expand \(a_1 a_2 a_3 > a_3^2 + a_1^2 a_4\) and 
\((a_1 a_4 - a_5) (a_1 a_2 a_3 - a_3^2 - a_1^2 a_4) > a_5 (a_1 a_2 - a_3)^2 + a_1 a_5^2\), not all the terms are positive, so the stability of \((0, E_6, 0, 0, 0, 0, 0)\) is defined by

\[
\begin{align*}
E_6 E*k2*k5*k7*k11 + E*E*k1*k2*k5*k7 &+ E6*E*k1*k2*k5*k7 + \\
E6*E*k1*k2*k7*k11 + E6*E*k1*k5*k7*k11 - E*S*k1*k2*k3*k5 &+ \\
E6*E*S*k1*k2*k3*k11 > 0,
\end{align*}
\]

\[
\begin{align*}
E6*E*k1*k2*k5*k7*k11 - E6*E*S*k1*k2*k3*k5*k11 > 0,
\end{align*}
\]

\[
\begin{align*}
(k2 + k5 + k7 + E6*k11 + Eo*k1 + S*k3)*(k2^2 + k5 + k2*k7 + k5*k7 &+ \\
E6*k2*k11 + E6*k5*k11 + E6*k7*k11 + Eo*k1*k2 + Eo*k1*k5 + \\
Eo*k1*k7 + S*k2*k3 + E6*Eo*k1*k11 + E6*S*k3*k11 + Eo*S*k1*k3) &-(k2^2 + k5 + k7 + E6*k11 + \\
E6*Eo*k1*k2*k5*k7 + E6*Eo*k1*k2*k7 + Eo*k1*k5*k7 + E6*Eo*k1*k2*k11 + \\
E6*Eo*k1*k5*k11 + E6*Eo*k1*k7*k11 + E6*S*k2*k3*k11 + \\
Eo*S*k1*k2*k3 + E6*Eo*S*k1*k3*k11) - (k2 + k5 + k7 + E6*k11 + \\
E6*Eo*k1*k2*k5*k7 &+ Eo*k1 + S*k3)^2*(E6*k2*k5*k7*k11 + Eo*k1*k2*k5*k7 + \\
E6*Eo*k1*k2*k5*k11 + E6*Eo*k1*k2*k7*k11 + E6*Eo*k1*k5*k7 &+ \\
E6*Eo*k1*k2*k3*k5 + E6*Eo*S*k1*k2*k3*k11) - (k2^2 + k5 + k7 + \\
E6*k2*k5*k11 + E6*k2*k7*k11 + E6*k5*k7*k11 + Eo*k1*k2*k5 + \\
Eo*k1*k2*k7 + Eo*k1*k5*k7 + E6*Eo*k1*k2*k11 + E6*Eo*k1*k5*k11 + \\
E6*Eo*k1*k7*k11 + E6*S*k2*k3*k11 + Eo*S*k1*k2*k3 + \\
E6*Eo*S*k1*k3*k11)^2 > 0,
\end{align*}
\]

and
Stability of \((0, E_6, 0, 0, 0, 0, A)\)

The characteristic polynomial of Equation C.9 is
\[
p(\lambda) = \lambda^7 + a_1 \lambda^6 + a_2 \lambda^5 + a_3 \lambda^4 + a_4 \lambda^3 + a_5 \lambda^2 \quad (B.3)
\]
where
\[
a_1 = k_2 + k_5 + k_7 + A*k_{10} + E_6*k_{11} + Eo*k_1 + S*k_3
\]
\[
a_2 = k_2*k_5 + k_2*k_7 + k_5*k_7 + A*k_{210} + E_{k210} + E_k211 + E_{6*Eo}*k_1*k_2*k_10 + A*k_5*k_{10} + E_{6*k211} +
\]
Stability Analysis for the Reduced System

E₆*k₅*k₁₁ + E₆*k₇*k₁₁ + E₀*k₁*k₂ + E₀*k₁*k₅ + E₀*k₁*k₇ + S*k₂*k₃ + A*E₆*k₁₀*k₁₁ + A*E₀*k₁*k₁₀ + E₆*E₀*k₁*k₁₁ + E₆*S*k₃*k₁₁ + E₀*S*k₁*k₃

a₃ = k₂*k₅*k₇ + A*k₂*k₅*k₁₀ + E₆*k₂*k₅*k₁₁ + E₆*k₂*k₇*k₁₁ + E₆*k₅*k₇*k₁₁ + E₀*k₁*k₂*k₅ + E₀*k₁*k₂*k₇ + E₀*k₁*k₅*k₇ + A*E₆*k₂*k₁₀*k₁₁ + A*E₆*k₅*k₁₀*k₁₁ + A*E₀*k₁*k₂*k₁₀ + A*E₀*k₁*k₅*k₁₀ + E₆*E₀*k₁*k₂*k₁₁ + E₆*E₀*k₁*k₅*k₁₁ + E₆*S*k₂*k₃*k₁₁ + E₀*S*k₁*k₂*k₃ + A*E₆*E₀*k₁*k₁₀*k₁₁ + E₆*E₀*S*k₁*k₃*k₁₁

