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A Mathematical Model of the Effect of Aspirin on Blood Clotting

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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_2, in the form of thromboxane B_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 the model accurately captures the steady-state chemical concentrations of interest in blood, both with and without aspirin treatment.

Acknowledgments

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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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Chapter 1

Introduction

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 G_2, or PGG_2 to prostaglandin H_2, or PGH_2. In the COX reaction, PGHS catalyzes the conversion of arachidonic acid to PGG_2. The product of the POX reaction, PGH_2 is then converted into TXA_2, 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 TXA_2 production by acting as a competitive inhibitor and irreversibly binding to the COX site of PGHS (Goltsov et al., 2010). Thus, production of PGG_2, and consequently PGH_2 and TXA_2, is inhibited.

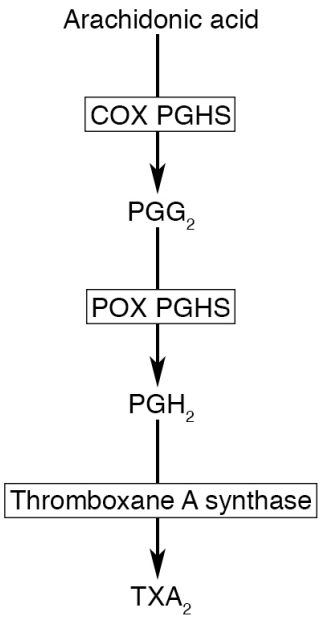

Figure 2.1 Chain of reactions of PGHS without aspirin.

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):

$$
\begin{aligned}
Fe(III) + PGG_2 &\xrightarrow{k_1} Fe(IV)PP^{\bullet} + PGH_2 \\
Fe(IV)PP^{\bullet} &\xrightarrow{k_2} Fe(IV)Tyr^{\bullet} \\
Fe(IV)Tyr^{\bullet} + AA &\xrightarrow{k_3} Fe(IV)AA^{\bullet}/Tyr \\
Fe(IV)AA^{\bullet}/Tyr + O_2 &\xrightarrow{k_4} Fe(IV)AAO_2{}^{\bullet}/Tyr \\
Fe(IV)AAO_2{}^{\bullet}/Tyr + O_2 &\xrightarrow{k_5} Fe(IV)Tyr^{\bullet} + PGG_2 \\
Fe(IV)PP^{\bullet} + FA &\xrightarrow{k_6} Fe(IV) + FA^{\bullet} \\
Fe(IV)Tyr^{\bullet} + FA &\xrightarrow{k_7} FE(IV) + FA^{\bullet} \\
Fe(IV) + FA &\xrightarrow{k_8} Fe(III) + FA^{\bullet}
\end{aligned}
\tag{3.1}
$$

where *Fe*'s denote different forms of the enzyme PGHS and $\bullet$ denotes radical chemistry, AA is arachidonic acid, O_2 is oxygen, and FA is ferulic acid.

We simplified these equations, omitting the radical chemistry and substrates oxygen and ferulic acid, to obtain:

$$\begin{aligned} E_1 + P &\xrightarrow{k_1} E_2 + H \\ E_2 &\xrightarrow{k_2} E_3 \\ E_3 + S &\xrightarrow{k_3} C_1 \xrightarrow{k_4} C_2 \xrightarrow{k_5} E_3 + P \\ E_2 &\xrightarrow{k_6} E_4 \\ E_3 &\xrightarrow{k_7} E_4 \\ E_4 &\xrightarrow{k_8} E_1 \end{aligned} \tag{3.2}$$

with the following variable definitions:

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

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

$$E_3 \xrightarrow{k_9} E_5, \tag{3.3}$$

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:

$$E^* + A \xrightarrow{k_{on,aspirin}} \hat{C}, \tag{3.4}$$

where A denotes aspirin and $\hat{C}$ is the PGHS-aspirin complex. Let $k_{on,aspirin}$ be called k_{10}.

From Wang et al. (2001), we deduced the relationship between PGH_2 and thromboxane:

$$E_6 + H \rightarrow X \rightarrow B, \tag{3.5}$$

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

$$\begin{aligned} E_6 + H &\underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} E' : H \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} C' \\ C' &\underset{k_{-c}}{\overset{k_c}{\rightleftharpoons}} HHT \\ C' &\underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} X, \end{aligned} \tag{3.6}$$

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

$$E_6 + H \overset{k_a}{\rightarrow} X. \tag{3.7}$$

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:

$$E_6 + H \overset{k_a}{\rightarrow} B, \tag{3.8}$$

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.

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

$$\begin{aligned}
E_1 + P &\xrightarrow{k_1} E_2 + H \\
E_2 &\xrightarrow{k_2} E_3 \\
E_3 + S &\xrightarrow{k_3} C_1 \xrightarrow{k_4} C_2 \xrightarrow{k_5} E_3 + P \\
E_2 &\xrightarrow{k_6} E_4 \\
E_3 &\xrightarrow{k_7} E_4 \\
E_4 &\xrightarrow{k_8} E_1 \\
E_3 &\xrightarrow{k_9} E_5 \\
E_3 + A &\xrightarrow{k_{10}} C_3 \\
E_6 + H &\xrightarrow{k_{11}} E_6 + B.
\end{aligned} \tag{3.9}$$

Converting these chemical equations to differential equations yielded

$$\begin{aligned}
\dot{E_1} &= -k_1 E_1 P + k_8 E_4 \\
\dot{E_2} &= k_1 E_1 P - E_2 \left(k_2 + k_6\right) \\
\dot{E_3} &= k_2 E_2 + k_5 C_2 - E_3 \left(k_3 S + k_7 + k_9 + k_{10} A\right) \\
\dot{E_4} &= k_6 E_2 + k_7 E_3 - k_8 E_4 \\
\dot{E_5} &= k_9 E_3 \\
\dot{E_6} &= 0 \\
\dot{C_1} &= k_3 E_3 S - k_4 C_1 \\
\dot{C_2} &= k_4 C_1 - k_5 C_2 \\
\dot{C_3} &= k_{10} E_3 A \\
\dot{P} &= -k_1 E_1 P + k_5 C_2 \\
\dot{H} &= k_1 E_1 P - k_{11} E_6 H \\
\dot{A} &= -k_{10} E_3 A \\
\dot{S} &= -k_3 E_3 S \\
\dot{B} &= k_{11} E_6 H
\end{aligned} \tag{3.10}$$

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 1.0867×10^{-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 1.7545×10^{-6} M that was converted from the reported 650 ng/mL using a molar mass of thromboxane B_2 of 370.48 g/mol.

Variables	Initial values (M)	Sources
E_1 (POX PGHS)	$10^{-8} - 10^{-6}$	Goltsov et al., 2010
E_2 (PGHS intermediate 1)	$10^{-8} - 10^{-6}$	
E_3 (COX PGHS)	$10^{-8} - 10^{-6}$	Goltsov et al., 2010
E_4 (PGHS intermediate 2)	$10^{-8} - 10^{-6}$	
E_5 (Inactive PGHS)	0	
E_6 (Thromboxane A synthase)	$10^{-8} - 10^{-6}$	
C_1 (PGHS-AA complex 1)	0	
C_2 (PGHS-AA complex 2)	0	
C_3 (PGHS-aspirin complex)	0	
P (PGG_2)	0	Gerrard et al., 1977
H (PGH_2)	0	Gerrard et al., 1977
A (Aspirin)	1.0867×10^{-4}	Feldman and Cryer, 1999
S (Arachidonic acid)	$10^{-9} - 10^{-7}$	Goltsov et al., 2010
B (TXB_2)	1.7545×10^{-6}	Feldman and Cryer, 1999

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

Table 3.2 Values for all rate constants.

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

$$A + B \xrightarrow{k} C + D, \tag{3.11}$$

the reaction rate is as follows:

$$\text{Reaction rate} = k[A][B], \tag{3.12}$$

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

$$k = \frac{\text{reaction rate}}{[A][B]}. \tag{3.13}$$

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

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.

Rate constants	Values	Units
k_1	1×10^8	$M^{-1}s^{-1}$
k_2	350	s^{-1}
k_3	1×10^6	$M^{-1}s^{-1}$
k_4	2.0×10^6	s^{-1}
k_5	2.0×10^6	s^{-1}
k_6	1.75×10^9	s^{-1}
k_7	1.0×10^9	s^{-1}
k_8	2.75×10^9	s^{-1}
k_9	5×10^{-2}	s^{-1}
k_{10}	10	$M^{-1}s^{-1}$
k_{11}	1.5×10^7	$M^{-1}s^{-1}$

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:

$$E_1 + P \xrightarrow{k_1} E_2 + H \tag{4.1}$$

$$E_2 \xrightarrow{k_2} E_3 \tag{4.2}$$

$$E_3 + S \xrightarrow{k_3} C_1 \xrightarrow{k_4} C_2 \xrightarrow{k_5} E_3 + P \tag{4.3}$$

$$E_2 \xrightarrow{k_6} E_4 \tag{4.4}$$

$$E_3 \xrightarrow{k_7} E_4 \tag{4.5}$$

$$E_4 \xrightarrow{k_8} E_1 \tag{4.6}$$

$$E_3 \xrightarrow{k_9} E_5 \tag{4.7}$$

$$E_3 + A \xrightarrow{k_{10}} C_3 \tag{4.8}$$

$$E_6 + H \xrightarrow{k_{11}} E_6 + B \tag{4.9}$$

We combined Equation 4.1 and Equation 4.2 to obtain

$$E_1 + P \xrightarrow{k_1} E_2 \xrightarrow{k_2} E_3 + H, \tag{4.10}$$

and reduced Equation 4.3 to

$$E_3 + S \xrightarrow{k_3} C_1 \xrightarrow{k_5} E_3 + P, \tag{4.11}$$

and combined Equation 4.5 and Equation 4.6 to obtain

$$E_3 \xrightarrow{k_7} E_1. \tag{4.12}$$

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:

$$\begin{aligned} E_1 + P &\xrightarrow{k_1} E_2 \xrightarrow{k_2} E_3 + H \\ E_3 + S &\xrightarrow{k_3} C_1 \xrightarrow{k_5} E_3 + P \\ E_3 &\xrightarrow{k_7} E_1 \\ E_3 &\xrightarrow{k_9} E_5 \\ E_3 + A &\xrightarrow{k_{10}} C_3 \\ E_6 + H &\xrightarrow{k_{11}} E_6 + B \end{aligned} \tag{4.13}$$

with the following corresponding differential equations:

$$\begin{aligned} \dot{E_1} &= k_7 E_3 - k_1 E_1 P \\ \dot{E_2} &= k_1 E_1 P - k_2 E_2 \\ \dot{E_3} &= k_2 E_2 + k_5 C_1 - k_3 E_3 S - k_7 E_3 - k_9 E_3 - k_{10} E_3 A \\ \dot{E_5} &= k_9 E_3 \\ \dot{E_6} &= 0 \\ \dot{C_1} &= k_3 E_3 S - k_5 C_1 \\ \dot{C_3} &= k_{10} E_3 A \\ \dot{P} &= k_5 C_1 - k_1 E_1 P \\ \dot{H} &= k_2 E_2 - k_{11} E_6 H \\ \dot{A} &= -k_{10} E_3 A \\ \dot{S} &= -k_3 E_3 S \\ \dot{B} &= k_{11} E_6 H \end{aligned} \tag{4.14}$$

