

Abstract

ZAGER, MICHAEL GARY. Modeling the Distribution and Metabolism of the Phytoestrogen Genistein in Rats. (Committee Chair: Hien T. Tran.)

Genistein is an endocrine-active compound found naturally in soy products, thereby making its main exposure route in humans dietary. It has been linked to various health effects, both beneficial and adverse. The liver is a major site of genistein transformation. Experimental data suggest genistein is mostly metabolized in the liver into its glucuronide form, and taken back into the gut lumen via biliary excretion. The data show a nontrivial, dose dependent delay in biliary excretion of genistein. After initial modeling attempts, it became clear that traditional physiologically-based pharmacokinetic (PBPK) modeling methods fail to accurately describe the observed data. Hence, we have developed several models that incorporate techniques not typically found in PBPK models to specifically simulate the observed dynamics.

The first of these models developed is based on delay differential equations (DDE), where the observed lag in biliary excretion is mathematically described by a time delay. This delay is a function of the concentration of genistein and time, since the data may also suggest that the lag diminishes over time. Existence and uniqueness of a solution to this nonlinear system of state-dependent delay differential equations was obtained. Several parameters in the model are experimentally unknown, including parameters in the delay function. To use the model for the prediction of tissue dose, we obtained these parameters

via an inverse problem formulation using a modified maximum likelihood cost function. The unconstrained optimization problem was solved by a Nelder-Mead algorithm. The nonlinear system of state-dependent delay equations was approximated by a three-stage, implicit, Runge-Kutta method, which has been shown to be an effective numerical method for solving these types of equations. Using a statistical hypothesis test, we showed that the delay model with the optimal set of parameters obtained is a statistically significant improvement over the PBPK model in simulating the experimental data.

Our second modeling approach was taken by considering a dispersion modeling technique originally proposed by Roberts and Rowland (43). Traditional modeling approaches for the liver have generally used the “well-stirred” model to describe events occurring in the liver. The well-stirred model cannot accommodate spatial variations, such as enzyme activity, hepatic cell permeability, and cellular transport inhibition. Our objective in this research effort is to develop a fully functioning model for the liver that simulates the distribution, metabolism and excretion of chemicals, and is able to accommodate spatial variations in biologically- and physiologically-based parameters. Our modeling strategy considers the liver as a series of two cylindrical tubes, one representing the blood vessel space and one representing the bile duct space, with a space of hepatocytes in between. Dispersion coefficients are adjusted to create a biologically relevant distribution of the concentration of genistein and its metabolites in the liver. Upon developing the equations and boundary conditions for this model, we established existence and uniqueness of a solution to the system, and continuous dependence of the solution on the initial data. A numerical code based on the finite element method was developed to solve the mixed system of nonlinear ordinary and partial differential equations. Parameter estimations were obtained via an inverse problem formulation.

MODELING THE DISTRIBUTION AND METABOLISM OF THE PHYTOESTROGEN GENISTEIN IN RATS

BY

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In memory of my grandparents Robert and Sally Myers
and my dear friend Bill Carroll, I thank you.

Biography

The author was born and raised in the Hudson River Valley of New York. He attended the State University of New York at New Paltz, where he obtained the degree of Bachelor of Arts in mathematics. Then, after a brief absence from academia, he returned, receiving the degree of Master of Science in computational mathematics from North Carolina State University in 1999. Then in 2003 he received the degree of doctorate of philosophy in computational mathematics, also from North Carolina State University. He has since accepted a postdoctoral fellowship from the University of North Carolina at Chapel Hill Curriculum in Toxicology, and will be conducting research at the National Health and Environmental Effects Research Laboratory of the U.S. Environmental Protection Agency in Research Triangle Park, NC.

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The first person I need to thank will come as a story. The first summer of my graduate career was fastly approaching. After seeing my fellow students scurrying around finding internships and research assistantships, I realized my plans to go to Myrtle Beach for three months was foiled. A couple weeks after I joined the scurry, I had no prospective opportunities, and was quite concerned. I came back from a weekend visit with the family on a Monday morning to find a message on my office chalkboard that I need to contact Dr. H.T. Tran ASAP. His name rang a bell, since he was one of my professors at the time. After checking my email, I saw another message explaining that I need to see Dr. Tran ASAP. After walking to the Math Department office, I found a note in my mailbox saying that I need to see Dr. Tran ASAP. I started to wonder what I did wrong, and was curious as to whether I was getting kicked out of school after one semester. Shortly after I reluctantly walked into his office, I realized he wanted me to go see a scientist at a place called CIIT in Research Triangle Park about a possible summer internship. My anxiety turned to joy, and I was on my way there in less than 10 minutes. Five years later, I have completed the work I started that summer at CIIT, which has resulted in a Ph.D. in mathematics. If it were not for Dr. H.T. Tran, I would not have had the tremendous opportunity I have enjoyed. As

my advisor, he has instilled in me invaluable skills that will inevitably serve to progress my career. I can't thank him enough for what he has taught me. I might also mention he is one of the best professors of mathematics I have ever had.

The story continues as I would like to thank Dr. Paul M. Schlosser for taking me in as his summer intern at CIIT and working with me, a graduate student with one semester of experience. The road was long, and I will never be able to express the depth of my appreciation for his insight, patience and ability to show me how to model biology. But, we never did play that round of golf.

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that originally lit the mathematical flame. Unfortunately, I do not recall their first names, so they will forever be inscribed in this document as “Mr. Wells” and “Mr. Murphy”.

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Chapter 1

Introduction

The presence of endocrine-active compounds (EAC) in the environment has become a concern to environmental biologists and toxicologists. Researchers have suggested that numerous health effects, both adverse and beneficial, may be caused by exposures to these compounds. The suggestions stem from observations that include field studies on wildlife and human cultures (1; 2; 3), controlled experiments on laboratory animals and human volunteers (4), and critical literature reviews (32). Phytoestrogens, a class of EAC that are naturally plant-derived, bind to the estrogen receptor as either agonists or antagonists (5). Because these chemicals are plant-derived, exposure to them is primarily through the diet. In particular, the phytoestrogen genistein is a significant component of these dietary exposures (6), including soybean-derived foods with high genistein concentrations such as tofu and soy-derived infant formula (7). Genistein is believed to have a variety of effects ranging from health benefits like cancer prevention (4; 8; 9; 10) to adverse effects such as endocrine disruption and promotion of carcinogenic tumor growth (11; 12; 29). Consequently, the health effects of genistein have been extensively studied. Endocrine- and cancer-related effects have been found in studies of soy-based infant formula (30; 33). The United States Environmental Protection Agency developed a pilot study using genistein, among other EAC, as part of a proposed multimillion dollar Endocrine Disruption Screening Program

designed to screen thousands of EAC and their health effects (22). Epidemiological studies show that Asian cultures with diets rich in soy products have a lower incidence of breast cancer than western cultures (13; 14; 15). Experimental studies have shown that treatment with genistein suppresses mammary tumor growth in rats and humans (16; 17). Experimental prostate cancer treatments using genistein are currently underway in the United States (18). Other studies have shown that genistein produces adverse endocrine-related effects in rats such as low birth weights and reduced anogenital distances (11; 12; 19). The National Toxicology Program conducted a multigenerational toxicity study on genistein in rats, and various additional studies are currently underway to complement that work. These studies include effects of neonatal treatment of genistein on mice and hormonal effects of dietary administration of genistein on rats (30; 31; 33).

Enterohepatic circulation is a process by which a chemical travels through the intestinal wall and into the liver via the portal vein. A portion of the chemical may subsequently re-enter the intestine via the bile duct after passing through the liver. It is possible for that portion, perhaps in part, to be re-absorbed through the intestinal wall and return to the liver. This process can continue indefinitely. For a thorough explanation of enterohepatic circulation, the reader is referred to (50). Genistein has been shown to undergo enterohepatic circulation (20; 21) and is extensively metabolized via glucuronidation. Most of the genistein excreted in the bile is in its glucuronidated form (20; 21). There is a period of time (delay) while genistein passes down the bile duct, when it is not available for re-absorption. Once excreted into the intestine, it is quickly cleaved from the conjugated form back into its parent form of pure genistein, after which it can be re-absorbed. We initially developed a preliminary PBPK model for rats that includes the simulation of biliary excretion (26). In attempting to calibrate the model, we observed that one of the data sets strongly suggests a concentration-dependent lag in biliary excretion of genistein (20). In particular, it appears that higher concentrations of genistein increase the time it takes for genistein to transit the

bile-duct, but that this increase in the delay is only temporary; i.e., there is an initial, dose-dependent reduction in biliary flow (increasing the delay time), but this response dissipates with time, until flow and the delay return to normal. Thus there is a transitory increase in the delay due to transit of the bile duct, which then decays back to its basal value, presumably as the tissues acclimate to the presence of genistein. The material excreted into the bile duct reaches the GI tract, where it becomes available for re-absorption (after this delay).

Physiologically based pharmacokinetic (PBPK) models are often developed to simulate the general dosimetry of a chemical. Due to the apparently intricate nature of the biliary excretion of genistein, PBPK models for the dosimetry of genistein have been difficult to develop based on traditional PBPK modeling approaches. The ordinary differential equations (ODE) used in standard PBPK models are unable to simulate such a delay without treating the liver in a special way, such as a series of compartments. That approach is really intermediary between two more ideal extremes: treating the biliary transit as a pure delay process, corresponding to plug-flow, and developing a fluid dispersion model using partial differential equations (PDEs). Numerical implementation of PDEs is more complex than including a simple delay, and it would be less straightforward to integrate PDEs with the ODEs describing the other physiological compartments. Since it also seemed possible that a simple delay could adequately describe the data, we therefore decided to first try that simpler approach.

In Chapter 2, we develop state-dependent delay differential equations (DDE) to incorporate the compartments and parameters necessary to simulate biliary excretion under the experimental conditions of the data set described in Sfakianos, et al. (20). The values of the unknown parameters are estimated by fitting the model to the biliary excretion data. Since the incorporation of any delay approximation increases the complexity of the model and number of parameters over what would exist without that, the statistical significance of the delay terms are tested, but in comparison to a standard model which uses neither DDEs

nor the rough compartmental approximation mentioned above. (That standard model is obtained simply by setting the delays in the DDE model to zero.) Theoretical existence and uniqueness of a solution to the equations and initial conditions is also established.

Then in Chapter 3, we develop and calibrate the fluid dispersion model, based on the work of Roberts and Rowland (43). We establish well-posedness of the model in Chapter 4, and present preliminary parameter estimation results in Chapter 5, including curve fittings for single dose and multiple-dose data sets. In Chapter 6 we discuss the performance of both models developed in this dissertation, issues and future directions of each model, including the possibility of the introduction of state-dependent parameters in the dispersion model.

Chapter 2

A Delayed Nonlinear PBPK Model

2.1 Model Development

In the work presented in Sfakianos et al. (20), rats were lightly anesthetized, fitted with bile duct cannulas to collect biliary excretions, and infused in the portal vein with one of four different dose rates of ^{14}C -labeled genistein dissolved in rat serum for 1 h. Measurements of the rate of excretion of ^{14}C -radioactivity were taken every 5 min from the beginning of the infusion through 65 min (Figure 2.1). Figure 2.1B is a magnification of the first 20 min into the infusions. Genistein first appears in the bile at the third measurement taken, 10 min (0.17 h) after the beginning of the infusion. At this time point, the *lowest* infusion rate yields the *highest* rate of genistein excretion. This trend continues through 15 min of infusion, and then after 45 min of infusion the highest infusion rate finally yields the highest excretion rate. Thus there is a delay between the start of the infusion and the time when the excretion rate responds proportionately to the dose.

The significance in these data is that Sfakianos (20) reports the only *in vivo* genistein experiment involving biliary excretion in the literature that includes multiple doses and time intervals in measurement short enough to capture the observed delay. All other published biliary excretion experiments are in time intervals of 20 min or longer, and no other

experiment includes more than one dose. Clearly, this delay would be very difficult to observe with time intervals as large as 20 min, and nearly impossible with a single genistein dose (Figure 2.1). Given the above experimental conditions, we formulated the model as

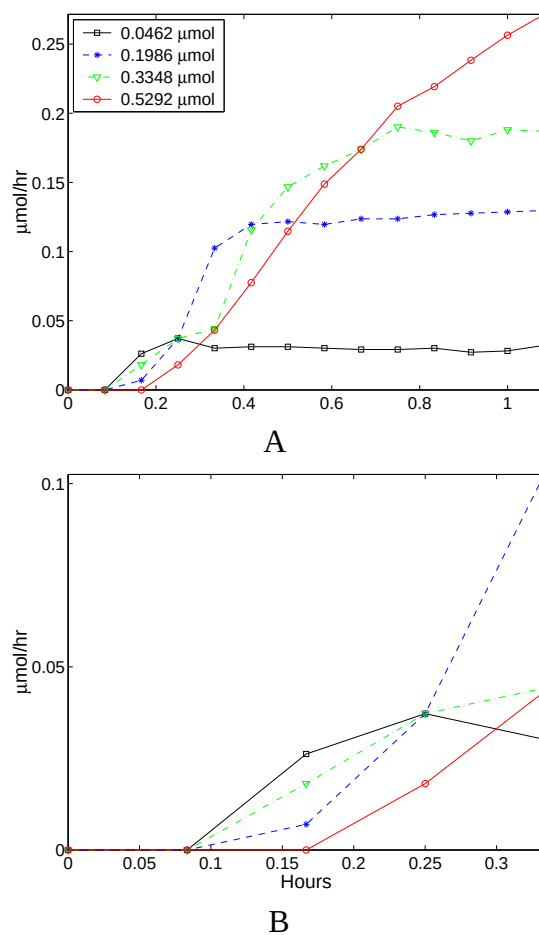


Figure 2.1: Dose- (or concentration-) dependent lag evident in genistein biliary excretion rate (20). Figure 2.1B is a magnification of Figure 2.1A, focusing on the first 20 min of reported excretion.

follows. Although active transport and elimination rates are likely saturable, we do not have data for these rates. Therefore, we use linear terms in the model as an approximation for these rates. In particular, the transport rates depend on the blood flow rates through the

particular tissues of each compartment. The values used for the blood flows were obtained from (23). The values for the partition coefficients were obtained from Plowchalk (24). The volumes of each compartment were calculated from the formula $volume = \frac{mass}{density}$ using values for densities and percentages of body mass listed in ILSI (23). Values and abbreviations used for the volumes are listed in Table 2.2. The elimination rates for the model are currently unavailable in the literature and therefore must be estimated. The abbreviations used for these rates are also given in Table 2.2. The values are listed as “estimated” and are provided in Section 2.3.

Experimental data suggest that genistein conjugation is saturable (20), that is, the rate at which conjugation occurs increases with the concentration of the compound being conjugated, but at high concentrations the rate asymptotically approaches an upper bound. Therefore Michaelis-Menten kinetics characterized by the parameters V_{max} and k_m are used to describe the rate. However, the values of these parameters are not available in the literature and must be estimated. V_{max} and k_m are usually approximated directly from the given data. But in this case, due to the delay present in the Sfakianos data, attempting to approximate these parameters using that data will potentially lead to poor estimates. Therefore, we chose to estimate the Michaelis-Menten parameters via the inverse problem discussed in Section 2.3. These parameters are listed in Table 2.2 as “estimated” and the values are provided in Section 2.3.

In the Sfakianos study, the authors reported that genistein is excreted in the bile, the vast majority of which is in its glucuronidated form, and they only report data for this metabolite. Although this and other studies suggest that traces of pure genistein and genistein sulfate may be found in the bile, they are negligible amounts (21) so we chose to ignore excretion of unconjugated genistein in the model. Another study has shown that genistein excreted in urine is in its pure and sulfated forms (21). Hence we have urinary elimination routes for pure and conjugated genistein in our model. We do not distinguish between the glucuronide and sulfate conjugates in the model.

With the above assumptions, the DDE model is split into two submodels; one part tracks pure genistein, and the other tracks conjugated genistein. Our resulting model is depicted in Figure 2.2, and the abbreviations used are defined in Table 2.1. The equations describing the model have the form:

$$\begin{aligned}
\frac{dC_{gen}^{pp}}{dt} &= \frac{Q_{pp}C_{gen}^B - \frac{Q_{pp}\mathcal{H}(C_{gen}^{pp})C_{gen}^{pp}}{P_{pp}}}{V_{pp}}, \\
\frac{dC_{gen}^{rp}}{dt} &= \frac{Q_{rp}C_{gen}^B - \frac{Q_{rp}\mathcal{H}(C_{gen}^{rp})C_{gen}^{rp}}{P_{rp}}}{V_{rp}} - k_{urine}^{gen}\mathcal{H}(C_{gen}^{rp})C_{gen}^{rp}, \\
\frac{dC_{gen}^l}{dt} &= \frac{Q_lC_{gen}^B - \frac{(Q_l+Q_{GI})\mathcal{H}(C_{gen}^l)C_{gen}^l}{P_l} + v_{inf}(t) + \frac{Q_{GI}\mathcal{H}(C_{gen}^{GI})C_{gen}^{GI}}{P_{rp}}}{V_l} - \frac{V_{max}\mathcal{H}(C_{gen}^l)C_{gen}^l}{k_m + \mathcal{H}(C_{gen}^l)C_{gen}^l}, \\
\frac{dC_{gen}^{GI}}{dt} &= \frac{Q_{GI}C_{gen}^B - \frac{Q_{GI}\mathcal{H}(C_{gen}^{GI})C_{gen}^{GI}}{P_{rp}}}{V_{GI}}, \\
\frac{dC_{con}^l}{dt} &= \frac{V_{max}\mathcal{H}(C_{gen}^l)C_{gen}^l}{k_m + \mathcal{H}(C_{gen}^l)C_{gen}^l} - k_{bile}\mathcal{H}(C_{con}^l)C_{con}^l - \frac{(Q_l+Q_{GI})(\mathcal{H}(C_{con}^l)C_{con}^l - \mathcal{H}(C_{con}^{ROB})C_{con}^{ROB})}{V_l}, \\
\frac{dC_{con}^{ROB}}{dt} &= \frac{(Q_l+Q_{GI})(\mathcal{H}(C_{con}^l)C_{con}^l - \mathcal{H}(C_{con}^{ROB})C_{con}^{ROB})}{V_{ROB}} - k_{urine}^{con}\mathcal{H}(C_{con}^{ROB})C_{con}^{ROB}, \\
\frac{dA_{con}^b}{dt} &= k_{bile}\mathcal{H}(C_{con}^l)C_{con}^l(t - d(t, C_{gen}^l))V_l, \\
C_{gen}^B &= \frac{\frac{Q_{rp}\mathcal{H}(C_{gen}^{rp})C_{gen}^{rp}}{P_{rp}} + \frac{Q_{pp}\mathcal{H}(C_{gen}^{pp})C_{gen}^{pp}}{P_{pp}} + \frac{(Q_l+Q_{GI})\mathcal{H}(C_{gen}^l)C_{gen}^l}{P_l}}{Q_t},
\end{aligned} \tag{2.1}$$

where the Heaviside function

$$\mathcal{H}(x) = \begin{cases} 1 & x \geq 0, \\ 0 & x < 0, \end{cases}$$

is adjoined to all state variables appearing on the right hand sides of the equations. The purpose of this is made clear in the next section. Justification for the introduction of the Heaviside function is in the fact that, in terms of the biology of the model, all concentrations of genistein and its conjugates are nonnegative for all times t . The abbreviations used for the rates, volumes, and other terms are defined in Table 2.2. Note that the volumes 0.012 L and 0.014 L for “blood” and “contents of GI tract” compartments, respectively, added to

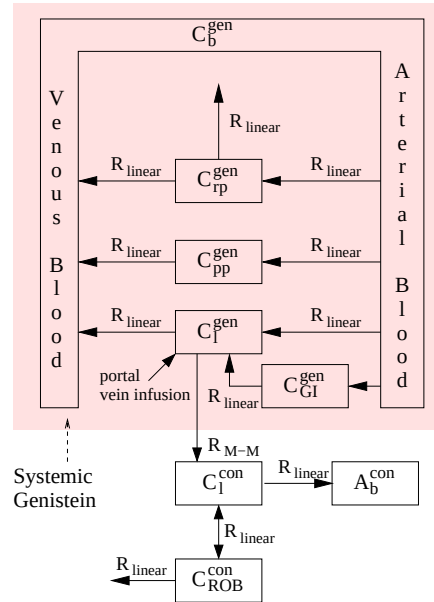


Figure 2.2: Genistein PBPK model with a state-dependent delay in the biliary excretion compartment (see Table 2.1 for descriptions of abbreviations).

the volumes in Table 2.2 for compartments tracking pure genistein in the model complete the mass (or volume) balance. (Blood volume is not listed in the table.) The volume for the ROB compartment (used in tracking genistein conjugates in the model) is obtained by subtracting the liver volume from the total rat volume.

We now describe the formulation of the venous infusion function $v_{inf}(t)$. When a chemical is infused into the portal vein, there is obviously a small but nonzero amount of time between the beginning of the infusion and the time at which the chemical reaches the liver at full dose, due to dispersion of the chemical in the bloodstream. A similar result occurs at the end of the infusion. Therefore we formulated the continuous function describing the venous infusions, $v_{inf}(t)$, by using a piecewise-defined cubic spline to capture the basic dynamics of this type of infusion. The formulation is as follows. A constant term c_v is multiplied to the spline function, where c_v is the constant venous infusion rate of GEN.

Table 2.1: Abbreviations defined for PBPK model.

Abbreviation	Description
C_{gen}^B	Concentration of pure genistein in plasma ($\mu\text{mol/hr}$)
C_{gen}^{rp}	Concentration of pure genistein in richly perfused tissues
C_{gen}^{pp}	Concentration of pure genistein in poorly perfused tissues
C_{gen}^l	Concentration of pure genistein in liver
C_{gen}^{GI}	Concentration of pure genistein in gastro-intestinal (GI) tissue
C_{con}^l	Concentration of genistein conjugates in liver
C_{con}^{ROB}	Concentration of genistein conjugates in rest of body (ROB)
A_{con}^b	Amount of genistein conjugates in bile - delayed compartment (μmol)
R_{linear}	Linear rate constant - see Figure 2.2 (hr^{-1})
R_{M-M}	Nonlinear (Michaelis-Menton) rate - see Figure 2.2 (hr^{-1})

The spline is defined as:

$$v_{inf}(t) = \begin{cases} c_v F(t) & 0 \leq t < \epsilon, \\ c_v G(t) & \epsilon \leq t \leq 1 - \epsilon, \\ c_v H(t) & 1 - \epsilon < t \leq 1, \\ c_v I(t) & 1 < t \leq 1 + \frac{5}{60}, \end{cases}$$

where

$$F(t) = \frac{-2}{\epsilon^3}t^3 + \frac{3}{\epsilon^2}t^2.$$

We first note that $F(0) = 0$, $\dot{F}(0) = \dot{F}(\epsilon) = 0$, and $F(\epsilon) = 1$; $G(t) = 1$, clearly with $G(\epsilon) = G(1 - \epsilon) = 1$ and $\dot{G}(\epsilon) = \dot{G}(1 - \epsilon) = 0$. In addition, the function $H(t)$ is given by

$$H(t) = \frac{2}{\epsilon^3}t^3 + \frac{3}{\epsilon^3}(\epsilon - 2)t^2 - \frac{6}{\epsilon^3}(\epsilon - 1)t + \frac{1}{\epsilon^3}(3\epsilon - 2),$$

where $H(1 - \epsilon) = 1$, $\dot{H}(1 - \epsilon) = \dot{H}(1) = 0$, and $H(1) = 0$. $I(t) = 0$, obviously with all values $\dot{I}$ equal to zero. The value of ϵ is chosen to accurately represent a typical venous

Table 2.2: Abbreviations defined for model equations.

Abbreviation	Description	Value
m_{rat}	Mass of rat (value from (20))	0.25 (kg)
Q_{pp}	Blood flow rate through poorly perfused tissue	$0.528Q_t$ (L/hr)
Q_{rp}	Blood flow rate through richly perfused tissue	$0.289Q_t$ (L/hr)
Q_l	Blood flow rate through liver	$0.03Q_t$ (L/hr)
Q_{GI}	Blood flow rate through GI tissue	$0.153Q_t$ (L/hr)
Q_t	Blood flow rate through heart (cardiac output)	$14.1(m_{rat})^{0.75}$ (L/hr)
P_{pp}	Blood/tissue partition coefficient for poorly perfused tissue	0.59
P_{rp}	Blood/tissue partition coefficient for richly perfused tissue	1.94
P_l	Blood/tissue partition coefficient for liver	3.61
V_{pp}	Volume of poorly perfused tissue	0.188 (L)
V_{rp}	Volume of richly perfused tissue	0.012 (L)
V_l	Volume of liver	0.0092 (L)
V_{GI}	Volume of GI tissue	0.011 (L)
V_{ROB}	Volume of ROB	0.2408 (L)
k_{urine}^{gen}	Linear rate term for pure genistein excretion in urine	estimated (hr^{-1})
k_{urine}^{con}	Linear rate term for genistein conjugate excretion in urine	estimated (hr^{-1})
k_{bile}	Linear rate term for genistein conjugate excretion in bile	estimated (hr^{-1})
V_{max}, k_m	Michaelis-Menton rate terms for saturable conjugation of genistein in liver	estimated ($\mu mol/L/hr, \mu mol/L$)
c_v	Venous infusion rate of pure genistein	varies (see Figure 2.1) ($\mu mol/hr$)
δ	Constant term used in adjusting venous infusion function $v_{inf}(t)$	500

Table 2.3: Abbreviations defined for model equations.

Abbreviation	Description	Value
t_{basal}	Constant term used as a basal biliary excretion delay in delay function $d(t, C_{gen}^l)$	estimated (hr)
c_{gen}	Constant term used as a genistein-specific delay in delay function $d(t, C_{gen}^l)$	estimated ($L \cdot hr / \mu mol$)
c_t	Constant term used in delay function $d(t, C_{gen}^l)$	estimated (hr)

infusion under the experimental conditions outlined in (20). The function is depicted in Figure 2.3. We now formulate the delay function $d(t, C_{gen}^l)$, which is given by

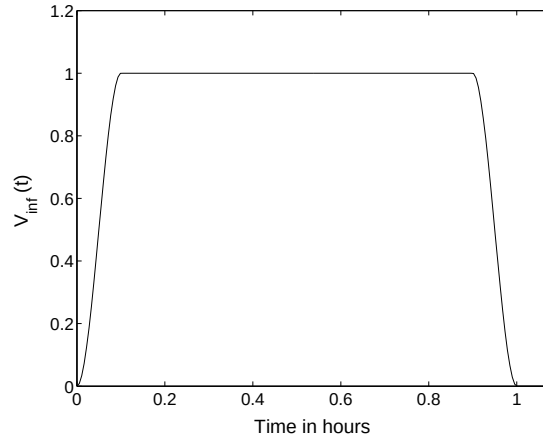


Figure 2.3: Venous infusion function $V_{inf}(t)$ with $c_v = 1$ and $\varepsilon = 0.1$. These values are used here for demonstration purposes and are not necessarily used in the model.

$$d(t, C_{gen}^l) = t_{basal} + c_{gen} \mathcal{H}(C_{gen}^l(t)) C_{gen}^l(t) e^{-\frac{t}{c_t}}. \quad (2.2)$$

By the nature of a bile duct cannulation, there is inevitably a delay in time from the beginning of biliary excretion of a chemical to the point where the bile has passed through

the complex array of bile duct canaliculi, the main bile duct, down the bile duct catheter, and finally into the collection cannula. This delay is labeled t_{basal} . Added to that constant delay is a genistein-specific constant c_{gen} , multiplied by the concentration of pure genistein ($C_{gen}^l(t)$) and a time-decaying term $e^{-\frac{t}{\alpha}}$. This last term is added because the Sfakianos data suggest that the delay diminishes after a certain amount of time has elapsed during the infusions, as seen in Figure 2.1. These considerations are taken into account in the formulation of the state-dependent delay function (2.2). The parameters t_{basal} , c_{gen} , and c_t are unknown and must be estimated. The values for these parameters are listed in Table 2.3 as “estimated” and are provided in Section 2.3.

Finally, the algebraic expression for the blood compartment C_{gen}^B is obtained by assuming a pseudo steady-state for the differential equation describing that compartment. Setting the equation equal to zero allows for an explicit solution for C_{gen}^B . This practice is standard in PBPK modeling and has the advantage of reducing the dimension of the system of equations by one. Computationally, this means our system of equations (2.1) can be numerically solved more efficiently.

2.2 Existence and Uniqueness

2.2.1 Local Existence and Uniqueness

In this section we prove local existence and uniqueness of a solution to our nonlinear state-dependent DDE based on the work of Driver (25). In his article, Driver presents a theorem for local existence and uniqueness of solutions to DDE of the type

$$\dot{y}_i(t) = f_i(t, \vec{y}(t), \vec{y}(g_2(t, \vec{y}(t))), \dots, \vec{y}(g_m(t, \vec{y}(t)))), \quad (2.3)$$

for $i = 1, \dots, n$, and each $g_j(t, \vec{y}(t)) \leq t$, $j = 2, \dots, m$.

If we define $g_1(t, \vec{y}(t)) \equiv t$, the above equation (2.3) can be written in vector form as follows:

$$\dot{\vec{y}}(t) = \vec{f}(t, \vec{y}(\vec{g}(t, \vec{y}(t)))),$$

where

$$\vec{y}(\vec{g}(t, \vec{y}(t))) = (\vec{y}(g_1(t, \vec{y}(t))), \vec{y}(g_2(t, \vec{y}(t))), \dots, \vec{y}(g_m(t, \vec{y}(t)))).$$

For the following development, we now introduce additional notation. We begin by defining

$$(t, Y) = (t, y_{11}, \dots, y_{1n}, y_{21}, \dots, y_{2n}, \dots, y_{m1}, \dots, y_{mn})$$

to be a vector in R^{mn+1} . Let $(t, \vec{y}) = (t, y_{11}, \dots, y_{1n})$ be the projection of (t, Y) into R^{n+1} , the space of the first $n+1$ coordinates of R^{mn+1} . Define

$$f(t, Y) = (f_1(t, Y), \dots, f_n(t, Y))$$

to be a real, n -dimensional, vector valued function over a domain $D \subset R^{mn+1}$, where D is open and connected. Let

$$\vec{g}(t, \vec{y}) = (g_1(t, \vec{y}), \dots, g_m(t, \vec{y}))$$

with $g_1(t, \vec{y}) = t$ be a real, m -dimensional, vector-valued function over domain D^* , the projection of D into R^{n+1} . For a vector $v = (v_1, \dots, v_n) \in R^n$, we define the norm $\|v\|$ by

$$\|v\| = \max_{1 \leq i \leq k} |v_i|,$$

where k can vary, depending on the dimension of the space. We will not emphasize what value k is when using the norm symbols, since it should be clear.

For a vector-valued function $\vec{h}(t, \vec{z})$ let the notation $\vec{h}(t, \vec{z}) \in (C, Lip)$ mean that throughout a region R , $\vec{h}(t, \vec{z})$ is continuous with respect to all variables and satisfies the Lipschitz condition $\|\vec{h}(t, \vec{z}) - \vec{h}(t, \hat{z})\| \leq A\|\vec{z} - \hat{z}\|$ whenever $(t, \vec{z}), (t, \hat{z}) \in R \times R^c$, where A is the Lipschitz constant and c is a positive integer.

Using these definitions, we now pose the following problem:

Problem P: Let $f(t, Y)$ be defined over the domain D and let $\vec{g}(t, \vec{y})$ be defined over D^* , with $g_1(t, \vec{y}) \equiv t$. For a fixed $t_0 \in R$, let $\alpha \in [-\infty, t_0]$ be some fixed constant. Let $\phi(t) = (\phi_1(t), \dots, \phi_n(t))$ be a real, n -dimensional, vector-valued function of t for $\alpha \leq t \leq t_0$. In the case that $\alpha = -\infty$, change all expressions of the form $\alpha \leq \dots$, here and throughout this section, into $\alpha < \dots$. Now, assuming that

- (i) $(t_0, \phi(t_0)) \in D^*$
- (ii) $\alpha \leq g_j(t_0, \phi(t_0)) \leq t_0$ for $j = 2, \dots, m$, and
- (iii) $(t_0, \phi(g(t_0, \phi(t_0)))) \in D$,

we seek a real, n -dimensional, vector-valued function $\vec{y}(t) = (y_1(t), \dots, y_n(t))$, defined for $\alpha \leq t < \beta \leq \infty$, such that

- (a) $\vec{y}(t) = \phi(t)$ for $\alpha \leq t \leq t_0$,
- (b) $\vec{y}(t)$ is continuous for $t_0 \leq t < \beta$,
- (c) $(t, \vec{y}(t)) \in D^*$ for $t_0 \leq t < \beta$,
- (d) $\alpha \leq g_j(t, \vec{y}(t)) \leq t$ for $t_0 \leq t < \beta, j = 1, \dots, m$,
- (e) $(t, y(g(t, \vec{y}(t)))) \in D$ for $t_0 \leq t < \beta$, and
- (f) $\dot{\vec{y}}(t) = \vec{f}(t, \vec{y}(g(t, \vec{y}(t))))$ for $t_0 \leq t < \beta$.

The following local existence and uniqueness result was proven in (25):

Theorem 2.1 Let $f(t, Y) \in (C, Lip)$ in each compact subset of D , let $\vec{g}(t, \vec{y}) \in (C, Lip)$ in each compact subset of D^* , let $\alpha \leq g_j(t, \vec{y}) \leq t$ for all $(t, \vec{y}) \in D^* \cap \{t \geq t_0\}$ ($j = 2, \dots, m$),

and let $\phi(t)$ be Lipschitz continuous on each finite subinterval of $[\alpha, t_0]$. Then there is a number $h > 0$ such that a unique solution of P exists for $\alpha \leq t < t_0 + h$.

To apply the above local existence and uniqueness result to our problem, we state several assumptions and observations about our model and introduce appropriate notation for our model equations, variables, and functions to correspond to the notation used above.

For our model, the initial condition is given by $\phi(t) = \vec{0}$ where $\phi : D_\phi \rightarrow R^7$ with $D_\phi = (-\infty, 0]$.

Defining $Y = (y_{11}, \dots, y_{17}, y_{21}, \dots, y_{27})$ to be a vector in R^{14} , and $\vec{y} = (y_{11}, \dots, y_{17})$, a vector in R^7 . That is, $y_{11}, \dots, y_{17}$ correspond to the 7 state variables of our model and $y_{21}, \dots, y_{27}$ correspond to the 7 state variables delayed by an amount of time admitted by the function $d(t, C_{gen}^l)$ defined in Section 2.1.

Now let $\vec{g} : D^* \rightarrow R \times R$ be defined by $\vec{g}(t, \vec{y}) = (g_1(t, \vec{y}), g_2(t, \vec{y}))$, where $g_1(t, \vec{y}) = t$, $g_2(t, \vec{y}) = t - d(t, C_{gen}^l)$, and the domain D^* is defined as follows:

$$D^* = R \times R^7.$$

If we define the following variables

$$\begin{aligned} y_{11} &= C_{gen}^{pp}(g_1(t, \vec{y})) & y_{21} &= C_{gen}^{pp}(g_2(t, \vec{y})) \\ y_{12} &= C_{gen}^{rp}(g_1(t, \vec{y})) & y_{22} &= C_{gen}^{rp}(g_2(t, \vec{y})) \\ y_{13} &= C_{gen}^l(g_1(t, \vec{y})) & y_{23} &= C_{gen}^l(g_2(t, \vec{y})) \\ y_{14} &= C_{gen}^{GI}(g_1(t, \vec{y})) & y_{24} &= C_{gen}^{GI}(g_2(t, \vec{y})) \\ y_{15} &= C_{con}^l(g_1(t, \vec{y})) & y_{25} &= C_{con}^l(g_2(t, \vec{y})) \\ y_{16} &= C_{con}^{ROB}(g_1(t, \vec{y})) & y_{26} &= C_{con}^{ROB}(g_2(t, \vec{y})) \\ y_{17} &= A_{con}^b(g_1(t, \vec{y})) & y_{27} &= A_{con}^b(g_2(t, \vec{y})), \end{aligned}$$

then the system (2.1) takes the form $\dot{\vec{y}} = \vec{f}(t, Y)$, where

$$\vec{f}(t, Y) = (f_1(t, Y), \dots, f_7(t, Y)). \quad (2.4)$$

Note that $\vec{f}(t, Y)$ is a real, 7-dimensional, vector-valued function over an open, connected subset D of $R \times R^{14}$ defined as follows:

$$D = R \times R^{14}.$$

We now state some trivial facts about our functions $\vec{f}(t, Y)$, $\vec{g}(t, \vec{y})$ and $\vec{\phi}(t)$ that are needed in order to apply Theorem 2.1. Note that we will drop the vector notation in the expressions for these functions from this point on. Also note the following:

$$(0, \phi(0)) \in D^*,$$

$$-\infty < g_2(0, \phi(0)) \leq 0,$$

$$(0, \phi(g_1(0, \phi(0))), \phi(g_2(0, \phi(0)))) \in D.$$

Also, since $\phi(t) = 0$ for $t \in D_\phi$, it follows that $\phi(t)$ is Lipschitz continuous on each finite subinterval of D_ϕ . Furthermore, we have the following:

Lemma 2.1 $-\infty < g_2(t, y) \leq t$ for all $(t, y) \in D^*$ such that $t \geq 0$.