a₄ = E₆*k₂*k₅*k₇*k₁₁ + E₀*k₁*k₂*k₅*k₇ + A*E₆*k₂*k₅*k₁₀*k₁₁ + A*E₀*k₁*k₂*k₅*k₁₀ + E₆*E₀*k₁*k₂*k₅*k₁₁ + E₆*E₀*k₁*k₂*k₇*k₁₁ + E₆*E₀*k₁*k₅*k₇*k₁₁ - E₀*S*k₁*k₂*k₃*k₅ + A*E₆*E₀*k₁*k₂*k₁₀*k₁₁ + A*E₆*E₀*k₁*k₅*k₁₀*k₁₁ + E₆*E₀*S*k₁*k₂*k₃*k₁₁ + E₆*E₀*S*k₁*k₂*k₃*k₅*k₁₁

a₅ = E₆*E₀*k₁*k₂*k₅*k₇*k₁₁ + A*E₆*E₀*k₁*k₂*k₅*k₁₀*k₁₁ - E₆*E₀*S*k₁*k₂*k₃*k₅*k₁₁.

The summary of the Routh Hurwitz Criteria gives the following requirements for stability of these two fixed points:

\[ a_1 > 0 \]
\[ a_2 > 0 \]
\[ a_3 > 0 \]
\[ a_4 > 0 \]
\[ a_5 > 0 \]

\[ a_1 a_2 a_3 > a_3^2 + a_1^2 a_4 \]
\[ (a_1 a_4 - a_5) (a_1 a_2 a_3 - a_3^2 - a_1^2 a_4) > a_5 (a_1 a_2 - a_3)^2 + a_1 a_5^2. \]

Because the rate constants, \( E_0 \), and \( S \) are all positive values, we know that \( a_1, a_2, \) and \( a_3 \) are positive. When we expand \( a_1 a_2 a_3 > a_3^2 + a_1^2 a_4 \) and \( (a_1 a_4 - a_5) (a_1 a_2 a_3 - a_3^2 - a_1^2 a_4) > a_5 (a_1 a_2 - a_3)^2 + a_1 a_5^2 \), not all the terms are positive, so the stability of \((0, E_6, 0, 0, 0, 0, A)\) is defined by

E₆*k₂*k₅*k₇*k₁₁ + E₀*k₁*k₂*k₅*k₇ + A*E₆*k₂*k₅*k₁₀*k₁₁ + A*E₀*k₁*k₂*k₅*k₁₀ + E₆*E₀*k₁*k₂*k₇*k₁₁ + E₆*E₀*k₁*k₅*k₇*k₁₁ - E₀*S*k₁*k₂*k₃*k₅ + A*E₆*E₀*k₁*k₂*k₃*k₅ + A*E₆*E₀*k₁*k₅*k₁₀*k₁₁ + A*E₆*E₀*S*k₁*k₂*k₃*k₁₁ + E₆*E₀*S*k₁*k₂*k₃*k₅*k₁₁ > 0.
Stability of $(0, E_6, 0, 0, 0, 0, A)$

$$E_6*E_6*O_k^1*k_1*k_2*k_5*k_7*k_11 + A*E_6*E_6*O_k^1*k_2*k_5*k_10*k_11 - E_6*E_6*O_k^1*k_2*k_3*k_5*k_11 > 0,$$

$$((k_2 + k_5 + k_7 + E_6*k_11 + E_6*k_1 + S*k_3) * (k_2 + k_5 + k_2 + k_7 + k_5 + k_7 + E_6*k_2*k_11 + E_6*k_5*k_11 + E_6*k_7*k_11 + E_6*E_6*O_k^1*k_12 + E_6*E_6*O_k^1*k_5*k_11 + E_6*E_6*O_k^1*k_7*k_11 + E_6*E_6*O_k^1*k_2*k_5 + E_6*E_6*O_k^1*k_2*k_7 + E_6*E_6*O_k^1*k_5*k_7 + E_6*E_6*O_k^1*k_7*k_11 + E_6*E_6*O_k^1*k_2*k_3 + E_6*E_6*O_k^1*k_3*k_11) - (k_2 + k_5 + k_7 + E_6*k_11 + E_6*E_6*O_k^1*k_3*k_11)^2 > 0,$$

and

$$- (E_6*E_6*O_k^1*k_2*k_5*k_7*k_11 - E_6*E_6*O_k^1*k_1*k_2*k_3*k_5*k_11)*(k_2 + k_5 + k_7 + E_6*k_11 + E_6*E_6*O_k^1*k_1 + S*k_3) * (k_2 + k_5 + k_2 + k_7 + k_5 + k_7 + E_6*k_2 + E_6*k_5 + E_6*k_7 + E_6*E_6*O_k^1*k_12 + E_6*E_6*O_k^1*k_2*k_5 + E_6*E_6*O_k^1*k_2*k_7 + E_6*E_6*O_k^1*k_5*k_7 + E_6*E_6*O_k^1*k_7*k_11 + E_6*E_6*O_k^1*k_2*k_3 + E_6*E_6*O_k^1*k_3*k_11) - (k_2 + k_5 + k_7 + E_6*k_2*k_5 + E_6*k_2*k_7 + E_6*k_5*k_7 + E_6*E_6*O_k^1*k_2*k_3 + E_6*E_6*O_k^1*k_3*k_11)^2 > 0.$$
Stability Analysis for the Reduced System