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:

$$E_o = E_1 + E_3 + E_2 + C_1 \tag{4.15}$$

and therefore defined E_1 as

$$E_1 = E_o - E_3 - E_2 - C_1 \tag{4.16}$$

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:

$$\begin{aligned}
\dot{E_3} &= k_2 E_2 + k_5 C_1 - E_3 \left(k_3 S + k_7 + k_{10} A\right) \\
\dot{E_6} &= 0 \\
\dot{E_2} &= k_1 \left(E_o - E_3 - E_2 - C_1\right) P - k_2 E_2 \\
\dot{C_1} &= k_3 E_3 S - k_5 C_1 \\
\dot{P} &= k_5 C_1 - k_1 \left(E_o - E_3 - E_2 - C_1\right) P \\
\dot{H} &= k_2 E_2 - k_{11} E_6 H \\
\dot{A} &= -k_{10} E_3 A
\end{aligned} \tag{4.17}$$

with fixed points of the form

$$\left(E_3, E_6, E_2, C_1, P, H, A\right). \tag{4.18}$$

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

$$\begin{array}{lll}(0,0,0,0,0,0,0) & (0,0,0,0,0,0,A) & (0,0,0,0,P,0,A)\\ (0,E_6,0,0,0,0,0) & (0,E_6,0,0,P,0,0) & (0,0,0,0,0,H,A)\\ (0,0,0,0,P,0,0) & (0,E_6,0,0,0,0,A) & (0,E_6,0,0,P,0,A)\\ (0,0,0,0,0,H,0) & (0,0,0,0,P,H,0) & (0,0,0,0,P,H,A).\end{array}$$

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 (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, when P is initially fixed at some value greater than zero, perturbations to E_o, and consequently to E_1, will cause the value of P to perturb away from that initial fixed value. Therefore, the following fixed points

$$\begin{array}{lll}(0,0,0,0,P,0,0) & (0,0,0,0,P,H,0) & (0,E_6,0,0,P,0,A)\\ (0,E_6,0,0,P,0,0) & (0,0,0,0,P,0,A) & (0,0,0,0,P,H,A),\end{array}$$

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

$$\begin{array}{lll}(0,0,0,0,0,0,0) & (0,0,0,0,0,H,A) & (0,0,0,0,0,H,0)\\ (0,E_6,0,0,0,0,A) & (0,E_6,0,0,0,0,0) & (0,0,0,0,0,0,A)\end{array}$$

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

$$J = \begin{bmatrix} -k_3S - k_7 - k_{10}A & 0 & k_2 & k_5 & 0 & 0 & 0\\ 0 & 0 & 0 & 0 & 0 & 0 & 0\\ 0 & 0 & -k_2 & 0 & k_1E_o & 0 & 0\\ k_3S & 0 & 0 & -k_5 & 0 & 0 & 0\\ 0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0\\ 0 & -k_{11}H & k_2 & 0 & 0 & -k_{11}E_6 & 0\\ -k_{10}A & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}. \tag{4.19}$$

4.2.3 Stability of $(0,0,0,0,0,0,0)$ and $(0,0,0,0,0,H,0)$

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

$$J_0 = \begin{bmatrix} -k_3S - k_7 & 0 & k_2 & k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_2 & 0 & k_1E_o & 0 & 0 \\ k_3S & 0 & 0 & -k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\ 0 & 0 & k_2 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}. \tag{4.20}$$

The characteristic polynomial of Equation 4.20 is

$$p(\lambda) = \lambda^7 + a_1\lambda^6 + a_2\lambda^5 + a_3\lambda^4 + a_4\lambda^3, \tag{4.21}$$

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 $(0,0,0,0,0,H,0)$ is

$$J_H = \begin{bmatrix} -k_3S - k_7 & 0 & k_2 & k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_2 & 0 & k_1E_o & 0 & 0 \\ k_3S & 0 & 0 & -k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\ 0 & -k_{11}H & k_2 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}, \tag{4.22}$$

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

$$k_1 k_2 k_5 E_o \left(k_7 - k_3 S\right) > 0$$
$$k_7 - k_3 S > 0.$$

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

$$\sigma = \frac{k_3 S}{k_7} - 1, \tag{4.23}$$

where the stability of the zero fixed point is dependent on the sign of σ, 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 discusses 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 appropriate 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 inactivation 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 σ was greater than zero and less than zero. A value of σ 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 σ 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, including arachidonic acid. We concluded that if arachidonic acid concentration 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 $(0,0,0,0,0,0,A)$, $(0,0,0,0,0,H,A)$, $(0,E_6,0,0,0,0,0)$, $(0,E_6,0,0,0,0,A)$

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

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

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_o, 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 $(0,0,0,0,0,0,A)$ is

$$J_A = \begin{bmatrix} -k_3S - k_7 - k_{10}A & 0 & k_2 & k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_2 & 0 & k_1E_o & 0 & 0 \\ k_3S & 0 & 0 & -k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\ 0 & 0 & k_2 & 0 & 0 & 0 & 0 \\ -k_{10}A & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}. \tag{4.24}$$

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

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

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

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

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

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

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 2.70×10^{-7} M, as determined in section 3.2.

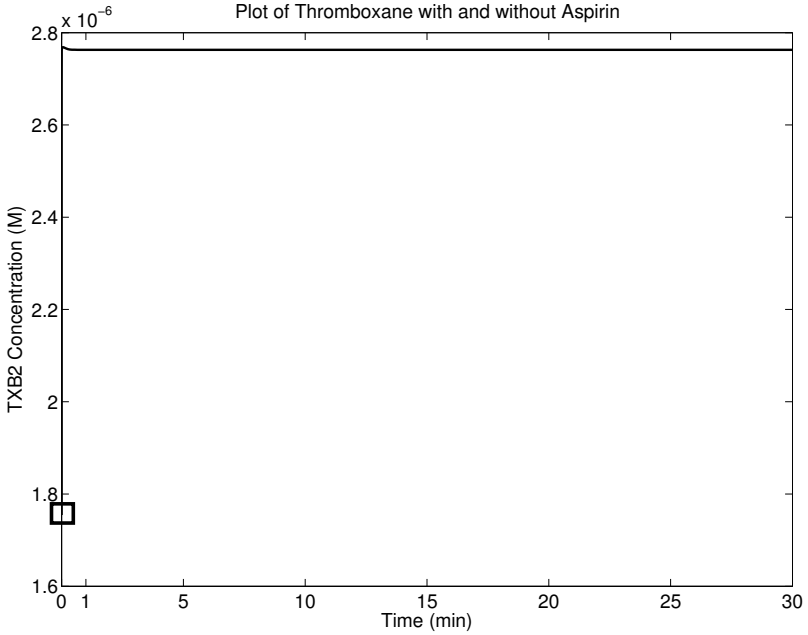

Figure 4.1 Concentration-time curve for TXB_2, with and without aspirin for the reduced system, with rate constants from Section 3.2.

When we used the original rate constants, the TXB_2 concentration increased from its starting concentration of 1.7545×10^{-6} M to a steady state value of 2.63×10^{-6} 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.

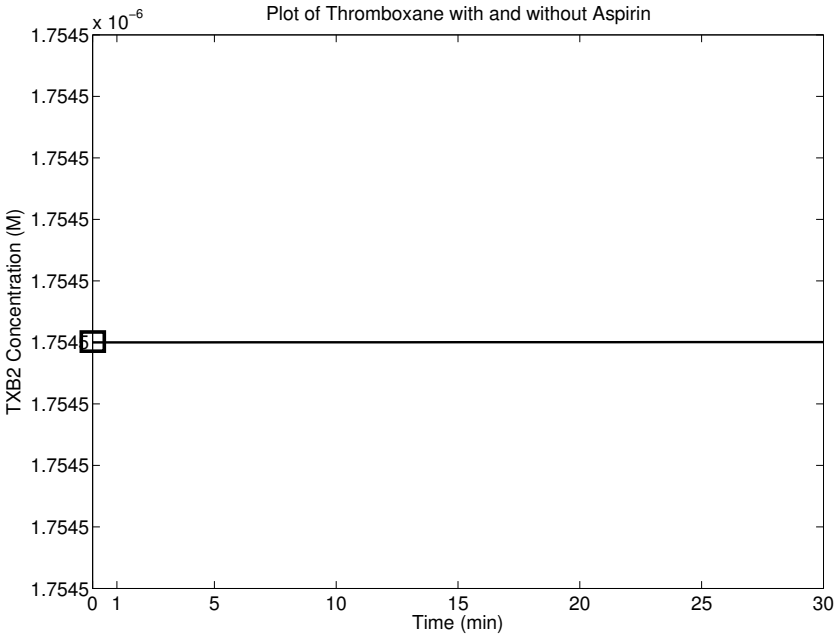

Figure 4.2 Concentration-time curve for TXB_2, with and without aspirin for the reduced system, with altered rate constants.

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:

$$
\begin{aligned}
\dot{E_1} &= -k_1 E_1 P + k_8 E_4 \\
\dot{E_2} &= k_1 E_1 P - E_2 \left(k_2 + k_6\right) \\
\dot{E_3} &= k_2 E_2 + k_5 C_2 - E_3 \left(k_3 S + k_7 + k_9 + k_{10} A\right) \\
\dot{E_4} &= k_6 E_2 + k_7 E_3 - k_8 E_4 \\
\dot{E_5} &= k_9 E_3 \\
\dot{E_6} &= 0 \\
\dot{C_1} &= k_3 E_3 S - k_4 C_1 \\
\dot{C_2} &= k_4 C_1 - k_5 C_2 \\
\dot{C_3} &= k_{10} E_3 A \\
\dot{P} &= -k_1 E_1 P + k_5 C_2 \\
\dot{H} &= k_1 E_1 P - k_{11} E_6 H \\
\dot{A} &= -k_{10} E_3 A \\
\dot{S} &= -k_3 E_3 S \\
\dot{B} &= k_{11} E_6 H
\end{aligned}
\tag{5.1}
$$

with the following variable definitions:

- $E_1 =$ POX PGHS
- $E_2 =$ PGHS intermediate 1
- $E_3 =$ COX PGHS
- $E_4 =$ PGHS intermediate 2
- $E_5 =$ inactive PGHS
- $C_1 =$ PGHS-AA complex 1
- $C_2 =$ PGHS-AA complex 2
- $C_3 =$ PGHS-aspirin complex
- $P = PGG_2$
- $H = PGH_2$
- $A =$ aspirin
- $S =$ arachidonic acid, or AA
- $B = TXB_2$

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:

$$E_o = E_1 + E_3 + E_2 + E_4 + C_1 + C_2 \tag{5.2}$$

and therefore defined E_1 as

$$E_1 = E_o - E_3 - E_2 - E_4 - C_1 - C_2 \tag{5.3}$$

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:

$$\begin{aligned}
\dot{E}_3 &= k_2E_2 + k_5C_1 - E_3\left(k_3S + k_7 + k_{10}A\right)\\
\dot{E}_6 &= 0\\
\dot{E}_2 &= k_1\left(E_o - E_3 - E_2 - E_4 - C_1 - C_2\right)P - E_2\left(k_2 + k_6\right)\\
\dot{E}_4 &= k_7E_3 + k_6E_2 - k_8E_4\\
\dot{C}_1 &= k_3SE_3 - k_4C_1\\
\dot{C}_2 &= k_4C_1 - k_5C_2\\
\dot{P} &= k_5C_2 - k_1\left(E_o - E_3 - E_2 - E_4 - C_1 - C_2\right)P\\
\dot{H} &= k_1\left(E_o - E_3 - E_2 - E_4 - C_1 - C_2\right)P - k_{11}E_6H\\
\dot{A} &= -k_{10}E_3A
\end{aligned} \tag{5.4}$$

with fixed points of the form

$$\left(E_3, E_6, E_2, E_4, C_1, C_2, P, H, A\right). \tag{5.5}$$

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

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

From our fixed points, we know that E_3, E_2, E_4, C_1, and $C_2 = 0$, making $E_0 = E_1$. If $E_0 = E_1 = 0$, 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

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

with $P > 0$ are always unstable. The stability of the remaining fixed points

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

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_o, 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

$$J = \begin{bmatrix} -k_3S - k_7 - k_{10}A & 0 & k_2 & 0 & 0 & k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_2 - k_6 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\ k_7 & 0 & k_6 & -k_8 & 0 & 0 & 0 & 0 & 0 \\ k_3S & 0 & 0 & 0 & -k_4 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_4 & -k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\ 0 & -k_{11}H & 0 & 0 & 0 & 0 & k_1E_o & -k_{11}E_6 & 0 \\ -k_{10}A & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}. \tag{5.6}$$

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 $2.70 \times 10^{-7}\ M$.