Proof $g_2(t, y) = t - (a + b\mathcal{H}(y_{13})y_{13}e^{-\frac{t}{c}})$, for positive constants a , b , and c . Clearly $t - (a + b\mathcal{H}(y_{13})y_{13}e^{-\frac{t}{c}}) \leq t$ for all $t \geq 0$, since $\mathcal{H}(y_{13})y_{13} \geq 0$.

Lemma 2.2 $g(t, y) \in (C, Lip)$ in each compact subset of D^* .

Proof If we show that both g_1 and g_2 are in (C, Lip) in every compact subset of D^* , then $g \in (C, Lip)$ in every compact subset of D^* . Note $g_1(t, y) = t$, being the identity function,

has the desired properties.

Now, since the functions $t \mapsto t$ and $t \mapsto e^{-\frac{t}{c}}$ are continuous on all compact sets, we have that $g_2(t, y) = t - (a + b\mathcal{H}(y_{15})y_{15}e^{-\frac{t}{c}})$ is continuous in t on all compact sets.

Let D' be a compact subset of D^* . Choose $(t, y), (t, \hat{y}) \in D'$. Then

$$\begin{aligned} \|g_2(t, y) - g_2(t, \hat{y})\| &= \|(t - (a + b\mathcal{H}(y_{15})y_{15}e^{-\frac{t}{c}})) - (t - (a + b\mathcal{H}(\hat{y}_{15})\hat{y}_{15}e^{-\frac{t}{c}}))\| \\ &= \|be^{-\frac{t}{c}}(\mathcal{H}(\hat{y}_{15})\hat{y}_{15} - \mathcal{H}(y_{15})y_{15})\| \end{aligned} \quad (2.5)$$

and since $be^{-\frac{t}{c}} > 0$ for all t ,

$$\begin{aligned} \|g_2(t, y) - g_2(t, \hat{y})\| &= be^{-\frac{t}{c}}\|\mathcal{H}(\hat{y}_{15})\hat{y}_{15} - \mathcal{H}(y_{15})y_{15}\| \leq be^0\|\mathcal{H}(\hat{y}_{15})\hat{y}_{15} - \mathcal{H}(y_{15})y_{15}\| \\ &\leq b\|\hat{y}_{15} - y_{15}\| \end{aligned}$$

for all $t \geq 0$. Hence g_2 is Lipschitz in y , and so it follows that g is Lipschitz in y . Therefore $g(t, y) \in (C, Lip)$ in each compact subset of D^* .

Lemma 2.3 $f(t, Y) \in (C, Lip)$ in each compact subset of D .

Proof By regrouping and renaming constants, it can be shown that $f(t, Y)$ can be rewritten as $f(t, Y) = (f_1(t, Y), \dots, f_7(t, Y))^T$, where

$$\begin{aligned} f_1(t, Y) &= c_1^{f_1}(k_1\mathcal{H}(y_{11})y_{11} + k_2\mathcal{H}(y_{12})y_{12} + k_3\mathcal{H}(y_{13})y_{13}) + c_2^{f_1}\mathcal{H}(y_{11})y_{11}, \\ f_2(t, Y) &= c_1^{f_2}(k_1\mathcal{H}(y_{11})y_{11} + k_2\mathcal{H}(y_{12})y_{12} + k_3\mathcal{H}(y_{13})y_{13}) + c_2^{f_2}\mathcal{H}(y_{12})y_{12}, \\ f_3(t, Y) &= c_1^{f_3}(k_1\mathcal{H}(y_{11})y_{11} + k_2\mathcal{H}(y_{12})y_{12} + k_3\mathcal{H}(y_{13})y_{13}) + c_2^{f_3}\mathcal{H}(y_{13})y_{13} \\ &\quad + v_{inf}(t) + c_3^{f_3}\mathcal{H}(y_{14})y_{14} + \frac{c_5^{f_3}\mathcal{H}(y_{13})y_{13}}{c_6^{f_3} + \mathcal{H}(y_{13})y_{13}}, \\ f_4(t, Y) &= c_1^{f_4}(k_1\mathcal{H}(y_{11})y_{11} + k_2\mathcal{H}(y_{12})y_{12} + k_3\mathcal{H}(y_{13})y_{13}) + c_2^{f_4}\mathcal{H}(y_{14})y_{14}, \end{aligned} \quad (2.6)$$

$$\begin{aligned}
f_5(t, Y) &= \frac{c_5^{f_3} \mathcal{H}(y_{13}) y_{13}}{c_6^{f_3} + \mathcal{H}(y_{13}) y_{13}} + c_1^{f_5} \mathcal{H}(y_{15}) y_{15} + c_2^{f_5} (\mathcal{H}(y_{15}) y_{15} - \mathcal{H}(y_{16}) y_{16}), \\
f_6(t, Y) &= c_1^{f_6} \mathcal{H}(y_{16}) y_{16} + c_2^{f_6} (\mathcal{H}(y_{15}) y_{15} - \mathcal{H}(y_{16}) y_{16}), \\
f_7(t, Y) &= c_1^{f_7} \mathcal{H}(y_{25}) y_{25},
\end{aligned}$$

where $c_1^{f_1} = \frac{Q_{pp}}{V_{pp}}$, $k_1 = \frac{Q_{pp}}{P_{pp} Q_t}$, and similarly for $c_i^{f_j}$ and k_i .

We will first show that f is continuous in t on every compact subset of $(-\infty, \infty)$. Note that the only explicit dependence of t in (2.6) lies in the function $v_{inf}(t)$ as defined in the previous section. But the construction of $v_{inf}(t)$ clearly implies continuity everywhere on the real line. Therefore it is continuous on every compact subset of $(-\infty, \infty)$, and hence so is f .

We now show f is Lipschitz continuous on each compact subset of D in each state variable. Note that if we show each f_i is Lipschitz continuous on all compact subsets of D , then our assertion follows. So let S be a compact subset of D , and choose $(t, Y), (t, \hat{Y}) \in S$.

We first consider f_3 . Note that

$$\begin{aligned}
\|f_3(t, Y) - f_3(t, \hat{Y})\| &= \left\| \left(c_1^{f_3} (k_1 \mathcal{H}(y_{11}) y_{11} + k_2 \mathcal{H}(y_{12}) y_{12} + k_3 \mathcal{H}(y_{13}) y_{13}) \right. \right. \\
&\quad \left. \left. + c_2^{f_3} \mathcal{H}(y_{13}) y_{13} + v_{inf}(t) + c_3^{f_3} \mathcal{H}(y_{14}) y_{14} + \frac{c_5^{f_3} \mathcal{H}(y_{13}) y_{13}}{c_6^{f_3} + \mathcal{H}(y_{13}) y_{13}} \right) \right. \\
&\quad \left. - \left(c_1^{f_3} (k_1 \mathcal{H}(\hat{y}_{11}) \hat{y}_{11} + k_2 \mathcal{H}(\hat{y}_{12}) \hat{y}_{12} + k_3 \mathcal{H}(\hat{y}_{13}) \hat{y}_{13}) \right. \right. \\
&\quad \left. \left. + c_2^{f_3} \mathcal{H}(\hat{y}_{13}) \hat{y}_{13} + v_{inf}(t) + c_3^{f_3} \mathcal{H}(\hat{y}_{14}) \hat{y}_{14} + \frac{c_5^{f_3} \mathcal{H}(\hat{y}_{13}) \hat{y}_{13}}{c_6^{f_3} + \mathcal{H}(\hat{y}_{13}) \hat{y}_{13}} \right) \right\| \\
&= \left\| c_1^{f_3} k_1 (\mathcal{H}(y_{11}) y_{11} - \mathcal{H}(\hat{y}_{11}) \hat{y}_{11}) + c_1^{f_3} k_2 (\mathcal{H}(y_{12}) y_{12} - \mathcal{H}(\hat{y}_{12}) \hat{y}_{12}) \right. \\
&\quad \left. + (c_1^{f_3} k_3 + c_2^{f_3}) (\mathcal{H}(y_{13}) y_{13} - \mathcal{H}(\hat{y}_{13}) \hat{y}_{13}) + c_3^{f_3} (\mathcal{H}(y_{14}) y_{14} \right. \\
&\quad \left. - \mathcal{H}(\hat{y}_{14}) \hat{y}_{14}) + c_5^{f_3} \left(\frac{\mathcal{H}(y_{13}) y_{13}}{c_6^{f_3} + \mathcal{H}(y_{13}) y_{13}} - \frac{\mathcal{H}(\hat{y}_{13}) \hat{y}_{13}}{c_6^{f_3} + \mathcal{H}(\hat{y}_{13}) \hat{y}_{13}} \right) \right\|
\end{aligned}$$

$$\begin{aligned}
&\leq c_1^{f_3} k_1 \|\mathcal{H}(y_{11})y_{11} - \mathcal{H}(\hat{y}_{11})\hat{y}_{11}\| + c_1^{f_3} k_2 \|\mathcal{H}(y_{12})y_{12} - \mathcal{H}(\hat{y}_{12})\hat{y}_{12}\| \\
&\quad + (c_1^{f_3} k_3 + c_2^{f_3}) \|\mathcal{H}(y_{13})y_{13} - \mathcal{H}(\hat{y}_{13})\hat{y}_{13}\| + c_3^{f_3} \|\mathcal{H}(y_{14})y_{14} \\
&\quad - \mathcal{H}(\hat{y}_{14})\hat{y}_{14}\| + c_5^{f_3} \left\| \frac{\mathcal{H}(y_{13})y_{13}}{c_6^{f_3} + \mathcal{H}(y_{13})y_{13}} - \frac{\mathcal{H}(\hat{y}_{13})\hat{y}_{13}}{c_6^{f_3} + \mathcal{H}(\hat{y}_{13})\hat{y}_{13}} \right\|.
\end{aligned}$$

Now note that

$$\begin{aligned}
&c_5^{f_3} \left\| \frac{\mathcal{H}(y_{13})y_{13}}{c_6^{f_3} + \mathcal{H}(y_{13})y_{13}} - \frac{\mathcal{H}(\hat{y}_{13})\hat{y}_{13}}{c_6^{f_3} + \mathcal{H}(\hat{y}_{13})\hat{y}_{13}} \right\| \\
&= c_5^{f_3} \left\| \frac{\mathcal{H}(y_{13})y_{13}(c_6^{f_3} + \mathcal{H}(\hat{y}_{13})\hat{y}_{13}) - \mathcal{H}(\hat{y}_{13})\hat{y}_{13}(c_6^{f_3} + \mathcal{H}(y_{13})y_{13})}{(c_6^{f_3} + \mathcal{H}(y_{13})y_{13})(c_6^{f_3} + \mathcal{H}(\hat{y}_{13})\hat{y}_{13})} \right\| \\
&= c_5^{f_3} \left\| \frac{c_6^{f_3}(\mathcal{H}(y_{13})y_{13} - \mathcal{H}(\hat{y}_{13})\hat{y}_{13})}{(c_6^{f_3} + \mathcal{H}(y_{13})y_{13})(c_6^{f_3} + \mathcal{H}(\hat{y}_{13})\hat{y}_{13})} \right\| \\
&\leq c_5^{f_3} \left\| \frac{c_6^{f_3}(\mathcal{H}(y_{13})y_{13} - \mathcal{H}(\hat{y}_{13})\hat{y}_{13})}{(c_6^{f_3})(c_6^{f_3})} \right\| \\
&= \frac{c_5^{f_3}}{c_6^{f_3}} \|\mathcal{H}(y_{13})y_{13} - \mathcal{H}(\hat{y}_{13})\hat{y}_{13}\| \leq \frac{c_5^{f_3}}{c_6^{f_3}} \|y_{13} - \hat{y}_{13}\|
\end{aligned}$$

with $c_5^{f_3}, c_6^{f_3} > 0$ and $\mathcal{H}(y_{13})y_{13}, \mathcal{H}(\hat{y}_{13})\hat{y}_{13} \geq 0$.

Hence, it follows that

$$\begin{aligned}
&\|f_3(t, Y) - f_3(t, \hat{Y})\| \leq c_1^{f_3} k_1 \|y_{11} - \hat{y}_{11}\| + c_1^{f_3} k_2 \|y_{12} - \hat{y}_{12}\| \\
&\quad + (c_1^{f_3} k_3 + c_2^{f_3}) \|y_{13} - \hat{y}_{13}\| + c_3^{f_3} \|y_{14} - \hat{y}_{14}\| + \frac{c_5^{f_3}}{c_6^{f_3}} \|y_{13} - \hat{y}_{13}\| \\
&\leq c_1^{f_3} k_1 \|Y - \hat{Y}\| + c_1^{f_3} k_2 \|Y - \hat{Y}\| + (c_1^{f_3} k_3 + c_2^{f_3}) \|Y - \hat{Y}\| + c_3^{f_3} \|Y - \hat{Y}\| \\
&\quad + \frac{c_5^{f_3}}{c_6^{f_3}} \|Y - \hat{Y}\| = \left(c_1^{f_3} k_1 + c_1^{f_3} k_2 + c_1^{f_3} k_3 + c_2^{f_3} + c_3^{f_3} + \frac{c_5^{f_3}}{c_6^{f_3}} \right) \|Y - \hat{Y}\|.
\end{aligned}$$

Let $A = c_1^{f_3}k_1 + c_1^{f_3}k_2 + c_1^{f_3}k_3 + c_2^{f_3} + c_3^{f_3} + \frac{c_5^{f_3}}{c_6^{f_3}}$. Noting that all terms in A are positive constants, we have $A > 0$. Hence, we have

$$||f_3(t, Y) - f_3(t, \hat{Y})|| \leq A ||Y - \hat{Y}||$$

which proves that f_3 is Lipschitz continuous on every compact subset of D , in all of its state variables, with Lipschitz constant A . We note that f_1 , f_2 , f_4 , f_5 and f_6 are all similar (simpler) cases to f_3 . Hence, it can be shown analogously that f_1 through f_6 are all Lipschitz continuous on every compact subset of D in all state variables. Recall $f_7(t, Y) = c_1^{f_7} \mathcal{H}(y_{25}) y_{25}$. It is trivial to show this function is Lipschitz continuous in y_{25} on all compact subsets of D . Therefore we have shown that $f \in (C, Lip)$ in each compact subset of D .

Now, with the aid of Lemmas 2.1-2.3 and applying Theorem 2.1, we obtain local existence and uniqueness of a solution to equation (2.1).

2.2.2 Extension of Existence and Uniqueness

Now that existence of a unique solution on an interval $-\infty < t < t_0 + h$ has been established, we wish to extend the interval in which the solution is guaranteed to exist as much as possible. Furthermore, we wish to be guaranteed that the solution remains unique throughout the extended interval. Although extensive research has led to various results in extending intervals of existence for ODE and DDE that have constant delays or bounded delays with dependence only in t (see (48) and (49)), we found few results for a class of DDE that include state-dependent delays. The following two theorems are presented and proven in (25) and will yield an extended interval of existence and uniqueness of a solution for the system (2.6).

Theorem 2.2 *Let $f(t, Y)$ be continuous in D , let $g(t, y)$ be continuous in D^* , let $\alpha \leq g_j(t, y) \leq t$ for all $(t, y) \in D^* \cap \{t \geq t_0\}$ ($j = 2, \dots, m$), and let $\phi(t)$ be continuous on $[\alpha, t_0]$. Then any solution, $y(t)$, of Problem P can be extended to $\alpha \leq t < \beta$, where $t_0 < \beta \leq \infty$ and for any compact set $F \subset D$ there is a sequence of numbers, $t_0 < t_1 < t_2 < \dots \rightarrow \beta$, such that $(t_i, y(g(t_i, y(t_i)))) \in D - F$ for $i = 1, 2, \dots$. Thus if β is finite and cannot be increased, we conclude that as $t \rightarrow \beta$, one of the following occurs:*

- (i) $\limsup \|y(g(t, y(t)))\| = \infty$, or
- (ii) $(t, y(g(t, y(t))))$ comes arbitrarily close to the boundary of D .

Theorem 2.3 *Let $f(t, Y) \in (C, Lip)$ in each compact subset of D , let $g(t, y) \in (C, Lip)$ in each compact subset of D^* , and let $\phi(t)$ be Lipschitz continuous on each finite subinterval of $[\alpha, t_0]$. Then for any interval $\alpha \leq t < \beta$, where $t_0 < \beta \leq \infty$, over which a solution, $y(t)$, of Problem P exists, the solution is unique.*

Note that the hypotheses in Theorems 2.2 and 2.3 are met by the system (2.6), since they are weaker, in both cases, than the hypotheses in Theorem 2.1, which we proved the system (2.6) meets. We conclude, therefore that the unique solution to our system can be extended from $\alpha > 0$ to a number β such that $\alpha < \beta \leq \infty$.

2.3 Inverse Problem

PBPK models require numerous parameters such as flow, metabolism, and elimination rate constants. In general, not all these parameters can be obtained from the literature or directly by experimental means. Therefore the unknown parameters must be estimated via, for example, the formulation of an inverse problem, that is, one compares experimental data (in our case given by (20)) to model predictions via a cost function and adjusts the

unknown parameters to achieve a minimal cost. The resulting “optimized” parameters yielding this minimal cost will be the values used in the model. Mathematically, the inverse problem is formulated as follows: let $\vec{q} = (q_1, \dots, q_n)$, where $q_1, \dots, q_n$ are our n unknown parameters. Then the inverse problem is defined as

$$q_{opt} = \min_{\vec{q} \in S} (J(\vec{q})),$$

where $S = \{\vec{q} \in R^n : q_k > 0, k = 1, 2, \dots, n\}$ and J is our cost function to be defined.

We considered several cost functions for this inverse problem, including least squares (LS), weighted least squares (WLS), and the maximum likelihood function (MLF). We compared the performances of these cost functions by using the optimized parameter set resulting from one function to obtain the cost this parameter set produces using the other two functions. With this comparison, we observed that the MLF consistently produced a parameter set that resulted in the best cost (lowest in the case of LS and WLS and highest in the case of MLF). Therefore we decided to use it exclusively throughout the estimation process. The MLF is defined as follows:

$$J(\vec{q}) = -\frac{1}{2} \sum_{i=1}^r [n_i (\log(2\pi) + 1) + \gamma_i \sum_{j=1}^{n_i} \log(f_{i,j}(\vec{q})) + n_i \log \left(\frac{1}{n_i} \sum_{j=1}^{n_i} \frac{(z_{i,j} - f_{i,j}(\vec{q}))^2}{(f_{i,j}(\vec{q}))^{\gamma_i}} \right)] , \quad (2.7)$$

where r is the number of different data types being used, i.e., biliary excretion *rate*, *cumulative* biliary excretion, urinary elimination rate, etc., n_i is the number of data points used in each data type, $z_{i,j}$ is each data point, where i corresponds to the data type and j corresponds to the time at which the data point represents, $f_{i,j}(\vec{q})$ is the model prediction at the time of the data point $z_{i,j}$, and finally each γ_i are the *heteroschedasticity terms* for each data type, which regulate how the cost for each data type is determined. As γ_i approaches zero, the cost function describes an absolute error model. As γ_i approaches two, the cost function describes a relative error model. Each γ_i is bounded between zero and two and is treated

as an unknown parameter to be optimized in the inverse problem. Hence $\{\gamma_1, \gamma_2, \dots\} \in \vec{q}$. For a derivation of (2.7), we refer the reader to (27). It is worth noting that in the derivation, it is assumed that there is no correlation between the data sets. Recall that in the two data sets we use, one set is obtained by integrating the data of the other. So, although the guarantee of the performance of the MLF cannot be made, we still decided to use it for our cost function since testing of the cost functions repeatedly showed the MLF to be the best choice.

In our problem we use two data types: the experimental data consisting of biliary excretion rate data and the cumulative biliary excretion data obtained by numerically integrating the excretion rate data. Although the two data sets are based on the same reported data, we observed that using two different types enhanced the performance of the MLF, yielding better data fits. So we have two heteroschedasticity terms, γ_1 and γ_2 , for the rate data and cumulative data, respectively.

The Nelder-Mead simplex-based method was the main optimization algorithm implemented for parameter optimization. This method proved to be most efficient generally leading to the lowest cost. The specific code used is *fminsearch* (MATLAB R11, The MathWorks, Inc.). The line search method, *fminunc*, also from MATLAB, was used as well. The results of the parameter estimation are listed in Table 2.4.

Model predictions of the experimental data using the optimized parameters are presented in Figures 2.4 and 2.5. In attempting to numerically solve the DDE, we found a high degree of stiffness. Therefore we implemented a three-stage, implicit, Runge-Kutta method (RADAR5, N. Guglielmi and E. Hairer, 2000), an effective numerical method for solving stiff, delay differential equations. In the cumulative biliary excretion depicted in Figure 2.4, the simulated excretion curves for the lower two doses (Figure 2.4A) fit the data well. Note the slight underestimation for the lower dose and an overestimation followed by an underestimation for the higher dose. In the case of the higher two doses (Figure 2.4B), a key characteristic of the data is a crossing of the two excretion curves. Although the

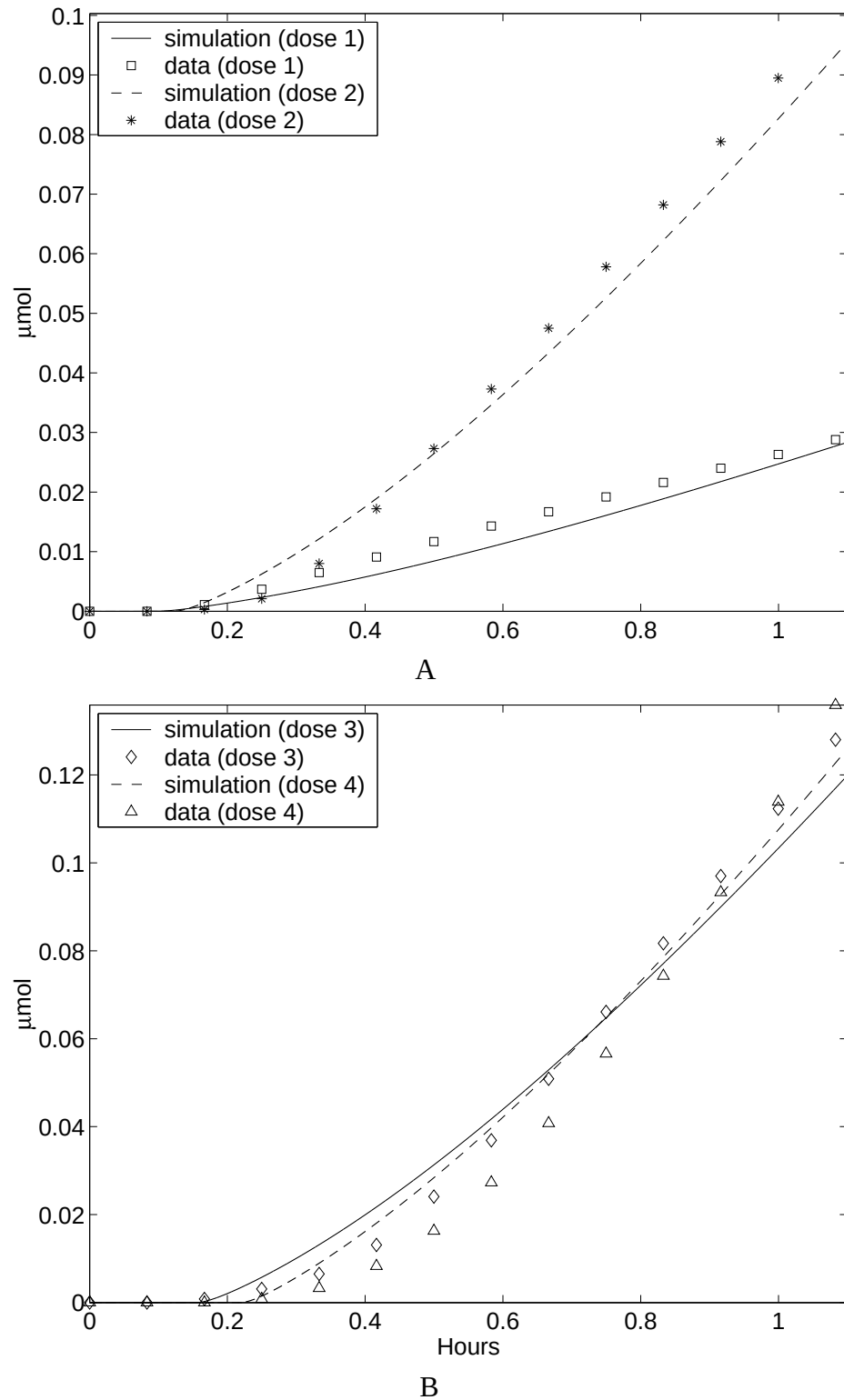


Figure 2.4: Cumulative biliary excretion of genistein glucuronide after a 1 h venous infusion of pure genistein.

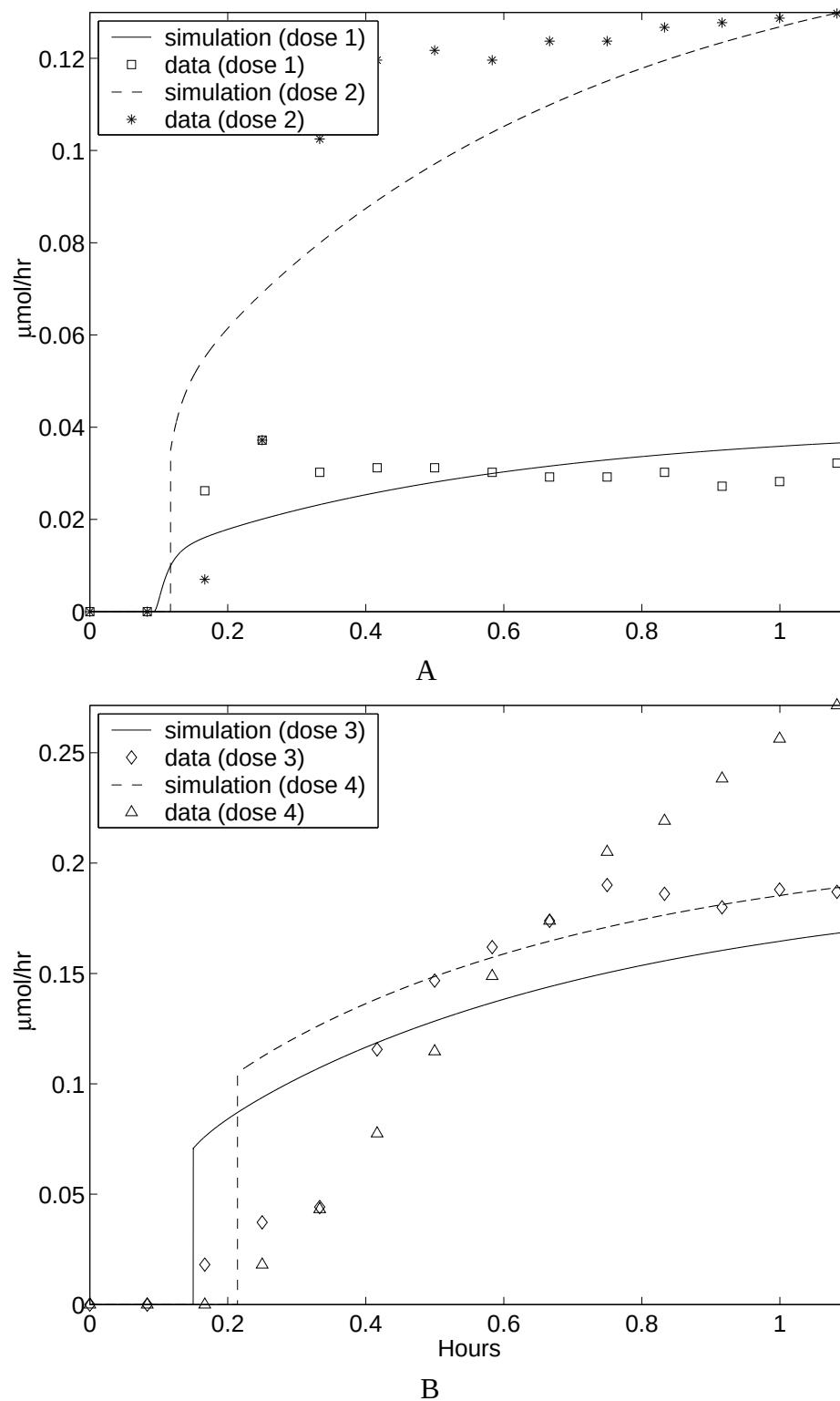


Figure 2.5: Biliary excretion rate of genistein glucuronide after a 1 h venous infusion of pure genistein.

Table 2.4: Resulting values from parameter optimization.

Parameter	Value
k_{urine}^{gen}	9.5 hr^{-1}
k_{urine}^{con}	0 hr^{-1}
k_{bile}	111.72 hr^{-1}
V_{max}	$25.25 \text{ } \mu\text{mol}/\text{L}/\text{hr}$
k_m	$0.6231 \text{ } \mu\text{mol}/\text{L}$
t_{basal}	0.0865 hr
c_{gen}	$0.0656 \text{ L} \cdot \text{hr}/\mu\text{mol}$
c_t	1.0107 hr
γ_1	2
γ_2	2

simulations begin with an underestimation and progress into a slight overestimation, they also cross, capturing this nontrivial characteristic of the state-dependent delay observed in the data.

The biliary excretion rate (i.e., the derivative of the cumulative excretion) is depicted in Figure 2.5. The fits to these data are not as close as the cumulative excretion data. This is to be expected since taking a derivative tends to amplify error. Note, however, that the fits to the data for the lower doses (Figure 2.5A) are better than for the upper doses. This possibly suggests a saturable process not accounted for in the model. However, the dynamics of the state dependence in the delay are more prominent in the derivative data, and the model decently characterizes these dynamics.

2.4 Statistical Significance of Delay Equations

In this section, we establish that our DDE model is a statistically significant improvement over the model based on standard ODE. To do this, we test the null hypothesis, that is, we compare the DDE model as described in the previous sections to the same model with the

delay set to zero.

Let $\vec{q}$ be the vector of parameters in the DDE model to be optimized. Clearly, $\vec{q}$ is of dimension 10 (Table 2.4). Recall the delay function

$$d(t, C_{gen}^l) = t_{basal} + c_{gen} C_{gen}^l(t) e^{-\frac{t}{c_t}}$$

with t_{basal} , c_{gen} , and c_t all elements of $\vec{q}$. To test the null hypothesis, we eliminate the delay function, thus eliminating three of the parameters in $\vec{q}$. We then optimize over $\vec{q}$, the vector of the remaining 7 unknown parameters, using a predetermined cost function (in this case, the MLF described in section 2.3). Then, using a test statistic U , we compare the optimal cost admitted by minimizer $\vec{q}^*$ to the optimal cost admitted by minimizer $\vec{q}$. Using the

Table 2.5: Resulting values from test statistic U .

$\chi^2(3)$		
Confidence Level (%)	α	Threshold Value τ
1%	0.99	0.115
10%	0.9	0.584
90%	0.1	6.251
95%	0.05	7.815
99%	0.01	11.34

developments in (28), we use the test statistic

$$U = r \left| \frac{J(\vec{q}^*) - J(\vec{q})}{J(\vec{q})} \right|,$$

where r is the number of data points in the data set. After obtaining U , we choose a significance level α and use $\chi^2(s)$ tables to obtain the threshold τ , where s is the number of degrees of freedom. If we find that $U > \tau$, then we may conclude that the null hypothesis

fails, i.e., the DDE model is a statistically significant improvement over the ODE model.

In our data set, $r = 112$. We found $J(\vec{q}) = 597.2634$ and $J(\vec{q}^*) = 402.0$. Recall that using the MLF, one wishes to maximize the likelihood. Hence, with the above numbers, $J(\vec{q})$ is “better” than $J(\vec{q}^*)$. Statistical significance is seen by the following. From our above equation, we compute the test statistic $U = 36.6162$. Noting that we have 3 degrees of freedom, we compute the thresholds for the stated confidence levels in Table 2.5. So we see that even with a 99% confidence level, $U > \tau$. Therefore the null hypothesis fails. This clearly shows a statistically significant improvement in simulating the data by implementing the DDE model.

Chapter 3

Dispersion Model Derivation

This chapter will cover the development of the genistein (GEN) dispersion model. In Section 3.1 we explore various biological issues that have to be addressed and we discuss what a dispersion model is, what it is used for, and several standing assumptions we make prior to the development of the model. Finally, we derive the model equations and boundary conditions in Sections 3.2 and 3.3.

3.1 Modeling Considerations

In modeling the liver with as sufficient biological accuracy to include spatial dependence in the equations, we need to understand the geometry of the liver and its functions from a spatial point of view.

The liver lobule is arguably the smallest element of the liver that is capable of performing all (or, at least, most) of the functions of the liver. Within a lobule there is a vast network of canals called sinusoids that blood travels through. The blood flows from the outer boundary of the lobule toward the center, where it is collected and exits through a larger vessel called the central vein. These sinusoids are lined with rows of cells called hepatocytes, where most metabolism is assumed to occur. The network of sinusoids and

hepatocytes form a loosely defined system of canals that bile travels through in the opposite direction of the blood. These canals are called bile duct canaliculi. For detailed illustrations and a thorough expansion of the function of the liver lobule, the reader is referred to (50).

We build our simplified model of the liver based on the rules outlined above. We construct a tube that represents the sinusoid structure with blood flowing in one direction. We construct another tube of equal length that represents the bile canaliculi with bile flowing in the opposite direction of the blood. We construct a row of hepatocytes separating these tubes, where portions of genistein are uptaken from the sinusoid into the hepatocytes, metabolized and excreted into the bile duct. Further details of this model are to follow, but first we need to address several relevant biological issues concerning this type of modeling.

The following considerations are addressed in attempt to maintain as much biological accuracy as possible in the development of the dispersion model, while simplifying where appropriate.

Issue 1 - GEN binds to albumin and alphafetoprotein (α FP) in rat serum (34).

Conclusion - This consideration raises an interesting point. Mendel (38) suggests the question of whether to track the total concentration of the chemical or the unbound portion when considering uptake into cells for metabolism. He proposes that the answer to this question is dependent on specific characteristics and properties of each specific chemical. Due to a lack of data in either direction for the case of GEN, and considering that unbound portions were used in the modeling of estradiol (E2) (39), we will use unbound portions of GEN when considering conjugation. This implies that we assume only unbound GEN is available for cell membrane crossing (and therefore conjugation).

Now, the concentration of α FP in an adult rat is approximately .25 nM (39). In the model for E2 by Plowchalk, binding to α FP was not tracked because the concentration is low enough that any bound amounts of chemical to α FP would be negligible and may be

omitted. Similarly, we will assume that GEN does not bind to α FP. This leaves us to track GEN binding to albumin.

According to the GEN model for humans developed in (34), the free fraction of total GEN concentration in human serum remains relatively constant beyond a total GEN concentration of 1000 nM. This is due to the fact that albumin controls the free fraction because the capacity of human serum hormone binding globulin (SHBG, the other main serum binding protein in human serum) is very small. So there is not enough SHBG in the system to affect the free fraction. In adult rat serum, α FP has a capacity comparatively low, similar to SHBG in humans. Hence, we shall assume the results are similar, that is, the free fraction of GEN remains constant through 1000 nM total GEN concentration in adult rat serum. Now, using the delay differential equation (DDE)-based GEN model of Zager, et. al (40), the total GEN serum concentrations, even during the largest infusion dose, do not exceed 1000 nM. Hence, in our model, we will assume the unbound fraction of GEN remains constant in serum.

Issue 2 - GEN does not regulate α FP concentrations (35).

Conclusion - We assume GEN does not regulate albumin concentrations either, due to a lack of any data in the literature suggesting otherwise. Furthermore, since the experiments (and hence the simulations) are on a one hour time scale, the effect would most likely be minimal within that amount of time.

Issue 3 - GEN binds to estrogen receptors (ER) in the liver and reproductive tissue (34).