\[ + E_0 k_1 k_2 + E_0 k_1 k_5 + E_0 k_1 k_7 + S k_2 k_3 + E_6 E_0 k_1 k_11 + E_6 S k_3 k_11 + E_0 S k_1 k_3) (k_2 k_5 k_7 + E_6 k_2 k_5 k_11 + E_6 k_2 k_7 k_11 + E_6 k_5 k_7 k_11 + E_0 k_1 k_2 k_5 + E_0 k_1 k_2 k_7 + E_0 k_1 k_5 k_7 + E_6 E_0 k_1 k_2 k_11 + E_6 E_0 k_1 k_5 k_11 + E_6 E_0 k_1 k_7 k_11 + E_6 S k_2 k_3 k_11 + E_0 S k_1 k_2 k_3 + E_6 E_0 S k_1 k_3 k_11) > 0. \]
Appendix C

Stability Analysis for the Full System

This section provides the reader with in depth criteria for the stability of the fixed points of the full system.

C.1 Stability of \((0,0,0,0,0,0,0,0,0)\) and \((0,0,0,0,0,0,0,H,0)\)

The Jacobian matrix at \((0,0,0,0,0,0,0,0,0)\) is

\[
J_0 = \begin{bmatrix}
-k_3 S - k_7 & 0 & k_2 & 0 & 0 & k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -k_2 - k_6 & 0 & 0 & 0 & k_1 E_o & 0 & 0 \\
k_7 & 0 & k_6 & -k_8 & 0 & 0 & 0 & 0 & 0 \\
k_3 S & 0 & 0 & 0 & -k_4 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & k_4 & -k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & k_5 & -k_1 E_o & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & k_1 E_o & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0
\end{bmatrix},
\]

(C.1)
Stability Analysis for the Full System

\[
J_H = \begin{bmatrix}
-k_3S - k_7 & 0 & k_2 & 0 & 0 & k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -k_2 - k_6 & 0 & 0 & 0 & k_1 E_o & 0 & 0 \\
k_7 & 0 & k_6 & -k_8 & 0 & 0 & 0 & 0 & 0 \\
k_3S & 0 & 0 & 0 & -k_4 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & k_4 & -k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & k_5 & -k_1 E_o & 0 & 0 \\
0 & -k_{11}H & 0 & 0 & 0 & 0 & k_1 E_o & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{bmatrix}
\] (C.2)

These two fixed points share a characteristic polynomial, which is

\[p(\lambda) = \lambda^9 + a_1 \lambda^8 + a_2 \lambda^7 + a_3 \lambda^6 + a_4 \lambda^5 + a_5 \lambda^4 + a_6 \lambda^3\] (C.3)

where

\[a_1 = k_2 + k_4 + k_5 + k_6 + k_7 + k_8 + E_o k_1 + S k_3\]

\[a_2 = k_2 k_4 + k_2 k_5 + k_2 k_7 + k_4 k_5 + k_2 k_8 + k_4 k_6 + k_4 k_7 + k_5 k_6 + k_4 k_8 + k_5 k_7 + k_6 k_7 + k_6 k_8 + k_7 k_8 + E_o k_1 k_2 + E_o k_1 k_4 + E_o k_1 k_5 + E_o k_1 k_6 + E_o k_1 k_7 + E_o k_1 k_8 + S k_2 k_3 + S k_3 k_4 + S k_3 k_5 + S k_3 k_6 + S k_3 k_8 + E_o S k_1 k_3\]

\[a_3 = k_2 k_4 k_5 + k_2 k_4 k_7 + k_2 k_4 k_8 + k_2 k_5 k_7 + k_2 k_5 k_8 + k_4 k_5 k_6 + k_4 k_5 k_7 + k_2 k_7 k_8 + k_4 k_5 k_8 + k_4 k_6 k_7 + k_4 k_6 k_8 + k_5 k_6 k_7 + k_4 k_7 k_8 + k_5 k_6 k_8 + k_5 k_7 k_8 + k_6 k_7 k_8 + E_o k_1 k_2 k_4 + E_o k_1 k_2 k_5 + E_o k_1 k_2 k_7 + E_o k_1 k_2 k_8 + E_o k_1 k_4 k_5 + E_o k_1 k_2 k_8 + E_o k_1 k_4 k_6 + E_o k_1 k_4 k_7 + E_o k_1 k_5 k_6 + E_o k_1 k_4 k_8 + E_o k_1 k_5 k_7 + E_o k_1 k_5 k_8 + E_o k_1 k_6 k_7 + E_o k_1 k_6 k_8 + E_o k_1 k_7 k_8 + S k_2 k_3 k_4 + S k_2 k_3 k_5 + S k_2 k_3 k_6 + S k_2 k_3 k_8 + S k_3 k_4 k_5 + S k_3 k_4 k_6 + S k_3 k_4 k_8 + S k_3 k_5 k_6 + S k_3 k_5 k_8 + S k_3 k_6 k_7 + S k_3 k_6 k_8 + S k_3 k_7 k_8 + S k_3 k_7 k_8 + E_o S k_1 k_2 k_3 + E_o S k_1 k_3 k_4 + E_o S k_1 k_3 k_5 + E_o S k_1 k_3 k_6 + E_o S k_1 k_3 k_8\]