Rate constants	Values	Units
k_1	8.5×10^6	$M^{-1}s^{-1}$
k_2	350	s^{-1}
k_3	1×10^6	$M^{-1}s^{-1}$
k_4	2.0×10^6	s^{-1}
k_5	2.0×10^6	s^{-1}
k_6	1.75×10^9	s^{-1}
k_7	1.0×10^9	s^{-1}
k_8	2.75×10^9	s^{-1}
k_9	5×10^{-2}	s^{-1}
k_{10}	9.99000305	$M^{-1}s^{-1}$
k_{11}	1.5×10^7	$M^{-1}s^{-1}$

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 $1.7545 \times 10^{-6}\ M$.

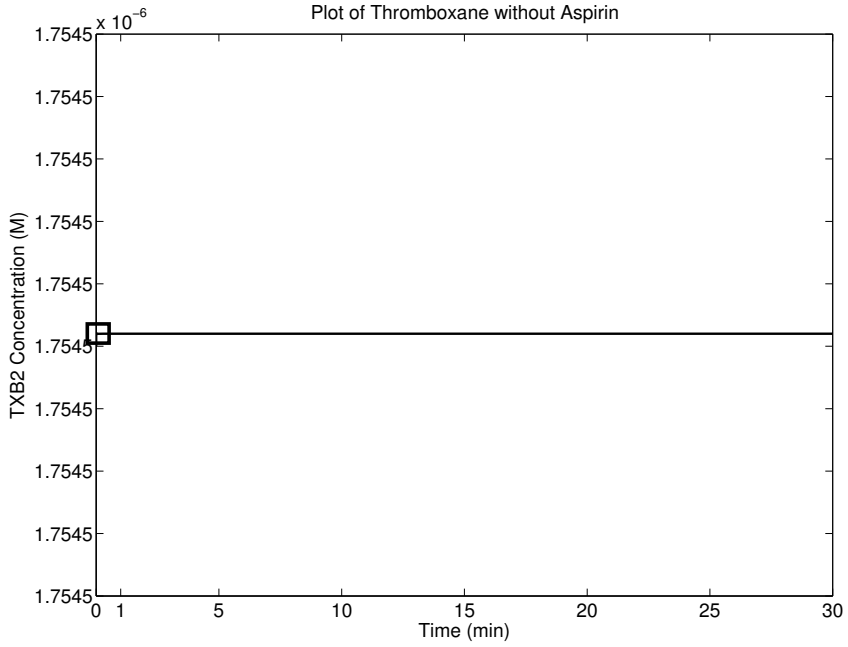

Figure 5.1 Concentration-time curve for TXB_2 without aspirin.

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 occured:

$$\begin{aligned} &\text{If } [A] > [S]\text{:} \\ &\qquad \dot{E_3} = k_2 E_2 + k_5 C_2 - E_3\left(k_9 + k_{10} A\right) \qquad (5.7) \\ &\text{Else:} \\ &\qquad \dot{E_3} = k_2 E_2 + k_5 C_2 - E_3\left(k_3 S + k_7 + k_9\right) \end{aligned}$$

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.

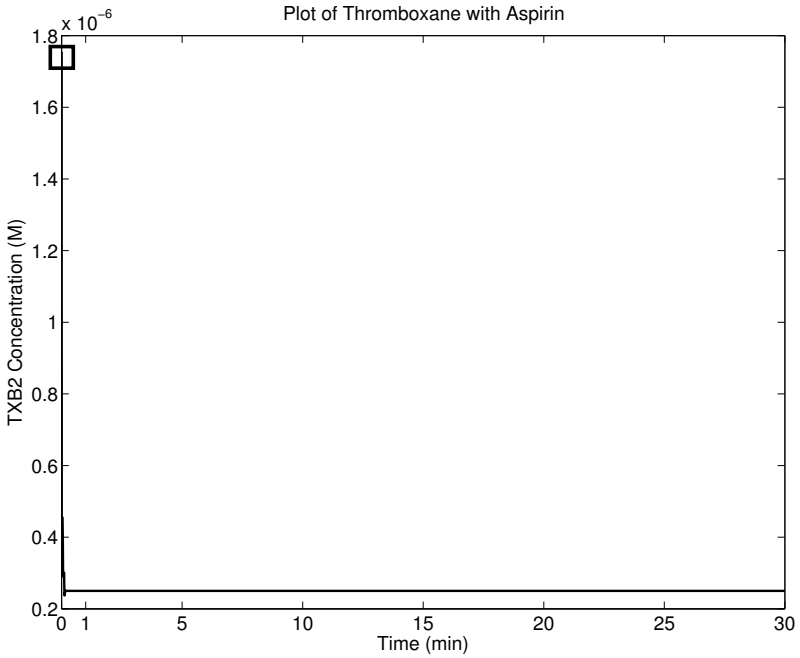

Figure 5.2 Concentration-time curve for TXB_2 with aspirin.

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 2.5×10^{-7} M, which was close to the predicted final concentration of TXB_2 of 2.70×10^{-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 2.70×10^{-7} M until 30 minutes after aspirin ingestion; at one minute after ingestion, 5 minutes, and 7.5 minutes, the TXB_2 concentrations were 1.552×10^{-6} M, 1.417×10^{-6} M, and 1.215×10^{-6} M, respectively (1999). However, our simulations showed a decrease to 2.5×10^{-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.

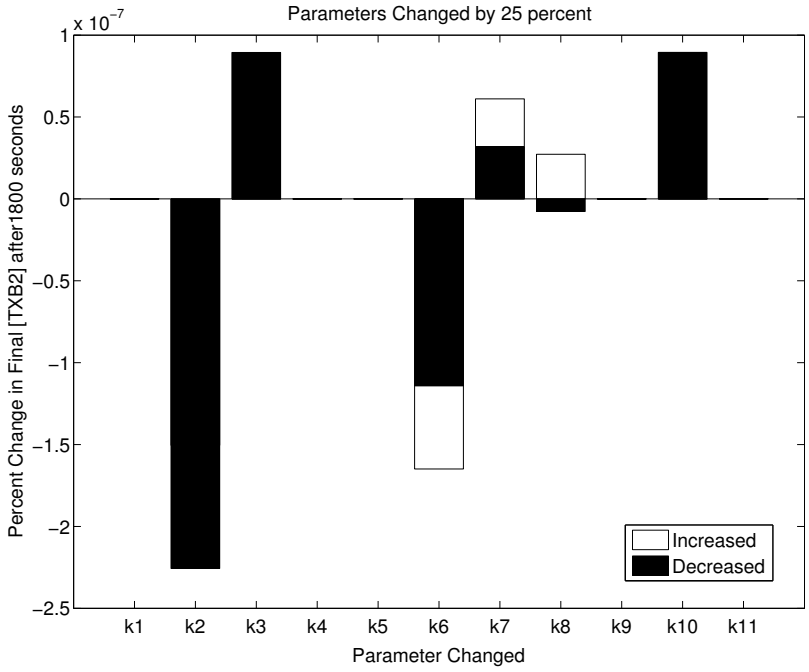

Figure 5.3 Sensitivity Analysis of Paramaters of the Dimensional System.

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:

$$\begin{aligned}
\dot{E_1} &= -E_1P + \frac{\lambda}{\mu}E_4 \\
\dot{E_2} &= \alpha E_1P - \beta E_2 \\
\dot{E_3} &= \sigma\eta E_2 + \frac{1}{\alpha}C_2 - E_3\left(S + \gamma + \delta + A\right) \\
\dot{E_4} &= \eta E_2 + \sigma\mu\gamma E_3 - \lambda E_4 \\
\dot{E_5} &= \kappa E_3 \\
\dot{E_6} &= 0 \\
\dot{C_1} &= \mu E_3 S - \eta C_1 \\
\dot{C_2} &= \rho C_1 - C_2 \\
\dot{C_3} &= \mu E_3 A \\
\dot{P} &= -\epsilon E_1P + \frac{\eta}{\sigma}C_2 \\
\dot{H} &= \theta E_1P - E_6H \\
\dot{A} &= \kappa E_3 A \\
\dot{S} &= -\rho E_3 S \\
\dot{B} &= \phi E_6 H
\end{aligned} \tag{5.8}$$

with the following unit-less coefficients:

- $\alpha = \frac{k_2}{k_4}$
- $\gamma = \frac{k_7}{k_5}$
- $\beta = \frac{k_2+k_6}{k_5}$
- $\delta = \frac{k_9}{k_5}$
- $\sigma = \frac{k_3}{k_1}$
- $\eta = \frac{k_4}{k_5}$

- $\lambda = \frac{k_8}{k_5}$
- $\kappa = \frac{k_2 k_{10}}{k_3 k_5}$
- $\mu = \frac{k_2}{k_5}$
- $\rho = \frac{k_6}{k_5}$
- $\epsilon = \frac{k_2}{k_5}$
- $\theta = \frac{k_{11}}{k_1}$
- $\phi = \frac{k_{10}}{k_{11}}$

5.4.1 Simulations

The results for the final concentration value of TXB_2 for the nondimensionalized system was the same as those for the dimensionalized system for both the case without aspirin and the case with aspirin.

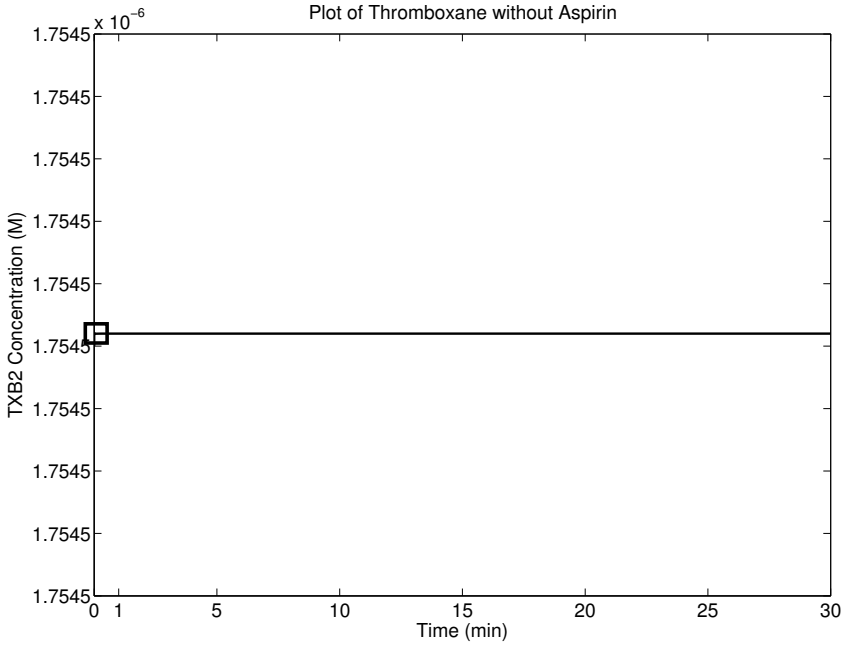

Figure 5.4 Concentration-time curve for TXB_2 without aspirin in the nondimensionalized system.