Conclusion - We will not track GEN-ER binding in the reproductive tissue considering that most GEN found in serum is in its glucuronidated form (which we assume does not bind to ER - see Issue 7), and since the reproductive tissue is a small percentage of total tissue

mass. We will now elaborate on this issue quantitatively.

The amount of ER in the liver is approximately 0.021 nmol/L (39). The volume of the liver of a 250 g rat is approximately 0.0092 L (23). Hence, in the liver, $[\text{ER}] = 2.3 \times 10^{-3} \text{ } \mu\text{mol/L} \approx 2.3 \text{ nM}$, including both bound and unbound ER. Note that the inverse of the GEN-ER dissociation constant (k_d),

$$\frac{1}{k_d} = \frac{[\text{GEN}_{\text{bound}} \cdot \text{ER}_{\text{bound}}]}{[\text{GEN}_{\text{free}}] \cdot [\text{ER}_{\text{free}}]}$$

from (41), and with

$$[\text{ER}_{\text{free}}] + [\text{GEN}_{\text{bound}} \cdot \text{ER}_{\text{bound}}] = [\text{ER}_{\text{total}}],$$

$$[\text{GEN}_{\text{free}}] + [\text{GEN}_{\text{bound}} \cdot \text{ER}_{\text{bound}}] = [\text{GEN}_{\text{total}}],$$

we have three equations. Now, again using the DDE model of (40), during the lowest infusion dose of GEN, the concentration of GEN in rat serum averages 25 nM . So let's assume $[\text{GEN}_{\text{total}}] = 25 \text{ nM}$. Also, the GEN-ER dissociation constant $k_d = 500 \text{ nM}$ (34). Hence, we now have

$$\frac{1}{500 \text{ nM}} = \frac{[\text{GEN}_{\text{bound}} \cdot \text{ER}_{\text{bound}}]}{[\text{GEN}_{\text{free}}] \cdot [\text{ER}_{\text{free}}]},$$

$$[\text{ER}_{\text{free}}] + [\text{GEN}_{\text{bound}} \cdot \text{ER}_{\text{bound}}] = 2.3 \text{ nM},$$

$$[\text{GEN}_{\text{free}}] + [\text{GEN}_{\text{bound}} \cdot \text{ER}_{\text{bound}}] = 25 \text{ nM}.$$

So we have three equations in three unknowns. From the first equation we have $[\text{GEN}_{\text{bound}} \cdot \text{ER}_{\text{bound}}] = \frac{[\text{GEN}_{\text{free}}] \cdot [\text{ER}_{\text{free}}]}{500 \text{ nM}}$. Substituting into the next two equations, we have

$$[\text{ER}_{\text{free}}] + \frac{[\text{GEN}_{\text{free}}] \cdot [\text{ER}_{\text{free}}]}{500 \text{ nM}} = 2.3 \text{ nM},$$

$$[GEN_{free}] + \frac{[GEN_{free}] \cdot [ER_{free}]}{500 \text{ nM}} = 25 \text{ nM},$$

and so we see that

$$[GEN_{free}] - [ER_{free}] = 25 \text{ nM} - 2.3 \text{ nM}.$$

Hence,

$$[ER_{free}] = [GEN_{free}] - 22.7 \text{ nM}.$$

Therefore, we have

$$[GEN_{free}] - 22.7 \text{ nM} + \frac{[GEN_{free}]^2 - 22.7 \text{ nM} \cdot [GEN_{free}]}{500 \text{ nM}} = 2.3 \text{ nM},$$

which simplifies to the quadratic equation,

$$[GEN_{free}]^2 + 477.3 \text{ nM} \cdot [GEN_{free}] - 12500 \text{ nM}^2 = 0.$$

Solving this equation, we obtain $[GEN_{free}] \approx 24.89 \text{ nM}$. Recalling that $[GEN_{total}] = 25 \text{ nM}$, we see that GEN binding to ER is negligible. We will not track this binding.

Issue 4 - Estradiol (E2) competes with GEN binding to estrogen receptors and serum proteins (34).

Conclusion - The concentration of albumin in rat serum is approximately $41 \times 10^4 \text{ nM}$ (39). Female rats cycle between 1 to 6 pg/mL of E2 (42). The molecular weight of E2 is 272.37 g/mol . Hence, 1 $\text{pg/mL} = 3.6715 \times 10^{-3} \text{ nM}$ of E2. So the highest concentration of E2 that female rat serum reaches is 6 $\text{pg/mL} = 2.203 \times 10^{-2} \text{ nM}$. Noting $[E2] \leq 2.203 \times 10^{-2} \text{ nM} \ll 41 \times 10^4 \text{ nM} = [\text{albumin}]$, we see that E2 cannot possibly displace enough albumin in serum to considerably affect GEN binding to albumin. So we will not track E2 binding to albumin.

Issue 5 - GEN is glucuronidated in the liver and intestinal wall, and this is a saturable process. Furthermore, GEN found in the bile is almost exclusively in its glucuronidated form (20).

Conclusion - We assume glucuronidation of GEN occurs in the hepatocytes within the liver. We will use a Michaelis-Menten term for the glucuronidation in the liver, and initially we will use a linear term for glucuronidation in the intestinal wall, which may be changed to a Michaelis-Menten term as needed.

Issue 6 - GEN glucuronide has been shown to be actively transported into the bile (36). It is also believed that most chemicals are excreted into bile by diffusion (37).

Conclusion - We will include a (linear) active transport term and a diffusion term for excretion of GEN-glucuronide into the bile.

Issue 7 - It has been shown that GEN glucuronide binds to blood proteins (internal CIIT studies - not published yet).

Conclusion - We compensate for conjugate binding in the model as we do for GEN. Due to a lack of data to suggest otherwise, and since ER concentrations are so low as not to have a considerable effect on the overall kinetics as pointed out above, we do not track any GEN conjugate-ER binding. Also, as with GEN, we assume only unbound conjugates cross cell membranes.

Issue 8 - Data suggest GEN does not affect bile flow (20).

Conclusion - We will assume GEN does not affect bile flow.

One other point that requires consideration is the following. Since the GEN infusions are given with GEN diluted in rat serum, is there a considerable amount of albumin being added to the rat as to increase the overall concentration in the rat considerably? To answer this question, we note that the infusions were all given at $4.5 \mu\text{L}/\text{min} = 270 \mu\text{L}/\text{hr} = 270 \mu\text{L}$ total. The plasma volume of a 250 g rat is approximately 10300 μL . So the amount of albumin infused is less than 2.6 percent of the amount in the rat. This negligible percentage will be ignored.

We now discuss the dispersion model and its benefits to modeling the liver. Let a *blood element* be defined as a volume considerably smaller than that of the liver but large enough to contain sufficient solute molecules, erythrocytes, etc., to enable continuous properties, such as concentration, to be well defined. We also define the *residence time* of an element to be the time spent by that element in the liver from entrance to exit. As blood elements travel through the liver, there is a natural distribution in the residence time of these elements due to the numerous paths available to them through the vast network of sinusoids in the liver, dispersion of material within the liver, etc. This distribution of residence times of elements is defined as the *residence time distribution*. Bile flow in the liver is a similar process and will be treated analogously. In light of this, we will use the developments of Roberts and Rowland (43) to represent the liver compartment in our model. In (43), a *dispersion model* is introduced and developed based on residence time distribution of solutes in the liver and on hepatic physiology. This dispersion model treats the process of dispersion as a differential process and represents the concentration-distance profile as a smooth variation. The spread in the residence time distribution of solute as it moves through the liver is mainly due to *axial dispersion*, which is described by the *axial dispersion number*, $\mathcal{D}$. The higher the value of $\mathcal{D}$, the greater the degree of axial dispersion. Now, axial dispersion is the result

of several events. These events include radial variations in flow velocity, variations in path length (as mentioned before), molecular diffusion, and mixing of blood at branch points and interconnections of sinusoids resulting in convective mixing in the direction of flow.

We will use the dispersion model in considering the sinusoidal/Disse spaces and the bile canaliculi/duct spaces of the liver. From this point on, we treat the sinusoid/Disse spaces, hepatocyte space and bile duct space as three distinct spaces (see Figure 3.1). The sinusoid and Disse spaces are treated as single volumes. Similarly, the hepatocyte space is treated as a single volume and the bile canaliculi/duct space is treated as a single volume as well.

In order to use the Roberts and Rowland developments, we need some further assumptions (all justified in (43)):

R1 Radial transport of solute is instantaneous, and thus at any point, distribution of solute between the sinusoid and Disse space is instantaneous.

R2 Axial diffusion of solute (between hepatocytes) can be ignored.

R3 The unbound fraction of total GEN concentration is constant within the sinusoidal and Disse space (although they may not be the same).

R4 Conditions do not vary across a section normal to flow. (This reduces the problem to one space dimension.)

All other compartments in the model are formulated as well-mixed compartments, theory of which is well developed. All flow rates to and from blood are approximated by linear terms, based on flow rates and partition coefficients obtained from the literature. All elimination rates in these compartments are initially estimated as linear as well, due to a lack of any data suggesting any strong saturable processes. The equations are formulated using mass-balance principles. The model is depicted in Figure 3.2.

3.2 Derivation of equations

We now derive the governing equations for the model. Descriptions and units of the variables and parameters are listed in Tables 3.1, 3.2 and 3.3. Note that the rest-of-body (ROB) compartments for pure GEN and GEN conjugates (CON) include tissue only which does not include systemic serum within organs.

Note from Table 3.3 that $f_{ss}^{G_u} = \frac{C_{ss}^{G_u}}{C_{ss}^{GEN}}$. Hence, we have

$$C_{ss}^{G_u} = f_{ss}^{G_u} C_{ss}^{GEN}.$$

Similarly, we have

$$C_{ss}^{C_u} = f_{ss}^{C_u} C_{ss}^{CON},$$

$$C_{l,s}^{G_u} = f_{l,s}^{G_u} C_{l,s}^{GEN},$$

$$C_{l,s}^{C_u} = f_{l,s}^{C_u} C_{l,s}^{CON},$$

$$C_{ss}^{C_u} = f_{ss}^{C_u} C_{ss}^{CON}.$$

Furthermore, in light of our assumption that only unbound GEN and CON can cross cell membranes, and since our ROB compartments are comprised of tissue *only*, we have that

$$C_{ROB}^{G_u} = C_{ROB}^{GEN},$$

$$C_{ROB}^{C_u} = C_{ROB}^{CON}.$$

Recall the venous infusion of GEN was administered in the serum. In Chapter 2, we explain the formulation of the continuously differentiable function $v_{inf}(t)$ to mathematically simulate the infusion. Here, the function $v_{inf}(t)$ will be added as a forcing function on the right hand side of the equation to be derived for the concentration of GEN in the systemic

Table 3.1: State variables for model.

Abbreviation	Description
C_{ROB}^{GEN}	Concentration of total GEN in ROB ($\mu\text{mol}/L$)
$C_{ROB}^{G_u}$	Concentration of unbound GEN in ROB
C_{ROB}^{CON}	Concentration of total CON in ROB
$C_{ROB}^{C_u}$	Concentration of unbound CON in ROB
C_{ss}^{GEN}	Concentration of total GEN in systemic serum
$C_{ss}^{G_u}$	Concentration of unbound GEN in systemic serum
C_{ss}^{CON}	Concentration of total CON in systemic serum
$C_{ss}^{C_u}$	Concentration of unbound CON in systemic serum
$C_{l,s}^{GEN}$	Concentration of total GEN in liver sinusoid space
$C_{l,s}^{G_u}$	Concentration of unbound GEN in liver sinusoid space
$C_{l,D}^{GEN}$	Concentration of total GEN in liver Disse space
$C_{l,D}^{G_u}$	Concentration of unbound GEN in liver Disse space
$C_{l,t}^{GEN}$	Concentration of total GEN in liver sinusoid extension
$C_{l,t}^{G_u}$	Concentration of unbound GEN in liver sinusoid extension
$C_{l,h}^{GEN}$	Concentration of total GEN in liver hepatocyte space
$C_{l,h}^{G_u}$	Concentration of unbound GEN in liver hepatocyte space
$C_{l,h}^{CON}$	Concentration of total CON in liver hepatocyte space
$C_{l,h}^{C_u}$	Concentration of unbound CON in liver hepatocyte space
$C_{l,s}^{CON}$	Concentration of total CON in liver sinusoid space
$C_{l,s}^{C_u}$	Concentration of unbound CON in liver sinusoid space
$C_{l,t}^{CON}$	Concentration of CON in liver sinusoid extension
$C_{l,t}^{C_u}$	Concentration of unbound CON in liver sinusoid extension
C_b^{CON}	Concentration of CON in bile duct space
C_{tube}^{CON}	Concentration of CON in bile extension tube

Table 3.2: Estimated parameters defined for model equations.

Abbreviation	Description
k_{ROB}^m	Linear rate term for GEN conjugation in GEN ROB compartment (hr^{-1})
k_{urine}^{GEN}	Linear rate term for GEN excretion in urine
k_{urine}^{CON}	Linear rate term for CON excretion in urine
k_{bile}	Linear rate term for active transport of CON into bile
V_{max}, k_m	Michaelis-Menton rate terms for saturable conjugation of GEN in liver ($\mu mol/L/hr$, $\mu mol/L$)
$\mathcal{D}_l$	Effective axial dispersion coefficient for liver ([area]/hr)
$\mathcal{D}_b$	Effective axial dispersion coefficient for bile duct
$\mathcal{D}_t$	Effective axial dispersion coefficient for bile extension tube ([area]/hr)
P_{GEN}	Permeability coefficient of hepatocyte to GEN (L/hr)
P_{CON}	Permeability coefficient of hepatocyte to CON

serum. To this end, we recall the definition of the infusion function $v_{inf}(t)$ as follows:

$$v_{inf}(t) = \begin{cases} c_v F(t) & 0 \leq t < \varepsilon, \\ c_v G(t) & \varepsilon \leq t \leq 1 - \varepsilon, \\ c_v H(t) & 1 - \varepsilon < t \leq 1, \\ c_v I(t) & 1 < t \leq 1 + \frac{5}{60}, \end{cases}$$

where we define

$$F(T) = \frac{-2}{\varepsilon^3} t^3 + \frac{3}{\varepsilon^2} t^2,$$

$$G(t) = 1,$$

$$H(t) = \frac{2}{\varepsilon^3} t^3 + \frac{3}{\varepsilon^3} (\varepsilon - 2) t^2 - \frac{6}{\varepsilon^3} (\varepsilon - 1) t + \frac{1}{\varepsilon^3} (3\varepsilon - 2),$$

and

$$I(t) = 0.$$

In the model, we set $\varepsilon = 0.01$.

Table 3.3: Known parameters defined for model equations.

Abbreviation	Description
m_{rat}	Mass of rat (g)
Q_l	Blood flow rate to liver (L/hr)
Q_b	Bile flow rate
V_{ss}	Volume of systemic serum (L)
$V_{l,s}$	Volume of liver sinusoid space
$V_{l,t}$	Volume of liver sinusoid space in extension tube
$V_{l,D}$	Volume of liver Disse space
$V_{l,Dt}$	Volume of liver Disse space in extension tube
$V_{l,h}$	Volume of liver hepatocyte space
V_b	Volume of bile duct space
V_{tube}	Volume of bile extension tube space
V_{ROB}	Volume of ROB compartment
v_l	Average fluid flow velocity in liver ([length]/hr)
v_b	Average fluid flow velocity in bile duct
$f_{ss}^{G_u}$	Free fraction of GEN in systemic serum $\frac{C_{ss}^{G_u}}{C_{ss}^{GEN}}$ (constant)
$f_{l,s}^{G_u}$	Free fraction of GEN in liver sinusoid space $\frac{C_{l,s}^{G_u}}{C_{l,s}^{GEN}}$
$f_{l,t}^{G_u}$	Free fraction of GEN in liver sinusoid space in extension tube
$f_{l,D}^{G_u}$	Free fraction of GEN in space of Disse $\frac{C_{l,D}^{G_u}}{C_{l,D}^{GEN}}$
$f_{l,Dt}^{G_u}$	Free fraction of GEN in space of Disse in extension tube
$f_{ss}^{C_u}$	Free fraction of CON in systemic serum $\frac{C_{ss}^{C_u}}{C_{ss}^{CON}}$
$f_{l,s}^{C_u}$	Free fraction of CON in liver sinusoid space $\frac{C_{l,s}^{C_u}}{C_{l,s}^{CON}}$
$f_{l,t}^{C_u}$	Free fraction of CON in liver sinusoid space in extension tube
$f_{l,D}^{C_u}$	Free fraction of CON in space of Disse $\frac{C_{l,D}^{C_u}}{C_{l,D}^{CON}}$
$f_{l,Dt}^{C_u}$	Free fraction of CON in space of Disse in extension tube
$\bar{L}$	Length of liver space and bile duct space [length]
$\bar{L}$	Length of sinusoid extension tube space
$-\hat{L}$	Length of bile extension tube space
k_1	$\frac{\bar{L}}{L}$ [constant]
k_2	$\frac{\bar{L}}{\hat{L}}$

We begin by formulating the mass-balance differential equations for the model based on Figure 3.2. In the next section we introduce an extension to the sinusoid tube of length $\bar{L}$. That is, the extension tube begins at length L and ends at length $L + \bar{L}$. It is at the end of this extension where the blood re-enters the systemic serum. We introduce equations for the concentration of genistein and genistein conjugates in this extension. The state variables are labeled, $C_{l,t}^{GEN}$ and $C_{l,t}^{CON}$. We derive the ROB and systemic serum compartments as follows:

$$\begin{aligned} \frac{dC_{ROB}^{GEN}}{dt} &= \frac{P_{GEN}}{V_{ROB}} (C_{ss}^{G_u} - C_{ROB}^{GEN}) - k_{urine}^{GEN} C_{ROB}^{GEN} - k_{ROB}^m C_{ROB}^{GEN} \\ &= \frac{P_{GEN}}{V_{ROB}} (f_{ss}^{G_u} C_{ss}^{GEN} - C_{ROB}^{GEN}) - k_{urine}^{GEN} C_{ROB}^{GEN} - k_{ROB}^m C_{ROB}^{GEN}, \end{aligned} \quad (3.1)$$

$$\begin{aligned} \frac{dC_{ROB}^{CON}}{dt} &= \frac{P_{CON}}{V_{ROB}} (C_{ss}^{C_u} - C_{ROB}^{CON}) - k_{urine}^{CON} C_{ROB}^{CON} + k_{ROB}^m C_{ROB}^{GEN} \\ &= \frac{P_{CON}}{V_{ROB}} (f_{ss}^{C_u} C_{ss}^{CON} - C_{ROB}^{CON}) - k_{urine}^{CON} C_{ROB}^{CON} + k_{ROB}^m C_{ROB}^{GEN}, \end{aligned} \quad (3.2)$$

$$\begin{aligned} \frac{dC_{ss}^{GEN}}{dt} &= \frac{P_{GEN}}{V_{ROB}} (C_{ROB}^{GEN} - C_{ss}^{G_u}) + \frac{Q_l}{V_{l,s}} (C_{l,t}^{GEN}(t, L + \bar{L}) - C_{ss}^{GEN}) \\ &\quad + \frac{1}{V_{ss}} v_{inf}(t) \\ &= \frac{P_{GEN}}{V_{ROB}} (C_{ROB}^{GEN} - f_{ss}^{G_u} C_{ss}^{GEN}) + \frac{Q_l}{V_{l,s}} (C_{l,t}^{GEN}(t, L + \bar{L}) - C_{ss}^{GEN}) \\ &\quad + \frac{1}{V_{ss}} v_{inf}(t), \end{aligned} \quad (3.3)$$

$$\begin{aligned}
\frac{dC_{ss}^{CON}}{dt} &= \frac{P_{CON}}{V_{ROB}}(C_{ROB}^{CON} - C_{ss}^{C_u}) + \frac{Q_l}{V_{l,s}}(C_{l,t}^{CON}(t, L + \bar{L}) - C_{ss}^{CON}) \\
&= \frac{P_{CON}}{V_{ROB}}(C_{ROB}^{CON} - f_{ss}^{C_u} C_{ss}^{CON}) + \frac{Q_l}{V_{l,s}}(C_{l,t}^{CON}(t, L + \bar{L}) - C_{ss}^{CON}),
\end{aligned} \tag{3.4}$$

where V_{ss} is the systemic serum volume. All state variables are defined in Table 3.1. Now, following the work presented in Banks, et al. (44), which is also based on the work of Roberts and Rowland, we treat the sinusoid and Disse spaces and the bile duct (canaliculi and larger bile ducts) spaces as cylinders of length L , as depicted in Figure 3.1. Consider

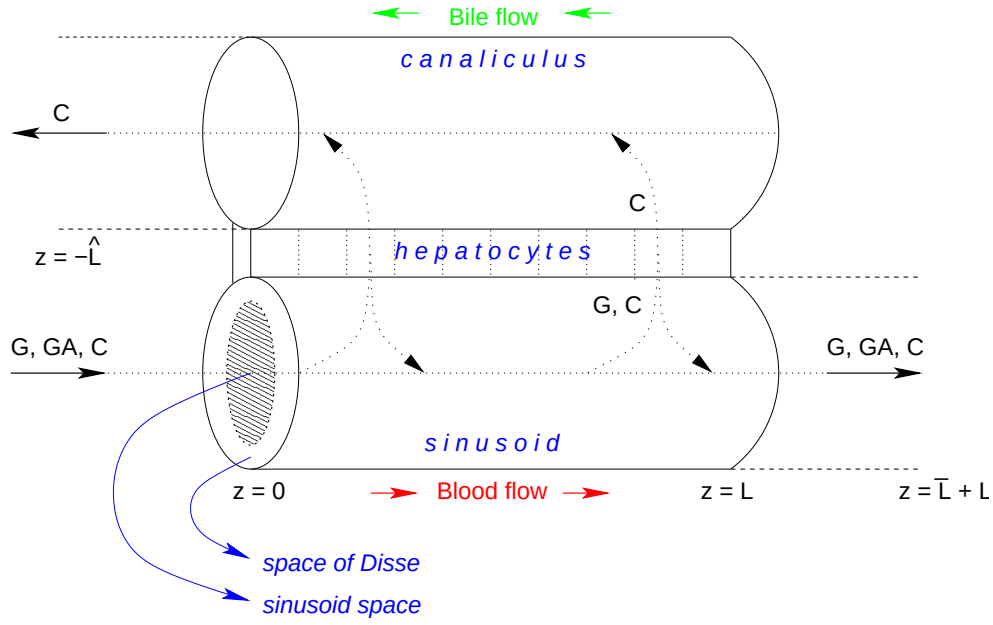


Figure 3.1: Liver submodel based on Roberts and Rowland (43).

an element of thickness δz at a position $z < L$ of the cylindrical sinusoid/Disse space (see

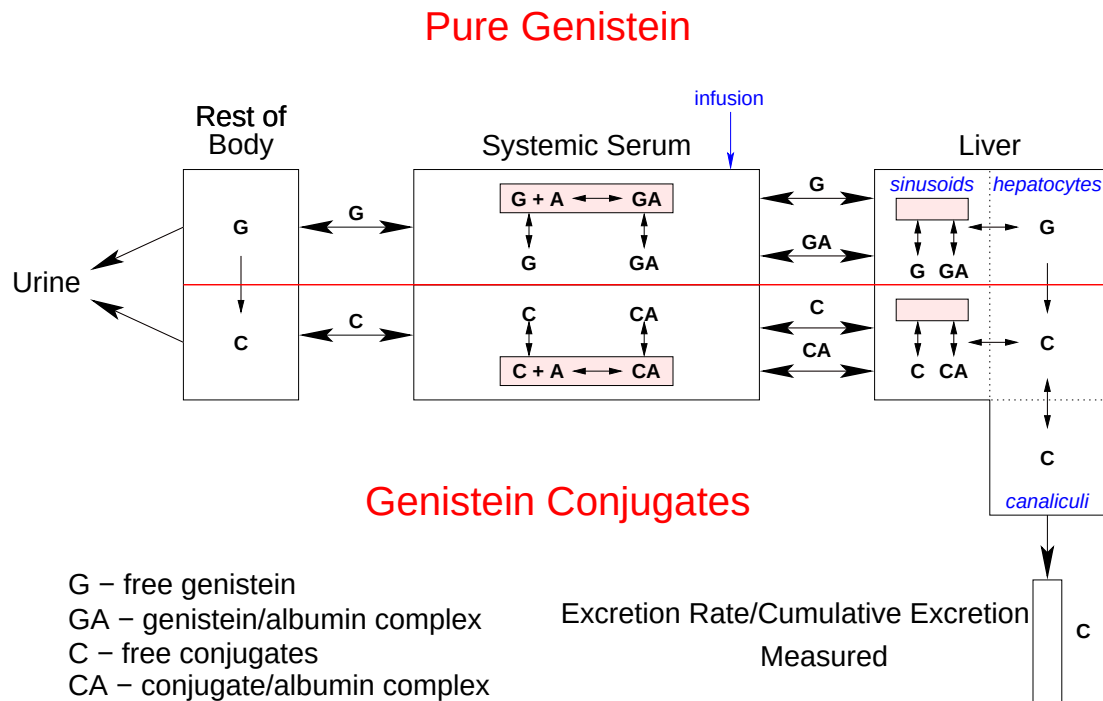


Figure 3.2: Model for GEN dosimetry in adult rats. Note that although the figure depicts the association and dissociation between GEN and albumin, these dynamics are not tracked in the model equations. In the model, we assume constant free fractions of GEN and CON (see Issue 1 in the previous section).

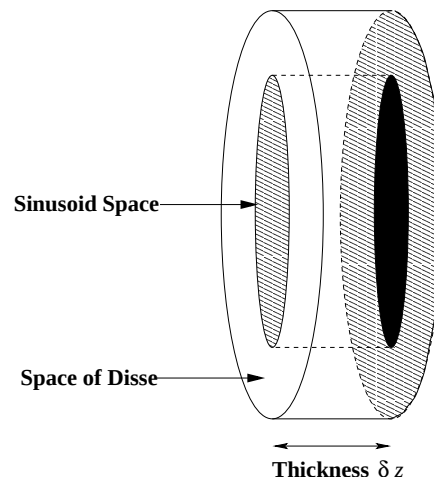


Figure 3.3: Sinusoid and Disse space/bile duct element of thickness δz .

Figure 3.3). Note that

$$\begin{pmatrix} \text{rate of change} \\ \text{of GEN} \\ \text{within element} \end{pmatrix} = \begin{pmatrix} \text{net rate of flow} \\ \text{of GEN} \\ \text{into element} \end{pmatrix} + \begin{pmatrix} \text{exchange rate} \\ \text{of GEN} \\ \text{within element} \end{pmatrix}$$

and following the work in (44),

$$\begin{pmatrix} \text{net rate of flow} \\ \text{of GEN} \\ \text{into element} \end{pmatrix} = V_{l,s}(z) \mathcal{D}_l \frac{\partial^2 C_{l,s}^{GEN}}{\partial z^2} - V_{l,s}(z) v_l \frac{\partial C_{l,s}^{GEN}}{\partial z},$$

where $V_{l,s}(z)$ is the volume of the sinusoidal space of the element. Now, with our assumption that GEN permeates across the hepatocyte cell membrane from the Disse space with a permeability coefficient of P_{GEN} , exchange of GEN in the sinusoid/Disse space element with the hepatocytes is given by:

$$\begin{aligned} \begin{pmatrix} \text{exchange rate} \\ \text{of GEN} \\ \text{within element} \end{pmatrix} &= P_{GEN}(C_{l,h}^{G_u} - C_{l,s}^{G_u}) \\ &= P_{GEN}(C_{l,h}^{GEN} - f_{l,s}^{G_u} C_{l,s}^{GEN}) \end{aligned}$$

since $C_{l,h}^{G_u} = C_{l,h}^{GEN}$, as described above. Hence, we have

$$\begin{aligned} \begin{pmatrix} \text{rate of change} \\ \text{of GEN} \\ \text{within element} \end{pmatrix} &= V_{l,s}(z) \mathcal{D}_l \frac{\partial^2 C_{l,s}^{GEN}}{\partial z^2} - V_{l,s}(z) v_l \frac{\partial C_{l,s}^{GEN}}{\partial z} \\ &\quad + P_{GEN}(C_{l,h}^{GEN} - f_{l,s}^{G_u} C_{l,s}^{GEN}). \end{aligned}$$

Assuming volumes do not change along the length L of the liver, we then obtain

$$\left(\begin{array}{c} \text{rate of change} \\ \text{of GEN in the} \\ \text{sinusoid/Disse space} \end{array} \right) = V_{l,s} \mathcal{D}_l \frac{\partial^2 C_{l,s}^{GEN}}{\partial z^2} - V_{l,s} v_l \frac{\partial C_{l,s}^{GEN}}{\partial z} + P_{GEN} (C_{l,h}^{GEN} - f_{l,s}^{G_u} C_{l,s}^{GEN}),$$

where $V_{l,s}$ is the volume of the sinusoidal space in the liver.

However, since GEN is present in sinusoidal serum and the Disse space, the rate of change of GEN in the sinusoid/Disse space is the sum of the rates of change in each of these two spaces, that is,

$$\left(\begin{array}{c} \text{rate of change} \\ \text{of GEN in the} \\ \text{sinusoid/Disse space} \end{array} \right) = V_{l,s} \frac{\partial C_{l,s}^{GEN}}{\partial t} + V_{l,D} \frac{\partial C_{l,D}^{GEN}}{\partial t} \quad (3.5)$$

Putting together (3.4) and (3.5), we have

$$\left(V_{l,s} + V_{l,D} \frac{f_{l,s}^{G_u}}{f_{l,D}^{G_u}} \right) \frac{\partial C_{l,s}^{GEN}}{\partial t} = V_{l,s} \mathcal{D}_l \frac{\partial^2 C_{l,s}^{GEN}}{\partial z^2} - V_{l,s} v_l \frac{\partial C_{l,s}^{GEN}}{\partial z} + P_{GEN} (C_{l,h}^{GEN} - f_{l,s}^{G_u} C_{l,s}^{GEN}). \quad (3.6)$$

Because of assumption R1, we have $C_{l,D}^{G_u} = C_{l,s}^{G_u}$. As stated above, we define the rate of diffusion of GEN between the space of Disse and the space of hepatocytes to be P_{GEN} . Also recall our assumption that metabolism of GEN in the hepatocytes is a saturable process, which is modeled by a Michaelis-Menten rate term for that process. So our mass balance

equation for GEN in the liver hepatocyte space is written as follows:

$$\begin{aligned}
 \frac{\partial C_{l,h}^{GEN}}{\partial t} &= \frac{P_{GEN}}{V_{l,h}}(C_{l,D}^{G_u} - C_{l,h}^{GEN}) - \frac{V_{max}C_{l,h}^{GEN}}{k_m + C_{l,h}^{GEN}} \\
 &= \frac{P_{GEN}}{V_{l,h}}(C_{l,s}^{G_u} - C_{l,h}^{GEN}) - \frac{V_{max}C_{l,h}^{GEN}}{k_m + C_{l,h}^{GEN}} \\
 &= \frac{P_{GEN}}{V_{l,h}}(f_{l,s}^{G_u}C_{l,s}^{GEN} - C_{l,h}^{GEN}) - \frac{V_{max}C_{l,h}^{GEN}}{k_m + C_{l,h}^{GEN}}. \tag{3.7}
 \end{aligned}$$

Recall that we assume biliary excretion is both an active and passive process. Hence, the exchange of CON into the bile from the hepatocytes can be expressed as a sum of two processes, namely a linear term for the active transport process and a passive diffusion term:

$$\left(\begin{array}{c} \text{exchange rate} \\ \text{of CON} \\ \text{within bile element} \end{array} \right) = P_{CON}(C_{l,h}^C - C_{l,b}^C) + k_{bile}C_{l,h}^C V_{l,h},$$

where P_{CON} is defined to be the permeability constant for diffusion of CON between the space of Disse and the space of hepatocytes. So, recalling $C_{l,h}^{G_u} = C_{l,h}^{CON}$, our mass-balance equation for CON in the hepatocyte space is given by

$$\frac{\partial C_{l,h}^C}{\partial t} = \frac{V_{max}C_{l,h}^{GEN}}{k_m + C_{l,h}^{GEN}} + \frac{P_{CON}}{V_{l,h}}(f_{l,s}^{G_u}C_{l,s}^C - C_{l,h}^C) - \left(\frac{P_{CON}}{V_{l,h}}(C_{l,h}^C - C_{l,b}^C) + k_{bile}C_{l,h}^C \right). \tag{3.8}$$

Similar to our derivation of equation (3.6), we now formulate a partial differential equation (PDE) for CON in the sinusoid/Disse space as follows. Note that

$$\left(\begin{array}{c} \text{rate of change} \\ \text{of CON} \\ \text{within element} \end{array} \right) = \left(\begin{array}{c} \text{net rate of flow} \\ \text{of CON} \\ \text{into element} \end{array} \right) + \left(\begin{array}{c} \text{exchange rate} \\ \text{of CON} \\ \text{within element} \end{array} \right),$$

where we have

$$\begin{aligned} \begin{pmatrix} \text{net rate of flow} \\ \text{of CON} \\ \text{into element} \end{pmatrix} &= V_{l,s}(z) \mathcal{D}_l \frac{\partial^2 C_{l,s}^C}{\partial z^2} - V_{l,s}(z) v_l \frac{\partial C_{l,s}^C}{\partial z}, \\ \begin{pmatrix} \text{exchange rate} \\ \text{of CON} \\ \text{within element} \end{pmatrix} &= P_{CON}(C_{l,h}^C - f_{l,s}^{C_u} C_{l,s}^C). \end{aligned}$$

Now, assuming volumes do not change, we obtain

$$\begin{pmatrix} \text{rate of change} \\ \text{of CON in the} \\ \text{sinusoid/Disse space} \end{pmatrix} = V_{l,s} \mathcal{D}_l \frac{\partial^2 C_{l,s}^C}{\partial z^2} - V_{l,s} v_l \frac{\partial C_{l,s}^C}{\partial z} + P_{CON}(C_{l,h}^C - f_{l,s}^{C_u} C_{l,s}^C). \quad (3.9)$$

Again, similar to the previous derivation, since CON are present in the sinusoidal and Disse spaces, we have

$$\begin{pmatrix} \text{rate of change} \\ \text{of CON in the} \\ \text{sinusoid/Disse space} \end{pmatrix} = V_{l,s} \frac{\partial C_{l,s}^C}{\partial t} + V_{l,D} \frac{\partial C_{l,D}^C}{\partial t}. \quad (3.10)$$

So putting together equations (3.9) and (3.10) yields

$$\left(V_{l,s} + \frac{f_{l,s}^{C_u}}{f_{l,D}^{C_u}} V_{l,D} \right) \frac{\partial C_{l,s}^C}{\partial t} = V_{l,s} \mathcal{D}_l \frac{\partial^2 C_{l,s}^C}{\partial z^2} - V_{l,s} v_l \frac{\partial C_{l,s}^C}{\partial z} + P_{CON}(C_{l,h}^C - f_{l,s}^{C_u} C_{l,s}^C). \quad (3.11)$$

We now formulate a PDE for CON in the bile duct, following the same procedure as above. Considering an element of the bile duct with thickness δz at a location $z < L$, we

have

$$\begin{pmatrix} \text{rate of change} \\ \text{of CON} \\ \text{within element} \end{pmatrix} = \begin{pmatrix} \text{net rate of flow} \\ \text{of CON} \\ \text{into element} \end{pmatrix} + \begin{pmatrix} \text{exchange rate} \\ \text{of CON} \\ \text{within element} \end{pmatrix}.$$

To this end, we note that

$$\begin{pmatrix} \text{net rate of flow} \\ \text{of CON} \\ \text{into element} \end{pmatrix} = V_{l,b}(z) \mathcal{D}_b \frac{\partial^2 C_{l,b}^C}{\partial z^2} + V_{l,b}(z) v_b \frac{\partial C_{l,b}^C}{\partial z},$$

$$\begin{pmatrix} \text{exchange rate} \\ \text{of CON} \\ \text{within element} \end{pmatrix} = P_{CON}(C_{l,h}^C - C_{l,b}^C) + k_{bile} C_{l,h}^C V_{l,h}(z).$$

Note the change of sign for the value of v_b , due to the opposite direction of bile flow to the blood flow. Putting these together and assuming no volume changes, we have

$$\begin{pmatrix} \text{rate of change} \\ \text{of CON} \\ \text{in the bile duct} \end{pmatrix} = V_{l,b} \mathcal{D}_b \frac{\partial^2 C_{l,b}^C}{\partial z^2} + V_{l,b} v_b \frac{\partial C_{l,b}^C}{\partial z} \quad (3.12)$$

$$+ P_{CON}(C_{l,h}^C - C_{l,b}^C) + k_{bile} C_{l,h}^C V_{l,h}.$$

Now, since the rate of change of CON in the bile duct is given by

$$\begin{pmatrix} \text{rate of change} \\ \text{of CON} \\ \text{in the bile duct} \end{pmatrix} = V_{l,b} \frac{\partial C_{l,b}^C}{\partial t}, \quad (3.13)$$

combining equations (3.13) and (3.14) yields

$$V_{l,b} \frac{\partial C_{l,b}^C}{\partial t} = V_{l,b} \mathcal{D}_b \frac{\partial^2 C_{l,b}^C}{\partial z^2} + V_{l,b} v_b \frac{\partial C_{l,b}^C}{\partial z} + P_{CON}(C_{l,h}^C - C_{l,b}^C) + k_{bile} C_{l,h}^C V_{l,h}. \quad (3.14)$$

Lastly, we consider the bile duct *outside the liver*. For lack of a better term, we will call this part of the bile duct the bile extension tube. Unlike the bile duct tube, this extended tube does not border any hepatocytes and is a single transport tube, rather than a network of bile duct canaliculi. To formulate the equation for this tube, we assume it extends out from the end of the bile duct from $z = 0$ to a length $z = -\hat{L}$. Recall the experimental data we desire to simulate is biliary excretion. For boundary condition purposes, this length $-\hat{L}$ is a very large number in magnitude, which we will discuss in the next section. In the model, we must choose a length less than $\hat{L}$ and compute the amount of CON in the extension tube at that point in order to simulate biliary excretion. This chosen length must represent not only the length of the bile duct outside the liver, but also the length of the canula attached to it that leads to the measuring device. To simplify the modeling process, we assume a homogeneous transition from the bile duct to the canula. That is, we assume the connection is seamless in that there is no flow or pressure disturbance in the bile while traveling through the duct and into the canula. Note that in the bile extension tube our dispersion number should be less than in the bile duct tube due to the differences between the two tubes, as stated above. That is, the extension tube represents a single transport tube away from the liver, as opposed to a network of canaliculi where we consider path differences, velocity differences, etc. Hence, with these considerations, we can formulate an equation for the extension tube from (3.14). Let C_{tube}^C be defined as the concentration of CON in the bile extension tube. Then, dropping the terms representing hepatocyte interaction and replacing the dispersion number, the equation for the bile extension tube becomes:

$$\frac{\partial C_{tube}^C}{\partial t} = \mathcal{D}_t \frac{\partial^2 C_{tube}^C}{\partial z^2} + v_b \frac{\partial C_{tube}^C}{\partial z}, \quad (3.15)$$

noting that the sign of the velocity term is negative as it is for the equation for the bile duct. This is because we are assuming the bile extension tube is simply a long tube with no outside interaction where flow begins at $x = 0$ and ends at some distance $x = -\hat{L}$.