\[a_4 = k_2 k_4 k_5 k_7 + k_2 k_4 k_5 k_8 + k_2 k_4 k_7 k_8 + k_2 k_4 k_7 k_8 + k_4 k_5 k_6 k_7 + k_4 k_5 k_6 k_8 + k_4 k_5 k_7 k_8 + k_4 k_6 k_7 k_8 + k_5 k_6 k_7 k_8 + E_o k_1 k_2 k_4 k_5 + E_o k_1 k_2 k_4 k_7 + E_o k_1 k_2 k_4 k_8 + E_o k_1 k_2 k_5 k_7 + E_o k_1 k_2 k_5 k_8 + E_o k_1 k_4 k_5 k_6 +\]
Stability of \((0, 0, 0, 0, 0, 0, A)\) and \((0, 0, 0, 0, 0, 0, H, A)\)

\[
\begin{align*}
E_o^*k_1*k_4*k_5*k_7 + E_o^*k_1*k_2*k_7*k_8 + E_o^*k_1*k_4*k_5*k_8 & + \\
E_o^*k_1*k_4*k_6*k_7 + E_o^*k_1*k_4*k_6*k_8 + E_o^*k_1*k_5*k_6*k_7 & + \\
E_o^*k_1*k_4*k_7*k_8 + E_o^*k_1*k_5*k_6*k_8 + E_o^*k_1*k_5*k_7*k_8 & + \\
E_o^*k_1*k_6*k_7*k_8 + S*k_2*k_3*k_4*k_8 + S*k_2*k_3*k_5*k_8 & + S*k_3*k_4*k_6*k_8 + \\
S*k_3*k_5*k_6*k_8 + E_o*S*k_1*k_2*k_3*k_4 & + E_o*S*k_1*k_2*k_3*k_5 & + & \\
E_o*S*k_1*k_2*k_3*k_4*k_6 + E_o*S*k_1*k_3*k_5*k_6 & + \\
E_o*S*k_1*k_3*k_4*k_8 + E_o*S*k_1*k_3*k_5*k_8 + E_o*S*k_1*k_3*k_6*k_8 & + \\
a_5 = k_2*k_4*k_5*k_7*k_8 + k_4*k_5*k_6*k_7*k_8 & + E_o*k_1*k_2*k_4*k_5*k_7 & + \\
E_o*k_1*k_2*k_4*k_5*k_8 + E_o*k_1*k_2*k_4*k_7*k_8 & + E_o*k_1*k_2*k_5*k_7*k_8 & + \\
E_o*k_1*k_4*k_5*k_6*k_7 + E_o*k_1*k_4*k_5*k_6*k_8 & + E_o*k_1*k_4*k_5*k_7*k_8 & + \\
E_o*k_1*k_4*k_6*k_7*k_8 & + E_o*k_1*k_5*k_6*k_7*k_8 & + E_o*S*k_1*k_2*k_3*k_4*k_5 & + \\
E_o*S*k_1*k_2*k_3*k_4*k_8 & + E_o*S*k_1*k_2*k_3*k_5*k_8 & + \\
E_o*S*k_1*k_3*k_4*k_6*k_8 & + E_o*S*k_1*k_3*k_5*k_6*k_8 & + \\
a_6 = E_o*k_1*k_2*k_4*k_5*k_7*k_8 & + E_o*k_1*k_4*k_5*k_6*k_7*k_8 & - \\
E_o*S*k_1*k_2*k_3*k_4*k_5 & + \\
\end{align*}
\]

C.2 Stability of \((0, 0, 0, 0, 0, 0, A)\) and \((0, 0, 0, 0, 0, 0, H, A)\)

The stabilities of these fixed points are functions of \(A\). The Jacobian matrix at \((0, 0, 0, 0, 0, 0, A)\) is

\[
J_A = \begin{bmatrix}
-k_3S - k_7 - k_{10}A & 0 & k_2 & 0 & 0 & k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & -k_2 - k_6 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\
k_7 & 0 & k_6 & -k_8 & 0 & 0 & 0 & 0 & 0 \\
k_3S & 0 & 0 & 0 & -k_4 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & k_4 & -k_5 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\
-k_{10}A & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\end{bmatrix},
\]

and the Jacobian matrix at \((0, 0, 0, 0, 0, 0, H, A)\) is
These two fixed points share a characteristic polynomial, which is

$$p(\lambda) = \lambda^9 + a_1 \lambda^8 + a_2 \lambda^7 + a_3 \lambda^6 + a_4 \lambda^5 + a_5 \lambda^4 + a_6 \lambda^3$$  \hspace{1cm} (C.6)
The stability of this fixed point is a function of $E_6$. The Jacobian matrix at $(0, E_6, 0, 0, 0, 0, 0, 0, 0)$ is
The characteristic polynomial for this fixed point is

\[ p(\lambda) = \lambda^9 + a_1 \lambda^8 + a_2 \lambda^7 + a_3 \lambda^6 + a_4 \lambda^5 + a_5 \lambda^4 + a_6 \lambda^3 + a_7 \lambda^2 \]  

where

\[ a_1 = k_2 + k_4 + k_5 + k_6 + k_7 + k_8 + E_6 k_11 + E_0 k_1 + S k_3 \]