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 TXB_2,

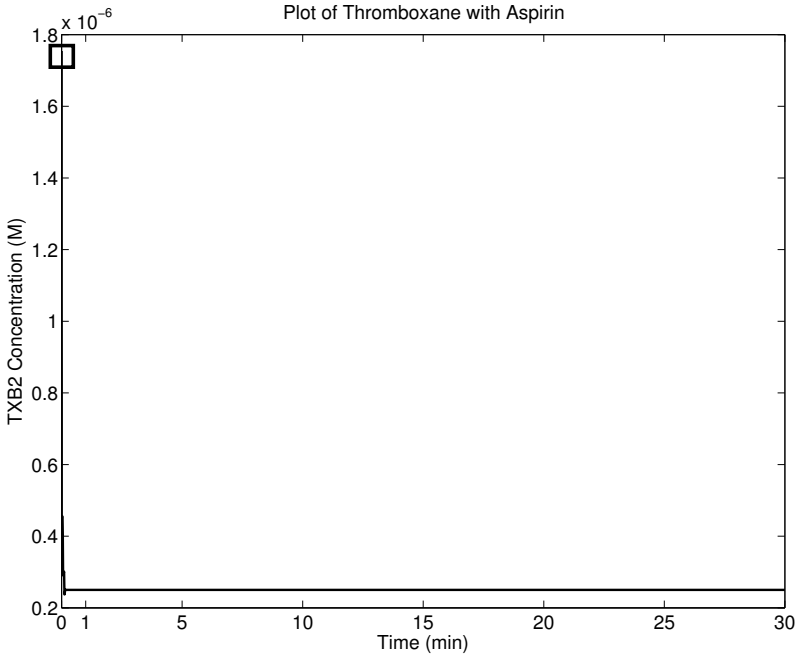

Figure 5.5 Concentration-time curve for TXB_2 with aspirin in the nondimensionalized system.

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 seen in Figure 5.6, which showed that the nondimensional parameters with the largest effect on the final concentration of TXB_2 were λ and $\frac{\lambda}{\mu}$. Considering that λ is $\frac{k_8}{k_5}$ and that $\frac{\lambda}{\mu}$ 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-

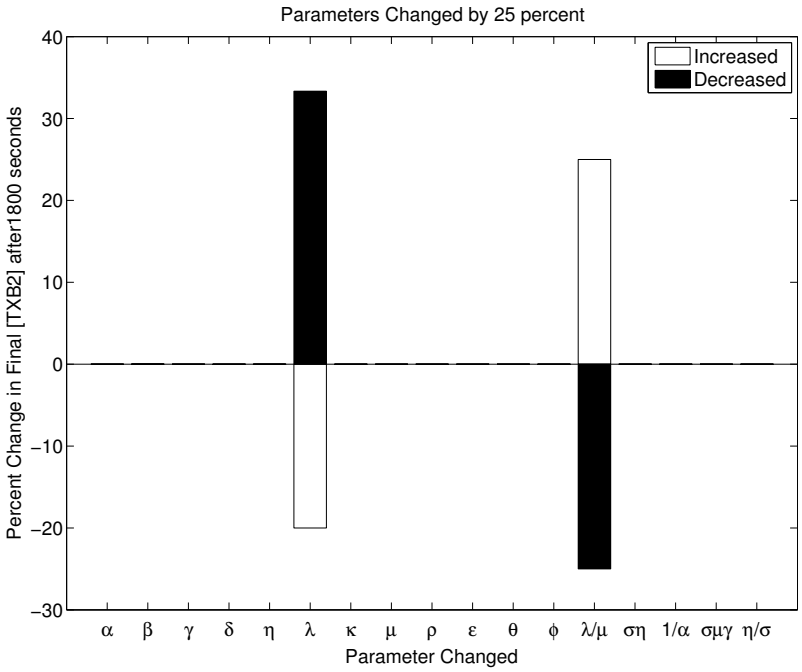

Figure 5.6 Sensitivity Analysis of Paramaters of the Nondimensionalized System.

sion 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 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

$$H_n = \begin{pmatrix} a_1 & 1 & 0 & 0 & \cdots & 0 \\ a_3 & a_2 & a_1 & 1 & \cdots & 0 \\ a_5 & a_4 & a_3 & a_2 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \ldots & \vdots \\ 0 & 0 & 0 & 0 & \cdots & a_n \end{pmatrix}$$

where $a_j = 0 if j > n$. 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:

$$detH_j > 0, j = 1, 2, \cdots, n.$$

Allen (2006) also provided a summary of the Routh Hurwitz Critera for $n = 2, 3, 4,$ and 5:

$$n = 2 : a_1 > 0 \text{ and } a_2 > 0$$
$$n = 3 : a_1 > 0, a_3 > 0, \text{ and } a_1 a_2 > a_3$$
$$n = 4 : a_1 > 0, a_3 > 0, a_4 > 0, \text{ and } a_1 a_2 a_3 > {a_3}^2 + {a_1}^2 a_4$$
$$n = 5 : a_i > 0, i = 1, 2, 3, 4, 5, a_1 a_2 a_3 > {a_3}^2 + {a_1}^2 a_4,$$
$$\text{and } (a_1 a_4 - a_5)\left(a_1 a_2 a_3 - {a_3}^2 - {a_1}^2 a_4\right) > a_5(a_1 a_2 - a_3)^2 + a_1 {a_5}^2.$$

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 Co (1999) to construct our Routh arrays, we provide the reader with a general Routh array from Purdue School of Engineering and Technology (2007), for polynomial

$$a_0 s^n + a^1 s^{n-1} + \cdots + a_{n-1} s + a_n = 0.$$

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

where

$$b_1 = \frac{a_1 a_2 - a_0 a_3}{a_1}$$
$$b_2 = \frac{a_1 a_4 - a_0 a_5}{a_1}$$
$$b_3 = \frac{a_1 a_6 - a_0 a_7}{a_1}$$

$$\vdots$$

$$c_1 = \frac{b_1 a_3 - a_1 b_2}{b_1}$$

$$c_2 = \frac{b_1 a_5 - a_1 b_3}{b_1}$$

$$c_3 = \frac{b_1 a_7 - a_1 b_4}{b_1}$$

$$\vdots$$

$$d_1 = \frac{c_1 b_2 - b_1 c_2}{c_1}$$

$$d_2 = \frac{c_1 b_3 - b_1 c_3}{c_1}$$

$$\vdots$$

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

B.1 Stability of $(0,0,0,0,0,0,A)$ and $(0,0,0,0,0,H,A)$

The characteristic polynomial of Equation C.4 and Equation C.4 is

$$p(\lambda) = \lambda^7 + a_1\lambda^6 + a_2\lambda^5 + a_3\lambda^4 + a_4\lambda^3 \tag{B.1}$$

where

```
a1 = k2 + k5 + k7 + A*k10 + Eo*k1 + S*k3

a2 = k2*k5 + k2*k7 + k5*k7 + A*k2*k10 + A*k5*k10 + Eo*k1*k2 +
Eo*k1*k5 + Eo*k1*k7 + S*k2*k3 + A*Eo*k1*k10 + Eo*S*k1*k3

a3 = k2*k5*k7 + A*k2*k5*k10 + Eo*k1*k2*k5 + Eo*k1*k2*k7 +
Eo*k1*k5*k7 + A*Eo*k1*k2*k10 + A*Eo*k1*k5*k10 +
Eo*S*k1*k2*k3

a4 = Eo*k1*k2*k5*k7 + A*Eo*k1*k2*k5*k10 - Eo*S*k1*k2*k3*k5.
```

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

$$a_1 > 0$$
$$a_3 > 0$$
$$a_4 > 0$$
$$a_1 a_2 a_3 > {a_3}^2 + {a_1}^2 a_4.$$

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 $a_1 a_2 a_3 > {a_3}^2 + {a_1}^2 a_4$, not all the terms are positive, so the stability of $(0, 0, 0, 0, 0, 0, A)$ and $(0, 0, 0, 0, 0, H, A)$ is defined by

```
Eo*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)*(k2 + k5 + 3*k7) + Eo*k1*(Eo*k1
*(8*k2*k5 + 3*k2*k7 + 3*k5*k7 + 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)) + Eo*k1*(Eo*k1*(k2^3 + 8*k2^2*k5
+ 4*k2^2*k7 + 8*k2*k5^2 + 16*k2*k5*k7 + 3*k2*k7^2  + k5^3 +
4*k5^2*k7 + 3*k5*k7^2 + Eo*k1*(k2^2 + 4*k2*k5 +2*k7*k2 + k5^2
+ 2*k7*k5)) + (2*k2 + 2*k5 + 3*k7)*(2*k2^2*k5 + k7*k2^2 +
2*k2*k5^2 + 4*k7*k2*k5 + k7*k5^2))+ k2*k5*(k2 + k5)*(k2*k5 +
2*k2*k7 + 2*k5*k7 + 3*k7^2)) + Eo*k1*(Eo*k1*(Eo*k1*(k2^2*k5 +
k2^2*k7 + k2*k5^2 + 4*k2*k5*k7 + k2*k7^2 + k5^2*k7 + k5*k7^2)
+ (k2 + k5 + k7)*(k2^2*k5 + k2^2*k7 + k2*k5^2 + 6*k2*k5*k7 +
k2*k7^2 + k5^2*k7 + k5*k7^2)) + k2^3*k5^2 + 4*k2^3*k5*k7 +
k2^3*k7^2 + k2^2*k5^3 + 8*k2^2*k5^2*k7 + 8*k2^2*k5*k7^2 +
k2^2*k7^3 + 4*k2*k5^3*k7 + 8*k2*k5^2*k7^2 + 4*k2*k5*k7^3 +
k5^3*k7^2 + k5^2*k7^3)+k2*k5*k7*(k5 + k7)*(k2 + k7)*(k2 + k5)
+ S*k3*(A*k10*(Eo*k1*(Eo*k1*(9*k2*k5 + 6*k2*k7 + 4*k5*k7 +
4*k2^2 + 2*k5^2 + Eo*k1(2*k2+k5))+ 2*k2^3 + 9*k2^2*k5 +
6*k7*k2^2 + 6*k2*k5^2 + 14*k7*k2*k5 + 2*k7*k5^2)+k2^3*k5 +
2*k2^2*k5^2 + 2*k2*k5^2*k7 + 4*k2^2*k5*k7+ S*k3*(Eo*k1*(2*k2*k5
+ 3*k2^2 + Eo*k1*(3*k2+k5))+ 2*k5*k2^2)+ A*k10*(Eo*k1*(3*k2^2 +
7*k2*k5 + k5^2 + Eo*k1*(3*k2+2*k5))+ k2*k5*(2*k2 + k5)))+ S*k3
*(k2 + Eo*k1)*(Eo*k1*(k2*k5 + 3*k2*k7 + k5*k7 + k2^2 + Eo*k1*k2
```

```
+ S*k2*k3)+ k2*k5*k7)+ Eo*k1*(Eo*k1*(Eo*k1*(k2*k5 + 2*k2*k7 +
k5*k7 + k2^2)+k2^3 + 2*k2^2*k5 + 4*k2^2*k7 + k2*k5^2 +
9*k2*k5*k7 +  3*k2*k7^2 +  2*k5^2*k7 + 2*k5*k7^2 ) + k2^3*k5 +
2*k2^3*k7 + k2^2*k5^2 + 9*k2^2*k5*k7 + 3*k2^2*k7^2 + 6*k2*k5^2*k7
+ 7*k2*k5*k7^2 + k5^2*k7^2)+ k2*k5*k7*(2*k2*k5 + 2*k2*k7 + k5*k7
+ k2^2)).
```