Equations (3.1-4), (3.6-8), (3.11), and (3.14-15) yield a system of ten ODE and PDE in ten unknowns. This system of equations is summarized as follows:

$$\left\{ \begin{array}{l} \frac{dC_{ROB}^{GEN}}{dt} = \frac{P_{GEN}}{V_{ROB}} (f_{ss}^{Gu} C_{ss}^{GEN} - C_{ROB}^{GEN}) - k_{urine}^{GEN} C_{ROB}^{GEN} \\ \quad - k_{ROB}^m C_{ROB}^{GEN}, \\ \frac{dC_{ROB}^{CON}}{dt} = \frac{P_{CON}}{V_{ROB}} (f_{ss}^{Cu} C_{ss}^{CON} - C_{ROB}^{CON}) - k_{urine}^{CON} C_{ROB}^{CON} \\ \quad + k_{ROB}^m C_{ROB}^{GEN}, \\ \frac{dC_{ss}^{GEN}}{dt} = \frac{P_{GEN}}{V_{ROB}} (C_{ROB}^{GEN} - f_{ss}^{Gu} C_{ss}^{GEN}) + \frac{Q_l}{V_{l,s}} (C_{l,t}^{GEN}(t, L + \bar{L}) \\ \quad - C_{ss}^{GEN}) + \frac{1}{V_{ss}} v_{inf}(t), \\ \frac{dC_{ss}^{CON}}{dt} = \frac{P_{CON}}{V_{ROB}} (C_{ROB}^{CON} - f_{ss}^{Cu} C_{ss}^{CON}) + \frac{Q_l}{V_{l,s}} (C_{l,t}^{CON}(t, L + \bar{L}) \\ \quad - C_{ss}^{CON}), \\ (V_{l,s} + \frac{f_{l,s}^{Gu}}{f_{l,D}^{Gu}} V_{l,D}) \frac{\partial C_{l,s}^{GEN}}{\partial t} = V_{l,s} \mathcal{D}_l \frac{\partial^2 C_{l,s}^{GEN}}{\partial z^2} - V_{l,s} v_l \frac{\partial C_{l,s}^{GEN}}{\partial z} \\ \quad + P_{GEN} (C_{l,h}^{GEN} - f_{l,s}^{Gu} C_{l,s}^{GEN}), \\ \frac{\partial C_{l,h}^{GEN}}{\partial t} = \frac{P_{GEN}}{V_{l,h}} (f_{l,s}^{Gu} C_{l,s}^{GEN} - C_{l,h}^{GEN}) - \frac{V_{max} C_{l,h}^{GEN}}{k_m + C_{l,h}^{GEN}}, \\ \frac{\partial C_{l,h}^C}{\partial t} = \frac{V_{max} C_{l,h}^{GEN}}{k_m + C_{l,h}^{GEN}} + \frac{P_{CON}}{V_{l,h}} (f_{l,s}^{Cu} C_{l,s}^C - C_{l,h}^C) \\ \quad - \left(\frac{P_{CON}}{V_{l,h}} (C_{l,h}^C - C_{l,b}^C) + k_{bile} C_{l,h}^C \right), \\ (V_{l,s} + \frac{f_{l,s}^{Cu}}{f_{l,D}^{Cu}} V_{l,D}) \frac{\partial C_{l,s}^C}{\partial t} = V_{l,s} \mathcal{D}_l \frac{\partial^2 C_{l,s}^C}{\partial z^2} - V_{l,s} v_l \frac{\partial C_{l,s}^C}{\partial z} \\ \quad + P_{CON} (C_{l,h}^C - f_{l,s}^{Cu} C_{l,s}^C), \\ V_{l,b} \frac{\partial C_{l,b}^C}{\partial t} = V_{l,b} \mathcal{D}_b \frac{\partial^2 C_{l,b}^C}{\partial z^2} + V_{l,b} v_b \frac{\partial C_{l,b}^C}{\partial z} + P_{CON} (C_{l,h}^C - C_{l,b}^C) \\ \quad + k_{bile} C_{l,h}^C V_{l,h}, \\ \frac{\partial C_{tube}^C}{\partial t} = \mathcal{D}_t \frac{\partial^2 C_{tube}^C}{\partial z^2} + v_b \frac{\partial C_{tube}^C}{\partial z}. \end{array} \right. \quad (3.16)$$

3.3 Boundary Conditions

3.3.1 Liver - Sinusoidal Cylinder

Since the two equations governing the concentration of GEN and CON in the liver compartment contain second order spatial derivatives, these equations require two boundary conditions each.

The boundary conditions at the entrance to the sinusoidal cylinder ($z = 0$) are simply the concentrations of GEN and CON in the systemic serum for all t . The boundary conditions take the form:

$$\begin{aligned} C_{l,s}^{GEN}(t,0) &= C_{ss}^{GEN}(t), \\ C_{l,s}^C(t,0) &= C_{ss}^C(t). \end{aligned} \tag{3.17}$$

Now, for the boundary conditions at the exit of the sinusoidal cylinder ($z = L$), we assume no significant events occur affecting the concentration of GEN or CON in the liver outside of the sinusoidal cylinder, which recall includes the sinusoidal, Disse and hepatocyte spaces. A common practice for boundary condition formulation in this case is to assume plug flow outside the sinusoid and set $\frac{\partial C_{l,s}^{GEN,C}}{\partial z} = 0$. This is known as the Dankwerts boundary condition and is used mostly in modeling tubular chemical reactors. Setting the gradient to zero is based on an argument in Danwerts's original paper (45) that fails to hold here due to the two way exchange of GEN and CON between the hepatocytes and sinusoid cylinder. So we abandon this approach and instead, we add a tube of length $\bar{L}$, where $\bar{L} \gg L$, onto the end of the sinusoid tube (at $z = L$), such that all of the properties of the sinusoid tube hold in this extended tube, but from $z = L$ to $z = L + \bar{L}$, there are no hepatocytes, thus no exchange of GEN or CON in the tube from L to $L + \bar{L}$. This assumption allows us to assume that at $z = L$, we have equality of concentrations and fluxes. We

choose a large enough value of $\bar{L}$ so that we may approximate our boundary conditions

$$\frac{\partial C_{l,t}^{GEN}}{\partial z}(t, L + \bar{L}) = 0, \quad \frac{\partial C_{l,t}^C}{\partial z}(t, L + \bar{L}) = 0, \quad (3.18)$$

where $C_{l,t}$ is the concentration in the extended sinusoid tube ($L \leq z \leq L + \bar{L}$). Then, upon formulating second order partial differential equations for the concentrations of GEN and CON in the extension, we can simply define the boundary conditions at $z = L$ as follows:

$$\begin{cases} C_{l,s}^{GEN}(t, L) &= C_{l,t}^{GEN}(t, L) \\ \mathcal{D}_l \frac{\partial C_{l,s}^{GEN}}{\partial z}(t, L) - v_l C_{l,s}^{GEN}(t, L) &= \mathcal{D}_l \frac{\partial C_{l,t}^{GEN}}{\partial z}(t, L) - v_l C_{l,t}^{GEN}(t, L), \end{cases} \quad (3.19)$$

$$\begin{cases} C_{l,s}^{CON}(t, L) &= C_{l,t}^{CON}(t, L) \\ \mathcal{D}_l \frac{\partial C_{l,s}^{CON}}{\partial z}(t, L) - v_l C_{l,s}^{CON}(t, L) &= \mathcal{D}_l \frac{\partial C_{l,t}^{CON}}{\partial z}(t, L) - v_l C_{l,t}^{CON}(t, L), \end{cases} \quad (3.20)$$

which yields the required eight total boundary conditions for equations (3.6), (3.11) and the two equations for the extension.

We now formulate the equations required for the concentrations of GEN and CON in the sinusoid tube extension. We begin by examining equations (3.6) and (3.11) and recalling that in the sinusoid tube extension we have no hepatocyte interaction, and hence no metabolism. Therefore, in the extension, equation (3.6) reduces to

$$\left(V_{l,t} + V_{l,Dt} \frac{f_{l,t}^{G_u}}{f_{l,Dt}^{G_u}} \right) \frac{\partial C_{l,t}^{GEN}}{\partial t} = V_{l,t} \mathcal{D}_l \frac{\partial^2 C_{l,t}^{GEN}}{\partial z^2} - V_{l,t} v_l \frac{\partial C_{l,t}^{GEN}}{\partial z} \quad (3.21)$$

and equation (3.11) reduces to

$$(V_{l,t} + V_{l,Dt} \frac{f_{l,t}^{C_u}}{f_{l,Dt}^{C_u}}) \frac{\partial C_{l,t}^C}{\partial t} = V_{l,t} \mathcal{D}_l \frac{\partial^2 C_{l,t}^C}{\partial z^2} - V_{l,t} v_l \frac{\partial C_{l,t}^C}{\partial z}, \quad (3.22)$$

where $V_{l,t}$ is the volume of the sinusoid space in the extension tube, $V_{l,Dt}$ is the volume of

the Disse space in the tube, $f_{l,t}^{G_u}$ is the free fraction of GEN in the sinusoid space in the tube, and $f_{l,Dt}^{G_u}$ is the free fraction of GEN in the Disse space of the tube and similar definitions are for the terms in the equation (3.22) for CON. Although, in general, one would use different dispersion coefficients for the concentrations of GEN and CON in the sinusoid extension tube from those used in the sinusoid tube, given our assumptions on the extension tube, we assume they are equal. Equations (3.21) and (3.22) will be added to the system of equations (3.16) to yield a system of 12 equations in 12 unknowns.

Note that this tube extension is mainly for computational purposes, and the value we desire for use in the rest of the model is located at $z = L + l$, where l is chosen to represent the length of the central vein extending out from the liver, recalling $\bar{L} \gg L$. It is here where we would normally use a flux term (convection and dispersion) for the values of the concentrations of GEN and CON entering back into the systemic serum. However, systemic serum is widely accepted to equilibrate rapidly. Therefore, the gradient of the concentration of a chemical in the extension tube will approach zero much sooner than the end of the long tube. This allows us to shorten the tube itself without compromising any substantial biological relevance. If we choose a value of $\bar{L}$ simply greater than L (say, twice as long), then we are able to choose the location that the blood re-enters the systemic serum to be the end of the extension tube. Although the gradient may not quite equal zero at the end of a tube of that length, it is a reasonable approximation and allowing the blood to re-enter the serum at the end of a tube of that length will not interfere with the dynamics of the rest of the system by adding a considerable transit time. Since the gradient is set to zero at the end of the extension tube, the dispersion terms do not appear in the equations (3.3) and (3.4).

3.3.2 Bile Duct Cylinder

Equations (3.14) and (3.15) are second order equations and also require two boundary conditions.

Since our model is designed so that the bile duct canaliculi begin at $z = L$ and recalling that flow is in the reverse direction of the blood in the sinusoids, we assume there is no flow of bile in the bile duct cylinder at $z = L$, and hence no flux across the boundary $z = L$. Hence, we obtain

$$0 = v_b C_b^C + \mathcal{D}_b \frac{\partial C_b^C}{\partial z}, \quad z = L. \quad (3.23)$$

For the boundary at $z = 0$, our situation is similar to the boundary condition described for the sinusoid tube at $z = L$. So we have

$$\begin{cases} C_b^C(t, 0) &= C_{tube}^C(t, 0) \\ \mathcal{D}_b \frac{\partial C_b^C}{\partial z}(t, 0) + v_b C_b^C(t, 0) &= \mathcal{D}_t \frac{\partial C_{tube}^C}{\partial z}(t, 0) + v_b C_{tube}^C(t, 0), \end{cases} \quad (3.24)$$

where C_{tube} is the concentration in the bile tube. As mentioned in the previous section, the value of $\hat{L}$ is chosen large enough so that we may approximate our boundary condition

$$\frac{\partial C_{tube}^{CON}}{\partial z}(t, -\hat{L}) = 0, \quad (3.25)$$

which yields the last of the four necessary boundary conditions.

Again, this tube extension is mainly for computational purposes, and the value we desire for use in the rest of the model is located at $z = -\hat{L}$, where $\hat{L}$ is chosen to represent the length of the main bile duct extending out from the liver to the point where the bile is delivered into the gut lumen. It is here where we use a flux term for the value of the concentration of CON being excreted to compute the biliary excretion rate via the following

differential equation to be added to the system:

$$\frac{dA_{exc}^{CON}}{dt} = Av_b C_{tube}^C(t, -\hat{l}) + A \mathcal{D}_t \frac{\partial C_{tube}^{CON}}{\partial z}(t, -\hat{l}), \quad (3.26)$$

where A is the cross-sectional area of the tube.

With equations (3.17-20) and (3.23-25), we have formulated boundary conditions for the system of equations (3.16), with the addition of equations (3.21), (3.22), and (3.26).

With the state variables defined in Table 3.1 and parameters in Tables 3.2 and 3.3, our new system of equations is given by:

$$\begin{aligned} \frac{dC_{ROB}^{GEN}}{dt} &= \frac{P_{GEN}}{V_{ROB}} (f_{ss}^{G_u} C_{ss}^{GEN} - C_{ROB}^{GEN}) - k_{urine}^{GEN} C_{ROB}^{GEN} \\ &\quad - k_{ROB}^m C_{ROB}^{GEN}, \\ \frac{dC_{ROB}^{CON}}{dt} &= \frac{P_{CON}}{V_{ROB}} (f_{ss}^{C_u} C_{ss}^{CON} - C_{ROB}^{CON}) - k_{urine}^{CON} C_{ROB}^{CON} \\ &\quad + k_{ROB}^m C_{ROB}^{GEN}, \\ \frac{dC_{ss}^{GEN}}{dt} &= \frac{P_{GEN}}{V_{ROB}} (C_{ROB}^{GEN} - f_{ss}^{G_u} C_{ss}^{GEN}) + \frac{Q_l}{V_{l,s}} (C_{l,t}^{GEN}(t, L + \bar{L}) \\ &\quad - C_{ss}^{GEN}) + \frac{1}{V_{ss}} v_{inf}(t), \\ \frac{dC_{ss}^{CON}}{dt} &= \frac{P_{CON}}{V_{ROB}} (C_{ROB}^{CON} - f_{ss}^{C_u} C_{ss}^{CON}) + \frac{Q_l}{V_{l,s}} (C_{l,t}^{CON}(t, L + \bar{L}) \\ &\quad - C_{ss}^{CON}), \\ (V_{l,s} + \frac{f_{l,s}^{G_u}}{f_{l,D}^{G_u}} V_{l,D}) \frac{\partial C_{l,s}^{GEN}}{\partial t} &= V_{l,s} \mathcal{D}_l \frac{\partial^2 C_{l,s}^{GEN}}{\partial z^2} - V_{l,s} v_l \frac{\partial C_{l,s}^{GEN}}{\partial z} \\ &\quad + P_{GEN} (C_{l,h}^{GEN} - f_{l,s}^{G_u} C_{l,s}^{GEN}), \\ \left(V_{l,t} + \frac{f_{l,t}^{G_u}}{f_{l,Dt}^{G_u}} V_{l,Dt} \right) \frac{\partial C_{l,t}^{GEN}}{\partial t} &= V_{l,t} \mathcal{D}_l \frac{\partial^2 C_{l,t}^{GEN}}{\partial z^2} - V_{l,t} v_l \frac{\partial C_{l,t}^{GEN}}{\partial z}, \end{aligned} \quad (3.27)$$

$$\begin{aligned}
\frac{\partial C_{l,h}^{GEN}}{\partial t} &= \frac{P_{GEN}}{V_{l,h}} (f_{l,s}^{G_u} C_{l,s}^{GEN} - C_{l,h}^{GEN}) - \frac{V_{max} C_{l,h}^{GEN}}{k_m + C_{l,h}^{GEN}}, \\
\frac{\partial C_{l,h}^C}{\partial t} &= \frac{V_{max} C_{l,h}^{GEN}}{k_m + C_{l,h}^{GEN}} + \frac{P_{CON}}{V_{l,h}} (f_{l,s}^{C_u} C_{l,s}^C - C_{l,h}^C) \\
&\quad - \left(\frac{P_{CON}}{V_{l,h}} (C_{l,h}^C - C_{l,b}^C) + k_{bile} C_{l,h}^C \right), \\
(V_{l,s} + \frac{f_{l,s}^{C_u}}{f_{l,D}^{C_u}} V_{l,D}) \frac{\partial C_{l,s}^C}{\partial t} &= V_{l,s} \mathcal{D}_l \frac{\partial^2 C_{l,s}^C}{\partial z^2} - V_{l,s} v_l \frac{\partial C_{l,s}^C}{\partial z} \\
&\quad + P_{CON} (C_{l,h}^C - f_{l,s}^{C_u} C_{l,s}^C), \\
(V_{l,t} + \frac{f_{l,t}^{C_u}}{f_{l,Dt}^{C_u}} V_{l,Dt}) \frac{\partial C_{l,t}^C}{\partial t} &= V_{l,t} \mathcal{D}_l \frac{\partial^2 C_{l,t}^C}{\partial z^2} - V_{l,t} v_l \frac{\partial C_{l,t}^C}{\partial z}, \\
V_{l,b} \frac{\partial C_{l,b}^C}{\partial t} &= V_{l,b} \mathcal{D}_b \frac{\partial^2 C_{l,b}^C}{\partial z^2} + V_{l,b} v_b \frac{\partial C_{l,b}^C}{\partial z} + P_{CON} (C_{l,h}^C - C_{l,b}^C) \\
&\quad + k_{bile} C_{l,h}^C V_{l,h}, \\
\frac{\partial C_{tube}^C}{\partial t} &= \mathcal{D}_t \frac{\partial^2 C_{tube}^C}{\partial z^2} + v_b \frac{\partial C_{tube}^C}{\partial z}, \\
\frac{dA_{exc}^{CON}}{dt} &= A v_b C_{tube}^{CON}(t, -\hat{l}) + A \mathcal{D}_t \frac{\partial C_{tube}^{CON}}{\partial z}(t, -\hat{l}).
\end{aligned}$$

Chapter 4

Well-posedness of Solutions

In this chapter we use results for a class of abstract nonlinear parabolic equations established in (46) to show existence, uniqueness and continuous dependence of the solutions to our mathematical model on the data. Specifically, we will define the state space of the equations derived for the dispersion model in Chapter 2, show the equations belong to the class of equations in (46) and satisfies the standing assumptions necessary to obtain existence, uniqueness and continuous dependence. It is important to note that state variable y_{13} defined in Chapter 5 is uncoupled with the rest of the system. Therefore, it can safely be dropped from our well-posedness analysis in the following sections.

4.1 State Space and Problem Formulation for the Dispersion Model

In this section we begin by rewriting the system of equations (3.27) in terms of a weak formulation. Details of this formulation may be found in Chapter 5.2. We introduce dimensionless spatial variable $x = \frac{z}{L}$ and use the following change of variables:

$$\begin{aligned}
y_1(t, x) &= C_{l,s}^{GEN}(t, Lx) - C_{ss}^{GEN}(t), \\
y_2(t, x) &= C_{l,h}^{GEN}(t, Lx), \\
y_3(t, x) &= C_{l,s}^{CON}(t, Lx) - C_{ss}^{CON}(t), \\
y_4(t, x) &= C_{l,h}^{CON}(t, Lx), \\
y_5(t, x) &= C_{l,b}^{CON}(t, Lx), \\
y_6(t, x) &= C_{l,t}^{GEN}(t, L + \bar{L}x), \\
y_7(t, x) &= C_{l,t}^{CON}(t, L + \bar{L}x), \\
y_8(t, x) &= C_{tube}^{CON}(t, \hat{L}(x - 1)), \\
y_9(t) &= C_{ROB}^{GEN}(t), \\
y_{10}(t) &= C_{ROB}^{CON}(t), \\
y_{11}(t) &= C_{ss}^{GEN}(t), \\
y_{12}(t) &= C_{ss}^{CON}(t),
\end{aligned} \tag{4.1}$$

where y_1 and y_3 satisfy homogeneous Dirichlet boundary conditions at $x = 0$. We multiply each i^{th} equation by a test function ϕ_i from an appropriate class of test functions, integrating in space over the first eight equations and integrating by parts in equations y_1 , y_3 and y_5 through y_8 . We define the usual L_2 inner product by

$$\langle f, g \rangle = \int_0^1 f(\zeta)g(\zeta)d\zeta.$$

We take all Hilbert spaces to have real scalar fields. We let $H = L_2(0, 1)$, $\hat{H} = H^1(0, 1)$ and $V = H_L^1(0, 1)$, where we define

$$H_L^1(0, 1) = \{\phi \in H^1(0, 1) | \phi(0) = 0\}$$

and V -norm

$$|\phi|_V = |\phi'|_H, \text{ for all } \phi \in V.$$

From this point on, we will denote the L_2 norm $|\cdot|_H$ simply by $|\cdot|$. Hence, for variables y_1 through y_8 , $|\cdot|$ denotes the L_2 norm and for variables y_9 through y_{12} , $|\cdot|$ obviously denotes the usual R -norm.

We define our state space

$$\mathcal{V} = V^2 \times \hat{H}^4 \times H^2 \times R^4$$

and the space

$$\mathcal{H} = H^8 \times R^4$$

with inner product and norms

$$\langle \psi, \phi \rangle_{\mathcal{H}} = \sum_{k=1}^8 \langle \psi_k, \phi_k \rangle + \sum_{n=9}^{12} \psi_n \phi_n, \text{ for all } \psi, \phi \in \mathcal{H},$$

$$|\phi|_{\mathcal{V}}^2 = |\phi_1|_V^2 + |\phi_2|^2 + |\phi_3|_V^2 + |\phi_4|^2 + \sum_{j=5}^8 |\phi_j|_{\hat{H}}^2 + \sum_{k=9}^{12} |\phi_k|^2, \text{ for } \phi \in \mathcal{V},$$

$$|\phi|_{\mathcal{H}}^2 = \sum_{j=1}^8 |\phi_j|^2 + \sum_{k=9}^{12} |\phi_k|^2, \text{ for } \phi \in \mathcal{H}.$$

The weak form of the system is as follows. We seek a solution $y(t) \in \mathcal{V}$ satisfying the initial condition $y(0) = \vec{0}$ and

$$\langle \dot{y}(t), \phi \rangle_{\mathcal{V}^*, \mathcal{V}} + \sigma(y(t), \phi) + \langle g(y(t)), \phi \rangle_{\mathcal{H}} = \langle f(t), \phi \rangle_{\mathcal{V}^*, \mathcal{V}}, \text{ for all } \phi \in \mathcal{V}, \quad (4.2)$$

where we have

$$y(t) = [y_1(t), \dots, y_{12}(t)],$$

$$f(t) = [-\frac{1}{V_{ss}}V_{inf}(t), 0, 0, 0, 0, 0, 0, 0, 0, 0, \frac{1}{V_{ss}}V_{inf}(t), 0]^T,$$

$$g(y) = [0, g_2(y_2), 0, -g_2(y_2), 0, 0, 0, 0, 0, 0, 0, 0],$$

$$g_2(y_2) = \begin{cases} \frac{V_{max}y_2}{k_m+y_2}, & y_2 \geq 0, \\ 0, & y_2 < 0, \end{cases}$$

and the sesquilinear form $\sigma : \mathcal{V} \times \mathcal{V} \rightarrow R$ is defined by

$$\begin{aligned} \sigma(\psi, \phi) = & -\frac{Q_l}{a_1 k_1} \mathcal{D}_l^N \psi'_6(0) \phi_1(1) + \frac{Q_l}{a_1} \psi_6(0) \phi_1(1) + \langle \frac{Q_l}{a_1} \mathcal{D}_l^N \psi'_1, \phi'_1 \rangle \\ & - \langle \frac{Q_l}{a_1} \psi_1, \phi'_1 \rangle - \langle \frac{P_{GEN}}{a_1} \psi_2, \phi_1 \rangle + \langle \frac{P_{GEN}}{a_1} f_{l,s}^{G_u} \psi_1, \phi_1 \rangle \\ & + \langle \frac{P_{GEN}}{a_1} f_{l,s}^{G_u} \psi_{11}, \phi_1 \rangle + \langle \frac{P_{GEN}}{V_{ROB}} \psi_9, \phi_1 \rangle - \langle \frac{P_{GEN}}{V_{ROB}} f_{ss}^{G_u} \psi_{11}, \phi_1 \rangle \\ & - \langle \frac{Q_l}{V_{l,s}} \psi_{11}, \phi_1 \rangle + \langle \frac{Q_l}{V_{l,s}} \psi_6(1), \phi_1 \rangle - \frac{Q_l}{a_1} \psi_{11} \phi_1(1) - \langle a_2 f_{l,s}^{G_u} \psi_1, \phi_2 \rangle \\ & + \langle a_2 \psi_2, \phi_2 \rangle - \langle a_2 f_{l,s}^{G_u} \psi_{11}, \phi_2 \rangle - \frac{Q_l}{a_3 k_1} \mathcal{D}_l^N \psi'_7(0) \phi_3(1) \\ & + \frac{Q_l}{a_3} \psi_7(0) \phi_3(1) + \langle \frac{Q_l}{a_3} \mathcal{D}_l^N \psi'_3, \phi'_3 \rangle - \langle \frac{Q_l}{a_3} \psi_3, \phi'_3 \rangle - \langle \frac{P_{CON}}{a_3} \psi_4, \phi_3 \rangle \\ & + \langle \frac{P_{CON}}{a_3} f_{l,s}^{C_u} \psi_3, \phi_3 \rangle + \langle \frac{P_{CON}}{a_3} f_{l,s}^{C_u} \psi_{12}, \phi_3 \rangle + \langle \frac{P_{CON}}{V_{ROB}} \psi_{10}, \phi_3 \rangle \\ & - \langle \frac{P_{CON}}{V_{ROB}} f_{ss}^{C_u} \psi_{12}, \phi_3 \rangle - \langle \frac{Q_l}{V_{l,s}} \psi_{12}, \phi_3 \rangle + \langle \frac{Q_l}{V_{l,s}} \psi_7(1), \phi_3 \rangle \\ & - \frac{Q_l}{a_3} \psi_{12} \phi_3(1) - \langle a_4 f_{l,s}^{C_u} \psi_3, \phi_4 \rangle + \langle 2a_4 \psi_4, \phi_4 \rangle - \langle a_4 f_{l,s}^{G_u} \psi_{12}, \phi_4 \rangle \\ & - \langle a_4 \psi_5, \phi_4 \rangle + \langle k_{bile} \psi_4, \phi_4 \rangle + \frac{Q_b}{V_{l,b}} \mathcal{D}_b^N \psi'_5(0) \phi_5(0) \\ & + \frac{Q_b}{V_{l,b}} \psi_8(1) \phi_5(0) + \langle \frac{Q_b}{V_{l,b}} \mathcal{D}_b^N \psi'_5, \phi'_5 \rangle + \langle \frac{Q_b}{V_{l,b}} \psi_5, \phi'_5 \rangle \\ & - \langle \frac{P_{CON}}{V_{l,b}} \psi_4, \phi_5 \rangle + \langle \frac{P_{CON}}{V_{l,b}} \psi_5, \phi_5 \rangle - \langle k_{bile} \frac{V_{l,h}}{V_{l,b}} \psi_4, \phi_5 \rangle \end{aligned} \quad (4.3)$$

$$\begin{aligned}
& + \frac{Q_l}{a_6} \psi_6(1) \phi_6(1) + \frac{Q_l}{a_6 k_1} \mathcal{D}_l^N \psi'_6(0) \phi_6(0) - \frac{Q_l}{a_6} \psi_1(1) \phi_6(0) \\
& + \langle \frac{Q_l k_1}{a_6} \mathcal{D}_l^N \psi'_6, \phi'_6 \rangle - \langle \frac{Q_l}{a_6} \psi_6, \phi'_6 \rangle - \frac{Q_l}{a_6} \psi_{11} \phi_6(0) + \frac{Q_l}{a_7} \psi_7(1) \phi_7(1) \\
& + \frac{Q_l}{a_7 k_1} \mathcal{D}_l^N \psi'_7(0) \phi_7(0) - \frac{Q_l}{a_7} \psi_3(1) \phi_7(0) + \langle \frac{Q_l k_1}{a_7} \mathcal{D}_l^N \psi'_7, \phi'_7 \rangle \\
& - \langle \frac{Q_l}{a_7} \psi_7, \phi'_7 \rangle - \frac{Q_l}{a_7} \psi_{12} \phi_7(0) - \frac{Q_b}{V_{tube}} \mathcal{D}_b^N \psi'_5(0) \phi_8(1) - \frac{Q_b}{V_{tube}} \psi_5(0) \phi_8(1) \\
& + \frac{Q_b}{V_{tube}} \psi_8(0) \phi_8(0) + \langle \frac{Q_b}{V_{tube}} \mathcal{D}_t^N \psi'_8, \phi'_8 \rangle + \langle \frac{Q_b}{V_{tube}} \psi_8, \phi'_8 \rangle \\
& - \frac{P_{GEN}}{V_{ROB}} f_{ss}^{G_u} \psi_{11} \phi_9 + \left(\frac{P_{GEN}}{V_{ROB}} + k_{urine}^{GEN} + k_{ROB}^m \right) \psi_9 \phi_9 \\
& - \frac{P_{CON}}{V_{ROB}} f_{ss}^{C_u} \psi_{12} \phi_{10} + \left(\frac{P_{CON}}{V_{ROB}} + k_{urine}^C \right) \psi_{10} \phi_{10} - k_{ROB}^m \psi_9 \phi_{10} \\
& - \frac{P_{GEN}}{V_{ROB}} (\psi_9 - f_{ss}^{G_u} \psi_{11}) \phi_{11} - \frac{Q_l}{V_{l,s}} \psi_6(x_0) \phi_{11} + \frac{Q_l}{V_{l,s}} \psi_{11} \phi_{11} \\
& - \frac{P_{CON}}{V_{ROB}} (\psi_{10} - f_{ss}^{C_u} \psi_{12}) \phi_{12} - \frac{Q_l}{V_{l,s}} \psi_7(x_0) \phi_{12} + \frac{Q_l}{V_{l,s}} \psi_{12} \phi_{12}.
\end{aligned}$$

Here, and in the following sections, $\dot{y} = \frac{\partial y}{\partial t}$, $\phi' = \frac{\partial \phi}{\partial x}$, and $\langle \cdot, \cdot \rangle_{\mathcal{V}^*, \mathcal{V}}$ denotes the usual duality product discussed in the next section.

4.2 Well-Posedness for a Class of Abstract Nonlinear Parabolic Systems

Consider the following system:

$$\begin{aligned}
\dot{y}(t) + \mathcal{A}y(t) + g(y(t)) &= f(t) \\
y(0) &= y_0,
\end{aligned} \tag{4.4}$$

in $\mathcal{V}^*$, for $t \in (0, T)$ with $T < \infty$. We assume $\mathcal{V}^*$, $\mathcal{V}$ and $\mathcal{H}$ are all separable, real Hilbert spaces forming a Gelfand triple which satisfies

$$\mathcal{V} \hookrightarrow \mathcal{H} \cong \mathcal{H}^* \hookrightarrow \mathcal{V}^*.$$

We denote the norms the spaces $\mathcal{V}^*$, $\mathcal{V}$ and $\mathcal{H}$ by $|\cdot|_{\mathcal{V}^*}$, $|\cdot|_{\mathcal{V}}$ and $|\cdot|$, respectively, and the inner product in $\mathcal{H}$ by $\langle \cdot, \cdot \rangle$. We assume the embedding $\mathcal{V} \hookrightarrow \mathcal{H}$ is dense and continuous, with

$$|\phi| \leq c|\phi|_{\mathcal{V}}$$

for all $\phi \in \mathcal{V}$. The duality product $\langle \cdot, \cdot \rangle_{\mathcal{V}^*, \mathcal{V}}$ is the extension by continuity of the inner product in $\mathcal{H}$ from $\mathcal{H} \times \mathcal{V}$ to $\mathcal{V}^* \times \mathcal{V}$.

We assume the operator $\mathcal{A}$ is defined in terms of a sesquilinear form $\sigma : \mathcal{V} \times \mathcal{V} \rightarrow \mathbb{R}$, i.e., we define $\mathcal{A} : \mathcal{V} \rightarrow \mathcal{V}^*$ by $\langle \mathcal{A}\phi, \psi \rangle_{\mathcal{V}^*, \mathcal{V}} = \sigma(\phi, \psi)$. With assumptions A1 and A2 made below on σ , it follows that $\mathcal{A} \in \mathcal{L}(\mathcal{V}, \mathcal{V}^*)$. For a complete discussion on this, inner product extensions and Gelfand triples, we refer the reader to (47).

We make the following standing assumptions on σ , f and g :

A1 The sesquilinear form σ is bounded in $\mathcal{V}$, i.e., there exists $\gamma_1 > 0$ such that

$$|\sigma(\psi, \phi)| \leq \gamma_1 |\psi|_{\mathcal{V}} |\phi|_{\mathcal{V}}, \quad \forall \psi, \phi \in \mathcal{V}.$$

A2 The sesquilinear form σ is elliptic on $\mathcal{V}$, that is, there exist $k > 0$ and $\lambda_0 > 0$ such that

$$\sigma(\phi, \phi) \geq k|\phi|_{\mathcal{V}}^2 - \lambda_0 |\phi|^2, \quad \forall \phi \in \mathcal{V}.$$

A3 The forcing function f satisfies

$$f \in L_2((0, T); \mathcal{V}^*).$$

A4 The nonlinear function $g : \mathcal{H} \rightarrow \mathcal{H}$ satisfies the following local Lipschitz condition: let $B_r(0) = \{u \in \mathcal{H} : |u| \leq r\}$ denote the ball of radius r centered around the origin in $\mathcal{H}$. Then given $r > 0$, there exists $L_{B_r} > 0$ such that

$$|g(\psi) - g(\phi)| \leq L_{B_r} |\psi - \phi|, \forall \psi, \phi \in B_r(0).$$

A5 There exist positive constants C_1 and C_2 such that

$$|g(\phi)| \leq C_1 |\phi| + C_2, \forall \phi \in \mathcal{H}.$$

We say that $y \in L_2((0, T); \mathcal{V})$ is a solution to the system (4.4) if it satisfies

$$\begin{aligned} \langle \dot{y}(t), \psi \rangle_{\mathcal{V}^*, \mathcal{V}} + \sigma(y(t), \psi) + \langle g(y(t)), \psi \rangle &= \langle f(t), \psi \rangle_{\mathcal{V}^*, \mathcal{V}} \\ y(0) &= y_0, \end{aligned} \quad (4.5)$$

for all $\psi \in \mathcal{V}$.