\[ a_2 = k_2 k_4 + k_2 k_5 + k_2 k_7 + k_4 k_5 + k_2 k_8 + k_4 k_6 + k_4 k_7 + k_5 k_6 + k_4 k_8 + k_5 k_7 + k_6 k_7 + k_6 k_8 + k_7 k_8 + E_6 k_2 k_11 + E_6 k_4 k_11 + E_6 k_5 k_11 + E_6 k_6 k_11 + E_6 k_7 k_11 + E_6 k_8 k_11 + E_6 k_1 k_2 + E_0 k_1 k_4 + E_0 k_1 k_5 + E_0 k_1 k_6 + E_0 k_1 k_7 + E_0 k_1 k_8 + E_0 k_1 k_9 + S k_2 k_3 + S k_3 k_4 + S k_3 k_5 + S k_3 k_6 + S k_3 k_8 + E_6 E_0 k_1 k_11 + E_6 S k_3 k_11 + E_0 S k_1 k_3 \]

\[ a_3 = k_2 k_4 k_5 + k_2 k_4 k_7 + k_2 k_4 k_8 + k_2 k_5 k_7 + k_2 k_5 k_8 + k_4 k_5 k_6 + k_4 k_5 k_7 + k_2 k_7 k_8 + k_4 k_5 k_8 + k_4 k_6 k_7 + k_4 k_6 k_8 + k_5 k_6 k_7 + k_5 k_6 k_8 + k_5 k_7 k_8 + k_6 k_7 k_8 + E_6 k_2 k_4 k_11 + E_6 k_2 k_5 k_11 + E_6 k_2 k_7 k_11 + E_6 k_4 k_5 k_11 + E_6 k_4 k_8 k_11 + E_6 k_4 k_6 k_11 + E_6 k_4 k_7 k_11 + E_6 k_5 k_6 k_11 + E_6 k_5 k_8 k_11 + E_6 k_5 k_7 k_11 + E_6 k_6 k_7 k_11 + E_6 k_6 k_8 k_11 + E_6 k_7 k_8 k_11 + E_0 k_1 k_2 k_4 + E_0 k_1 k_2 k_5 + E_0 k_1 k_2 k_7 + E_0 k_1 k_4 k_5 + E_0 k_1 k_4 k_8 + E_0 k_1 k_5 k_7 + E_0 k_1 k_5 k_8 + E_0 k_1 k_6 k_7 + E_0 k_1 k_6 k_8 + E_0 k_1 k_7 k_8 + S k_2 k_3 k_4 + S k_2 k_3 k_5 + S k_2 k_3 k_8 + S k_3 k_4 k_6 + S k_3 k_5 k_6 + S k_3 k_4 k_8 + S k_3 k_5 k_8 + S k_3 k_6 k_8 + E_6 E_0 k_1 k_2 k_11 + E_6 E_0 k_1 k_4 k_11 + E_6 E_0 k_1 k_5 k_11 + E_6 E_0 k_1 k_6 k_11 + E_6 E_0 k_1 k_7 k_11 + \]
Stability of \((0, E_0, 0, 0, 0, 0, 0, 0)\)
\[ a_6 = E_6 k_2 k_4 k_5 k_7 k_8 k_{11} + E_6 k_4 k_5 k_6 k_7 k_8 k_{11} + \]
\[ E_0 k_1 k_2 k_5 k_7 k_8 + E_0 k_1 k_4 k_5 k_6 k_7 k_8 + \]
\[ E_6 E_0 k_1 k_2 k_4 k_5 k_7 k_{11} + E_6 E_0 k_1 k_2 k_4 k_5 k_8 k_{11} + \]
\[ E_6 E_0 k_1 k_2 k_4 k_7 k_8 k_{11} + E_6 E_0 k_1 k_2 k_5 k_7 k_8 k_{11} + \]
\[ E_6 E_0 k_1 k_4 k_5 k_6 k_7 k_{11} + E_6 E_0 k_1 k_4 k_5 k_8 k_{11} + \]
\[ a_7 = E_6 E_0 k_1 k_2 k_4 k_5 k_7 k_8 k_{11} + \]
\[ E_6 E_0 k_1 k_4 k_5 k_6 k_7 k_8 k_{11} - E_6 E_0 S k_1 k_2 k_3 k_4 k_5 k_8 k_{11} \]

C.4 Stability of \((0, E_6, 0, 0, 0, 0, 0, 0, A)\)

The stability of this fixed point is a function of \(E_6\) and \(A\). The Jacobian matrix at \((0, E_6, 0, 0, 0, 0, 0, 0, A)\) is
Stability of $(0, E_6, 0, 0, 0, 0, 0, A)$