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

```
a1 = k2 + k5 + k7 + E6*k11 + Eo*k1 + S*k3

a2 = k2*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

a3 = k2*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

a4 = 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*k11 - Eo*S*k1*k2*k3*k5 +
E6*Eo*S*k1*k2*k3*k11

a5 = E6*Eo*k1*k2*k5*k7*k11 - E6*Eo*S*k1*k2*k3*k5*k11.
```

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

$$\begin{gathered} a_1 > 0 \\ a_2 > 0 \\ a_3 > 0 \\ a_4 > 0 \\ a_5 > 0 \\ a_1 a_2 a_3 > {a_3}^2 + {a_1}^2 a_4 \\ (a_1 a_4 - a_5)\left(a_1 a_2 a_3 - {a_3}^2 - {a_1}^2 a_4\right) > a_5 (a_1 a_2 - a_3)^2 + a_1 {a_5}^2. \end{gathered}$$

Because the rate constants, E_o, 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 $(a_1 a_4 - a_5)\left(a_1 a_2 a_3 - {a_3}^2 - {a_1}^2 a_4\right) > a_5 (a_1 a_2 - a_3)^2 + a_1 {a_5}^2$, not all the terms are positive, so the stability of $(0, E_6, 0, 0, 0, 0)$ is defined by

```
E6*k2*k5*k7*k11 + E*k1*k2*k5*k7 + E6*E*k1*k2*k5*k11 +
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,

E6*E*k1*k2*k5*k7*k11 - E6*E*S*k1*k2*k3*k5*k11 > 0,

(k2 + k5 + k7 + E6*k11 + Eo*k1 + S*k3)*(k2*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*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) - (k2 + k5 + k7 + E6*k11 +
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*k11
- Eo*S*k1*k2*k3*k5 + E6*Eo*S*k1*k2*k3*k11) - (k2*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,
```

and

```
- (E6*Eo*k1*k2*k5*k7*k11 - E6*Eo*S*k1*k2*k3*k5*k11)*(k2*k5*k7
- (k2 + k5 + k7 + E6*k11 + Eo*k1 + S*k3)*(k2*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) + 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 -
(E6*Eo*k1*k2*k5*k7*k11 - E6*Eo*S*k1*k2*k3*k5*k11)^2*(k2 +
k5 + k7 + E6*k11 + Eo*k1 + S*k3) - ((k2 + k5 + k7 + E6*k11 +
Eo*k1 + S*k3)*(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*k11
- Eo*S*k1*k2*k3*k5 + E6*Eo*S*k1*k2*k3*k11)
- E6*Eo*k1*k2*k5*k7*k11 + E6*Eo*S*k1*k2*k3*k5*k11)*((k2*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 + (k2 + k5 + k7 + E6*k11 + 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*k11 - Eo*S*k1*k2*k3*k5 +
E6*Eo*S*k1*k2*k3*k11) - (k2 + k5 + k7 + E6*k11 + Eo*k1
+ S*k3)*(k2*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*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)) > 0.
```

B.3 Stability of $(0, E_6, 0, 0, 0, 0, A)$

The characteristic polynomial of Equation C.9 is

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

where

```
a1 = k2 + k5 + k7 + A*k10 + E6*k11 + Eo*k1 + S*k3

a2 = k2*k5 + k2*k7 + k5*k7 + A*k2*k10 + A*k5*k10 + E6*k2*k11 +
```

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

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

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

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

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

$$\begin{gathered} a_1 > 0 \\ a_2 > 0 \\ a_3 > 0 \\ a_4 > 0 \\ a_5 > 0 \\ a_1 a_2 a_3 > {a_3}^2 + {a_1}^2 a_4 \\ (a_1 a_4 - a_5)\left(a_1 a_2 a_3 - {a_3}^2 - {a_1}^2 a_4\right) > a_5(a_1 a_2 - a_3)^2 + a_1 {a_5}^2. \end{gathered}$$

Because the rate constants, E_o, 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 $(a_1 a_4 - a_5)\left(a_1 a_2 a_3 - {a_3}^2 - {a_1}^2 a_4\right) > a_5(a_1 a_2 - a_3)^2 + a_1 {a_5}^2$, not all the terms are positive, so the stability of $(0, E_6, 0, 0, 0, A)$ is defined by

```
E6*k2*k5*k7*k11 + Eo*k1*k2*k5*k7 + A*E6*k2*k5*k10*k11 +
A*Eo*k1*k2*k5*k10 + E6*Eo*k1*k2*k5*k11 + E6*Eo*k1*k2*k7*k11 +
E6*Eo*k1*k5*k7*k11 - Eo*S*k1*k2*k3*k5 + A*E6*Eo*k1*k2*k10*k11 +
A*E6*Eo*k1*k5*k10*k11 + E6*Eo*S*k1*k2*k3*k11 > 0,
```

```
E6*Eo*k1*k2*k5*k7*k11 + A*E6*Eo*k1*k2*k5*k10*k11 -
E6*Eo*S*k1*k2*k3*k5*k11 > 0,

((k2 + k5 + k7 + E6*k11 + Eo*k1 + S*k3)*(k2*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*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) - (k2 +
k5 + k7 + E6*k11 + 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*k11 - Eo*S*k1*k2*k3*k5 + E6*Eo*S*k1*k2*k3*k11)
- (k2*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,
```

and

```
- (E6*Eo*k1*k2*k5*k7*k11 - E6*Eo*S*k1*k2*k3*k5*k11)*(k2*k5*k7
- (k2 + k5 + k7 + E6*k11 + Eo*k1 + S*k3)*(k2*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)
+ 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 - (E6*Eo*k1*k2*k5*k7*k11
- E6*Eo*S*k1*k2*k3*k5*k11)^2*(k2 + k5 + k7 + E6*k11 + Eo*k1 +
S*k3) - ((k2 + k5 + k7 + E6*k11 + Eo*k1 + S*k3)*(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*k11 - Eo*S*k1*k2*k3*k5 + E6*Eo*S*k1*k2*k3*k11)
- E6*Eo*k1*k2*k5*k7*k11 + E6*Eo*S*k1*k2*k3*k5*k11)*((k2*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 + (k2 + k5 + k7 + E6*k11 + 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*k11 - Eo*S*k1*k2*k3*k5 +
E6*Eo*S*k1*k2*k3*k11) - (k2 + k5 + k7 + E6*k11 + Eo*k1 +
S*k3)*(k2*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*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)) > 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

$$J_0 = \begin{bmatrix} -k_3S - k_7 & 0 & k_2 & 0 & 0 & k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_2 - k_6 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\ k_7 & 0 & k_6 & -k_8 & 0 & 0 & 0 & 0 & 0 \\ k_3S & 0 & 0 & 0 & -k_4 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_4 & -k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}, \tag{C.1}$$

and the Jacobian matrix at $(0,0,0,0,0,0,0,H,0)$ is

$$J_H = \begin{bmatrix} -k_3S - k_7 & 0 & k_2 & 0 & 0 & k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_2 - k_6 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\ k_7 & 0 & k_6 & -k_8 & 0 & 0 & 0 & 0 & 0 \\ k_3S & 0 & 0 & 0 & -k_4 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_4 & -k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\ 0 & -k_{11}H & 0 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}. \tag{C.2}$$

These two fixed points share a characteristic polynomial, which is

$$p(\lambda) = \lambda^9 + a_1\lambda^8 + a_2\lambda^7 + a_3\lambda^6 + a_4\lambda^5 + a_5\lambda^4 + a_6\lambda^3 \tag{C.3}$$

where

```
a1 = k2 + k4 + k5 + k6 + k7 + k8 + Eo*k1 + S*k3

a2 = k2*k4 + k2*k5 + k2*k7 + k4*k5 + k2*k8 + k4*k6 + k4*k7 +
k5*k6 + k4*k8 + k5*k7 + k5*k8 + k6*k7 + k6*k8 + k7*k8 +
Eo*k1*k2 + Eo*k1*k4 + Eo*k1*k5 + Eo*k1*k6 + Eo*k1*k7 +
Eo*k1*k8 + S*k2*k3 + S*k3*k4 + S*k3*k5 + S*k3*k6 + S*k3*k8 +
Eo*S*k1*k3

a3 = k2*k4*k5 + k2*k4*k7 + k2*k4*k8 + k2*k5*k7 + k2*k5*k8 +
k4*k5*k6 + k4*k5*k7 + k2*k7*k8 + k4*k5*k8 + k4*k6*k7 + k4*k6*k8
+ k5*k6*k7 + k4*k7*k8 + k5*k6*k8 + k5*k7*k8 + k6*k7*k8 +
Eo*k1*k2*k4 + Eo*k1*k2*k5 + Eo*k1*k2*k7 + Eo*k1*k4*k5 +
Eo*k1*k2*k8 + Eo*k1*k4*k6 + Eo*k1*k4*k7 + Eo*k1*k5*k6 +
Eo*k1*k4*k8 + Eo*k1*k5*k7 + Eo*k1*k5*k8 + Eo*k1*k6*k7 +
Eo*k1*k6*k8 + Eo*k1*k7*k8 + S*k2*k3*k4 + S*k2*k3*k5 +
S*k2*k3*k8 + S*k3*k4*k6 + S*k3*k5*k6 + S*k3*k4*k8 + S*k3*k5*k8
+ S*k3*k6*k8 + Eo*S*k1*k2*k3 + Eo*S*k1*k3*k4 + Eo*S*k1*k3*k5
+ Eo*S*k1*k3*k6 + Eo*S*k1*k3*k8

a4 = k2*k4*k5*k7 + k2*k4*k5*k8 + k2*k4*k7*k8 + k2*k5*k7*k8 +
k4*k5*k6*k7 + k4*k5*k6*k8 + k4*k5*k7*k8 + k4*k6*k7*k8 +
k5*k6*k7*k8 + Eo*k1*k2*k4*k5 + Eo*k1*k2*k4*k7 + Eo*k1*k2*k4*k8
+ Eo*k1*k2*k5*k7 + Eo*k1*k2*k5*k8 + Eo*k1*k4*k5*k6 +
```

```
Eo*k1*k4*k5*k7 + Eo*k1*k2*k7*k8 + Eo*k1*k4*k5*k8 +
Eo*k1*k4*k6*k7 + Eo*k1*k4*k6*k8 + Eo*k1*k5*k6*k7 +
Eo*k1*k4*k7*k8 + Eo*k1*k5*k6*k8 + Eo*k1*k5*k7*k8 +
Eo*k1*k6*k7*k8 + S*k2*k3*k4*k8 + S*k2*k3*k5*k8 + S*k3*k4*k6*k8
+ S*k3*k5*k6*k8 + Eo*S*k1*k2*k3*k4 + Eo*S*k1*k2*k3*k5 +
Eo*S*k1*k2*k3*k8 + Eo*S*k1*k3*k4*k6 + Eo*S*k1*k3*k5*k6 +
Eo*S*k1*k3*k4*k8 + Eo*S*k1*k3*k5*k8 + Eo*S*k1*k3*k6*k8

a5 = k2*k4*k5*k7*k8 + k4*k5*k6*k7*k8 + Eo*k1*k2*k4*k5*k7 +
Eo*k1*k2*k4*k5*k8 + Eo*k1*k2*k4*k7*k8 + Eo*k1*k2*k5*k7*k8 +
Eo*k1*k4*k5*k6*k7 + Eo*k1*k4*k5*k6*k8 + Eo*k1*k4*k5*k7*k8 +
Eo*k1*k4*k6*k7*k8 + Eo*k1*k5*k6*k7*k8 - Eo*S*k1*k2*k3*k4*k5 +
Eo*S*k1*k2*k3*k4*k8 + Eo*S*k1*k2*k3*k5*k8 +
Eo*S*k1*k3*k4*k6*k8 + Eo*S*k1*k3*k5*k6*k8

a6 = Eo*k1*k2*k4*k5*k7*k8 + Eo*k1*k4*k5*k6*k7*k8 -
Eo*S*k1*k2*k3*k4*k5*k8
```

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

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

$$J_A = \begin{bmatrix} -k_3S - k_7 - k_{10}A & 0 & k_2 & 0 & 0 & k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_2 - k_6 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\ k_7 & 0 & k_6 & -k_8 & 0 & 0 & 0 & 0 & 0 \\ k_3S & 0 & 0 & 0 & -k_4 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_4 & -k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\ -k_{10}A & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}, \tag{C.4}$$

and the Jacobian matrix at $(0,0,0,0,0,0,0,H,A)$ is

$$J_{H,A} = \begin{bmatrix} -k_3S - k_7 - k_{10}A & 0 & k_2 & 0 & 0 & k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_2 - k_6 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\ k_7 & 0 & k_6 & -k_8 & 0 & 0 & 0 & 0 & 0 \\ k_3S & 0 & 0 & 0 & -k_4 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_4 & -k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\ 0 & -k_{11}H & 0 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\ -k_{10}A & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}. \tag{C.5}$$

These two fixed points share a characteristic polynomial, which is

$$p(\lambda) = \lambda^9 + a_1\lambda^8 + a_2\lambda^7 + a_3\lambda^6 + a_4\lambda^5 + a_5\lambda^4 + a_6\lambda^3 \tag{C.6}$$

where