Theorem 4.1 Under assumptions A1 - A5 with $y_0 \in \mathcal{H}$, the system (4.5) has a unique global solution $y \in L_2((0, T); \mathcal{V}) \cap C([0, T]; \mathcal{H})$ which depends continuously on the data (y_0, f) .

This theorem is presented and proven in (46).

4.3 Well-Posedness of the Dispersion Model

In this section we verify that the dispersion model fits into the class of systems discussed in Section 4.2.

Consider the weak formulation of the dispersion model (4.2) with the definitions of the spaces, inner products and norms outlined in Section 4.2. The spaces $\mathcal{V}$ and $\mathcal{H}$ form a Gelfand triple with the duality product $\langle \cdot, \cdot \rangle_{\mathcal{V}^*, \mathcal{V}}$. Below are proofs that assumptions A1

- A5 hold in the case of the weak formulation of the dispersion model. Once proof that the assumptions hold is given, we may apply Theorem 4.1 to establish the global existence, uniqueness and continuous dependence of a solution for the dispersion model.

Proof of A1 Note that

$$|\phi|_{\mathcal{V}} \geq |\phi_i| \geq |\phi_i(x)|, \forall x, i.$$

With the above inequalities and (4.3), clearly γ_1 can be found so that A1 holds.

Proof of A2 From (4.3), we have

$$\begin{aligned} \sigma(\phi, \phi) = & -\frac{Q_l}{a_1 k_1} \mathcal{D}_l^N \phi'_6(0) \phi_1(1) + \frac{Q_l}{a_1} \phi_6(0) \phi_1(1) + \frac{Q_l}{a_1} \mathcal{D}_l^N |\phi_1|_{\mathcal{V}}^2 \\ & - \frac{Q_l}{a_1} \langle \phi_1, \phi'_1 \rangle - \frac{P_{GEN}}{a_1} \langle \phi_2, \phi_1 \rangle + \frac{P_{GEN}}{a_1} f_{l,s}^{G_u} |\phi_1|^2 \\ & + \frac{P_{GEN}}{a_1} f_{l,s}^{G_u} \langle \phi_{11}, \phi_1 \rangle + \frac{P_{GEN}}{V_{ROB}} \langle \phi_9, \phi_1 \rangle - \frac{P_{GEN}}{V_{ROB}} f_{ss}^{G_u} \langle \phi_{11}, \phi_1 \rangle \\ & - \frac{Q_l}{V_{l,s}} \langle \phi_{11}, \phi_1 \rangle + \frac{Q_l}{V_{l,s}} \langle \phi_6(1), \phi_1 \rangle - \frac{Q_l}{a_1} \phi_{11} \phi_1(1) - a_2 f_{l,s}^{G_u} \langle \phi_1, \phi_2 \rangle \\ & + a_2 |\phi_2|^2 - a_2 f_{l,s}^{G_u} \langle \phi_{11}, \phi_2 \rangle - \frac{Q_l}{a_3 k_1} \mathcal{D}_l^N \phi'_7(0) \phi_3(1) + \frac{Q_l}{a_3} \phi_7(0) \phi_3(1) \\ & + \frac{Q_l}{a_3} \mathcal{D}_l^N |\phi_3|_{\mathcal{V}}^2 - \frac{Q_l}{a_3} \langle \phi_3, \phi'_3 \rangle - \frac{P_{CON}}{a_3} \langle \phi_4, \phi_3 \rangle \\ & + \frac{P_{CON}}{a_3} f_{l,s}^{C_u} |\phi_3|^2 + \frac{P_{CON}}{a_3} f_{l,s}^{C_u} \langle \phi_{12}, \phi_3 \rangle + \frac{P_{CON}}{V_{ROB}} \langle \phi_{10}, \phi_3 \rangle \\ & - \frac{P_{CON}}{V_{ROB}} f_{ss}^{C_u} \langle \phi_{12}, \phi_3 \rangle - \frac{Q_l}{V_{l,s}} \langle \phi_{12}, \phi_3 \rangle + \frac{Q_l}{V_{l,s}} \langle \phi_7(1), \phi_3 \rangle \\ & - \frac{Q_l}{a_3} \phi_{12} \phi_3(1) - a_4 f_{l,s}^{C_u} \langle \phi_3, \phi_4 \rangle + 2a_4 |\phi_4|^2 - a_4 f_{l,s}^{G_u} \langle \phi_{12}, \phi_4 \rangle \\ & - a_4 \langle \phi_5, \phi_4 \rangle + k_{bile} |\phi_4|^2 + \frac{Q_b}{V_{l,b}} \mathcal{D}_b^N \phi'_5(0) \phi_5(0) \\ & + \frac{Q_b}{V_{l,b}} \phi_8(1) \phi_5(0) + \frac{Q_b}{V_{l,b}} \mathcal{D}_b^N |\phi_5|_{\mathcal{V}}^2 + \frac{Q_b}{V_{l,b}} \langle \phi_5, \phi'_5 \rangle \\ & - \frac{P_{CON}}{V_{l,b}} \langle \phi_4, \phi_5 \rangle + \frac{P_{CON}}{V_{l,b}} |\phi_5|^2 - k_{bile} \frac{V_{l,h}}{V_{l,b}} \langle \phi_4, \phi_5 \rangle \end{aligned} \quad (4.6)$$

$$\begin{aligned}
& + \frac{Q_l}{a_6} \phi_6^2(1) + \frac{Q_l k_1}{a_6} \mathcal{D}_l^N \phi_6'(0) \phi_6(0) - \frac{Q_l}{a_6} \phi_1(1) \phi_6(0) \\
& + \frac{Q_l k_1}{a_6} \mathcal{D}_l^N |\phi_6|_V^2 - \frac{Q_l}{a_6} \langle \phi_6, \phi_6' \rangle - \frac{Q_l}{a_6} \phi_{11} \phi_6(0) + \frac{Q_l}{a_7} \phi_7^2(1) \\
& + \frac{Q_l k_1}{a_7} \mathcal{D}_l^N \phi_7'(0) \phi_7(0) - \frac{Q_l}{a_7} \phi_3(1) \phi_7(0) + \frac{Q_l k_1}{a_7} \mathcal{D}_l^N |\phi_7|_V^2 \\
& - \frac{Q_l}{a_7} \langle \phi_7, \phi_7' \rangle - \frac{Q_l}{a_7} \phi_{12} \phi_7(0) - \frac{Q_b}{V_{tube}} \mathcal{D}_b^N \phi_5'(0) \phi_8(1) - \frac{Q_b}{V_{tube}} \phi_5(0) \phi_8(1) \\
& + \frac{Q_b}{V_{tube}} \phi_8^2(0) + \frac{Q_b}{V_{tube}} \mathcal{D}_t^N |\phi_8|_V^2 + \frac{Q_b}{V_{tube}} \langle \phi_8, \phi_8' \rangle \\
& - \frac{P_{GEN}}{V_{ROB}} f_{ss}^{G_u} \phi_{11} \phi_9 + \left(\frac{P_{GEN}}{V_{ROB}} + k_{urine}^{GEN} + k_{ROB}^m \right) \phi_9^2 \\
& - \frac{P_{CON}}{V_{ROB}} f_{ss}^{C_u} \phi_{12} \phi_{10} + \left(\frac{P_{CON}}{V_{ROB}} + k_{urine}^C \right) \phi_{10}^2 - k_{ROB}^m \phi_9 \phi_{10} \\
& - \frac{P_{GEN}}{V_{ROB}} \phi_9 \phi_{11} + \frac{P_{GEN}}{V_{ROB}} f_{ss}^{G_u} \phi_{11}^2 - \frac{Q_l}{V_{l,s}} \phi_6(x_0) \phi_{11} + \frac{Q_l}{V_{l,s}} \phi_{11}^2 \\
& - \frac{P_{CON}}{V_{ROB}} \phi_{10} \phi_{12} + \frac{P_{CON}}{V_{ROB}} f_{ss}^{C_u} \phi_{12}^2 - \frac{Q_l}{V_{l,s}} \phi_7(x_0) \phi_{12} + \frac{Q_l}{V_{l,s}} \phi_{12}^2.
\end{aligned}$$

Using the Cauchy-Schwartz inequality and noting that $2ab \leq a^2 + b^2$, $\forall a, b \in R$, we have

$$\begin{aligned}
\frac{Q_l}{a_6} k_1 \mathcal{D}_l^N \phi_6'(0) \phi_1(1) & \leq \left(\frac{Q_l}{a_6} k_1 \mathcal{D}_l^N \right)^{\frac{1}{2}} |\phi_6'| \left(\frac{Q_l}{a_6} k_1 \mathcal{D}_l^N \right)^{\frac{1}{2}} |\phi_1| \\
& \leq \frac{Q_l}{2a_6} k_1 \mathcal{D}_l^N |\phi_6|_V^2 + \frac{Q_l}{2a_6} k_1 \mathcal{D}_l^N |\phi_1|^2.
\end{aligned}$$

Similarly,

$$\begin{aligned}
\frac{Q_l}{a_1} \langle \phi_1, \phi_1' \rangle & \leq \frac{Q_l}{\mathcal{D}_l^N a_1} |\phi_1|^2 + \frac{Q_l \mathcal{D}_l^N}{4a_1} |\phi_1|_V^2, \\
\frac{Q_l}{a_7} k_1 \mathcal{D}_l^N \phi_7'(0) \phi_3(1) & \leq \frac{Q_l}{2a_7} k_1 \mathcal{D}_l^N |\phi_7|_V^2 + \frac{Q_l}{2a_7} k_1 \mathcal{D}_l^N |\phi_3|^2,
\end{aligned}$$

$$\begin{aligned}
\frac{Q_l}{a_3} \langle \phi_3, \phi'_3 \rangle &\leq \frac{Q_l}{\mathcal{D}_l^N a_3} |\phi_3|^2 + \frac{Q_l \mathcal{D}_l^N}{4a_3} |\phi_3|_V^2, \\
\frac{Q_l}{a_6} \langle \phi_6, \phi'_6 \rangle &\leq \frac{Q_l}{2a_6} k_1 \mathcal{D}_l^N |\phi_6|_V^2 + \frac{Q_l}{2a_6 k_1 \mathcal{D}_l^N} |\phi_6|^2, \\
\frac{Q_l}{a_7} \langle \phi_7, \phi'_7 \rangle &\leq \frac{Q_l}{2a_7} k_1 \mathcal{D}_l^N |\phi_7|_V^2 + \frac{Q_l}{2a_7 k_1 \mathcal{D}_l^N} |\phi_7|^2, \\
\frac{Q_b}{V_{l,b}} \mathcal{D}_b^N \phi'_5(0) \phi_8(1) &\leq \frac{Q_b \mathcal{D}_b^N}{V_{l,b}} |\phi_5|_V^2 + \frac{Q_b \mathcal{D}_b^N}{4V_{l,b}} |\phi_8|^2.
\end{aligned}$$

Using the same techniques, we see that for any $f_1, f_2 \in H, g_1, g_2 \in R, a > 0$,

$$a \langle f_1, f_2 \rangle \leq \frac{a}{2} |f_1|^2 + \frac{a}{2} |f_2|^2.$$

Also note that

$$\begin{aligned}
a f_1^2(x) &\leq a |f_1|^2, \\
a g_1 g_2 &\leq \frac{a}{2} |g_1|_R^2 + \frac{a}{2} |g_2|_R^2, \\
a |g_1| |g_2| &\leq \frac{a}{2} |g_1|_R^2 + \frac{a}{2} |g_2|_R^2, \\
a f_1(x) g_2 &\leq \frac{a}{2} |f_1|^2 + \frac{a}{2} |g_2|_R^2.
\end{aligned}$$

Hence, we have

$$\begin{aligned}
\sigma(\phi, \phi) &\geq \frac{3\mathcal{D}_l^N Q_l}{4a_1} |\phi_1|_V^2 + a_2 |\phi_2|^2 + \frac{3\mathcal{D}_l^N Q_l}{4a_3} |\phi_3|_V^2 + 2a_4 |\phi_4|^2 + \frac{P_{CON}}{V_{l,b}} |\phi_5|^2 + \frac{Q_l}{a_6} |\phi_6|^2 \\
&\quad + \frac{Q_l}{a_7} |\phi_7|^2 + \frac{Q_b}{V_{tube}} |\phi_8|^2 + \left(\frac{P_{GEN}}{V_{ROB}} + k_{urine}^{GEN} + k_{ROB}^m \right) \phi_9^2
\end{aligned}$$

$$\begin{aligned}
& + \left(\frac{P_{CON}}{V_{ROB}} + k_{urine}^C \right) \phi_{10}^2 + \frac{Q_l}{V_{l,s}} \phi_{11}^2 + \frac{Q_l}{V_{l,s}} \phi_{12}^2 \\
& - \left(\frac{Q_l}{\mathcal{D}_l^N a_1} + \frac{Q_l}{2V_{l,s}} + \frac{P_{GEN}}{2a_1} + \frac{P_{GEN} f_{ss}^{G_u}}{2V_{ROB}} + \frac{a_2 f_{l,s}^{G_u}}{2} + \frac{Q_l \mathcal{D}_l^N}{2a_1 k_1} + \frac{Q_l}{a_1} \right) |\phi_1|^2 \\
& - \left(\frac{P_{GEN}}{2a_1} + a_2 f_{l,s}^{G_u} \right) |\phi_2|^2 \\
& - \left(\frac{2Q_l}{\mathcal{D}_l^N a_3} + \frac{Q_l}{2V_{l,s}} + \frac{P_{CON}}{2a_3} + \frac{P_{CON} f_{ss}^{C_u}}{2V_{ROB}} + \frac{a_4 f_{l,s}^{C_u}}{2} + \frac{Q_l \mathcal{D}_l^N}{2a_3 k_1} + \frac{Q_l}{a_3} \right) |\phi_3|^2 \\
& - \left(a_4 f_{l,s}^{C_u} + \frac{a_4}{2} + \frac{P_{CON}}{2a_3} + \frac{P_{CON}}{2V_{l,b}} + \frac{k_{bile} V_{l,h}}{2V_{l,b}} \right) |\phi_4|^2 \\
& - \left(\frac{Q_b}{2V_{l,b}} + \frac{P_{CON}}{2V_{l,b}} + \frac{k_{bile} V_{l,h}}{2V_{l,b}} \right) |\phi_5|^2 - \left(\frac{Q_l}{a_6} + \frac{Q_l}{2V_{l,s}} + \frac{Q_l}{2a_6 k_1 \mathcal{D}_l^N} \right) |\phi_6|^2 \\
& - \left(\frac{Q_l}{a_7} + \frac{Q_l}{2V_{l,s}} + \frac{Q_l}{2a_7 k_1 \mathcal{D}_l^N} \right) |\phi_7|^2 - \left(\frac{Q_b \mathcal{D}_b^N}{4V_{tube}} + \frac{Q_b}{2V_{tube}} \right) |\phi_8|^2 \\
& - \left(\frac{P_{GEN}}{2V_{ROB}} (1 + f_{ss}^{G_u}) + \frac{k_{ROB}^m}{2} \right) |\phi_9|^2 - \left(\frac{P_{CON}}{2V_{ROB}} (1 + f_{ss}^{C_u}) + \frac{k_{ROB}^m}{2} \right) |\phi_{10}|^2 \\
& - \left(\frac{P_{GEN} f_{ss}^{G_u}}{V_{ROB}} + \frac{Q_l}{2V_{l,s}} + \frac{a_2 f_{l,s}^{G_u}}{2} + \frac{P_{GEN}}{2V_{ROB}} + \frac{Q_l}{2V_{l,s}} + \frac{Q_l}{2a_1} + \frac{Q_l}{2a_6} \right) |\phi_{11}|^2 \\
& - \left(\frac{P_{CON} f_{ss}^{C_u}}{V_{ROB}} + \frac{Q_l}{2V_{l,s}} + \frac{a_4 f_{l,s}^{C_u}}{2} + \frac{P_{CON}}{2V_{ROB}} + \frac{Q_l}{2V_{l,s}} + \frac{Q_l}{2a_3} + \frac{Q_l}{2a_7} \right) |\phi_{12}|^2.
\end{aligned}$$

Choosing the appropriate min for k_1 and max for λ_0 , we have shown the assertion holds.

Proof of A3 Note that $v_{inf}(t) \in L_2((0, T), \mathcal{V}^*)$ for any $T > 0$, since $v_{inf}(t)$ is a continuously differentiable, piecewise polynomial. Therefore, this condition is satisfied.

Proof of A4 Let $r > 0$ be given. Let $\phi, \psi \in B_r(0)$. Recalling that all entries in the function $g(\cdot)$ are zero except entries 2 and 4, we have

$$|g(\phi) - g(\psi)|^2 = 2|g_2(\phi_2) - g_2(\psi_2)|^2,$$

which yields

$$|g(\phi) - g(\psi)| = 2^{\frac{1}{2}} |g_2(\phi_2) - g_2(\psi_2)|.$$

In light of this, we may limit our scope to $g_2(\cdot)$ in our proof. We will give this proof in four parts.

1) Assume $\phi_2, \psi_2 \geq 0$. Then, recalling the definition of $g_2(\cdot)$ and dividing the top and bottom by k_m yields

$$\begin{aligned} |g_2(\phi_2) - g_2(\psi_2)| &= \frac{V_{max}}{k_m} \left| \frac{\phi_2}{1 + \frac{\phi_2}{k_m}} - \frac{\psi_2}{1 + \frac{\psi_2}{k_m}} \right| \\ &= \frac{V_{max}}{k_m} \left| \frac{\phi_2 - \psi_2}{(1 + \frac{\phi_2}{k_m})(1 + \frac{\psi_2}{k_m})} \right| \\ &\leq \frac{V_{max}}{k_m} |\phi_2 - \psi_2| \leq \frac{V_{max}}{k_m} |\phi - \psi|. \end{aligned}$$

If we let $L_{B_r} = 2^{\frac{1}{2}} \frac{V_{max}}{k_m}$, the assertion is proven for this part.

2) Assume $\phi_2 \geq 0$ and $\psi_2 < 0$. Again, dividing the top and bottom of g_2 by k_m , we have

$$\begin{aligned} |g_2(\phi_2) - g_2(\psi_2)| &= |g_2(\phi_2)| = \frac{V_{max}}{k_m} \left| \frac{\phi_2}{1 + \frac{\phi_2}{k_m}} \right| \\ &\leq \frac{V_{max}}{k_m} |\phi_2| \leq \frac{V_{max}}{k_m} |\phi_2 - \psi_2| \leq \frac{V_{max}}{k_m} |\phi - \psi|. \end{aligned} \tag{4.7}$$

Letting $L_{B_r} = 2^{\frac{1}{2}} \frac{V_{max}}{k_m}$, the assertion is proven for this part.

3) Assume $\phi_2 < 0$ and $\psi_2 \geq 0$. This proof is identical to the above proof.

4) Assume $\phi_2, \psi_2 < 0$. Then

$$|g_2(\phi_2) - g_2(\psi_2)| = 0 \leq V_{max} |\phi - \psi|.$$

Letting $L_{B_r} = 2^{\frac{1}{2}} V_{max}$, the assertion is proven for this part, and the condition is satisfied.

Proof of A5 As in the proof of A4, we must only concern ourselves with $g_2(\cdot)$, that is,

$$|g(\phi)| = 2^{\frac{1}{2}} |g_2(\phi_2)|,$$

for any $\phi \in \mathcal{H}$. So we only need to show that $|g_2(\phi_2)| \leq C_1 |\phi| + C_2$. We will break the proof into two parts.

1) Assume $\phi_2 \geq 0$. Then

$$\begin{aligned} |g_2(\phi_2)| &= \left| \frac{V_{max}\phi_2}{k_m + \phi_2} \right| = \frac{V_{max}}{k_m} \left| \frac{\phi_2}{1 + \frac{\phi_2}{k_m}} \right| \\ &\leq \frac{V_{max}}{k_m} |\phi_2| \leq \frac{V_{max}}{k_m} |\phi|. \end{aligned}$$

2) Assume $\phi_2 < 0$. Then

$$|g_2(\phi_2)| = 0 \leq \frac{V_{max}}{k_m} |\phi|.$$

Hence, in both cases we have $|g_2(\phi_2)| \leq \frac{V_{max}}{k_m} |\phi|$. Letting C_2 be any positive number, the condition is satisfied.

So with A1-A5 satisfied, there exists a unique solution to our weak system that depends continuously on the initial condition and forcing function.

Chapter 5

Model Simulation

5.1 Model Simplification

We begin with changes of variables and labeling. Recall L is our assumed length of the liver sinusoid and bile duct cylinders, $\bar{L}$ and $\hat{L}$ are the lengths of the sinusoid and bile extension tubes, respectively, and $x = z/L$. We define:

$$\begin{aligned} y_1(t, x) &= C_{l,s}^{GEN}(t, Lx) - C_{ss}^{GEN}(t), \\ y_2(t, x) &= C_{l,h}^{GEN}(t, Lx), \\ y_3(t, x) &= C_{l,s}^{CON}(t, Lx) - C_{ss}^{CON}(t), \\ y_4(t, x) &= C_{l,h}^{CON}(t, Lx), \\ y_5(t, x) &= C_{l,b}^{CON}(t, Lx), \\ y_6(t, x) &= C_{l,t}^{GEN}(t, L + \bar{L}x), \\ y_7(t, x) &= C_{l,t}^{CON}(t, L + \bar{L}x), \\ y_8(t, x) &= C_{tube}^{CON}(t, \hat{L}(x - 1)), \end{aligned} \tag{5.1}$$

$$\begin{aligned}
y_9(t) &= C_{ROB}^{GEN}(t), \\
y_{10}(t) &= C_{ROB}^{CON}(t), \\
y_{11}(t) &= C_{ss}^{GEN}(t), \\
y_{12}(t) &= C_{ss}^{CON}(t), \\
y_{13}(t) &= A_{exc}^{CON}(t),
\end{aligned}$$

where, $x \in [0, 1]$. In addition,

$$\frac{\partial}{\partial x} = L \frac{\partial}{\partial z}, \quad \frac{\partial^2}{\partial x^2} = L^2 \frac{\partial^2}{\partial z^2}.$$

for $y_1 - y_5$,

$$\frac{\partial}{\partial x} = \bar{L} \frac{\partial}{\partial z}, \quad \frac{\partial^2}{\partial x^2} = \bar{L}^2 \frac{\partial^2}{\partial z^2}.$$

for y_6 and y_7 , and

$$\frac{\partial}{\partial x} = \hat{L} \frac{\partial}{\partial z}, \quad \frac{\partial^2}{\partial x^2} = \hat{L}^2 \frac{\partial^2}{\partial z^2}$$

for y_8 . Note that y_1 and y_3 are introduced to assure homogeneity in the Dirichlet boundary conditions.

For a function $y(t, z)$, we use the notations $\dot{y} := \frac{\partial y}{\partial t}$ and $y' := \frac{\partial y}{\partial x}$. So now with the definitions of (5.1), we have

$$\begin{aligned}
C_{l,s}^{GEN} &= y_1 + y_{11}, \\
C_{l,s}^C &= y_3 + y_{12},
\end{aligned} \tag{5.2}$$

and also

$$\begin{aligned}
\frac{\partial}{\partial x}(C_{l,s}^{GEN}) &= \frac{\partial}{\partial x}(y_1) + \frac{\partial}{\partial x}(y_{11}) = y'_1, \\
\frac{\partial}{\partial x}(C_{l,s}^C) &= \frac{\partial}{\partial x}(y_3) + \frac{\partial}{\partial x}(y_{12}) = y'_3.
\end{aligned} \tag{5.3}$$

5.2 Weak form of Equations

We now begin deriving the weak form of our system of differential equations (3.27), noting the change in the order of equations and changes of variables due to the definitions given in (5.1):

$$\left(V_{l,s} + V_{l,D} \frac{f_{l,s}^{G_u}}{f_{l,D}^{G_u}} \right) (\dot{y}_1 + \dot{y}_{11}) = \frac{V_{l,s} \mathcal{D}_l}{L^2} y_1'' - \frac{V_{l,s} v_l}{L} y_1' \quad (5.4)$$

$$+ P_{GEN}(y_2 - f_{l,s}^{G_u}(y_1 + y_{11})),$$

$$\frac{\partial y_2}{\partial t} = \frac{P_{GEN}}{V_{l,h}} (f_{l,s}^{G_u}(y_1 + y_{11}) - y_2) - g(y_2), \quad (5.5)$$

$$\left(V_{l,s} + \frac{f_{l,s}^{C_u}}{f_{l,D}^{C_u}} V_{l,D} \right) (\dot{y}_3 + \dot{y}_{12}) = \frac{V_{l,s} \mathcal{D}_l}{L^2} y_3'' - \frac{V_{l,s} v_l}{L} y_3' \quad (5.6)$$

$$+ P_{CON}(y_4 - f_{l,s}^{C_u}(y_3 + y_{12})),$$

$$\dot{y}_4 = g(y_2) + \frac{P_{CON}}{V_{l,h}} (f_{l,s}^{C_u}(y_3 + y_{12}) - y_4) \quad (5.7)$$

$$- \left(\frac{P_{CON}}{V_{l,h}} (y_4 - y_5) + k_{bile} y_4 \right),$$

$$\dot{y}_5 = \frac{\mathcal{D}_b y_5''}{L^2} + \frac{v_b y_5'}{L} + \frac{P_{CON}}{V_{l,b}} (y_4 - y_5) + \frac{k_{bile} V_{l,h}}{V_{l,b}} y_4, \quad (5.8)$$

$$\left(V_{l,t} + \frac{f_{l,t}^{G_u}}{f_{l,Dt}^{G_u}} V_{l,Dt} \right) \dot{y}_6(t, x) = \frac{V_{l,t} \mathcal{D}_l}{L^2} y_6'' - \frac{V_{l,t} v_l}{L} y_6', \quad (5.9)$$

$$\left(V_{l,t} + \frac{f_{l,t}^{C_u}}{f_{l,Dt}^{C_u}} V_{l,Dt} \right) \dot{y}_7(t, x) = \frac{V_{l,t} \mathcal{D}_l}{L^2} y_7'' - \frac{V_{l,t} v_l}{L} y_7', \quad (5.10)$$

$$\dot{y}_8 = \mathcal{D}_t \frac{1}{\bar{L}^2} y_8'' + v_b \frac{1}{\bar{L}} y_8', \quad (5.11)$$

$$\dot{y}_9 = \frac{P_{GEN}}{V_{ROB}} (f_{ss}^{G_u} y_{11} - y_9) - k_{urine}^{GEN} y_9 - k_{ROB}^m y_9, \quad (5.12)$$

$$\dot{y}_{10} = \frac{P_{CON}}{V_{ROB}} (f_{ss}^{Cu} y_{12} - y_{10}) - k_{urine}^{CON} y_{10} + k_{ROB}^m y_9, \quad (5.13)$$

$$\begin{aligned} \dot{y}_{11} = & \frac{P_{GEN}}{V_{ROB}} (y_9 - f_{ss}^{Gu} y_{11}) + \frac{Q_l}{V_{l,s}} (y_6(t, 1) - y_{11}) \\ & + \frac{1}{V_{ss}} v_{inf}(t), \end{aligned} \quad (5.14)$$

$$\dot{y}_{12} = \frac{P_{CON}}{V_{ROB}} (y_{10} - f_{ss}^{Cu} y_{12}) + \frac{Q_l}{V_{l,s}} (y_7(t, 1) - y_{12}), \quad (5.15)$$

$$\dot{y}_{13} = A v_b y_8(t, x_1) + \frac{A \mathcal{D}_t}{\hat{L}} y_8'(t, x_1), \quad (5.16)$$

where we define

$$g(y_i) = \begin{cases} \frac{V_{max} y_i}{km + y_i}, & y_i \geq 0, \\ 0, & y_i < 0, \end{cases} \quad (5.17)$$

and we have $x_1 = 1 - \frac{\hat{L}}{L}$.

We begin with equation (5.4). If we let $A_{l,s}$ be defined as the cross-sectional area of the liver sinusoid cylinder, then we have $V_{l,s} = A_{l,s} L$, where L is the length of the cylinder. Hence,

$$\frac{V_{l,s}}{L} = A_{l,s}. \quad (5.18)$$

Since $v_l = \frac{Q_l}{A_{l,s}}$, we have

$$A_{l,s} v_l = Q_l. \quad (5.19)$$

Therefore,

$$\frac{V_{l,s}}{L} v_l = Q_l. \quad (5.20)$$

From (5.18) and (5.19), we also obtain

$$\frac{V_{l,s} \mathcal{D}_l}{L^2 Q_l} = \frac{A_{l,s} \mathcal{D}_l}{L Q_l} := \mathcal{D}_l^N,$$

where $\mathcal{D}_l^N$ is the axial dispersion number defined by Roberts and Rowland (43). Hence, we

have

$$\frac{V_{l,s}\mathcal{D}_l}{L^2} = Q_l\mathcal{D}_l^N. \quad (5.21)$$

For convenience, we will let the sinusoid extension tube volume $V_{l,t} = k_1 V_{l,s}$ and the bile tube volume $V_{tube} = k_2 V_{l,b}$, where k_1 and k_2 are constants. It follows that $k_1 = \bar{L}/L$, $k_2 = \hat{L}/L$, and

$$\frac{V_{l,t}}{L} = \frac{V_{l,s}}{L}, \quad \frac{V_{l,t}}{L} = \frac{k_1 V_{l,s}}{L}, \quad \frac{V_{l,t}}{L^2} = \frac{k_1 V_{l,s}}{L^2}, \quad (5.22)$$

$$\frac{V_{l,t}}{L^2} = \frac{V_{l,s}}{LL} = \frac{1}{k_1} \frac{V_{l,t}}{LL} = \frac{1}{k_1} \frac{V_{l,s}}{L^2}, \quad (5.23)$$

$$\frac{V_{tube}}{\hat{L}} = \frac{V_{l,b}}{L}, \quad \frac{V_{tube}}{L} = \frac{k_2 V_{l,b}}{L}, \quad \frac{V_{tube}}{L^2} = \frac{k_2 V_{l,b}}{L^2}, \quad (5.24)$$

$$\frac{V_{tube}}{\hat{L}^2} = \frac{V_{l,b}}{LL} = \frac{1}{k_2} \frac{V_{tube}}{LL} = \frac{1}{k_2} \frac{V_{l,b}}{L^2}. \quad (5.25)$$

Let $a_1 = V_{l,s} + V_{l,D} \frac{f_{l,s}^{Gu}}{f_{l,D}^{Gu}}$. Then

$$\dot{y}_1 + \dot{y}_{11} = \frac{\mathcal{D}_l^N Q_l}{a_1} y_1'' - \frac{Q_l}{a_1} y_1' + \frac{P_{GEN}}{a_1} (y_2 - f_{l,s}^{Gu} (y_1 + y_{11})). \quad (5.26)$$

Multiplying equation (5.26) by a test function ϕ_1 and taking the L_2 inner product yields

$$\begin{aligned} \langle \dot{y}_1, \phi_1 \rangle + \langle \dot{y}_{11}, \phi_1 \rangle &= \langle \frac{\mathcal{D}_l^N Q_l}{a_1} y_1'', \phi_1 \rangle - \langle \frac{Q_l}{a_1} y_1', \phi_1 \rangle + \langle \frac{P_{GEN}}{a_1} y_2, \phi_1 \rangle \\ &\quad - \langle \frac{P_{GEN}}{a_1} f_{l,s}^{Gu} y_1, \phi_1 \rangle - \langle \frac{P_{GEN}}{a_1} f_{l,s}^{Gu} y_{11}, \phi_1 \rangle. \end{aligned} \quad (5.27)$$

Integrating the first two terms on the right side of equation (5.27) by parts, using the fact

that $\dot{y}_{11}$ is known, and considering the natural boundary conditions, we have

$$\begin{aligned}
\langle \dot{y}_1, \phi_1 \rangle &= \frac{Q_l}{a_1} ((\mathcal{D}_l^N y'_1 - y_1) \phi_1)(t, 1) - \langle \frac{\mathcal{D}_l^N Q_l}{a_1} y'_1, \phi'_1 \rangle + \langle \frac{Q_l}{a_1} y_1, \phi'_1 \rangle \\
&+ \langle \frac{P_{GEN}}{a_1} y_2, \phi_1 \rangle - \langle \frac{P_{GEN}}{a_1} f_{l,s}^{Gu} y_1, \phi_1 \rangle - \langle \frac{P_{GEN}}{a_1} f_{l,s}^{Gu} y_{11}, \phi_1 \rangle \\
&- \langle \frac{P_{GEN}}{V_{ROB}} y_9, \phi_1 \rangle + \langle \frac{P_{GEN}}{V_{ROB}} f_{ss}^{Gu} y_{11}, \phi_1 \rangle - \langle \frac{Q_l}{V_{l,s}} y_6(t, 1), \phi_1 \rangle \\
&+ \langle \frac{Q_l}{V_{l,s}} y_{11}, \phi_1 \rangle - \langle \frac{1}{V_{ss}} V_{inf}(t), \phi_1 \rangle.
\end{aligned} \tag{5.28}$$

Recalling that at $z = L$, we have equality of flux between the sinusoid and the extension tube cylinders. Enforcing that boundary condition in equation (5.28) yields

$$\begin{aligned}
\langle \dot{y}_1, \phi_1 \rangle &= \frac{Q_l}{a_6 k_1} (\mathcal{D}_l^N y'_6(t, 0) - y_6(t, 0)) \phi_1(1) - \langle \frac{\mathcal{D}_l^N Q_l}{a_1} y'_1, \phi'_1 \rangle + \langle \frac{Q_l}{a_1} y_1, \phi'_1 \rangle \\
&+ \langle \frac{P_{GEN}}{a_1} y_2, \phi_1 \rangle - \langle \frac{P_{GEN}}{a_1} f_{l,s}^{Gu} y_1, \phi_1 \rangle - \langle \frac{P_{GEN}}{a_1} f_{l,s}^{Gu} y_{11}, \phi_1 \rangle \\
&- \langle \frac{P_{GEN}}{V_{ROB}} y_9, \phi_1 \rangle + \langle \frac{P_{GEN}}{V_{ROB}} f_{ss}^{Gu} y_{11}, \phi_1 \rangle - \langle \frac{Q_l}{V_{l,s}} y_6(t, 1), \phi_1 \rangle \\
&+ \langle \frac{Q_l}{V_{l,s}} y_{11}, \phi_1 \rangle + \frac{Q_l}{a_1} y_{11} \phi_1(1) - \langle \frac{1}{V_{ss}} V_{inf}(t), \phi_1 \rangle,
\end{aligned} \tag{5.29}$$

where $a_6 = V_{l,t} + \frac{f_{l,t}^{Gu}}{f_{l,Dt}^{Gu}} V_{l,Dt}$.

Now, letting $a_2 = \frac{P_{GEN}}{V_{l,h}}$, equation (5.5) becomes

$$\dot{y}_2 = a_2 f_{l,s}^{Gu} (y_1 + y_{11}) - a_2 y_2 - g(y_2). \tag{5.30}$$

Multiplying by test function ϕ_2 and taking the inner product yields

$$\begin{aligned}
\langle \dot{y}_2, \phi_2 \rangle &= \langle a_2 f_{l,s}^{Gu} y_1, \phi_2 \rangle - \langle a_2 y_2, \phi_2 \rangle \\
&- \langle g(y_2), \phi_2 \rangle + \langle a_2 f_{l,s}^{Gu} y_{11}, \phi_2 \rangle.
\end{aligned} \tag{5.31}$$

For equation (5.6), we let $a_3 = V_{l,s} + \frac{f_{l,s}^{Cu}}{f_{l,d}^{Cu}} V_{l,D}$ and multiply by a test function ϕ_3 . Deriving the weak form of this equation is identical to that of equation (5.26). Also note that by letting $a_4 = \frac{P_{CON}}{V_{l,h}}$ and multiplying equation (5.7) by a test function ϕ_4 , the derivation of the weak form of equation (5.7) is similar to that of equation (5.30).