The characteristic polynomial for this fixed point is

$$p(\lambda) = \lambda^9 + a_1 \lambda^8 + a_2 \lambda^7 + a_3 \lambda^6 + a_4 \lambda^5 + a_5 \lambda^4 + a_6 \lambda^3 + a_7 \lambda^2$$  \hspace{1cm} (C.10)
Stability Analysis for the Full System

\[
\begin{align*}
E_0k_1k_4k_8 & + E_0k_1k_5k_7 + E_0k_1k_5k_8 + E_0k_1k_6k_7 + \\
E_0k_1k_6k_8 & + E_0k_1k_7k_8 + S_k_2k_3k_4 + S_k_2k_3k_5 + \\
S_k_2k_3k_8 & + S_k_3k_4k_6 + S_k_3k_5k_6 + S_k_3k_4k_8 + S_k_3k_5k_8 + \\
S_k_3k_5k_6 & + A*e_6k_2k_10k_11 + A*e_6k_4k_10k_11 + \\
A*e_6k_5k_10k_11 & + A*e_6k_6k_10k_11 + A*e_6k_8k_10k_11 + \\
A*e_6k_1k_2k_10 & + A*e_6k_1k_4k_10 + A*e_6k_1k_5k_10 + \\
A*e_6k_1k_6k_10 & + A*e_6k_1k_8k_10 + E_6E_0k_1k_2k_11 + \\
E_6E_0k_1k_4k_11 & + E_6E_0k_1k_5k_11 + E_6E_0k_1k_6k_11 + \\
E_6E_0k_1k_7k_11 & + E_6E_0k_1k_8k_11 + E_6S_k_2k_3k_11 + \\
E_6S_k_3k_4k_11 & + E_6S_k_3k_5k_11 + E_6S_k_3k_6k_11 + \\
E_6S_k_3k_8k_11 & + E_6S_k_4k_1k_2k_3 + E_6S_k_1k_3k_4 + \\
E_6S_k_1k_3k_5 & + E_6S_k_1k_3k_6 + E_6S_k_1k_3k_8 + \\
A*e_6E_0k_1k_10k_11 & + E_6E_0S_k_1k_3k_11.
\end{align*}
\]

\[
a_4 = k_2k_4k_5k_7 + k_2k_4k_5k_8 + k_2k_4k_7k_8 + k_2k_5k_7k_8 +
\]

\[
k_4k_5k_6k_7 + k_4k_5k_6k_8 + k_4k_5k_7k_8 + k_4k_5k_7k_8 +
\]

\[
k_5k_6k_7k_8 + A_k_2k_4k_5k_10 + A_k_2k_4k_8k_10 + A_k_2k_5k_8k_10 +
\]

\[
A_k_4k_5k_6k_10 + A_k_4k_5k_8k_10 + A_k_4k_6k_8k_10 +
\]

\[
A_k_5k_6k_8k_10 + E_6E_0k_2k_4k_5k_11 + E_6E_0k_2k_4k_7k_11 +
\]

\[
E_6E_0k_2k_4k_8k_11 + E_6E_0k_2k_5k_7k_11 + E_6E_0k_2k_5k_8k_11 +
\]

\[
E_6E_0k_5k_6k_7k_11 + E_6E_0k_5k_6k_8k_11 + E_6E_0k_5k_6k_8k_11 +
\]

\[
E_6E_0k_5k_7k_8k_11 + E_6E_0k_5k_7k_8k_11 + E_6E_0k_5k_7k_8k_11 +
\]

\[
E_6E_0k_1k_2k_4k_5k_11 + E_6E_0k_1k_2k_4k_5k_7 +
\]

\[
E_6E_0k_1k_2k_4k_5k_8 + E_6E_0k_1k_2k_4k_5k_8 +
\]

\[
S_k_2k_3k_4k_5k_8 + S_k_2k_3k_5k_6k_8 + S_k_3k_4k_6k_8 + S_k_3k_5k_6k_8 +
\]

\[
A*e_6k_2k_4k_10k_11 + A*e_6k_2k_5k_10k_11 + A*e_6k_4k_5k_10k_11 +
\]

\[
A*e_6k_4k_5k_10k_11 + A*e_6k_4k_6k_10k_11 + A*e_6k_5k_6k_10k_11 +
\]

\[
A*e_6k_4k_6k_10k_11 + A*e_6k_4k_8k_10k_11 + A*e_6k_6k_8k_10k_11 +
\]

\[
A*e_6k_1k_2k_4k_10 + A*e_6k_1k_2k_5k_10 + A*e_6k_1k_4k_5k_10 +
\]

\[
A*e_6k_1k_2k_8k_10 + A*e_6k_1k_4k_6k_10 + A*e_6k_1k_5k_6k_10 +
\]

\[
A*e_6k_1k_4k_8k_10 + A*e_6k_1k_5k_8k_10 + A*e_6k_1k_6k_8k_10 +
\]

\[
E_6E_0k_1k_2k_4k_11 + E_6E_0k_1k_2k_5k_11 + E_6E_0k_1k_2k_7k_11 +
\]

\[
E_6E_0k_1k_4k_5k_11 + E_6E_0k_1k_4k_5k_11 + E_6E_0k_1k_4k_6k_11 +
\]

\[
E_6E_0k_1k_4k_6k_11 + E_6E_0k_1k_4k_8k_11 +
\]

\[
E_6E_0k_1k_5k_7k_11 + E_6E_0k_1k_5k_8k_11 + E_6E_0k_1k_5k_7k_11 +
\]

\[
E_6E_0k_1k_5k_8k_11 + E_6E_0k_1k_5k_7k_11 + E_6E_0k_1k_5k_7k_11 +
\]

\[
E_6E_0k_1k_5k_6k_8 + E_6E_0k_1k_5k_7k_8 + E_6E_0k_1k_5k_7k_8 +
\]

\[
S_k_2k_3k_4k_8 + S_k_2k_3k_5k_8 + S_k_3k_4k_6k_8 + S_k_3k_5k_6k_8 +
\]
Stability of $\left(0, E_6, 0, 0, 0, 0, 0, 0, A\right)$