```
a1 = k2 + k4 + k5 + k6 + k7 + k8 + A*k10 + Eo*k1 + S*k3

a2 = k2*k4 + k2*k5 + k2*k7 + k4*k5 + k2*k8 + k4*k6 + k4*k7 +
k5*k6 + k4*k8 + k5*k7 + k5*k8 + k6*k7 + k6*k8 + k7*k8 + A*k2*k10
+ A*k4*k10 + A*k5*k10 + A*k6*k10 + A*k8*k10 + Eo*k1*k2 +
Eo*k1*k4 + Eo*k1*k5 + Eo*k1*k6 + Eo*k1*k7 + Eo*k1*k8 + S*k2*k3
+ S*k3*k4 + S*k3*k5 + S*k3*k6 + S*k3*k8 + A*Eo*k1*k10 +
Eo*S*k1*k3

a3 = k2*k4*k5 + k2*k4*k7 + k2*k4*k8 + k2*k5*k7 + k2*k5*k8 +
k4*k5*k6 + k4*k5*k7 + k2*k7*k8 + k4*k5*k8 + k4*k6*k7 +
k4*k6*k8 + k5*k6*k7 + k4*k7*k8 + k5*k6*k8 + k5*k7*k8 + k6*k7*k8
+ A*k2*k4*k10 + A*k2*k5*k10 + A*k4*k5*k10 + A*k2*k8*k10 +
A*k4*k6*k10 + A*k5*k6*k10 + A*k4*k8*k10 + A*k5*k8*k10 +
A*k6*k8*k10 + Eo*k1*k2*k4 + Eo*k1*k2*k5 + Eo*k1*k2*k7 +
Eo*k1*k4*k5 + Eo*k1*k2*k8 + Eo*k1*k4*k6 + Eo*k1*k4*k7 +
Eo*k1*k5*k6 + Eo*k1*k4*k8 + Eo*k1*k5*k7 + Eo*k1*k5*k8 +
Eo*k1*k6*k7 + Eo*k1*k6*k8 + Eo*k1*k7*k8 + S*k2*k3*k4 +
S*k2*k3*k5 + S*k2*k3*k8 + S*k3*k4*k6 + S*k3*k5*k6 + S*k3*k4*k8 +
S*k3*k5*k8 + S*k3*k6*k8 + A*Eo*k1*k2*k10 + A*Eo*k1*k4*k10 +
A*Eo*k1*k5*k10 + A*Eo*k1*k6*k10 + A*Eo*k1*k8*k10 +
Eo*S*k1*k2*k3 + Eo*S*k1*k3*k4 + Eo*S*k1*k3*k5 + Eo*S*k1*k3*k6
+ Eo*S*k1*k3*k8
```

```
a4 = k2*k4*k5*k7 + k2*k4*k5*k8 + k2*k4*k7*k8 + k2*k5*k7*k8 +
k4*k5*k6*k7 + k4*k5*k6*k8 + k4*k5*k7*k8 + k4*k6*k7*k8 +
k5*k6*k7*k8 + A*k2*k4*k5*k10 + A*k2*k4*k8*k10 + A*k2*k5*k8*k10
+ A*k4*k5*k6*k10 + A*k4*k5*k8*k10 + A*k4*k6*k8*k10 +
A*k5*k6*k8*k10 + Eo*k1*k2*k4*k5 + Eo*k1*k2*k4*k7 +
Eo*k1*k2*k4*k8 + Eo*k1*k2*k5*k7 + Eo*k1*k2*k5*k8 +
Eo*k1*k4*k5*k6 + Eo*k1*k4*k5*k7 + Eo*k1*k2*k7*k8 +
Eo*k1*k4*k5*k8 + Eo*k1*k4*k6*k7 + Eo*k1*k4*k6*k8 +
Eo*k1*k5*k6*k7 + Eo*k1*k4*k7*k8 + Eo*k1*k5*k6*k8 +
Eo*k1*k5*k7*k8 + Eo*k1*k6*k7*k8 + S*k2*k3*k4*k8 + S*k2*k3*k5*k8
+ S*k3*k4*k6*k8 + S*k3*k5*k6*k8 + A*Eo*k1*k2*k4*k10 +
A*Eo*k1*k2*k5*k10 + A*Eo*k1*k4*k5*k10 + A*Eo*k1*k2*k8*k10 +
A*Eo*k1*k4*k6*k10 + A*Eo*k1*k5*k6*k10 + A*Eo*k1*k4*k8*k10 +
A*Eo*k1*k5*k8*k10 + A*Eo*k1*k6*k8*k10 + Eo*S*k1*k2*k3*k4 +
Eo*S*k1*k2*k3*k5 + Eo*S*k1*k2*k3*k8 + Eo*S*k1*k3*k4*k6 +
Eo*S*k1*k3*k5*k6 + Eo*S*k1*k3*k4*k8 + Eo*S*k1*k3*k5*k8 +
Eo*S*k1*k3*k6*k8

a5 = k2*k4*k5*k7*k8 + k4*k5*k6*k7*k8 + A*k2*k4*k5*k8*k10 +
A*k4*k5*k6*k8*k10 + Eo*k1*k2*k4*k5*k7 + Eo*k1*k2*k4*k5*k8 +
Eo*k1*k2*k4*k7*k8 + Eo*k1*k2*k5*k7*k8 + Eo*k1*k4*k5*k6*k7 +
Eo*k1*k4*k5*k6*k8 + Eo*k1*k4*k5*k7*k8 + Eo*k1*k4*k6*k7*k8 +
Eo*k1*k5*k6*k7*k8 - Eo*S*k1*k2*k3*k4*k5 + Eo*S*k1*k2*k3*k4*k8 +
Eo*S*k1*k2*k3*k5*k8 + Eo*S*k1*k3*k4*k6*k8 + Eo*S*k1*k3*k5*k6*k8
+ A*Eo*k1*k2*k4*k5*k10 + A*Eo*k1*k2*k4*k8*k10 +
A*Eo*k1*k2*k5*k8*k10 + A*Eo*k1*k4*k5*k6*k10 +
A*Eo*k1*k4*k5*k8*k10 + A*Eo*k1*k4*k6*k8*k10 +
A*Eo*k1*k5*k6*k8*k10

a6 = Eo*k1*k2*k4*k5*k7*k8 + Eo*k1*k4*k5*k6*k7*k8 +
A*Eo*k1*k2*k4*k5*k8*k10 + A*Eo*k1*k4*k5*k6*k8*k10 -
Eo*S*k1*k2*k3*k4*k5*k8
```

C.3 Stability of $(0, E_6, 0, 0, 0, 0, 0, 0, 0)$

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

$$J_{E_6} = \begin{bmatrix} -k_3S - k_7 & 0 & k_2 & 0 & 0 & k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_2 - k_6 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\ k_7 & 0 & k_6 & -k_8 & 0 & 0 & 0 & 0 & 0 \\ k_3S & 0 & 0 & 0 & -k_4 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_4 & -k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & k_1E_o & -k_{11}E_6 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}. \tag{C.7}$$

The characteristic polynomial for this fixed point is

$$p(\lambda) = \lambda^9 + a_1\lambda^8 + a_2\lambda^7 + a_3\lambda^6 + a_4\lambda^5 + a_5\lambda^4 + a_6\lambda^3 + a_7\lambda^2 \tag{C.8}$$

where

```
a1 = k2 + k4 + k5 + k6 + k7 + k8 + E6*k11 + Eo*k1 + S*k3

a2 = k2*k4 + k2*k5 + k2*k7 + k4*k5 + k2*k8 + k4*k6 + k4*k7 +
k5*k6 + k4*k8 + k5*k7 + k5*k8 + k6*k7 + k6*k8 + k7*k8 +
E6*k2*k11 + E6*k4*k11 + E6*k5*k11 + E6*k6*k11 + E6*k7*k11 +
E6*k8*k11 + Eo*k1*k2 + Eo*k1*k4 + Eo*k1*k5 + Eo*k1*k6 +
Eo*k1*k7 + Eo*k1*k8 + S*k2*k3 + S*k3*k4 + S*k3*k5 + S*k3*k6
+ S*k3*k8 + E6*Eo*k1*k11 + E6*S*k3*k11 + Eo*S*k1*k3

a3 = k2*k4*k5 + k2*k4*k7 + k2*k4*k8 + k2*k5*k7 + k2*k5*k8 +
k4*k5*k6 + k4*k5*k7 + k2*k7*k8 + k4*k5*k8 + k4*k6*k7 +
k4*k6*k8 + k5*k6*k7 + k4*k7*k8 + k5*k6*k8 + k5*k7*k8 +
k6*k7*k8 + E6*k2*k4*k11 + E6*k2*k5*k11 + E6*k2*k7*k11 +
E6*k4*k5*k11 + E6*k2*k8*k11 + E6*k4*k6*k11 + E6*k4*k7*k11 +
E6*k5*k6*k11 + E6*k4*k8*k11 + E6*k5*k7*k11 + E6*k5*k8*k11 +
E6*k6*k7*k11 + E6*k6*k8*k11 + E6*k7*k8*k11 + Eo*k1*k2*k4 +
Eo*k1*k2*k5 + Eo*k1*k2*k7 + Eo*k1*k4*k5 + Eo*k1*k2*k8 +
Eo*k1*k4*k6 + Eo*k1*k4*k7 + Eo*k1*k5*k6 + Eo*k1*k4*k8 +
Eo*k1*k5*k7 + Eo*k1*k5*k8 + Eo*k1*k6*k7 + Eo*k1*k6*k8 +
Eo*k1*k7*k8 + S*k2*k3*k4 + S*k2*k3*k5 + S*k2*k3*k8 +
S*k3*k4*k6 + S*k3*k5*k6 + S*k3*k4*k8 + S*k3*k5*k8 +
S*k3*k6*k8 + E6*Eo*k1*k2*k11 + E6*Eo*k1*k4*k11 +
E6*Eo*k1*k5*k11 + E6*Eo*k1*k6*k11 + E6*Eo*k1*k7*k11 +
```