For equation (5.8), we observe similarity to equations (5.20) and (5.21), yielding

$$\frac{V_{l,b} \mathcal{D}_b}{L^2} = Q_b \mathcal{D}_b^N, \quad \frac{V_{l,b} v_b}{L} = Q_b,$$

where $\mathcal{D}_b^N$, the axial dispersion number, is defined similar to $\mathcal{D}_l^N$. Therefore, we obtain

$$\dot{y}_5 = \frac{Q_b \mathcal{D}_b^N}{V_{l,b}} y_5'' + \frac{Q_b}{V_{l,b}} y_5' + \frac{P_{CON}}{V_{l,b}} (y_4 - y_5) + k_{bile} \frac{V_{l,h}}{V_{l,b}} y_4. \quad (5.32)$$

Multiplying (5.32) by test function ϕ_5 , taking the inner product, and integrating the first two terms on the right side by parts yields

$$\begin{aligned} \langle \dot{y}_5, \phi_5 \rangle &= -\frac{Q_b \mathcal{D}_b^N}{V_{l,b}} y_5'(t, 0) \phi_5(0) - \frac{Q_b}{V_{l,b}} y_5(t, 1) \phi_5(0) - \langle \frac{Q_b \mathcal{D}_b^N}{V_{l,b}} y_5', \phi_5' \rangle \\ &\quad - \langle \frac{Q_b}{V_{l,b}} y_5, \phi_5' \rangle + \langle \frac{1}{V_{l,b}} (P_{CON} + k_{bile} V_{l,h}) y_4, \phi_5 \rangle \\ &\quad - \langle \frac{P_{CON}}{V_{l,b}} y_5, \phi_5 \rangle, \end{aligned} \quad (5.33)$$

where we use the zero flux boundary condition at $z = L$ and the equality of concentrations boundary condition between the bile duct and bile extension tube cylinders at $z = 0$.

For equation (5.9), we use (5.22) and (5.23) to obtain

$$\dot{y}_6 = \frac{Q_l}{a_6 k_1} \mathcal{D}_l^N y_6'' - \frac{Q_l}{a_6} y_6'. \quad (5.34)$$

Multiplying by a test function ϕ_6 , integrating by parts, using the zero gradient boundary condition at $z = L + \bar{L}$, and using the equality of concentrations between the sinusoid and

extension tube cylinders boundary condition at $z = L$ yields

$$\begin{aligned} \langle \dot{y}_6, \phi_6 \rangle = & -\frac{Q_l}{a_6} y_6(t, 1) \phi_6(1) - \frac{Q_l \mathcal{D}_l^N}{k_1 a_6} y_6'(t, 0) \phi_6(0) + \frac{Q_l}{a_6} y_1(t, 1) \phi_6(0) \\ & - \langle \frac{Q_l \mathcal{D}_l^N}{k_1 a_6} y_6', \phi_6' \rangle + \langle \frac{Q_l}{a_6} y_6, \phi_6' \rangle + \frac{Q_l}{a_6} y_{11} \phi_6(0). \end{aligned} \quad (5.35)$$

If we let $a_7 = V_{l,t} + \frac{f_{l,t}^{Cu}}{f_{l,Dt}^{Cu}} V_{l,Dt}$, multiply by a test function ϕ_7 , and again use (5.22) and (5.23), the derivation of the weak form of equation (5.10) is identical to that of (5.34).

Now, using (5.24) and (5.25) equation (5.11) can be written as

$$\dot{y}_8 = \frac{Q_b}{V_{tube}} \mathcal{D}_t^N y_8'' + \frac{Q_b}{V_{tube}} y_8'.$$

To put the equation into weak form, we multiply by a test function ϕ_8 and use the equality of flux boundary condition at $z = 0$ and the zero gradient boundary condition at $z = -\hat{L}$ to obtain

$$\begin{aligned} \langle \dot{y}_8, \phi_8 \rangle = & \frac{Q_b}{V_{tube}} ((\mathcal{D}_b^N y_5'(t, 0) + y_5(t, 0)) \phi_8(1) - \frac{Q_b}{V_{tube}} y_8(t, 0) \phi_8(0) \\ & - \langle \frac{Q_b}{V_{tube}} \mathcal{D}_t^N y_8', \phi_8' \rangle - \langle \frac{Q_b}{V_{tube}} y_8, \phi_8' \rangle. \end{aligned} \quad (5.36)$$

The last five equations (5.12-5.16) do not contain spatial derivatives, and so our system of

equations in weak form is given by:

$$\begin{aligned}
 \langle \dot{y}_1, \phi_1 \rangle = & \frac{Q_l}{a_1} (\mathcal{D}_l^N \frac{1}{k_1} y'_6(t, 0) - y_6(t, 0)) \phi_1(1) - \langle \frac{\mathcal{D}_l^N Q_l}{a_1} y'_1, \phi'_1 \rangle \\
 & + \langle \frac{Q_l}{a_1} y_1, \phi'_1 \rangle + \langle \frac{P_{GEN}}{a_1} y_2, \phi_1 \rangle - \langle \frac{P_{GEN}}{a_1} f_{l,s}^{Gu} y_1, \phi_1 \rangle \\
 & - \langle \frac{P_{GEN}}{a_1} f_{l,s}^{Gu} y_{11}, \phi_1 \rangle - \langle \frac{P_{GEN}}{V_{ROB}} y_9, \phi_1 \rangle + \langle \frac{P_{GEN}}{V_{ROB}} f_{ss}^{Gu} y_{11}, \phi_1 \rangle \\
 & - \langle \frac{Q_l}{V_{l,s}} y_6(t, 1), \phi_1 \rangle + \langle \frac{Q_l}{V_{l,s}} y_{11}, \phi_1 \rangle + \frac{Q_l}{a_1} y_{11} \phi_1(1) \\
 & - \langle \frac{1}{V_{ss}} V_{inf}(t), \phi_1 \rangle,
 \end{aligned} \tag{5.37}$$

$$\begin{aligned}
 \langle \dot{y}_2, \phi_2 \rangle = & \langle a_2 f_{l,s}^{Gu} y_1, \phi_2 \rangle - \langle a_2 y_2, \phi_2 \rangle - \langle g(y_2), \phi_2 \rangle \\
 & + \langle a_2 f_{l,s}^{Gu} y_{11}, \phi_2 \rangle,
 \end{aligned} \tag{5.38}$$

$$\begin{aligned}
 \langle \dot{y}_3, \phi_3 \rangle = & \frac{Q_l}{a_3} (\mathcal{D}_l^N \frac{1}{k_1} y'_7(t, 0) - y_7(t, 0)) \phi_3(1) - \langle \frac{\mathcal{D}_l^N Q_l}{a_3} y'_3, \phi'_3 \rangle \\
 & + \langle \frac{Q_l}{a_3} y_3, \phi'_3 \rangle + \langle \frac{P_{CON}}{a_3} y_4, \phi_3 \rangle - \langle \frac{P_{CON}}{a_3} f_{l,s}^{Cu} y_3, \phi_3 \rangle \\
 & - \langle \frac{P_{CON}}{a_3} f_{l,s}^{Cu} y_{12}, \phi_3 \rangle - \langle \frac{P_{CON}}{V_{ROB}} y_{10}, \phi_3 \rangle + \langle \frac{P_{CON}}{V_{ROB}} f_{ss}^{Cu} y_{12}, \phi_3 \rangle \\
 & - \langle \frac{Q_l}{V_{l,s}} y_7(t, 1), \phi_3 \rangle + \langle \frac{Q_l}{V_{l,s}} y_{12}, \phi_3 \rangle + \frac{Q_l}{a_3} y_{12} \phi_3(1),
 \end{aligned} \tag{5.39}$$

$$\begin{aligned}
 \langle \dot{y}_4, \phi_4 \rangle = & \langle a_4 f_{l,s}^{Cu} y_3, \phi_4 \rangle - \langle 2a_4 y_4, \phi_4 \rangle + \langle a_4 y_5, \phi_4 \rangle \\
 & - \langle k_{bile} y_4, \phi_4 \rangle + \langle g(y_2), \phi_4 \rangle + \langle a_4 f_{l,s}^{Cu} y_{12}, \phi_4 \rangle,
 \end{aligned} \tag{5.40}$$

$$\begin{aligned}
 \langle \dot{y}_5, \phi_5 \rangle = & - \frac{Q_b \mathcal{D}_b^N}{V_{l,b}} y'_5(t, 0) \phi_5(0) - \frac{Q_b}{V_{l,b}} y_8(t, 1) \phi_5(0) - \langle \frac{Q_b \mathcal{D}_b^N}{V_{l,b}} y'_5, \phi'_5 \rangle \\
 & - \langle \frac{Q_b}{V_{l,b}} y_5, \phi'_5 \rangle + \langle \frac{1}{V_{l,b}} (P_{CON} + k_{bile} V_{l,h}) y_4, \phi_5 \rangle \\
 & - \langle \frac{P_{CON}}{V_{l,b}} y_5, \phi_5 \rangle,
 \end{aligned} \tag{5.41}$$

$$\begin{aligned}
 \langle \dot{y}_6, \phi_6 \rangle = & - \frac{Q_l}{a_6} y_6(t, 1) \phi_6(1) - \frac{Q_l \mathcal{D}_l^N}{k_1 a_6} y'_6(t, 0) \phi_6(0) + \frac{Q_l}{a_6} y_1(t, 1) \phi_6(0) \\
 & - \langle \frac{Q_l \mathcal{D}_l^N}{k_1 a_6} y'_6, \phi'_6 \rangle + \langle \frac{Q_l}{a_6} y_6, \phi'_6 \rangle + \frac{Q_l}{a_6} y_{11} \phi_6(0),
 \end{aligned} \tag{5.42}$$

$$\langle \dot{y}_7, \phi_7 \rangle = -\frac{Q_l}{a_7} y_7(t, 1) \phi_7(1) - \frac{Q_l \mathcal{D}_l^N}{k_1 a_7} y_7'(t, 0) \phi_7(0) + \frac{Q_l}{a_7} y_3(t, 1) \phi_7(0) \quad (5.43)$$

$$\begin{aligned} & - \langle \frac{Q_l \mathcal{D}_l^N}{k_1 a_7} y_7', \phi_7' \rangle + \langle \frac{Q_l}{a_7} y_7, \phi_7' \rangle + \frac{Q_l}{a_7} y_{12} \phi_7(0), \\ \langle \dot{y}_8, \phi_8 \rangle &= \frac{Q_b}{V_{tube}} ((\mathcal{D}_b^N y_5'(t, 0) + y_5(t, 0)) \phi_8(1) - \frac{Q_b}{V_{tube}} y_8(t, 0) \phi_8(0) \end{aligned} \quad (5.44)$$

$$\begin{aligned} & - \langle \frac{Q_b}{V_{tube}} \mathcal{D}_t^N y_8', \phi_8' \rangle - \langle \frac{Q_b}{V_{tube}} y_8, \phi_8' \rangle, \\ \dot{y}_9 &= \frac{P_{GEN}}{V_{ROB}} f_{ss}^{Gu} y_{11} - (\frac{P_{GEN}}{V_{ROB}} + k_{urine}^{GEN} + k_{ROB}^m) y_9, \end{aligned} \quad (5.45)$$

$$\dot{y}_{10} = \frac{P_{CON}}{V_{ROB}} (f_{ss}^{Cu} y_{12} - y_{10}) + k_{ROB}^m y_9 - k_{urine}^C y_{10}, \quad (5.46)$$

$$\dot{y}_{11} = \frac{P_{GEN}}{V_{ROB}} (y_9 - f_{ss}^{Gu} y_{11}) + \frac{Q_l}{V_{l,s}} (y_6(t, 1) - y_{11}) + \frac{1}{V_{ss}} v_{inf}(t), \quad (5.47)$$

$$\dot{y}_{12} = \frac{P_{CON}}{V_{ROB}} (y_{10} - f_{ss}^{Cu} y_{12}) + \frac{Q_l}{V_{l,s}} (y_7(t, 1) - y_{12}), \quad (5.48)$$

$$\dot{y}_{13} = Q_b y_8(t, x_1) + \mathcal{D}_t^N Q_b y_8'(t, x_1), \quad (5.49)$$

noting that $\frac{A \mathcal{D}_l}{\tilde{L}} = \mathcal{D}_t^N Q_b$.

5.3 Formulation of Finite Element Method

5.3.1 Basis Elements

We partition the interval $[0, 1]$ into N subintervals of length h , where $h = \frac{1}{N}$, by $0 = x_0 < x_1 < \dots < x_N = 1$, with $x_i = ih$, $i = 0, 1, \dots, N$.

Using the weak form of the equations (5.37-5.49), we formulate the Galerkin method

with the following finite element approximations:

$$\begin{aligned}
 y_1^N(t, x) &= \sum_{j=1}^N \alpha_j(t) \phi_j(x), \\
 y_2^N(t, x) &= \sum_{j=0}^N \beta_j(t) \phi_j(x), \\
 y_3^N(t, x) &= \sum_{j=1}^N \gamma_j(t) \phi_j(x), \\
 y_4^N(t, x) &= \sum_{j=0}^N \eta_j(t) \phi_j(x), \\
 y_5^N(t, x) &= \sum_{j=0}^N \xi_j(t) \phi_j(x), \\
 y_6^N(t, x) &= \sum_{j=0}^N \rho_j(t) \phi_j(x), \\
 y_7^N(t, x) &= \sum_{j=0}^N \omega_j(t) \phi_j(x), \\
 y_8^N(t, x) &= \sum_{j=0}^N \theta_j(t) \phi_j(x),
 \end{aligned} \tag{5.50}$$

where the basis elements $\phi_j(x)$, $j = 0, \dots, N$ are the linear hat functions defined as follows:

$$\phi_j(x) = \begin{cases} \frac{x-x_{j-1}}{h}, & x_{j-1} \leq x \leq x_j, \\ \frac{x_{j+1}-x}{h}, & x_j \leq x \leq x_{j+1}, \\ 0, & 0 \leq x \leq x_{j-1} \text{ or } x_{j+1} \leq x \leq 1, \end{cases} \tag{5.51}$$

such that

$$\phi_j'(x) = \begin{cases} \frac{1}{h}, & x_{j-1} \leq x \leq x_j, \\ \frac{-1}{h}, & x_j \leq x \leq x_{j+1}, \\ 0, & 0 \leq x \leq x_{j-1} \text{ or } x_{j+1} \leq x \leq 1. \end{cases} \tag{5.52}$$

Note that in (5.50), the indices beginning with 1 enforce the essential boundary conditions

on those equations.

Also note the following inner products with corresponding values:

$$\langle \phi_j, \phi_i \rangle = \begin{cases} 0, & |i-j| \geq 2 \\ \frac{h}{6}, & |i-j| = 1 \\ \frac{2h}{3}, & 0 < i=j < N \\ \frac{h}{3}, & i=j=0, N, \end{cases}$$

$$\langle \phi'_j, \phi'_i \rangle = \begin{cases} 0, & |i-j| \geq 2 \\ \frac{-1}{h}, & |i-j| = 1 \\ \frac{2}{h}, & 0 < i=j < N \\ \frac{1}{h}, & i=j=0, N, \end{cases}$$

$$\langle \phi_j, \phi'_i \rangle = \begin{cases} 0, & |i-j| \geq 2 \\ \frac{1}{2}, & i=j+1 \\ -\frac{1}{2}, & i=j-1 \\ 0, & 0 < i=j < N \\ \frac{1}{2}, & i=j=N \\ -\frac{1}{2}, & i=j=0. \end{cases}$$

5.3.2 Finite Element Approximations

We now substitute the functions in (5.50) into the system of equations (5.37-5.49) to obtain the matrix/vector form of our equations.

Making the substitutions described above, using equation (5.37), for each i such that

$i = 1 \cdots N$, we have

$$\begin{aligned}
\sum_1^N \dot{\alpha}_j(t) < \phi_j, \phi_i > = & \frac{Q_l}{k_1 a_1} \mathcal{D}_l^N \left(\sum_0^N \rho_j(t) \phi_j'(0) \right) \phi_i(1) - \frac{Q_l}{a_1} \left(\sum_0^N \rho_j(t) \phi_j(0) \right) \phi_i(1) \\
& - \frac{Q_l}{a_1} \mathcal{D}_l^N \sum_1^N \alpha_j(t) < \phi_j', \phi_i' > + \frac{Q_l}{a_1} \sum_1^N \alpha_j(t) < \phi_j, \phi_i' > \\
& + \frac{P_{GEN}}{a_1} \sum_0^N \beta_j(t) < \phi_j, \phi_i > - \frac{f_{l,s}^{G_u} P_{GEN}}{a_1} \sum_1^N \alpha_j(t) < \phi_j, \phi_i > \\
& - \frac{f_{l,s}^{G_u} P_{GEN}}{a_1} y_{11}(t) < 1, \phi_i > - \frac{P_{GEN}}{V_{ROB}} y_9 < 1, \phi_i > \\
& + \frac{P_{GEN}}{V_{ROB}} f_{ss}^{G_u} y_{11} < 1, \phi_i > - \frac{Q_l}{V_{l,s}} \sum_0^N \rho_j(t) \phi_j(1) < 1, \phi_i > \\
& + \frac{Q_l}{V_{l,s}} y_{11} < 1, \phi_i > + \frac{Q_l}{a_1} y_{11} \phi_i(1) \\
& - \frac{1}{V_{ss}} V_{inf}(t) < 1, \phi_i >, \tag{5.53}
\end{aligned}$$

and then in matrix-vector form, we have

$$\begin{aligned}
\bar{M} \dot{\vec{\alpha}}(t) = & \left(\frac{-Q_l}{a_1} \mathcal{D}_l^N \bar{A} + \frac{Q_l}{a_1} \bar{K} - \frac{f_{l,s}^{G_u} P_{GEN}}{a_1} \bar{M} \right) \vec{\alpha}(t) + \frac{P_{GEN}}{a_1} \tilde{M} \vec{\beta}(t) \\
& - \frac{P_{GEN}}{V_{ROB}} y_9 \vec{\Phi}_N + \left(\frac{f_{ss}^{G_u} P_{GEN}}{V_{ROB}} + \frac{Q_l}{V_{l,s}} - \frac{f_{l,s}^{G_u} P_{GEN}}{a_1} \right) y_{11}(t) \vec{\Phi}_N \\
& + \frac{Q_l}{k_1 a_1} \mathcal{D}_l^N (\rho_0(t) \phi_0'(0) + \rho_1(t) \phi_1'(0)) \vec{e}_N - \frac{Q_l}{a_1} \rho_0(t) \phi_0(0) \vec{e}_N \\
& - \frac{Q_l}{V_{l,s}} \phi_N(1) \rho_N(t) \vec{\Phi}_N + \frac{Q_l}{a_1} y_{11} \vec{e}_N - \frac{1}{V_{ss}} V_{inf}(t) \vec{\Phi}_N, \tag{5.54}
\end{aligned}$$

where the definitions of the constant matrices $\bar{M}$, $\bar{K}$ and $\bar{A}$, and constant vectors $\vec{\Phi}_N$ and $\vec{e}_N$, and all other constant matrices and vectors are found at the end of this section.

Following the same steps taken to obtain (5.54) from (5.37), we can form a similar

matrix-vector formulation for equation (5.39). Hence, we have

$$\begin{aligned}
 \vec{M}\dot{\vec{\gamma}}(t) = & \left(\frac{-Q_l}{a_3} \mathcal{D}_l^N \bar{A} + \frac{Q_l}{a_3} \bar{K} - \frac{f_{l,s}^{C_u} P_{CON}}{a_3} \bar{M} \right) \vec{\gamma}(t) + \frac{P_{CON}}{a_3} \vec{M} \vec{\eta}(t) \\
 & - \frac{P_{CON}}{V_{ROB}} y_{10} \vec{\Phi}_N + \left(\frac{f_{ss}^{C_u} P_{CON}}{V_{ROB}} + \frac{Q_l}{V_{l,s}} - \frac{f_{l,s}^{C_u} P_{CON}}{a_3} \right) y_{12}(t) \vec{\Phi}_N \\
 & + \frac{Q_l}{k_1 a_3} \mathcal{D}_l^N (\omega_0(t) \phi'_0(0) + \omega_1(t) \phi'_1(0)) \vec{e}_N - \frac{Q_l}{a_3} \omega_0(t) \phi_0(0) \vec{e}_N \\
 & - \frac{Q_l}{V_{l,s}} \phi_N(1) \omega_N(t) \vec{\Phi}_N + \frac{Q_l}{a_3} y_{12} \vec{e}_N.
 \end{aligned} \tag{5.55}$$

Using equation (5.38), for each i such that $i = 0 \cdots N$, we have

$$\begin{aligned}
 \sum_0^N \dot{\beta}_j(t) \langle \phi_j, \phi_i \rangle = & a_2 f_{l,s}^{G_u} \sum_1^N \alpha_j(t) \langle \phi_j, \phi_i \rangle - a_2 \sum_0^N \beta_j(t) \langle \phi_j, \phi_i \rangle \\
 & - \left\langle g \left(\sum_0^N \beta_j(t) \phi_j \right), \phi_i \right\rangle + a_2 f_{l,s}^{G_u} y_{11} \langle 1, \phi_i \rangle.
 \end{aligned}$$

In matrix-vector form, we have

$$\vec{M} \dot{\vec{\beta}}(t) = a_2 f_{l,s}^{G_u} \vec{M} \vec{\alpha}(t) - a_2 \vec{M} \vec{\beta}(t) - \vec{G}_m(\vec{\beta}(t)) + a_2 f_{l,s}^{G_u} y_{11}(t) \vec{\Phi}_{N+1}, \tag{5.56}$$

where we define, for $\vec{x}(t)$ an $N+1$ dimensional vector, the $N+1$ dimensional vector-valued function

$$[\vec{G}_m(\vec{x}(t))]_i = \left\langle g \left(\sum_0^N x_j(t) \phi_j \right), \phi_i \right\rangle, \tag{5.57}$$

for each $i = 0, \cdots, N$.

The matrix-vector form of (5.40) is obtained in a similar fashion as for (5.38), and is

given by

$$\begin{aligned} M\dot{\vec{\eta}}(t) = & -(2a_4 + k_{bile})M\vec{\eta}(t) + a_4 f_{l,s}^{Cu} \tilde{M}\vec{\gamma}(t) + a_4 M\vec{\xi}(t) + \vec{G}_m(\vec{\beta}(t)) \\ & + a_4 f_{l,s}^{Cu} y_{12}(t) \vec{\Phi}_{N+1}. \end{aligned} \quad (5.58)$$

For equation (5.41), for each i such that $i = 0 \cdots N$, we have

$$\begin{aligned} \sum_0^N \dot{\xi}_j(t) < \phi_j, \phi_i > = & -\frac{Q_b \mathcal{D}_b^N}{V_{l,b}} \left(\sum_0^N \xi_j(t) \phi'_j(0) \right) \phi_i(0) - \frac{Q_b}{V_{l,b}} \left(\sum_0^N \theta_j(t) \phi_j(1) \right) \phi_i(0) \\ & - \frac{Q_b}{V_{l,b}} \mathcal{D}_b^N \sum_0^N \xi_j(t) < \phi'_j, \phi'_i > - \frac{Q_b}{V_{l,b}} \sum_0^N \xi_j(t) < \phi_j, \phi'_i > \\ & + \frac{P_{CON}}{V_{l,b}} \sum_0^N \eta_j(t) < \phi_j, \phi_i > - \frac{P_{CON}}{V_{l,b}} \sum_0^N \xi_j(t) < \phi_j, \phi_i > \\ & + \frac{k_{bile} V_{l,h}}{V_{l,b}} \sum_0^N \eta_j(t) < \phi_j, \phi_i >, \end{aligned} \quad (5.59)$$

and in matrix-vector form:

$$\begin{aligned} M\dot{\vec{\xi}}(t) = & \left(\frac{-Q_b}{V_{l,b}} \mathcal{D}_b^N A - \frac{Q_b}{V_{l,b}} K - \frac{P_{CON}}{V_{l,b}} M \right) \vec{\xi}(t) \\ & + \left(\frac{P_{CON}}{V_{l,b}} M + \frac{k_{bile} V_{l,h}}{V_{l,b}} M \right) \vec{\eta}(t) \\ & - \frac{Q_b \mathcal{D}_b^N}{V_{l,b}} (\xi_0(t) \phi'_0(0) + \xi_1(t) \phi'_1(0)) \vec{d}_{N+1} - \frac{Q_b}{V_{l,b}} \theta_N(t) \phi_N(1) \vec{d}_{N+1}. \end{aligned} \quad (5.60)$$

In the case for equation (5.42), for each i such that $i = 0 \cdots N$, we have

$$\begin{aligned} \sum_0^N \dot{\rho}_j(t) \langle \phi_j, \phi_i \rangle &= -\frac{Q_l}{a_6} \left(\sum_0^N \rho_j(t) \phi_j(1) \right) \phi_i(1) - \frac{Q_l}{k_1 a_6} \mathcal{D}_l^N \sum_0^N \rho_j(t) \phi_j'(0) \phi_i(0) \\ &\quad + \frac{Q_l}{a_6} \sum_1^N \alpha_j(t) \phi_j(1) \phi_i(0) - \frac{Q_l}{k_1 a_6} \mathcal{D}_l^N \sum_0^N \rho_j(t) \langle \phi_j', \phi_i' \rangle \\ &\quad + \frac{Q_l}{a_6} \sum_0^N \rho_j(t) \langle \phi_j, \phi_i' \rangle + \frac{Q_l}{a_6} y_{11} \phi_i(0), \end{aligned} \quad (5.61)$$

and in matrix-vector form:

$$\begin{aligned} M \dot{\vec{\rho}}(t) &= \left(-\frac{Q_l}{k_1 a_6} \mathcal{D}_l^N A + \frac{Q_l}{a_6} K \right) \vec{\rho}(t) - \frac{Q_l}{a_6} \rho_N(t) \phi_N(1) \vec{e}_{N+1} \\ &\quad - \frac{Q_l}{k_1 a_6} \mathcal{D}_l^N (\rho_0(t) \phi_0'(0) + \rho_1(t) \phi_1'(0)) \vec{d}_{N+1} \\ &\quad + \frac{Q_l}{a_6} (\alpha_N(t) \phi_N(1)) \vec{d}_{N+1} + \frac{Q_l}{a_6} y_{11} \vec{d}_{N+1}. \end{aligned} \quad (5.62)$$

Obtaining the matrix-vector form of equation (5.43) is identical to that of equation (5.42), as shown above, and is given by

$$\begin{aligned} M \dot{\vec{\omega}}(t) &= \left(-\frac{Q_l}{k_1 a_7} \mathcal{D}_l^N A + \frac{Q_l}{a_7} K \right) \vec{\omega}(t) - \frac{Q_l}{a_7} \omega_N(t) \phi_N(1) \vec{e}_{N+1} \\ &\quad - \frac{Q_l}{k_1 a_7} \mathcal{D}_l^N (\omega_0(t) \phi_0'(0) + \omega_1(t) \phi_1'(0)) \vec{d}_{N+1} \\ &\quad + \frac{Q_l}{a_7} (\gamma_N(t) \phi_N(1)) \vec{d}_{N+1} + \frac{Q_l}{a_7} y_{12} \vec{d}_{N+1}. \end{aligned} \quad (5.63)$$

For equation (5.44), for each i such that $i = 0 \cdots N$, we have

$$\begin{aligned} \sum_0^N \dot{\theta}_j(t) \langle \phi_j, \phi_i \rangle &= \frac{Q_b}{V_{tube}} \mathcal{D}_b^N \left(\sum_0^N \xi_j(t) \phi_j'(0) \right) \phi_i(1) + \frac{Q_b}{V_{tube}} \left(\sum_0^N \xi_j(t) \phi_j(0) \right) \phi_i(1) \\ &\quad - \frac{Q_b}{V_{tube}} \left(\sum_0^N \theta_j(t) \phi_j(0) \right) \phi_i(0) - \frac{Q_b}{V_{tube}} \mathcal{D}_t^N \sum_0^N \theta_j(t) \langle \phi_j', \phi_i' \rangle \\ &\quad - \frac{Q_b}{V_{tube}} \sum_0^N \theta_j(t) \langle \phi_j, \phi_i' \rangle, \end{aligned} \quad (5.64)$$

and the matrix-vector form is given by:

$$\begin{aligned} M \dot{\vec{\theta}}(t) &= \left(\frac{-Q_b}{V_{tube}} \mathcal{D}_t^N A - \frac{Q_b}{V_{tube}} K \right) \vec{\theta}(t) + \frac{Q_b}{V_{tube}} \mathcal{D}_b^N (\xi_0(t) \phi_0'(0)) \\ &\quad + \xi_1(t) \phi_1'(0) \vec{e}_{N+1} + \frac{Q_b}{V_{tube}} \xi_0(t) \phi_0(0) \vec{e}_{N+1} \\ &\quad - \frac{Q_b}{V_{tube}} \theta_0(t) \phi_0(0) \vec{d}_{N+1}. \end{aligned} \quad (5.65)$$

Recalling that we are not multiplying by any test functions and integrating, making the finite element substitutions for equations (5.45-5.49) is straightforward:

$$\begin{aligned} \dot{y}_9 &= \left(\frac{-P_{GEN}}{V_{ROB}} - k_{urine}^{GEN} - k_{ROB}^m \right) y_9 + \frac{P_{GEN}}{V_{ROB}} f_{ss}^{Gu} y_{11}, \\ \dot{y}_{10} &= k_{ROB}^m y_9 - \left(\frac{P_{CON}}{V_{ROB}} + k_{urine} \right) y_{10} + \frac{P_{CON}}{V_{ROB}} f_{ss}^{Cu} y_{12}, \\ \dot{y}_{11} &= \frac{Q_l}{V_{l,s}} \phi_N(1) \rho_N(t) + \frac{P_{GEN}}{V_{ROB}} y_9 \\ &\quad - \left(\frac{Q_l}{V_{l,s}} + \frac{P_{GEN}}{V_{ROB}} f_{ss}^{Gu} \right) y_{11} + \frac{1}{V_{ss}} v_{inf}(t), \\ \dot{y}_{12} &= \frac{Q_l}{V_{l,s}} \phi_N(1) \omega_N(t) + \frac{P_{CON}}{V_{ROB}} y_{10} - \left(\frac{Q_l}{V_{l,s}} + \frac{P_{CON}}{V_{ROB}} f_{ss}^{Cu} \right) y_{12}, \\ \dot{y}_{13} &= Q_b [\phi_0(x_1) \cdots \phi_N(x_1)] \vec{\theta}(t) \\ &\quad + Q_b \mathcal{D}_t^N [\phi_0'(x_1) \cdots \phi_N'(x_1)] \vec{\theta}(t) \end{aligned} \quad (5.66)$$

The definitions for the constant matrices and vectors above are as follows:

$$\begin{aligned}(M)_{ij} &= \langle \phi_j, \phi_i \rangle, \quad 1 \leq i, j \leq N+1, \\ (\bar{M})_{ij} &= \langle \phi_j, \phi_i \rangle, \quad 1 \leq i, j \leq N, \\ (\tilde{M})_{ij} &= \langle \phi_j, \phi_i \rangle, \quad 1 \leq i \leq N, \quad 1 \leq j \leq N+1, \\ (\tilde{\tilde{M}})_{ij} &= \langle \phi_j, \phi_i \rangle, \quad 1 \leq i \leq N+1, \quad 1 \leq j \leq N,\end{aligned}$$

$$\begin{aligned}(A)_{ij} &= \langle \phi'_j, \phi'_i \rangle, \quad 1 \leq i, j \leq N+1, \\ (\bar{A})_{ij} &= \langle \phi'_j, \phi'_i \rangle, \quad 1 \leq i, j \leq N, \\ (K)_{ij} &= \langle \phi_j, \phi'_i \rangle, \quad 1 \leq i, j \leq N+1, \\ (\bar{K})_{ij} &= \langle \phi_j, \phi'_i \rangle, \quad 1 \leq i, j \leq N, \\ (\vec{\Phi}_N)_i &= \langle 1, \phi_i \rangle, \quad 1 \leq i \leq N, \\ (\vec{\Phi}_{N+1})_i &= \langle 1, \phi_i \rangle, \quad 1 \leq i \leq N+1, \\ (\vec{e}_N)_i &= \begin{cases} 1, & i = N \\ 0, & \text{otherwise,} \end{cases} \\ (\vec{e}_{N+1})_i &= \begin{cases} 1, & i = N+1 \\ 0, & \text{otherwise,} \end{cases} \\ (\vec{d}_{N+1})_i &= \begin{cases} 1, & i = 0 \\ 0, & \text{otherwise.} \end{cases}\end{aligned}$$

5.3.3 Semi-Discrete Problem

With the formulation of equations (5.54 - 5.66), we may now develop the semi-discrete system as follows.