\[ a_5 = k_2k_4k_5k_7k_8 + k_4k_5k_6k_7k_8 + A k_2k_4k_5k_8k_{10} + A k_4k_5k_6k_8k_{10} + E_6k_2k_4k_5k_7k_{11} + E_6k_2k_4k_5k_8k_{11} + E_6k_2k_4k_7k_8k_{11} + E_6k_2k_5k_6k_7k_{11} + E_6k_4k_5k_6k_7k_{11} + E_6k_4k_5k_7k_8k_{11} + E_6k_4k_6k_7k_8k_{11} + E_6k_5k_6k_7k_8k_{11} + E_6k_5k_6k_8k_{11} + E_6k_5k_7k_8k_{11} + E_6k_5k_8k_{11} + E_6k_6k_7k_8k_{11} + E_6k_6k_8k_{11} + E_6k_7k_8k_{11} + E_6k_8k_{11} + E_6k_9k_{11} + E_6k_{10}k_{11} + E_6k_{11}k_{11} + E_6k_{12}k_{11}; \]

\[ a_6 = E_6k_2k_4k_5k_6k_7k_8 + E_6k_2k_4k_5k_7k_8 + E_6k_2k_4k_6k_7k_8 + E_6k_2k_5k_6k_7k_8 + E_6k_3k_4k_6k_7k_8 + E_6k_3k_5k_6k_7k_8 + E_6k_4k_5k_6k_7k_8 + E_6k_5k_6k_7k_8 + E_6k_6k_7k_8 + E_6k_7k_8; \]

\[ a_7 = E_6k_2k_4k_5k_6k_7k_8 + E_6k_2k_4k_5k_7k_8 + E_6k_2k_4k_6k_7k_8 + E_6k_2k_5k_6k_7k_8 + E_6k_3k_4k_6k_7k_8 + E_6k_3k_5k_6k_7k_8 + E_6k_4k_5k_6k_7k_8 + E_6k_5k_6k_7k_8 + E_6k_6k_7k_8 + E_6k_7k_8; \]

\[ a_8 = E_6k_2k_4k_5k_6k_7k_8 + E_6k_2k_4k_5k_7k_8 + E_6k_2k_4k_6k_7k_8 + E_6k_2k_5k_6k_7k_8 + E_6k_3k_4k_6k_7k_8 + E_6k_3k_5k_6k_7k_8 + E_6k_4k_5k_6k_7k_8 + E_6k_5k_6k_7k_8 + E_6k_6k_7k_8 + E_6k_7k_8; \]

\[ a_9 = E_6k_2k_4k_5k_6k_7k_8 + E_6k_2k_4k_5k_7k_8 + E_6k_2k_4k_6k_7k_8 + E_6k_2k_5k_6k_7k_8 + E_6k_3k_4k_6k_7k_8 + E_6k_3k_5k_6k_7k_8 + E_6k_4k_5k_6k_7k_8 + E_6k_5k_6k_7k_8 + E_6k_6k_7k_8 + E_6k_7k_8; \]

\[ a_{10} = E_6k_2k_4k_5k_6k_7k_8 + E_6k_2k_4k_5k_7k_8 + E_6k_2k_4k_6k_7k_8 + E_6k_2k_5k_6k_7k_8 + E_6k_3k_4k_6k_7k_8 + E_6k_3k_5k_6k_7k_8 + E_6k_4k_5k_6k_7k_8 + E_6k_5k_6k_7k_8 + E_6k_6k_7k_8 + E_6k_7k_8; \]
Stability Analysis for the Full System

A*E6*E0*k1*k4*k5*k10*k11 + A*E6*E0*k1*k2*k8*k10*k11 +
A*E6*E0*k1*k4*k6*k10*k11 + A*E6*E0*k1*k5*k6*k10*k11 +
A*E6*E0*k1*k4*k8*k10*k11 + A*E6*E0*k1*k5*k8*k10*k11 +
A*E6*E0*k1*k6*k8*k10*k11 + E6*Eo*S*k1*k2*k3*k4*k11 +
E6*Eo*S*k1*k3*k5*k6*k11 + E6*Eo*S*k1*k3*k5*k8*k11 +
E6*Eo*S*k1*k3*k6*k8*k11 + E6*Eo*S*k1*k3*k5*k8*k11 +
E6*Eo*S*k1*k3*k6*k8*k11