```
E6*Eo*k1*k8*k11 + E6*S*k2*k3*k11 + E6*S*k3*k4*k11 +
E6*S*k3*k5*k11 + E6*S*k3*k6*k11 + E6*S*k3*k8*k11 +
Eo*S*k1*k2*k3 + Eo*S*k1*k3*k4 + Eo*S*k1*k3*k5 +
Eo*S*k1*k3*k6 + Eo*S*k1*k3*k8 + E6*Eo*S*k1*k3*k11

a4 = k2*k4*k5*k7 + k2*k4*k5*k8 + k2*k4*k7*k8 + k2*k5*k7*k8 +
k4*k5*k6*k7 + k4*k5*k6*k8 + k4*k5*k7*k8 + k4*k6*k7*k8 +
k5*k6*k7*k8 + E6*k2*k4*k5*k11 + E6*k2*k4*k7*k11 +
E6*k2*k4*k8*k11 + E6*k2*k5*k7*k11 + E6*k2*k5*k8*k11 +
E6*k4*k5*k6*k11 + E6*k4*k5*k7*k11 + E6*k2*k7*k8*k11 +
E6*k4*k5*k8*k11 + E6*k4*k6*k7*k11 + E6*k4*k6*k8*k11 +
E6*k5*k6*k7*k11 + E6*k4*k7*k8*k11 + E6*k5*k6*k8*k11 +
E6*k5*k7*k8*k11 + E6*k6*k7*k8*k11 + Eo*k1*k2*k4*k5 +
Eo*k1*k2*k4*k7 + Eo*k1*k2*k4*k8 + Eo*k1*k2*k5*k7 +
Eo*k1*k2*k5*k8 + Eo*k1*k4*k5*k6 + Eo*k1*k4*k5*k7 +
Eo*k1*k2*k7*k8 + Eo*k1*k4*k5*k8 + Eo*k1*k4*k6*k7 +
Eo*k1*k4*k6*k8 + Eo*k1*k5*k6*k7 + Eo*k1*k4*k7*k8 +
Eo*k1*k5*k6*k8 + Eo*k1*k5*k7*k8 + Eo*k1*k6*k7*k8 +
S*k2*k3*k4*k8 + S*k2*k3*k5*k8 + S*k3*k4*k6*k8 + S*k3*k5*k6*k8
+ E6*Eo*k1*k2*k4*k11 + E6*Eo*k1*k2*k5*k11 +
E6*Eo*k1*k2*k7*k11 + E6*Eo*k1*k4*k5*k11 + E6*Eo*k1*k2*k8*k11
+ E6*Eo*k1*k4*k6*k11 + E6*Eo*k1*k4*k7*k11 +
E6*Eo*k1*k5*k6*k11 + E6*Eo*k1*k4*k8*k11 + E6*Eo*k1*k5*k7*k11
+ E6*Eo*k1*k5*k8*k11 + E6*Eo*k1*k6*k7*k11 + E6*Eo*k1*k6*k8*k11
+ E6*Eo*k1*k7*k8*k11 + E6*S*k2*k3*k4*k11 + E6*S*k2*k3*k5*k11 +
E6*S*k2*k3*k8*k11 + E6*S*k3*k4*k6*k11 + E6*S*k3*k5*k6*k11 +
E6*S*k3*k4*k8*k11 + E6*S*k3*k5*k8*k11 + E6*S*k3*k6*k8*k11 +
Eo*S*k1*k2*k3*k4 + Eo*S*k1*k2*k3*k5 + Eo*S*k1*k2*k3*k8 +
Eo*S*k1*k3*k4*k6 + Eo*S*k1*k3*k5*k6 + Eo*S*k1*k3*k4*k8 +
Eo*S*k1*k3*k5*k8 + Eo*S*k1*k3*k6*k8 + E6*Eo*S*k1*k2*k3*k11 +
E6*Eo*S*k1*k3*k4*k11 + E6*Eo*S*k1*k3*k5*k11 +
E6*Eo*S*k1*k3*k6*k11 + E6*Eo*S*k1*k3*k8*k11

a5 = k2*k4*k5*k7*k8 + k4*k5*k6*k7*k8 + E6*k2*k4*k5*k7*k11 +
E6*k2*k4*k5*k8*k11 + E6*k2*k4*k7*k8*k11 + E6*k2*k5*k7*k8*k11 +
E6*k4*k5*k6*k7*k11 + E6*k4*k5*k6*k8*k11 + E6*k4*k5*k7*k8*k11 +
E6*k4*k6*k7*k8*k11 + E6*k5*k6*k7*k8*k11 + Eo*k1*k2*k4*k5*k7 +
Eo*k1*k2*k4*k5*k8 + Eo*k1*k2*k4*k7*k8 + Eo*k1*k2*k5*k7*k8 +
Eo*k1*k4*k5*k6*k7 +  Eo*k1*k4*k5*k6*k8 + Eo*k1*k4*k5*k7*k8 +
Eo*k1*k4*k6*k7*k8 + Eo*k1*k5*k6*k7*k8 + E6*Eo*k1*k2*k4*k5*k11 +
```

```
E6*Eo*k1*k2*k4*k7*k11 + E6*Eo*k1*k2*k4*k8*k11 +
E6*Eo*k1*k2*k5*k7*k11 + E6*Eo*k1*k2*k5*k8*k11 +
E6*Eo*k1*k4*k5*k6*k11 + E6*Eo*k1*k4*k5*k7*k11 +
E6*Eo*k1*k2*k7*k8*k11 + E6*Eo*k1*k4*k5*k8*k11 +
E6*Eo*k1*k4*k6*k7*k11 + E6*Eo*k1*k4*k6*k8*k11 +
E6*Eo*k1*k5*k6*k7*k11 + E6*Eo*k1*k4*k7*k8*k11 +
E6*Eo*k1*k5*k6*k8*k11 + E6*Eo*k1*k5*k7*k8*k11 +
E6*Eo*k1*k6*k7*k8*k11 + E6*S*k2*k3*k4*k8*k11 +
E6*S*k2*k3*k5*k8*k11 + E6*S*k3*k4*k6*k8*k11 +
E6*S*k3*k5*k6*k8*k11 - Eo*S*k1*k2*k3*k4*k5 +
Eo*S*k1*k2*k3*k4*k8 + Eo*S*k1*k2*k3*k5*k8 + Eo*S*k1*k3*k4*k6*k8
+ Eo*S*k1*k3*k5*k6*k8 + E6*Eo*S*k1*k2*k3*k4*k11 +
E6*Eo*S*k1*k2*k3*k5*k11 + E6*Eo*S*k1*k2*k3*k8*k11 +
E6*Eo*S*k1*k3*k4*k6*k11 + E6*Eo*S*k1*k3*k5*k6*k11 +
E6*Eo*S*k1*k3*k4*k8*k11 + E6*Eo*S*k1*k3*k5*k8*k11 +
E6*Eo*S*k1*k3*k6*k8*k11

a6 = 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 +
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 -
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

a7 = E6*Eo*k1*k2*k4*k5*k7*k8*k11 +
E6*Eo*k1*k4*k5*k6*k7*k8*k11 - E6*Eo*S*k1*k2*k3*k4*k5*k8*k11
```

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

$$J_{E_6,A} = \begin{bmatrix} -k_3S - k_7 - k_{10}A & 0 & k_2 & 0 & 0 & k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -k_2 - k_6 & 0 & 0 & 0 & k_1E_o & 0 & 0 \\ k_7 & 0 & k_6 & -k_8 & 0 & 0 & 0 & 0 & 0 \\ k_3S & 0 & 0 & 0 & -k_4 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & k_4 & -k_5 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & k_5 & -k_1E_o & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & k_1E_o & -k_{11}E_6 & 0 \\ -k_{10}A & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix}. \tag{C.9}$$

The characteristic polynomial for this fixed point is

$$p(\lambda) = \lambda^9 + a_1\lambda^8 + a_2\lambda^7 + a_3\lambda^6 + a_4\lambda^5 + a_5\lambda^4 + a_6\lambda^3 + a_7\lambda^2 \tag{C.10}$$

where

```
a1 = k2 + k4 + k5 + k6 + k7 + k8 + A*k10 + E6*k11 + Eo*k1 +
S*k3

a2 = k2*k4 + k2*k5 + k2*k7 + k4*k5 + k2*k8 + k4*k6 + k4*k7 +
k5*k6 + k4*k8 + k5*k7 + k5*k8 + k6*k7 + k6*k8 + k7*k8 +
A*k2*k10 + A*k4*k10 + A*k5*k10 + A*k6*k10 + A*k8*k10 +
E6*k2*k11 + E6*k4*k11 + E6*k5*k11 + E6*k6*k11 + E6*k7*k11
+ E6*k8*k11 + Eo*k1*k2 + Eo*k1*k4 + Eo*k1*k5 + Eo*k1*k6 +
Eo*k1*k7 + Eo*k1*k8 + S*k2*k3 + S*k3*k4 + S*k3*k5 + S*k3*k6
+ S*k3*k8 + A*E6*k10*k11 + A*Eo*k1*k10 + E6*Eo*k1*k11 +
E6*S*k3*k11 + Eo*S*k1*k3

a3 = k2*k4*k5 + k2*k4*k7 + k2*k4*k8 + k2*k5*k7 + k2*k5*k8 +
k4*k5*k6 + k4*k5*k7 + k2*k7*k8 + k4*k5*k8 + k4*k6*k7 +
k4*k6*k8 + k5*k6*k7 + k4*k7*k8 + k5*k6*k8 + k5*k7*k8 +
k6*k7*k8 + A*k2*k4*k10 + A*k2*k5*k10 + A*k4*k5*k10 +
A*k2*k8*k10 + A*k4*k6*k10 + A*k5*k6*k10 + A*k4*k8*k10 +
A*k5*k8*k10 + A*k6*k8*k10 + E6*k2*k4*k11 + E6*k2*k5*k11 +
E6*k2*k7*k11 + E6*k4*k5*k11 + E6*k2*k8*k11 + E6*k4*k6*k11
+ E6*k4*k7*k11 + E6*k5*k6*k11 + E6*k4*k8*k11 + E6*k5*k7*k11
+ E6*k5*k8*k11 + E6*k6*k7*k11 + E6*k6*k8*k11 + E6*k7*k8*k11
+ Eo*k1*k2*k4 + Eo*k1*k2*k5 + Eo*k1*k2*k7 + Eo*k1*k4*k5 +
Eo*k1*k2*k8 + Eo*k1*k4*k6 + Eo*k1*k4*k7 + Eo*k1*k5*k6 +
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Eo*k1*k4*k8 + Eo*k1*k5*k7 + Eo*k1*k5*k8 + Eo*k1*k6*k7 +
Eo*k1*k6*k8 + Eo*k1*k7*k8 + S*k2*k3*k4 + S*k2*k3*k5 +
S*k2*k3*k8 + S*k3*k4*k6 + S*k3*k5*k6 + S*k3*k4*k8 + S*k3*k5*k8
+ S*k3*k6*k8 + A*E6*k2*k10*k11 + A*E6*k4*k10*k11 +
A*E6*k5*k10*k11 + A*E6*k6*k10*k11 + A*E6*k8*k10*k11 +
A*Eo*k1*k2*k10 + A*Eo*k1*k4*k10 + A*Eo*k1*k5*k10 +
A*Eo*k1*k6*k10 + A*Eo*k1*k8*k10 + E6*Eo*k1*k2*k11 +
E6*Eo*k1*k4*k11 + E6*Eo*k1*k5*k11 + E6*Eo*k1*k6*k11 +
E6*Eo*k1*k7*k11 + E6*Eo*k1*k8*k11 + E6*S*k2*k3*k11 +
E6*S*k3*k4*k11 + E6*S*k3*k5*k11 + E6*S*k3*k6*k11 +
E6*S*k3*k8*k11 + Eo*S*k1*k2*k3 + Eo*S*k1*k3*k4 +
Eo*S*k1*k3*k5 + Eo*S*k1*k3*k6 + Eo*S*k1*k3*k8 +
A*E6*Eo*k1*k10*k11 + E6*Eo*S*k1*k3*k11