Let

$$y^N(t) = [(\vec{\alpha}(t))^T, (\vec{\beta}(t))^T, (\vec{\gamma}(t))^T, (\vec{\eta}(t))^T, (\vec{\xi}(t))^T, (\vec{\rho}(t))^T, (\vec{\omega}(t))^T, (\vec{\theta}(t))^T, \\ y_9(t), y_{10}(t), y_{11}(t), y_{12}(t), y_{13}(t)]^T.$$

Then the finite dimensional system to be solved is given by

$$\mathcal{M}\dot{\vec{y}}^N(t) = \mathcal{A}\vec{y}^N(t) + \mathcal{G}(\vec{y}^N(t)) + \mathcal{F}(t), \quad (5.67)$$

where we define

$$\mathcal{M} = \begin{bmatrix} \bar{M} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & M & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & \bar{M} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & M & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & M & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & M & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & M & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & M & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & I, \end{bmatrix}$$

with

$$\mathcal{A} = \begin{bmatrix} \mathcal{A}_{11} & \frac{P_{GEN}}{a_1} \tilde{M} & 0 & 0 & 0 & \mathcal{A}_{16} & 0 & 0 & \mathcal{A}_{19} \\ a_2 f_{l,s}^{G_u} \tilde{M} & -a_2 M & 0 & 0 & 0 & 0 & 0 & 0 & \mathcal{A}_{29} \\ 0 & 0 & \mathcal{A}_{33} & \frac{P_{CON}}{a_3} \tilde{M} & 0 & 0 & \mathcal{A}_{37} & 0 & \mathcal{A}_{39} \\ 0 & 0 & a_4 f_{l,s}^{C_u} \tilde{M} & \mathcal{A}_{44} & a_4 M & 0 & 0 & 0 & \mathcal{A}_{49} \\ 0 & 0 & 0 & \mathcal{A}_{54} & \mathcal{A}_{55} & 0 & 0 & \mathcal{A}_{58} & 0 \\ \mathcal{A}_{61} & 0 & 0 & 0 & 0 & \mathcal{A}_{66} & 0 & 0 & \mathcal{A}_{69} \\ 0 & 0 & \mathcal{A}_{73} & 0 & 0 & 0 & \mathcal{A}_{77} & 0 & \mathcal{A}_{79} \\ 0 & 0 & 0 & 0 & \mathcal{A}_{85} & 0 & 0 & \mathcal{A}_{88} & 0 \\ 0 & 0 & 0 & 0 & 0 & \mathcal{A}_{96} & \mathcal{A}_{97} & \mathcal{A}_{98} & \mathcal{A}_{99} \end{bmatrix}$$

and

$$\mathcal{A}_{11} = \frac{-Q_l}{a_1} \mathcal{D}_l^N \bar{A} + \frac{Q_l}{a_1} \bar{K} - f_{l,s}^{G_u} \frac{P_{GEN}}{a_1} \bar{M},$$

$$\mathcal{A}_{16} = \left[\left(\frac{Q_l}{k_1 a_1} \mathcal{D}_l^N \phi'_0(0) - \frac{Q_l}{a_1} \phi_0(0) \right) \vec{e}_N \frac{Q_l}{k_1 a_1} \mathcal{D}_l^N \phi'_1(0) \vec{e}_N \vec{0} \cdots \vec{0} - \frac{Q_l}{V_{l,s}} \vec{\Phi}_N \right],$$

$$\mathcal{A}_{19} = \left[-\frac{P_{GEN}}{V_{ROB}} \vec{\Phi}_N \vec{0} \left(\left(f_{ss}^{G_u} \frac{P_{GEN}}{V_{ROB}} + \frac{Q_l}{V_{l,s}} - f_{l,s}^{G_u} \frac{P_{GEN}}{a_1} \right) \vec{\Phi}_N + \frac{Q_l}{a_1} \vec{e}_N \right) \vec{0} \vec{0} \right],$$

$$\mathcal{A}_{29} = \left[\vec{0} \vec{0} a_2 f_{l,s}^{G_u} \vec{\Phi}_{N+1} \vec{0} \vec{0} \right],$$

$$\mathcal{A}_{33} = \frac{-Q_l}{a_3} \mathcal{D}_l^N \bar{A} + \frac{Q_l}{a_3} \bar{K} - f_{l,s}^{C_u} \frac{P_{CON}}{a_3} \bar{M},$$

$$\mathcal{A}_{37} = \left[\left(\frac{Q_l}{k_1 a_3} \mathcal{D}_l^N \phi'_0(0) - \frac{Q_l}{a_3} \phi_0(0) \right) \vec{e}_N \frac{Q_l}{k_1 a_3} \mathcal{D}_l^N \phi'_1(0) \vec{e}_N \vec{0} \cdots \vec{0} - \frac{Q_l}{V_{l,s}} \vec{\Phi}_N \right],$$

$$\mathcal{A}_{39} = \left[\vec{0} - \frac{P_{CON}}{V_{ROB}} \vec{\Phi}_N \vec{0} \left(\left(f_{ss}^{C_u} \frac{P_{CON}}{V_{ROB}} + \frac{Q_l}{V_{l,s}} - f_{l,s}^{C_u} \frac{P_{CON}}{a_3} \right) \vec{\Phi}_N + \frac{Q_l}{a_3} \vec{e}_N \right) \vec{0} \right],$$

$$\mathcal{A}_{44} = -(2a_4 + k_{bile})M,$$

$$\mathcal{A}_{49} = \left[\vec{0} \vec{0} \vec{0} a_4 f_{l,s}^{C_u} \vec{\Phi}_{N+1} \vec{0} \right],$$

$$\mathcal{A}_{54} = \frac{P_{CON}}{V_{l,b}}M + \frac{k_{bile}V_{l,h}}{V_{l,b}}M,$$

$$\mathcal{A}_{55} = \frac{-Q_b}{V_{l,b}}\mathcal{D}_b^N A - \frac{Q_b}{V_{l,b}}K - \frac{P_{CON}}{V_{l,b}}M + B_{55},$$

$$\mathcal{A}_{58} = \left[\vec{0} \cdots \vec{0} - \frac{Q_b}{V_{l,b}}\phi_N(1)\vec{d}_{N+1} \right],$$

$$\mathcal{A}_{61} = \left[\vec{0} \cdots \vec{0} \frac{Q_l}{a_6}\phi_N(1)\vec{d}_{N+1} \right],$$

$$\mathcal{A}_{66} = \frac{-Q_l}{k_1 a_6}\mathcal{D}_l^N A + \frac{Q_l}{a_6}K + B_{66},$$

$$\mathcal{A}_{69} = \left[\vec{0} \vec{0} \frac{Q_l}{a_6}\vec{d}_{N+1} \vec{0} \vec{0} \right],$$

$$\mathcal{A}_{73} = \left[\vec{0} \cdots \vec{0} \frac{Q_l}{a_7}\phi_N(1)\vec{d}_{N+1} \right],$$

$$\mathcal{A}_{77} = \frac{-Q_l}{k_1 a_7}\mathcal{D}_l^N A + \frac{Q_l}{a_7}K + B_{77},$$

$$\mathcal{A}_{79} = \left[\vec{0} \vec{0} \vec{0} \frac{Q_l}{a_7}\vec{d}_{N+1} \vec{0} \right],$$

$$\mathcal{A}_{85} = \left[\frac{Q_b}{V_{tube}}(\mathcal{D}_b^N \phi'_0(0) + \phi_0(0))\vec{e}_{N+1} \frac{Q_b}{V_{tube}}\mathcal{D}_b^N \phi'_1(0)\vec{e}_{N+1} \vec{0} \cdots \vec{0} \right],$$

$$\mathcal{A}_{88} = \frac{-Q_b}{V_{tube}}\mathcal{D}_t^N A - \frac{Q_b}{V_{tube}}K + B_{88},$$

$$\begin{aligned}
\mathcal{A}_{96} &= \frac{Q_l}{V_{l,s}} \begin{bmatrix} 0 & \cdots & 0 \\ 0 & \cdots & 0 \\ 0 & \cdots & \phi_N(1) \\ 0 & \cdots & 0 \\ 0 & \cdots & 0 \end{bmatrix}, \\
\mathcal{A}_{97} &= \frac{Q_l}{V_{l,s}} \begin{bmatrix} 0 & \cdots & 0 \\ 0 & \cdots & 0 \\ 0 & \cdots & 0 \\ 0 & \cdots & \phi_N(1) \\ 0 & \cdots & 0 \end{bmatrix}, \\
\mathcal{A}_{98} &= Q_b \begin{bmatrix} 0 & \cdots & 0 \\ 0 & \cdots & 0 \\ 0 & \cdots & 0 \\ 0 & \cdots & 0 \\ \phi_0(x_1) & \cdots & \phi_N(x_1) \end{bmatrix} + Q_b \mathcal{D}_t^N \begin{bmatrix} 0 & \cdots & 0 \\ 0 & \cdots & 0 \\ 0 & \cdots & 0 \\ 0 & \cdots & 0 \\ \phi'_0(x_1) & \cdots & \phi'_N(x_1) \end{bmatrix}, \\
\mathcal{A}_{99} &= \begin{bmatrix} \mathcal{A}_{99}^{11} & 0 & \frac{P_{GEN} f_{ss}^{Gu}}{V_{ROB}} & 0 & 0 \\ k_{ROB}^m \left(\frac{-P_{CON}}{V_{ROB}} - k_{urine}^C \right) & 0 & 0 & \frac{P_{CON} f_{ss}^{Cu}}{V_{ROB}} & 0 \\ \frac{P_{GEN}}{V_{ROB}} & 0 & \left(-\frac{Q_l}{V_{l,s}} - \frac{P_{GEN} f_{ss}^{Gu}}{V_{ROB}} \right) & 0 & 0 \\ 0 & \frac{P_{CON}}{V_{ROB}} & 0 & \left(-\frac{Q_l}{V_{l,s}} - \frac{P_{CON} f_{ss}^{Cu}}{V_{ROB}} \right) & 0 \\ 0 & 0 & 0 & 0 & 0 \end{bmatrix},
\end{aligned}$$

and

$$B_{55} = \left[-\frac{Q_b}{V_{l,b}} \mathcal{D}_b^N \phi'_0(0) \vec{d}_{N+1} - \frac{Q_b}{V_{l,b}} \mathcal{D}_b^N \phi'_1(0) \vec{d}_{N+1} \vec{0} \cdots \vec{0} \right],$$

$$\begin{aligned}
B_{66} &= \left[-\frac{Q_l}{k_1 a_6} \mathcal{D}_l^N \phi'_0(0) \vec{d}_{N+1} - \frac{Q_l}{k_1 a_6} \mathcal{D}_l^N \phi'_1(0) \vec{d}_{N+1} \vec{0} \cdots \vec{0} - \frac{Q_l}{a_6} \phi_N(1) \vec{e}_{N+1} \right], \\
B_{77} &= \left[-\frac{Q_l}{k_1 a_7} \mathcal{D}_l^N \phi'_0(0) \vec{d}_{N+1} - \frac{Q_l}{k_1 a_7} \mathcal{D}_l^N \phi'_1(0) \vec{d}_{N+1} \vec{0} \cdots \vec{0} - \frac{Q_l}{a_7} \phi_N(1) \vec{e}_{N+1} \right], \\
B_{88} &= \left[-\frac{Q_b}{V_{tube}} \phi_0(0) \vec{d}_{N+1} \vec{0} \cdots \vec{0} \right], \\
\mathcal{A}_{99}^{11} &= \left(\frac{-P_{GEN}}{V_{ROB}} - k_{urine}^{GEN} - k_{ROB}^m \right)
\end{aligned}$$

and the functions

$$\begin{aligned}
\mathcal{G}(\vec{y}^N(t)) &= \left[\vec{0}_N - \vec{G}_m(\vec{\beta}(t)) \vec{0}_N \vec{G}_m(\vec{\beta}(t)) \vec{0}_{N+1} \vec{0}_{N+1} \vec{0}_{N+1} \vec{0}_{N+1} 0 0 0 0 0 \right]^T, \\
\mathcal{F}(t) &= \left[-\frac{1}{V_{ss}} v_{inf}(t) \vec{\Phi}_N \vec{0}_{N+1} \vec{0}_N \vec{0}_{N+1} \vec{0}_{N+1} \vec{0}_{N+1} \vec{0}_{N+1} \vec{0}_{N+1} 0 0 \frac{1}{V_{ss}} v_{inf}(t) 0 0 \right]^T.
\end{aligned}$$

5.4 Inverse Problem

The formulation of the inverse problem for the dispersion model is almost identical to that of the DDE model, outlined in Section 2.3. The inverse problem is defined in same way, and is given by:

$$q_{opt}^{\vec{}} = \min_{\vec{q} \in S} (J(\vec{q})),$$

where $S = \{\vec{q} \in R^n : q_k > 0, k = 1, 2, \dots, n\}$ and J is our cost function.

In the DDE model, we used the MLF for the cost function, introducing two more parameters to be optimized. In the case of the dispersion model, we decided to use the single data set comprising of the biliary excretion rate data only, and hence used the standard least squares cost function, given by:

$$J(\vec{q}) = \sum_{j=1}^n (z_j - f_j(\vec{q}))^2,$$

where each z_j is a data point, j corresponds to the time at which the data point represents, $f_{i,j}(\vec{q})$ is the model prediction at the time of the data point $z_{i,j}$. As will be discussed in Section 6.2.2, we ultimately wish to implement the MLF for this model, however for our preliminary optimization results, the performance of the least squares cost function is satisfactory.

For the same reasons as in the DDE model, the Nelder-Mead simplex-based method was the optimization algorithm implemented for parameter optimization. Since the biliary excretion rate are the original experimental data in (20), we used that data set (as opposed to the cumulative biliary excretion data) to compare the dispersion model to.

5.5 Numerical Methods

In this section we describe the numerical procedure used in obtaining the solutions for our dispersion model. From the proof of Theorem 4.1 (see (46)), we are guaranteed of theoretical convergence of the semi-discrete problem to the solution of the infinite-dimensional model equations (5.37-5.49) as $N \rightarrow \infty$. All entries in the matrices $\mathcal{M}$ and $\mathcal{A}$ that contain certain combinations of inner products of basis elements were calculated analytically, as shown in Section 5.3.1.

5.5.1 Implementation

The computer code was written and ran in MATLAB, Version 6.1.0.450, Release 12.1 (The Mathworks, Inc., Natick, MA). Computations were performed on a Red Hat Linux based Dell Precision 420 with dual Intel Pentium III 1 GHz processors and a Red Hat Linux based Micron Millennia with a single Intel Pentium III 1.4 GHz processor. We implemented the MATLAB ODE solver *ode15s* to solve the system of equations in the semi-discrete problem. This is a variable order, variable time-step solver designed for stiff systems of

ODE. Since the stiffness of our semi-discrete system varies with parameter values, this was the solver of choice in our problem. The MATLAB optimization routine *fminsearch*, which is based on a simplex-based method, was implemented for the parameter estimations.

5.5.2 Convergence of the Numerical Scheme

As was established in the proof of Theorem 4.1 in (46), we are guaranteed theoretical convergence of solutions for the finite dimensional Galerkin approximation to the solution of the infinite dimensional system. This convergence is seen in Figure 5.1, where we fix the parameters and vary N from 12 to 96 elements.

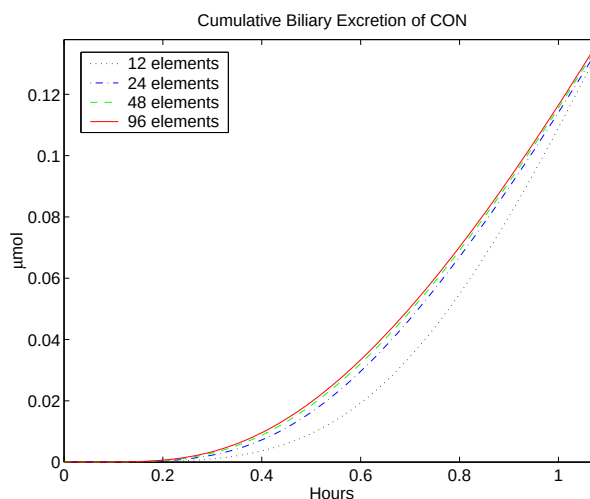


Figure 5.1: Demonstration of convergence of the Galerkin approximations to the infinite dimensional solution.

5.6 Simulations

We estimated the unknown parameters for each single dose of the four doses of genistein administered. The results of these estimations are in Section 5.6.2. We also estimated a

parameter set using all four doses. That is, we generated a cost after running the model using each of the four doses and comparing the model to the data set. The results of this estimation are in Section 5.6.4. In each case, we noticed that setting the free-fractions to the values obtained in Section 3.1 did not allow for enough free genistein to be uptaken, metabolized and excreted into the bile for any relevant comparison to the data. Therefore, for our preliminary parameter estimations all free-fraction coefficients were set to .98. Further discussion about this can be found in Section 6.2.2. We also set the dispersion numbers for the bile duct and the bile extension tube equal, for simplicity. In the future, this will not generally be the case.

5.6.1 The Need for Nonlinearity

When we replace the Michaelis-Menten rate term for the saturable process of genistein metabolism with a linear rate term, the plots of the data versus simulation strongly suggest that a saturable term is, indeed, needed. Since experimental data show that genistein metabolism is saturable, this was naturally the rate for which we chose to use a Michaelis-Menten term. In Figure 5.2A, we simulated the lowest dose of genistein administered using the estimated parameters for the lowest dose, where the estimated parameters used were with a linear metabolism term. In Figure 5.2B, we simulated the highest dose administered using the estimated parameters for the lowest dose. Clearly the model overpredicts the data for the highest dose by a large margin in Figure 5.2B, while accurately predicting the data for the lowest dose in Figure 5.2A. Now, as seen in the plots of Section 5.6.4, the saturable metabolism term corrects this problem to a point, but further correction is needed. In this case, the model may suggest that even more saturable terms may be necessary.

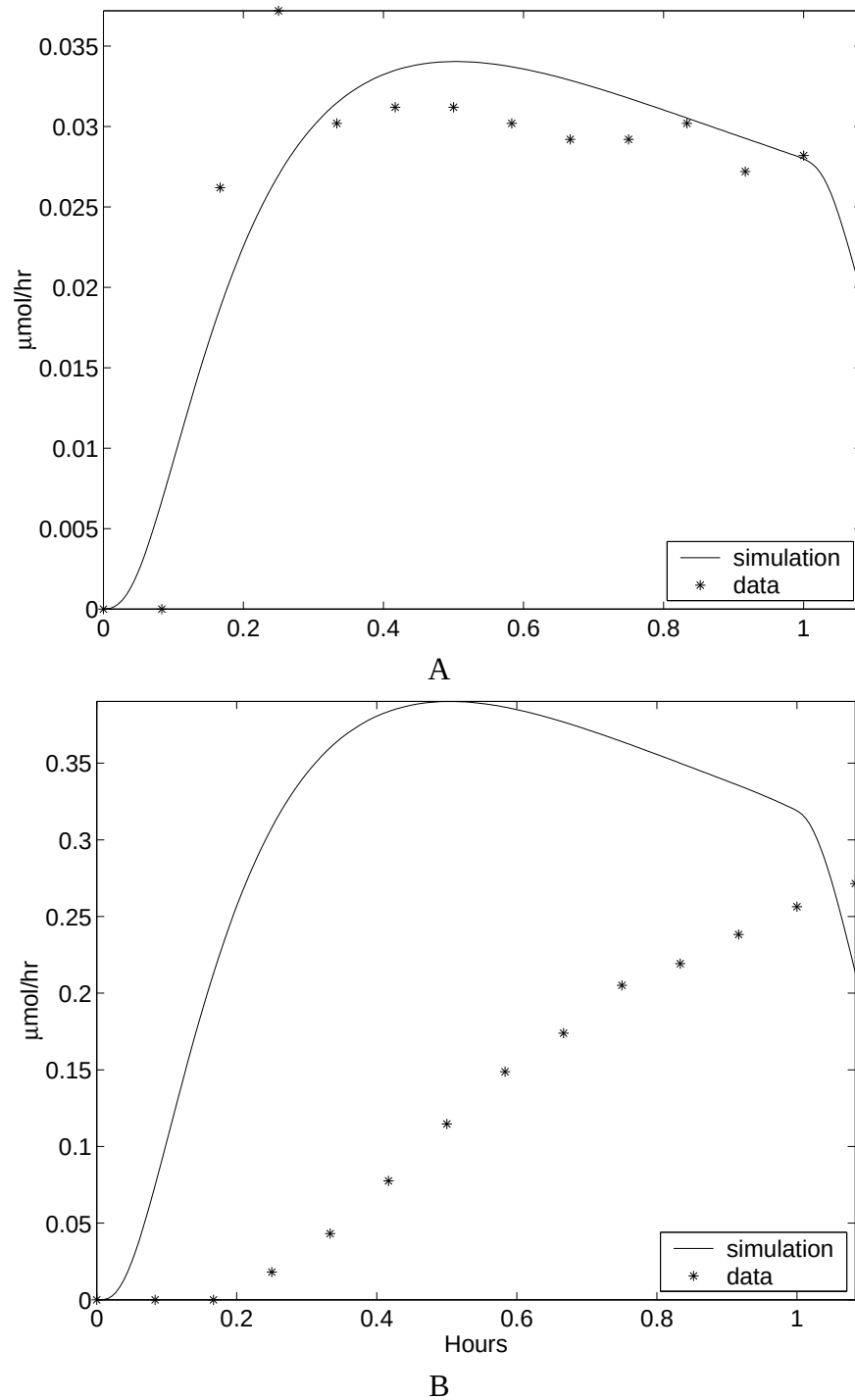


Figure 5.2: Figure A: Simulation of biliary excretion rate using the lowest dose with the estimated parameters for the lowest dose. Figure B: Simulation of biliary excretion rate using the largest dose with the estimated parameters for the lowest dose.

5.6.2 Single Dose Simulations

We estimated the unknown parameters (Table 3.2) using the excretion rate data for each single dose of genistein. The following plots show various state variables of the model, the most noteworthy of is the biliary excretion rates, whereas those plots represent the comparison between the model and the original experimental data. Plots of the state variables for GEN and CON in the ROB and systemic serum compartments are shown for Doses 1 and 4. Table 5.1 following the figures lists the estimated parameters used to obtain the simulations shown for each of the four doses. For purposes of minimizing the number of parameters needed to be estimated, we simplified the model in the following manner. First, we set the metabolism term for the ROB compartment $k_m^{ROB} = 0$. Recall that this term is to account for genistein metabolism in the intestinal wall. But since the experimental conditions require a venous infusion of genistein, as opposed to a bolus oral dose or a gut infusion, and since the rats were bile duct-cannulated, the amount of genistein being conjugated in the intestinal wall under these conditions is likely negligible. Since there are no urinary elimination data (with the correct experimental conditions) and changing the values of these parameters simply requires an adjustment of the other estimated parameters to maintain an accurate simulation of the data, we fixed the urinary elimination rate constants to $k_{urine}^{GEN} = 4.0$ and $k_{urine}^{CON} = 0$. Lastly, we set the dispersion numbers for the bile duct space and the bile extension tube equal, i.e., $\mathcal{D}_b^N = \mathcal{D}_t^N$. These simplifications, and the choice of using a least squares cost function over the MLF discussed in Chapter 2 reduces the number of estimated parameters from 13 to 7. We discuss the variations in the parameter sets in Section 5.6.4.

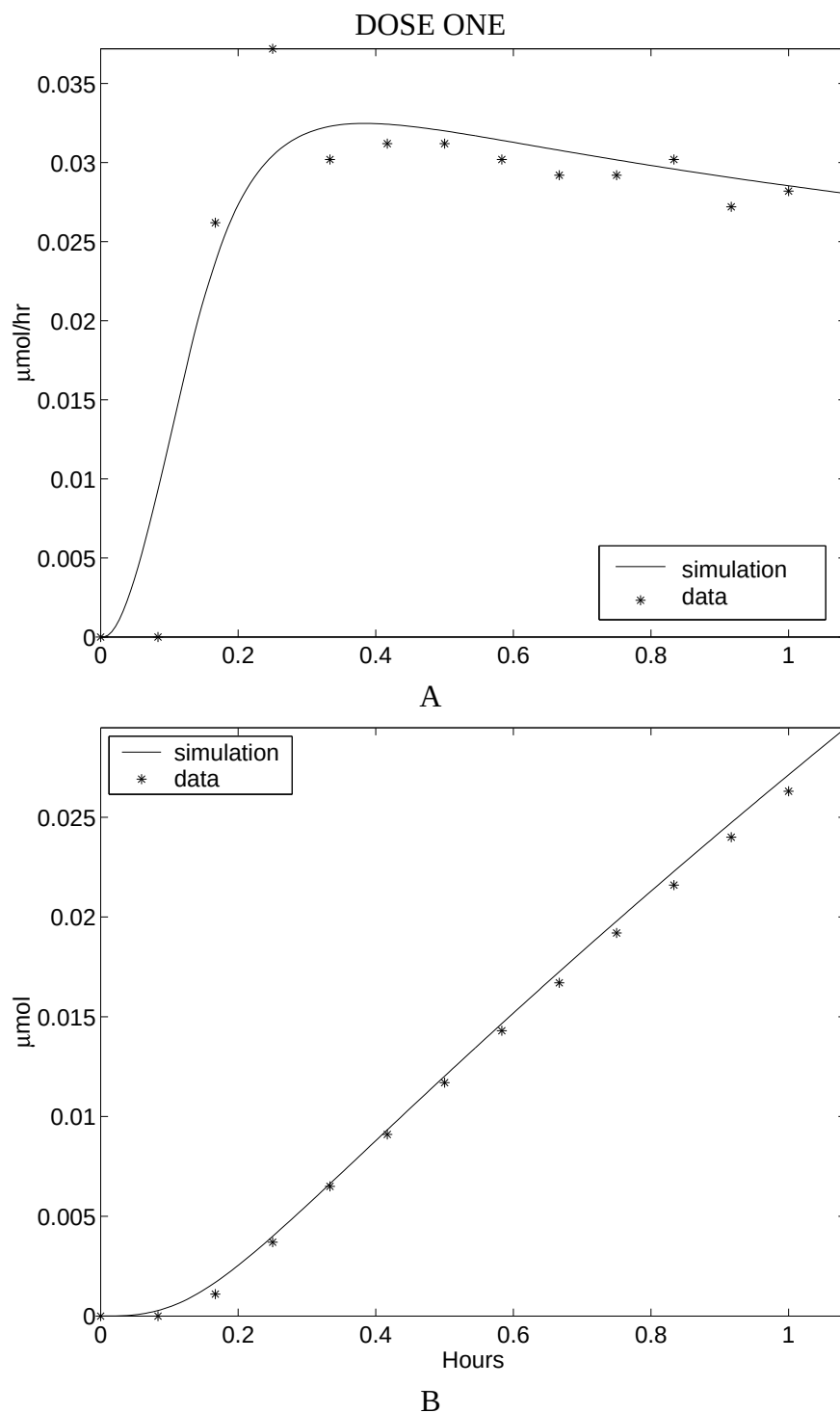


Figure 5.3: (Dose 1) A: Biliary excretion rate of conjugates after a 1 hour dose of pure genistein. B: Cumulative biliary excretion of conjugates after a 1 hour dose of pure genistein.

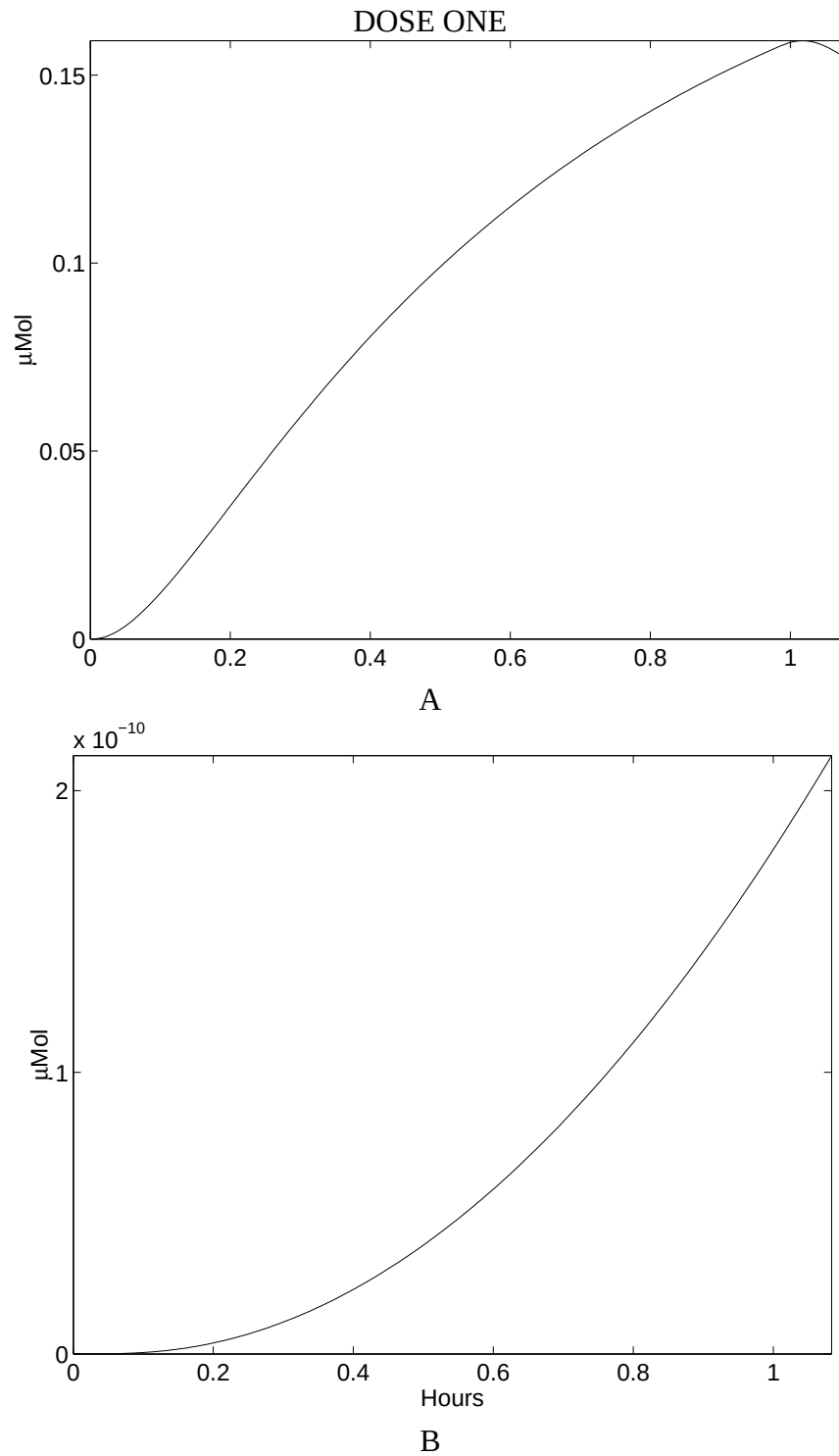


Figure 5.4: (Dose 1) A: Genistein concentration in ROB compartment after a 1 hour dose of pure genistein. B: Conjugates concentration in ROB compartment after a 1 hour dose of pure genistein.

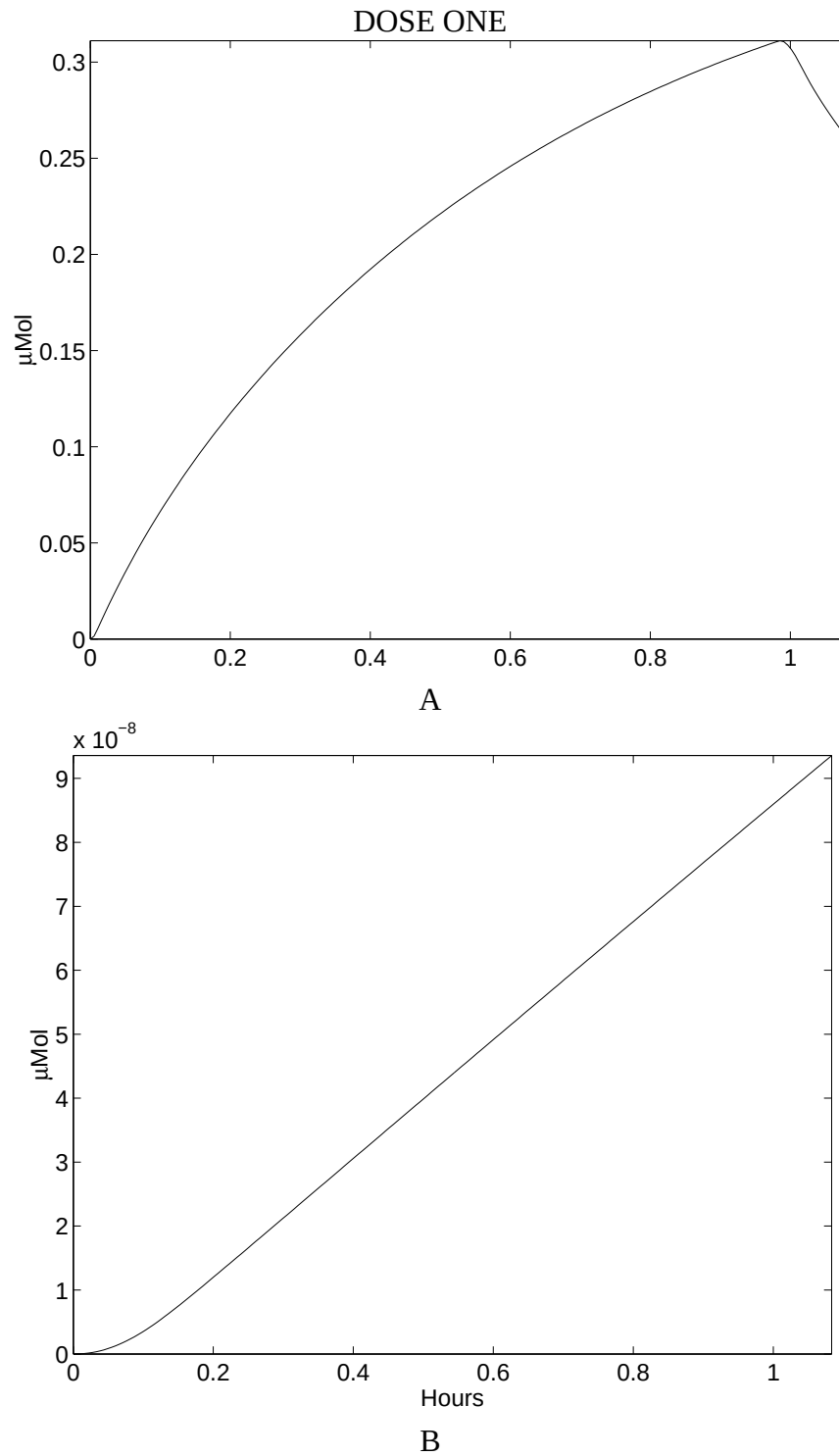


Figure 5.5: (Dose 1) A: Genistein concentration in systemic serum compartment after a 1 hour dose of pure genistein. B: Conjugates concentration in systemic serum compartment after a 1 hour dose of pure genistein.

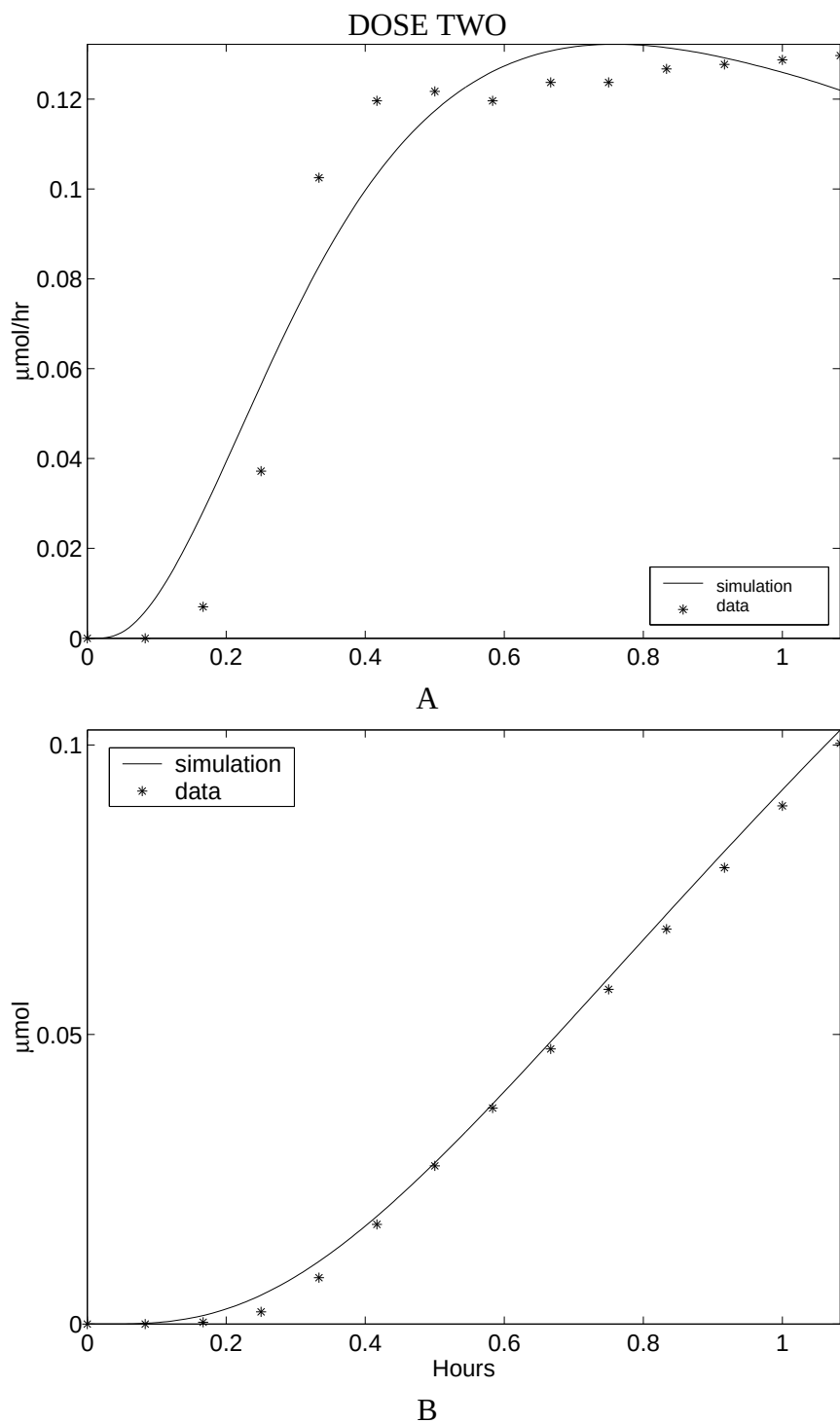


Figure 5.6: (Dose 2) A: Biliary excretion rate of conjugates after a 1 hour dose of pure genistein. B: Cumulative biliary excretion of conjugates after a 1 hour dose of pure genistein.