\[ a_6 = E6*k2*k4*k5*k7*k8*k11 + E6*k4*k5*k6*k7*k8*k11 + \]
\[ Eo*k1*k2*k4*k5*k7*k8 + Eo*k1*k4*k5*k6*k7*k8 + \]
A*E6*k2*k4*k5*k8*k10*k11 + A*E6*k4*k5*k6*k8*k10*k11 +
A*Eo*k1*k2*k4*k5*k8*k10 + A*Eo*k1*k4*k5*k6*k8*k10 +
E6*Eo*k1*k2*k4*k5*k7*k11 + E6*Eo*k1*k2*k4*k5*k8*k11 +
E6*Eo*k1*k2*k4*k7*k8*k11 + E6*Eo*k1*k2*k5*k7*k8*k11 +
E6*Eo*k1*k4*k5*k6*k7*k11 + E6*Eo*k1*k4*k5*k6*k8*k11 +
E6*Eo*k1*k4*k5*k7*k8*k11 + E6*Eo*k1*k4*k6*k7*k8*k11 +
E6*Eo*k1*k5*k6*k7*k8*k11 - Eo*S*k1*k2*k3*k4*k5*k8 +
A*E6*Eo*k1*k2*k4*k5*k10*k11 + A*E6*Eo*k1*k2*k4*k8*k10*k11 +
A*E6*Eo*k1*k2*k5*k8*k10*k11 + A*E6*Eo*k1*k4*k5*k6*k10*k11 +
A*E6*Eo*k1*k3*k5*k8*k10*k11 + A*E6*Eo*k1*k4*k6*k8*k10*k11 +
A*E6*Eo*k1*k5*k6*k8*k10*k11 - E6*Eo*S*k1*k2*k3*k4*k5*k11 +
E6*Eo*S*k1*k2*k3*k4*k8*k11 + E6*Eo*S*k1*k2*k3*k5*k8*k11 +
E6*Eo*S*k1*k3*k4*k6*k8*k11 + E6*Eo*S*k1*k3*k5*k6*k8*k11

\[ a_7 = E6*Eo*k1*k2*k4*k5*k7*k8*k11 + E6*Eo*k1*k4*k5*k6*k7*k8*k11 + \]
\[ A*E6*Eo*k1*k2*k4*k5*k8*k10*k11 + A*E6*Eo*k1*k2*k4*k5*k8*k10*k11 + \]
A*E6*Eo*k1*k2*k4*k5*k8*k10*k11

C.5 Applying the Routh-Hurwitz Criterion

The fixed points \((0, 0, 0, 0, 0, 0, 0, 0, 0, H, 0)\), \((0, 0, 0, 0, 0, 0, 0, 0, 0, H, A)\), and \((0, 0, 0, 0, 0, 0, 0, 0, 0, H, A)\) result in a Routh array for \(n = 6\).
Applying the Routh-Hurwitz Criterion

The Routh array for the above fixed points is

\[ J = \begin{bmatrix}
  a_6 & a_4 & a_2 & 0 \\
  a_5 & a_3 & a_1 & 0 \\
  A & B & 0 & 0 \\
  C & D & 0 & 0 \\
  E & 0 & 0 & 0 \\
  D & 0 & 0 & 0 \\
  D & 0 & 0 & 0 \\
\end{bmatrix} \]  \hspace{1cm} (C.11)

where

\[ A = \frac{a_5 a_4 - a_3 a_6}{a_5} \]
\[ B = \frac{a_3 a_2 - a_1 a_4}{a_5} \]
\[ C = a_3 - \frac{a_5 (a_3 a_2 - a_1 a_4)}{a_5 a^4 - a_3 a^6} \]
\[ D = \frac{a_1 (a_3 a_2 - a_1 a_4)}{a_5 a^4 - a_3 a^6} \]
\[ E = \frac{a_3 (a_1 a_4 - a_2 a_3) (a_1 a_6 - a_2 a_5 - a_3 a_6 + a_4 a_5)}{a_3 a^2 a_6 - a_1 a_4 a_5 + a_2 a_3 a_5 - a_3 a_4 a_5} \]

and we want \( a_6, a_5, A, C, E, \) and \( D \) to all be either positive or negative.

The fixed points \((0, E_6, 0, 0, 0, 0, 0, 0, 0)\) and \((0, E_6, 0, 0, 0, 0, 0, 0, A)\) result in a Routh array for \( n = 7 \):

\[ J = \begin{bmatrix}
  a_7 & a_5 & a_3 & a_1 & 0 \\
  a_6 & a_4 & a_2 & 0 & 0 \\
  A & B & C & 0 & 0 \\
  D & E & 0 & 0 & 0 \\
  F & G & 0 & 0 & 0 \\
  H & 0 & 0 & 0 & 0 \\
  G & 0 & 0 & 0 & 0 \\
  G & 0 & 0 & 0 & 0 \\
\end{bmatrix} \]  \hspace{1cm} (C.12)
Stability Analysis for the Full System

where

\[
A = \frac{a_6a_5 - a_4a_7}{a_6}
\]

\[
B = \frac{a_4a_3 - a_2a_5}{a_6}
\]

\[
C = \frac{a_1a_2}{a_6}
\]

\[
D = a_4 - \frac{a_6(a_3a_4 - a_2a_5)}{a_5a_6 - a_4a_7}
\]

\[
E = \frac{a_2(a_1a_4 + a_2a_5 - a_3a_4)}{a_4a_7 - a_5a_6}
\]

\[
F = \frac{a_4(a_2(a_7(a_2a_5 - a_3a_4 - a_4a_5 + a_1a_4) - a_5a_6(a_1 + a_3 - a_5)) + a_3a_4(a_3a_6 + a_4a_7 - a_5a_6))}{a_6(a_4a_7 + a_3a_6) - a_5a_6(a_2 + a_4)}
\]

\[
G = \frac{-a_1a_2^2(a_1a_4 + a_2a_5 - a_3a_4)}{(a_4a_7 - a_5a_6)^2}
\]

\[
H = \frac{EF - DG}{F}
\]

and we want \(a_7, a_6, A, D, F, H\), and \(G\) to all be either positive or negative.


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