a4 = k2*k4*k5*k7 + k2*k4*k5*k8 + k2*k4*k7*k8 + k2*k5*k7*k8 +
k4*k5*k6*k7 + k4*k5*k6*k8 + k4*k5*k7*k8 + k4*k6*k7*k8 +
k5*k6*k7*k8 + A*k2*k4*k5*k10 + A*k2*k4*k8*k10 + A*k2*k5*k8*k10
+ A*k4*k5*k6*k10 + A*k4*k5*k8*k10 + A*k4*k6*k8*k10 +
A*k5*k6*k8*k10 + E6*k2*k4*k5*k11 + E6*k2*k4*k7*k11 +
E6*k2*k4*k8*k11 + E6*k2*k5*k7*k11 + E6*k2*k5*k8*k11 +
E6*k4*k5*k6*k11 + E6*k4*k5*k7*k11 + E6*k2*k7*k8*k11 +
E6*k4*k5*k8*k11 + E6*k4*k6*k7*k11 + E6*k4*k6*k8*k11 +
E6*k5*k6*k7*k11 + E6*k4*k7*k8*k11 + E6*k5*k6*k8*k11 +
E6*k5*k7*k8*k11 + E6*k6*k7*k8*k11 + Eo*k1*k2*k4*k5 +
Eo*k1*k2*k4*k7 + Eo*k1*k2*k4*k8 + Eo*k1*k2*k5*k7 +
Eo*k1*k2*k5*k8 + Eo*k1*k4*k5*k6 + Eo*k1*k4*k5*k7 +
Eo*k1*k2*k7*k8 + Eo*k1*k4*k5*k8 + Eo*k1*k4*k6*k7 +
Eo*k1*k4*k6*k8 + Eo*k1*k5*k6*k7 + Eo*k1*k4*k7*k8 +
Eo*k1*k5*k6*k8 + Eo*k1*k5*k7*k8 + Eo*k1*k6*k7*k8 +
S*k2*k3*k4*k8 + S*k2*k3*k5*k8 + S*k3*k4*k6*k8 + S*k3*k5*k6*k8 +
A*E6*k2*k4*k10*k11 + A*E6*k2*k5*k10*k11 + A*E6*k4*k5*k10*k11
+ A*E6*k2*k8*k10*k11 + A*E6*k4*k6*k10*k11 + A*E6*k5*k6*k10*k11
+ A*E6*k4*k8*k10*k11 + A*E6*k5*k8*k10*k11 + A*E6*k6*k8*k10*k11
+ A*Eo*k1*k2*k4*k10 + A*Eo*k1*k2*k5*k10 + A*Eo*k1*k4*k5*k10 +
A*Eo*k1*k2*k8*k10 + A*Eo*k1*k4*k6*k10 + A*Eo*k1*k5*k6*k10 +
A*Eo*k1*k4*k8*k10 + A*Eo*k1*k5*k8*k10 + A*Eo*k1*k6*k8*k10 +
E6*Eo*k1*k2*k4*k11 + E6*Eo*k1*k2*k5*k11 + E6*Eo*k1*k2*k7*k11 +
E6*Eo*k1*k4*k5*k11 + E6*Eo*k1*k2*k8*k11 + E6*Eo*k1*k4*k6*k11 +
E6*Eo*k1*k4*k7*k11 + E6*Eo*k1*k5*k6*k11 + E6*Eo*k1*k4*k8*k11 +
E6*Eo*k1*k5*k7*k11 + E6*Eo*k1*k5*k8*k11 + E6*Eo*k1*k6*k7*k11 +
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E6*Eo*k1*k6*k8*k11 + E6*Eo*k1*k7*k8*k11 + E6*S*k2*k3*k4*k11 +
E6*S*k2*k3*k5*k11 + E6*S*k2*k3*k8*k11 + E6*S*k3*k4*k6*k11 +
E6*S*k3*k5*k6*k11 + E6*S*k3*k4*k8*k11 + E6*S*k3*k5*k8*k11 +
E6*S*k3*k6*k8*k11 + Eo*S*k1*k2*k3*k4 + Eo*S*k1*k2*k3*k5 +
Eo*S*k1*k2*k3*k8 + Eo*S*k1*k3*k4*k6 + Eo*S*k1*k3*k5*k6 +
Eo*S*k1*k3*k4*k8 + Eo*S*k1*k3*k5*k8 + Eo*S*k1*k3*k6*k8 +
A*E6*Eo*k1*k2*k10*k11 + A*E6*Eo*k1*k4*k10*k11
+ A*E6*Eo*k1*k5*k10*k11 + A*E6*Eo*k1*k6*k10*k11 +
A*E6*Eo*k1*k8*k10*k11 + E6*Eo*S*k1*k2*k3*k11 +
E6*Eo*S*k1*k3*k4*k11 + E6*Eo*S*k1*k3*k5*k11 +
E6*Eo*S*k1*k3*k6*k11 + E6*Eo*S*k1*k3*k8*k11

a5 = k2*k4*k5*k7*k8 + k4*k5*k6*k7*k8 + A*k2*k4*k5*k8*k10 +
A*k4*k5*k6*k8*k10 + E6*k2*k4*k5*k7*k11 + E6*k2*k4*k5*k8*k11 +
E6*k2*k4*k7*k8*k11 + E6*k2*k5*k7*k8*k11 + E6*k4*k5*k6*k7*k11 +
E6*k4*k5*k6*k8*k11 + E6*k4*k5*k7*k8*k11 + E6*k4*k6*k7*k8*k11 +
E6*k5*k6*k7*k8*k11 + Eo*k1*k2*k4*k5*k7 + Eo*k1*k2*k4*k5*k8 +
Eo*k1*k2*k4*k7*k8 + Eo*k1*k2*k5*k7*k8 + Eo*k1*k4*k5*k6*k7 +
Eo*k1*k4*k5*k6*k8 + Eo*k1*k4*k5*k7*k8 + Eo*k1*k4*k6*k7*k8 +
Eo*k1*k5*k6*k7*k8 + E6*Eo*k1*k2*k4*k5*k11 +
E6*Eo*k1*k2*k4*k7*k11 + E6*Eo*k1*k2*k4*k8*k11 +
E6*Eo*k1*k2*k5*k7*k11 + E6*Eo*k1*k2*k5*k8*k11 +
E6*Eo*k1*k4*k5*k6*k11 + E6*Eo*k1*k4*k5*k7*k11 +
E6*Eo*k1*k2*k7*k8*k11 + E6*Eo*k1*k4*k5*k8*k11 +
E6*Eo*k1*k4*k6*k7*k11 + E6*Eo*k1*k4*k6*k8*k11 +
E6*Eo*k1*k5*k6*k7*k11 + E6*Eo*k1*k4*k7*k8*k11 +
E6*Eo*k1*k5*k6*k8*k11 + E6*Eo*k1*k5*k7*k8*k11 +
E6*Eo*k1*k6*k7*k8*k11 + E6*S*k2*k3*k4*k8*k11 +
E6*S*k2*k3*k5*k8*k11 + E6*S*k3*k4*k6*k8*k11 +
E6*S*k3*k5*k6*k8*k11 - Eo*S*k1*k2*k3*k4*k5 +
Eo*S*k1*k2*k3*k4*k8 + Eo*S*k1*k2*k3*k5*k8 +
Eo*S*k1*k3*k4*k6*k8 + Eo*S*k1*k3*k5*k6*k8 +
A*E6*k2*k4*k5*k10*k11 + A*E6*k2*k4*k8*k10*k11 +
A*E6*k2*k5*k8*k10*k11 + A*E6*k4*k5*k6*k10*k11 +
A*E6*k4*k5*k8*k10*k11 + A*E6*k4*k6*k8*k10*k11 +
A*E6*k5*k6*k8*k10*k11 + A*Eo*k1*k2*k4*k5*k10 +
A*Eo*k1*k2*k4*k8*k10 + A*Eo*k1*k2*k5*k8*k10
+ A*Eo*k1*k4*k5*k6*k10 + A*Eo*k1*k4*k5*k8*k10
+ A*Eo*k1*k4*k6*k8*k10 + A*Eo*k1*k5*k6*k8*k10 +
A*E6*Eo*k1*k2*k4*k10*k11 + A*E6*Eo*k1*k2*k5*k10*k11 +
```

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

a6 = 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*k4*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

a7 = 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*k4*k5*k6*k8*k10*k11 - E6*Eo*S*k1*k2*k3*k4*k5*k8*k11
```

C.5 Applying the Routh-Hurwitz Criterion

The fixed points $(0,0,0,0,0,0,0,0,0)$, $(0,0,0,0,0,0,0,0,H,0)$, $(0,0,0,0,0,0,0,0,0,A)$, and $(0,0,0,0,0,0,0,0,H,A)$ result in a Routh array for $n = 6$.

The Routh array for the above fixed points is

$$J = \begin{bmatrix} a_6 & a_4 & a_2 & 0 \\ a_5 & a_3 & a_1 & 0 \\ A & B & 0 & 0 \\ C & D & 0 & 0 \\ E & 0 & 0 & 0 \\ D & 0 & 0 & 0 \\ D & 0 & 0 & 0 \end{bmatrix} \tag{C.11}$$

where

$$\begin{aligned} A &= \frac{a_5 a_4 - a_3 a_6}{a_5} \\ B &= \frac{a_3 a_2 - a_1 a_4}{a_5} \\ C &= a_3 - \frac{a_5 \left(a_3 a_2 - a_1 a_4\right)}{a_5 a_4 - a_3 a_6} \\ D &= \frac{a_1 \left(a_3 a_2 - a_1 a_4\right)}{a_5 a_4 - a_3 a_6} \\ E &= \frac{a_3 \left(a_1 a_4 - a_2 a_3\right) \left(a_1 a_6 - a_2 a_5 - a_3 a_6 + a_4 a_5\right)}{{a_3}^2 a_6 - a_1 a_4 a_5 + a_2 a_3 a_5 - a_3 a_4 a_5}, \end{aligned}$$

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 $n = 7$:

$$J = \begin{bmatrix} a_7 & a_5 & a_3 & a_1 & 0 \\ a_6 & a_4 & a_2 & 0 & 0 \\ A & B & C & 0 & 0 \\ D & E & 0 & 0 & 0 \\ F & G & 0 & 0 & 0 \\ H & 0 & 0 & 0 & 0 \\ G & 0 & 0 & 0 & 0 \\ G & 0 & 0 & 0 & 0 \end{bmatrix} \tag{C.12}$$

where

$$A = \frac{a_6 a_5 - a_4 a_7}{a_6}$$
$$B = \frac{a_4 a_3 - a_2 a_5}{a_6}$$
$$C = \frac{a_1 a_2}{a_6}$$
$$D = a_4 - \frac{a_6 \left(a_3 a_4 - a_2 a_5\right)}{a_5 a_6 - a_4 a_7}$$
$$E = \frac{a_2 \left(a_1 a_4 + a_2 a_5 - a_3 a_4\right)}{a_4 a_7 - a_5 a_6}$$
$$F = \frac{a_4 \left(a_2 \left(a_7 \left(a_2 a_5 - a_3 a_4 - a_4 a_5 + a_1 a_4\right) - a_5 a_6 \left(a_1 + a_3 - a_5\right)\right) + a_3 a_4 \left(a_3 a_6 + a_4 a_7 - a_5 a_6\right)\right)}{a_6 \left(a_4 \left(a_4 a_7 + a_3 a_6\right) - a_5 a_6 \left(a_2 + a_4\right)\right)}$$
$$G = \frac{-a_1 {a_2}^2 \left(a_1 a_4 + a_2 a_5 - a_3 a_4\right)}{\left(a_4 a_7 - a_5 a_6\right)^2}$$
$$H = \frac{EF - DG}{F}$$

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

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