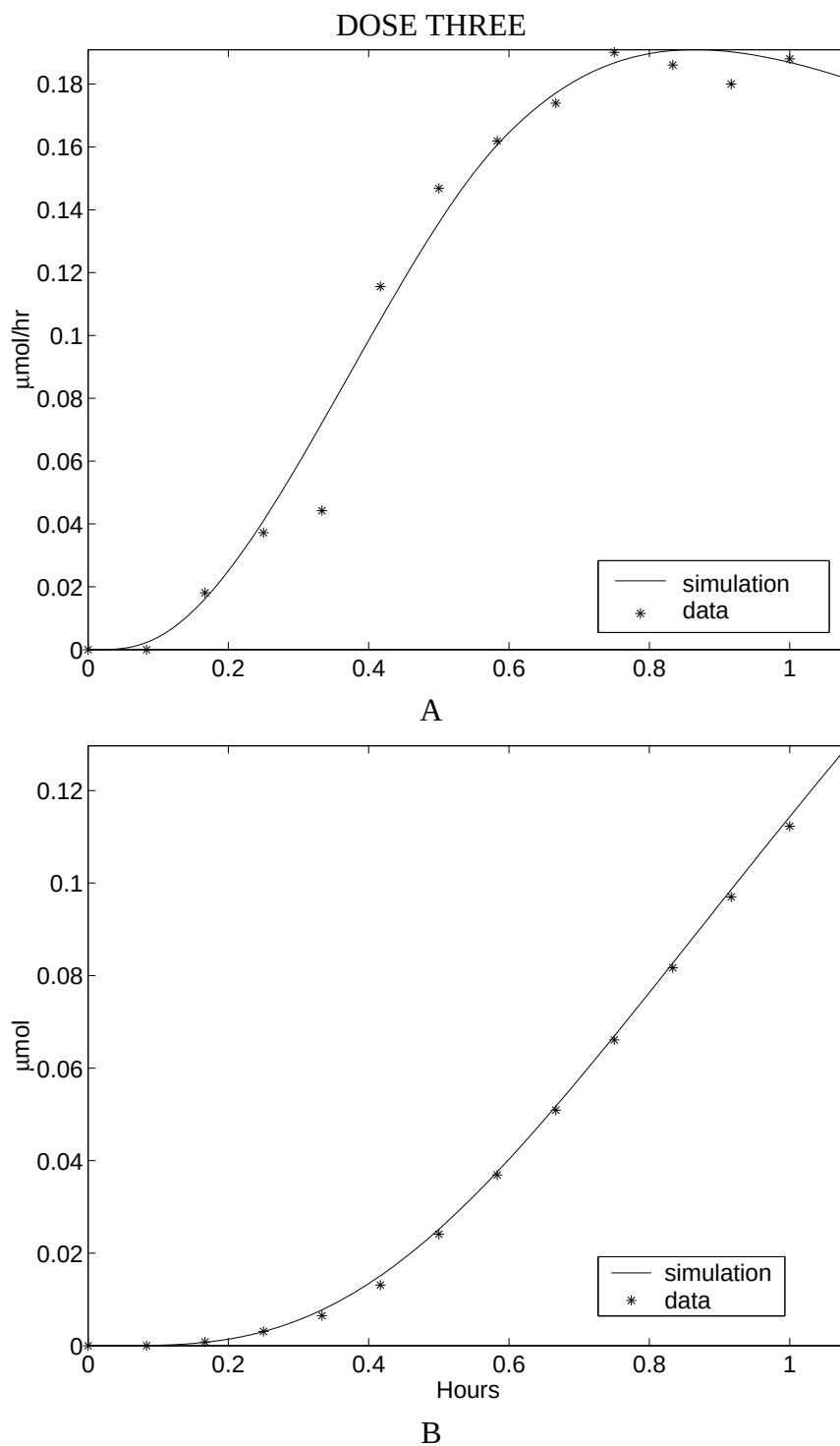


Figure 5.7: (Dose 3) A: Biliary excretion rate of conjugates after a 1 hour dose of pure genistein. B: Cumulative biliary excretion of conjugates after a 1 hour dose of pure genistein.

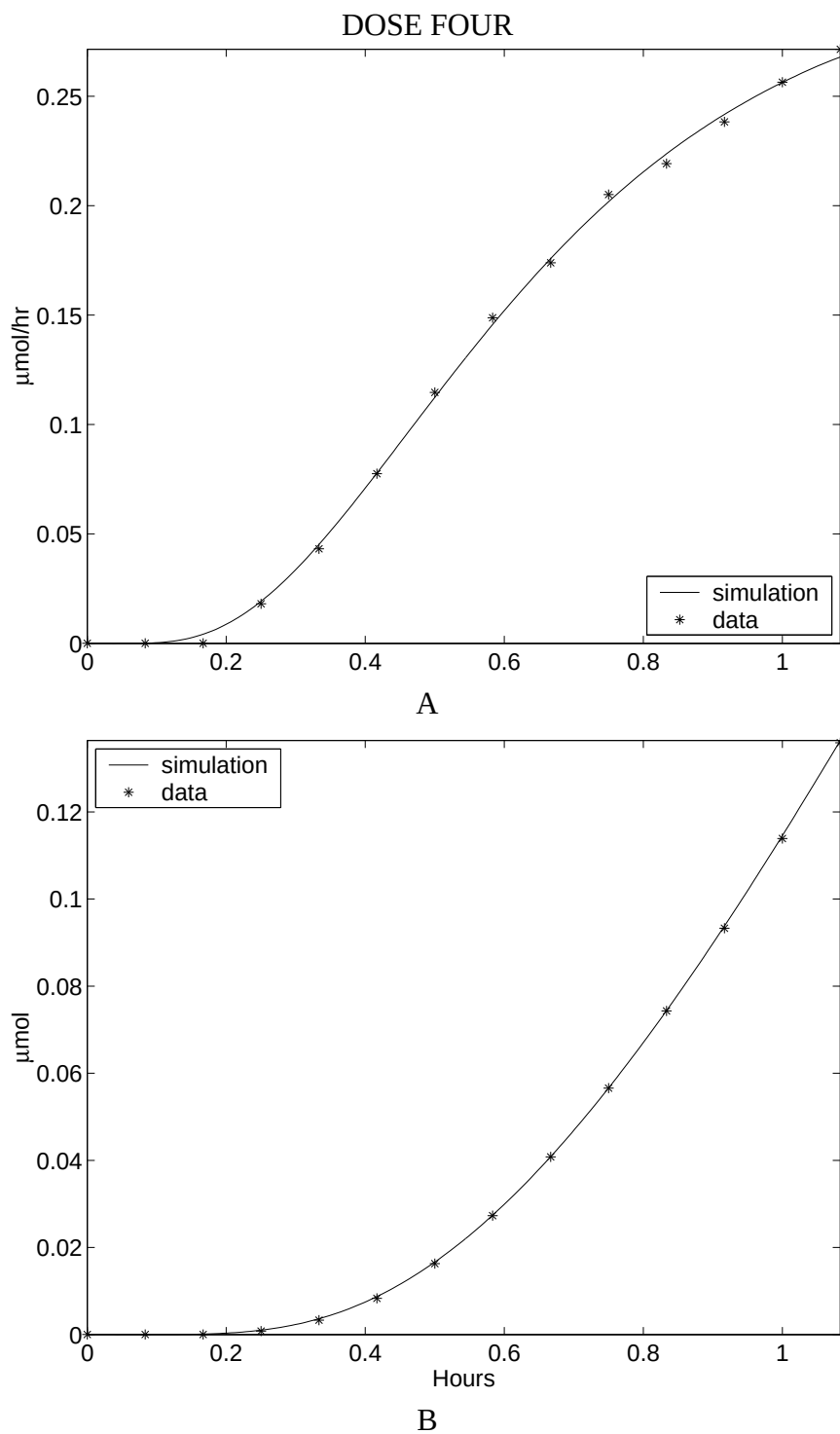


Figure 5.8: (Dose 4) A: Biliary excretion rate of conjugates after a 1 hour dose of pure genistein. B: Cumulative biliary excretion of conjugates after a 1 hour dose of pure genistein.

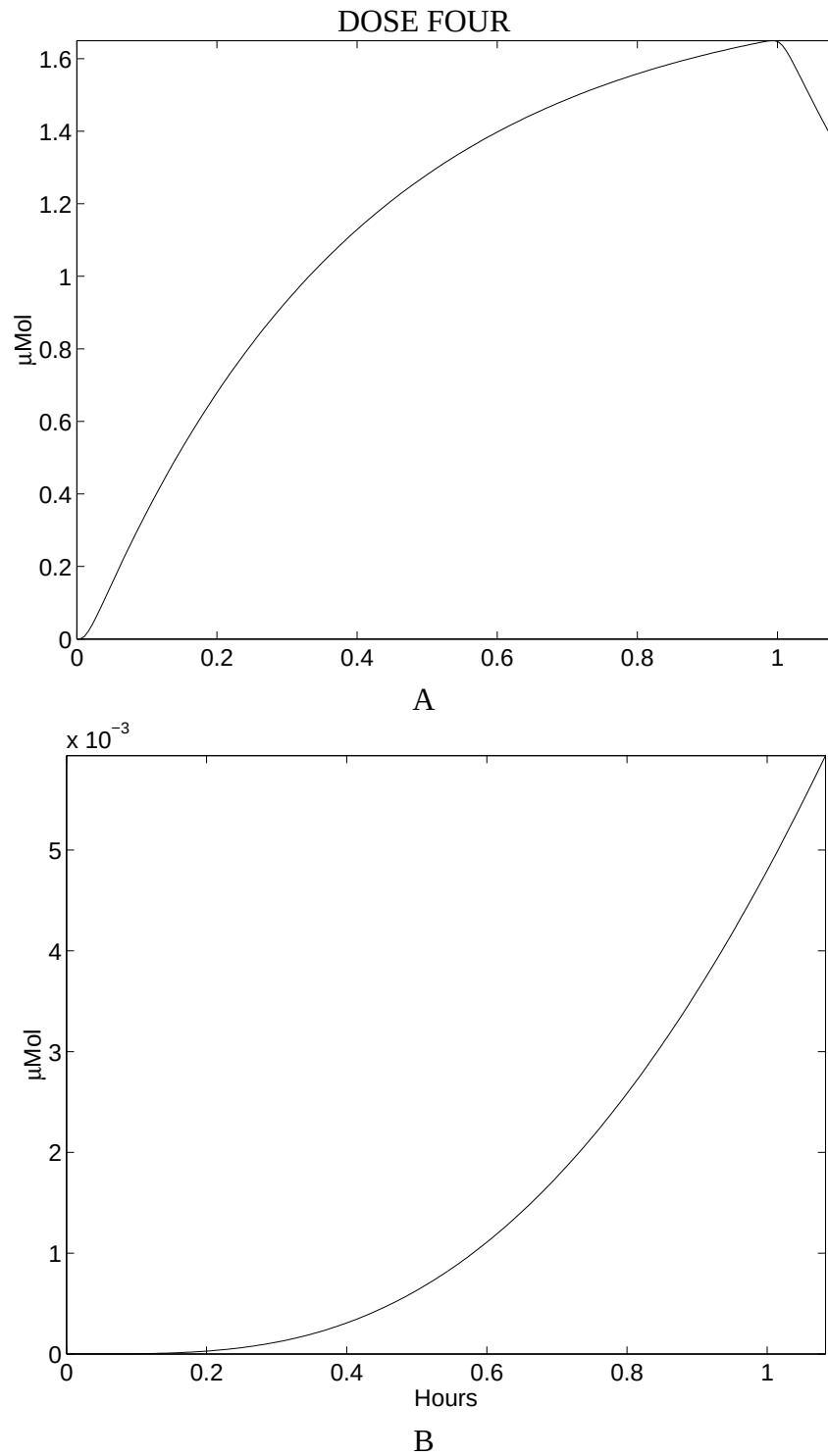


Figure 5.9: (Dose 4) A: Genistein concentration in ROB compartment after a 1 hour dose of pure genistein. B: Conjugates concentration in ROB compartment after a 1 hour dose of pure genistein.

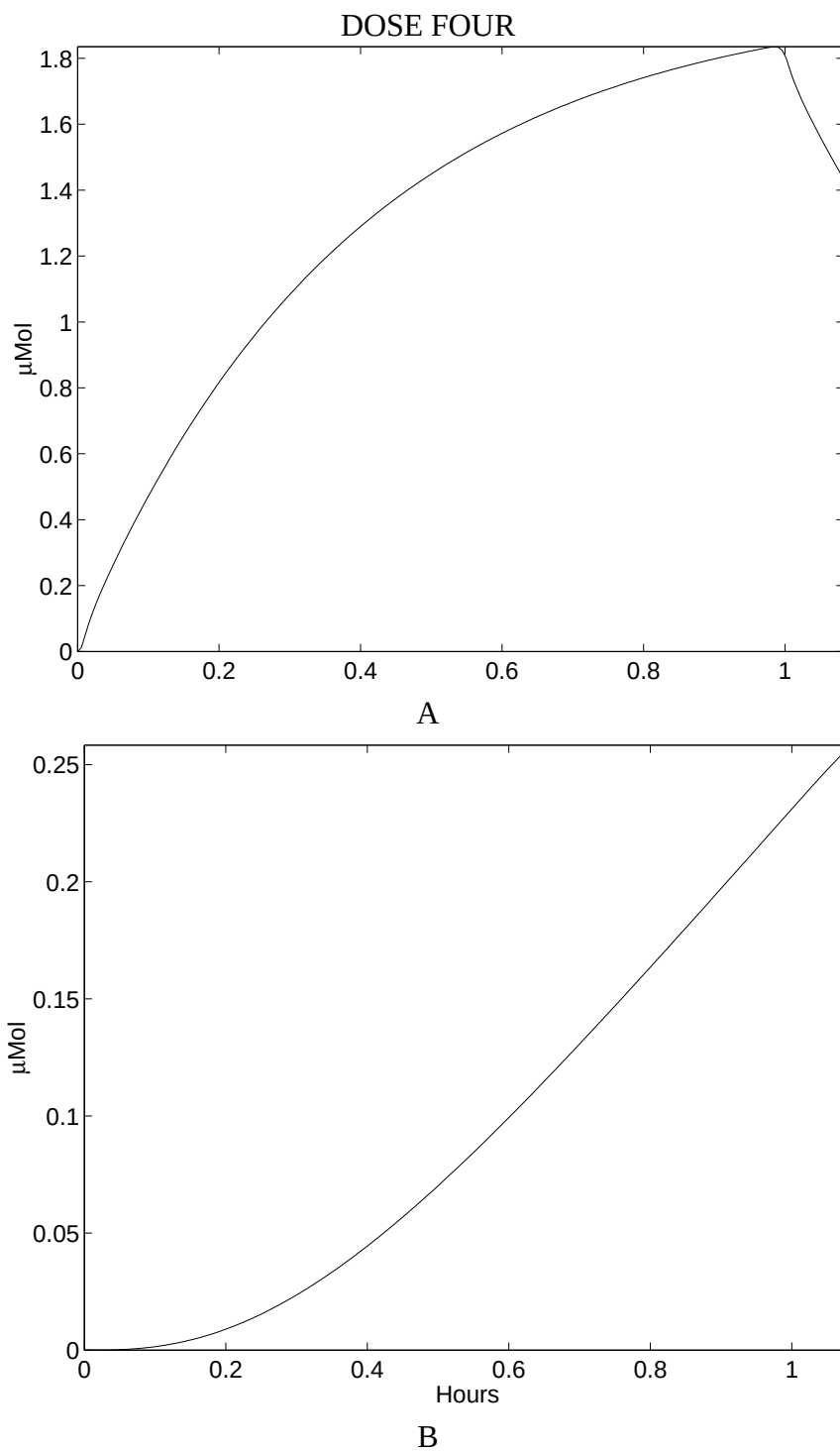


Figure 5.10: (Dose 4) A: Genistein concentration in systemic serum compartment after a 1 hour dose of pure genistein. B: Conjugates concentration in systemic serum compartment after a 1 hour dose of pure genistein.

Table 5.1: Resulting values from parameter optimization for the dispersion model.

Dose 1	0.0462 $\mu\text{mol/hr}$
Parameter	Value
k_{bile}	$8.2147 \times 10^3 \text{ hr}^{-1}$
V_{max}	$12.7045 \mu\text{mol/L/hr}$
k_m	$0.0006 \mu\text{mol/L}$
P_{GEN}	1.074 L/hr
P_{CON}	0.001 L/hr
$\mathcal{D}_l^N$	9.0506×10^3
$\mathcal{D}_b^N$	13.2303
Dose 2	0.1986 $\mu\text{mol/hr}$
Parameter	Value
k_{bile}	41.3181 hr^{-1}
V_{max}	$88.347 \mu\text{mol/L/hr}$
k_m	$0.013 \mu\text{mol/L}$
P_{GEN}	1.1381 L/hr
P_{CON}	0.0021 L/hr
$\mathcal{D}_l^N$	600.2301
$\mathcal{D}_b^N$	2.6281
Dose 3	0.3348 $\mu\text{mol/hr}$
Parameter	Value
k_{bile}	7.2836 hr^{-1}
V_{max}	$215.8068 \mu\text{mol/L/hr}$
k_m	$0.0019 \mu\text{mol/L}$
P_{GEN}	1.1481 L/hr
P_{CON}	0.0037 L/hr
$\mathcal{D}_l^N$	1.5336×10^3
$\mathcal{D}_b^N$	2.0193
Dose 4	0.5292 $\mu\text{mol/hr}$
Parameter	Value
k_{bile}	9.1104 hr^{-1}
V_{max}	$4.0332 \times 10^{14} \mu\text{mol/L/hr}$
k_m	$5.4588 \times 10^9 \mu\text{mol/L}$
P_{GEN}	10.2633 L/hr
P_{CON}	0.013 L/hr
$\mathcal{D}_l^N$	4.1285×10^3
$\mathcal{D}_b^N$	0.149

5.6.3 Parameter Comparison - Single Dose

In this section we examine the parameters and the differences between doses in Table 5.1.

Figure 5.11 depicts the simulation results for Dose 4 using several values of V_{max} and k_m . Figure 5.11B shows that the original value of $V_{max} = 4.0332 \times 10^{14}$ can easily be lowered to a value of 10^4 without any considerable loss of goodness of fit, noting that “goodness of fit” is defined in this case as merely an eyeball examination. However, if V_{max} is lowered enough, goodness of fit is considerably lost (Figure 5.11C). Noting that the value of V_{max} tends to increase with increasing doses of genistein, one may be compelled to think that V_{max} is a dose- or concentration-dependent parameter. However, V_{max} represents the largest rate at which the liver can metabolize genistein. From a biological standpoint, it is unlikely that genistein is able to increase the saturation point of its own metabolism. A more reasonable explanation is that, although genistein metabolism is saturable (as experimental data suggest), its saturation point is relatively high. Therefore, as the doses become smaller, the value of V_{max} necessary to accurately predict the data becomes smaller. However, if we raise the value of V_{max} at the lower doses, the data become overpredicted by the model. This suggests that other parameters are affected by this change, and perhaps these other parameters are concentration dependent.

Figure 5.12 compares the concentration of conjugates in the bile extension tube between Dose 1 and Dose 4. The difference in the dynamics seen in Figure 5.12 is nontrivial, and may suggest a dose- or concentration-dependence in the parameter $\mathcal{D}_b^N$. If this is the case, the biological meaning is not completely clear. It seems unlikely that genistein could affect the viscosity of blood serum, however it would seem unreasonable to rule it out. A more realistic possibility is that genistein causes a slowdown in bile flow, in which case we may see a lower concentration of conjugates toward the end of the extension tube for higher concentrations of genistein over time. That is exactly what Figure 5.12 depicts. Another explanation is that setting $\mathcal{D}_b^N = \mathcal{D}_t^N$ (as described in the previous section) causes this effect.

In fact, another effect noticed by that equality is the drop in the simulated biliary excretion rate in the first three doses. This is caused by the value of the dispersion number for the bile (and, by equality, the extension tube) being high enough to force a decrease in the rate of excretion when the concentration gradient (with respect to space) begins to decrease. This can be seen in the equation (3.26), where the rate is dependent on the concentration of conjugates and the gradient of the concentration in the extension tube. This decrease may not be biologically exact, and lowering the value of $\mathcal{D}_t^N$ would rid the model of this characteristic by allowing the concentration term to dominate the equation.

However, the fact remains that the model suggests there is a clear concentration dependent change in the parameter $\mathcal{D}_b^N$ or $\mathcal{D}_t^N$. But since $\mathcal{D}_t^N$ is the dispersion number for the extension tube, where only transport is occurring, the more likely candidate for dependence is $\mathcal{D}_b^N$. More discussion on this topic is found in Section 6.2.

Note the considerable difference in magnitude between $\mathcal{D}_l^N$ and $\mathcal{D}_b^N$ for all four parameter sets. Recall that $\mathcal{D}_l^N$ is the dispersion number for blood. Experimentally, it is widely accepted that chemicals equilibrate very quickly in systemic blood. In fact, in traditional PBPK modeling the common practice is to assume a pseudo-steady state for the blood ODE and solve for the state variable algebraically. Therefore, the rather large values found in all four parameter sets is not considered biologically unrealistic.

Notice the values for the parameter k_{bile} . The pattern seen suggests that at low doses, k_{bile} must remain fairly small in order to allow the model to fit the data. However, at large doses, k_{bile} becomes larger. Although the larger values can be lowered slightly without losing significant accuracy in the model simulations, there still remains a clear pattern of the value of k_{bile} increasing as the dose decreases. Again, this observation suggests another possibility of concentration dependence in certain parameters of the model.

Lastly, note the considerably high value of $k_m = 5.4588 \times 10^9$ in the parameter set for Dose 4. This value does not coincide with the estimated values for the other three doses. However, as seen in Figure 5.11A, this high value can be lowered to a value of $k_m = 10^{-10}$

without losing any considerable accuracy in the simulation. This is not alarming, since our inverse problem is an unconstrained inverse problem and hence, the parameter values are free to increase without bound. So, although the value of $k_m = 5.4588 \times 10^9$ may be (at least) locally optimal, clearly a value as low as $k_m = 10^{-10}$ still yields accuracy. Noting that, we see that the parameters k_m , P_{GEN} and P_{CON} do not vary greatly among parameter sets. This suggests that these parameters are probably not dose- or concentration-dependent.

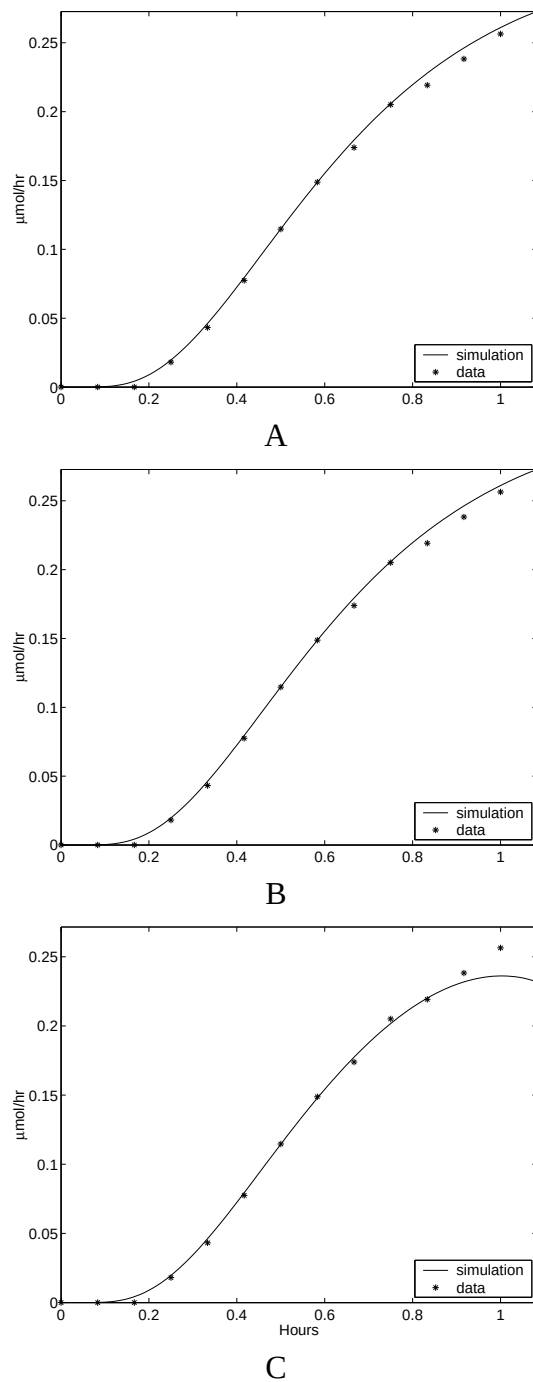


Figure 5.11: (Dose 4) A: Biliary excretion rate of conjugates with $k_m = 10^{-10}$. B: Biliary excretion rate of conjugates with $V_{max} = 10^4$ and $k_m = 10^{-6}$. C: Biliary excretion rate of conjugates with $V_{max} = 10^3$ and $k_m = 10^{-6}$.

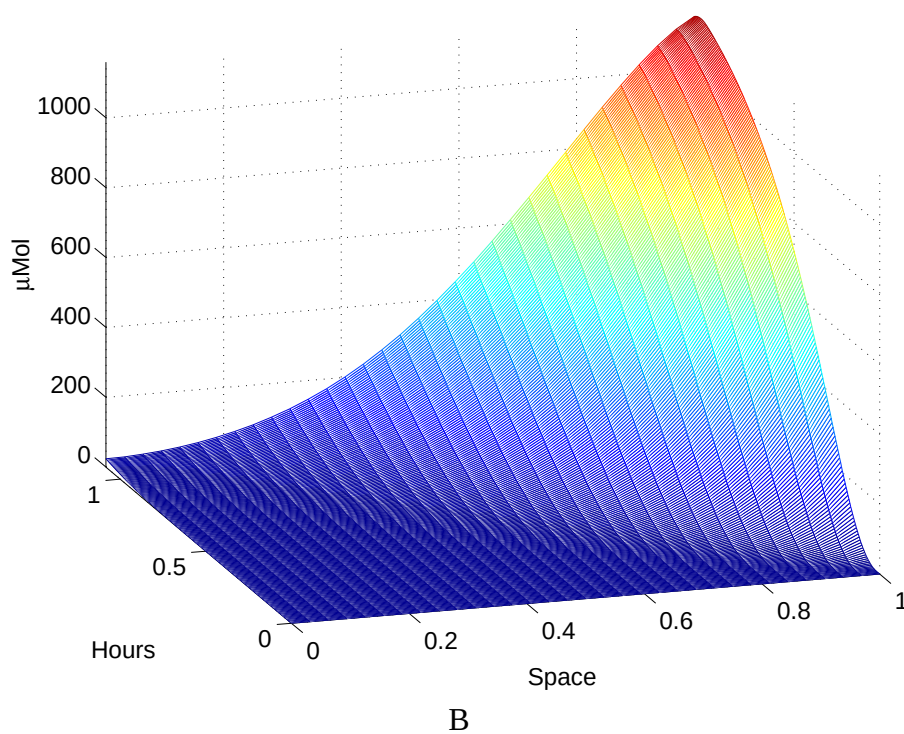
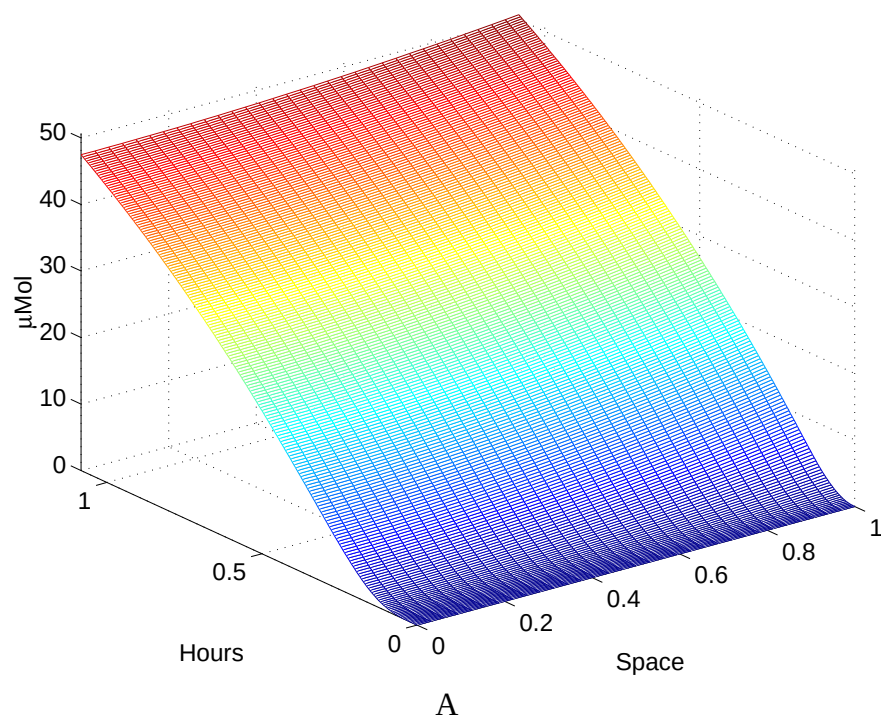


Figure 5.12: A: Concentration of genistein glucuronide in the bile extension tube using Dose 1. B: Concentration of genistein glucuronide in the bile extension tube using Dose 4.

5.6.4 Multiple Dose

In this section we present the preliminary results of the parameter estimation for the 4-dose simulations. In this case we generate a cost by simulating one dose after another, cumulatively adding the cost of each data comparison. These results are preliminary in that, as discussed in Section 6.2, numerous augmentations can be made to the model to improve the multi-dose performance. Unfortunately, most (if not all) of the augmentations introduce substantial nonlinearities to the model, which create difficulties, both numerically and theoretically. Therefore, we did not pursue those courses of action for this dissertation, but they are ideal directions for the future.

Figures 5.13A and 5.14A depict the biliary excretion rates. The simulations demonstrate the model's ability to reproduce the relatively smooth curves suggested by the experimental data. This qualitative behavior of the model is undoubtedly a crucial feature in accurately reproducing the data. This is seen in the single-dose simulations as well. Figures 5.15A and 5.15B show a comparison between model predictions using the single dose parameters and the multi-dose parameters. Values for the parameters obtained are listed in Table 5.2. Although several parameters have the characteristic high values as seen in the single dose parameter sets, these high values are able to be lowered to more reasonable levels, as in the case of the single-dose estimations.

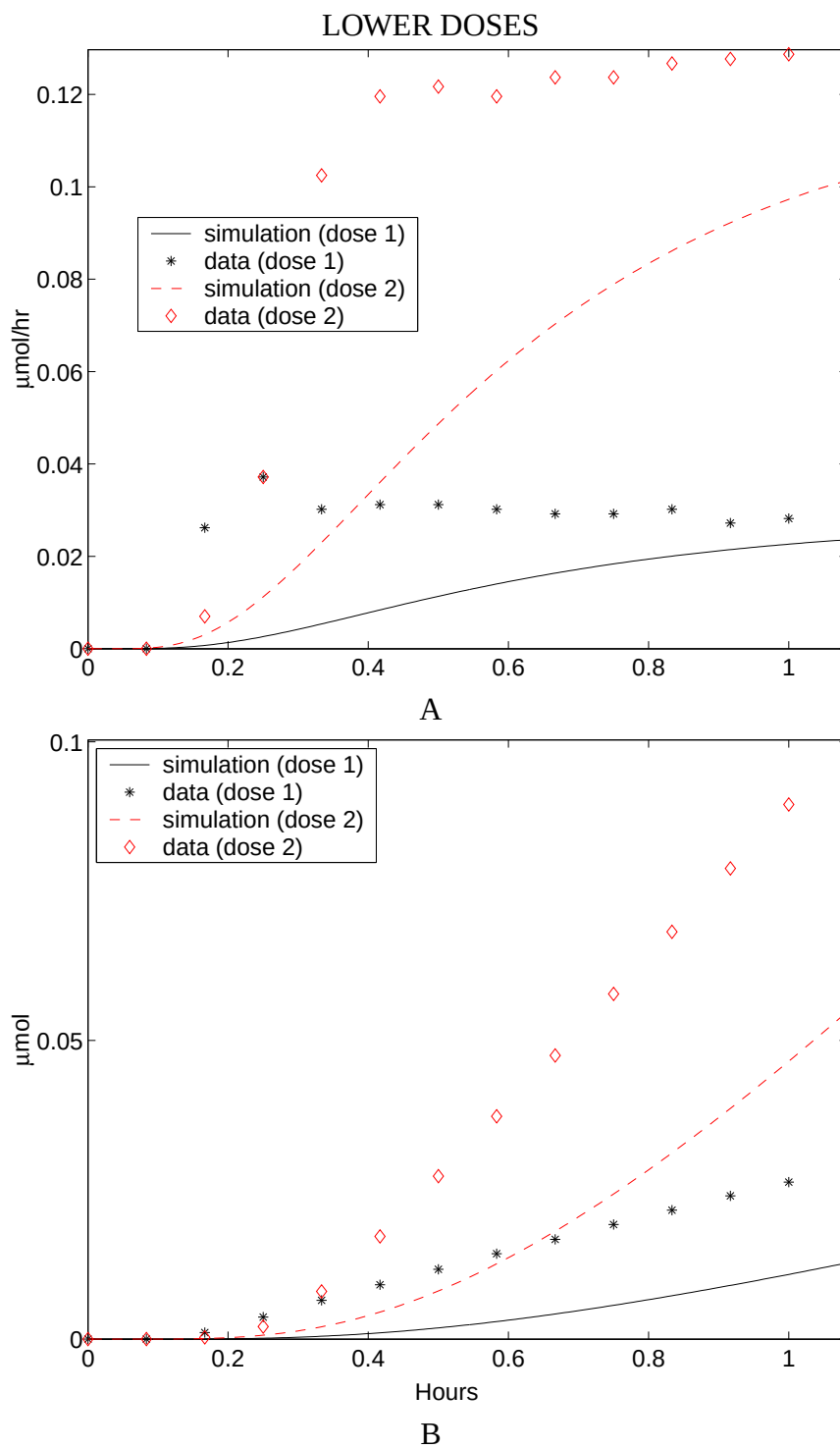


Figure 5.13: (Lower Doses) A: Biliary excretion rate of conjugates after a 1 hour dose of pure genistein, Doses 1 and 2. B: Cumulative biliary excretion of conjugates after a 1 hour dose of pure genistein, Doses 1 and 2.

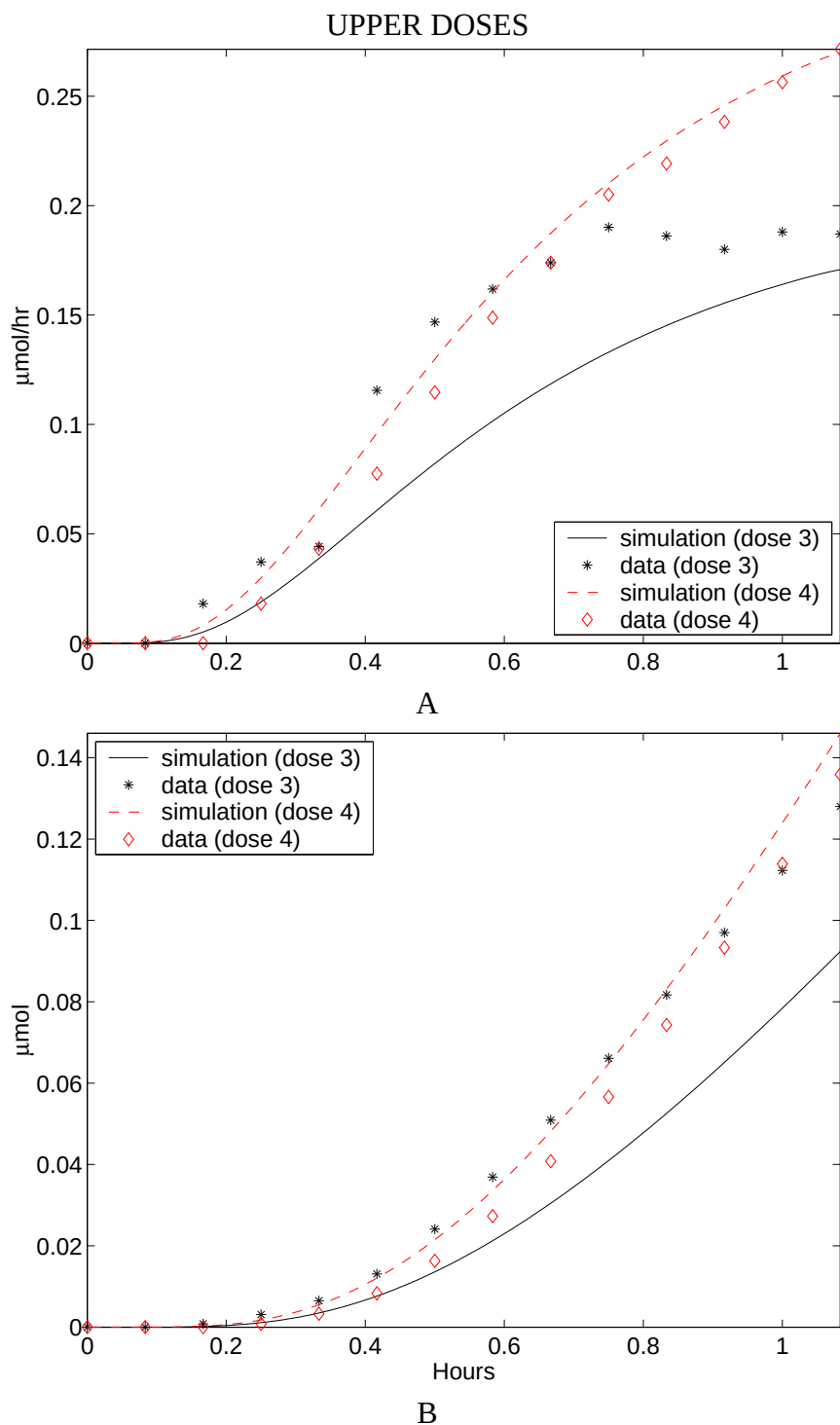


Figure 5.14: (Upper Doses) A: Biliary excretion rate of conjugates after a 1 hour dose of pure genistein, Doses 3 and 4. B: Cumulative biliary excretion of conjugates after a 1 hour dose of pure genistein, Doses 3 and 4.

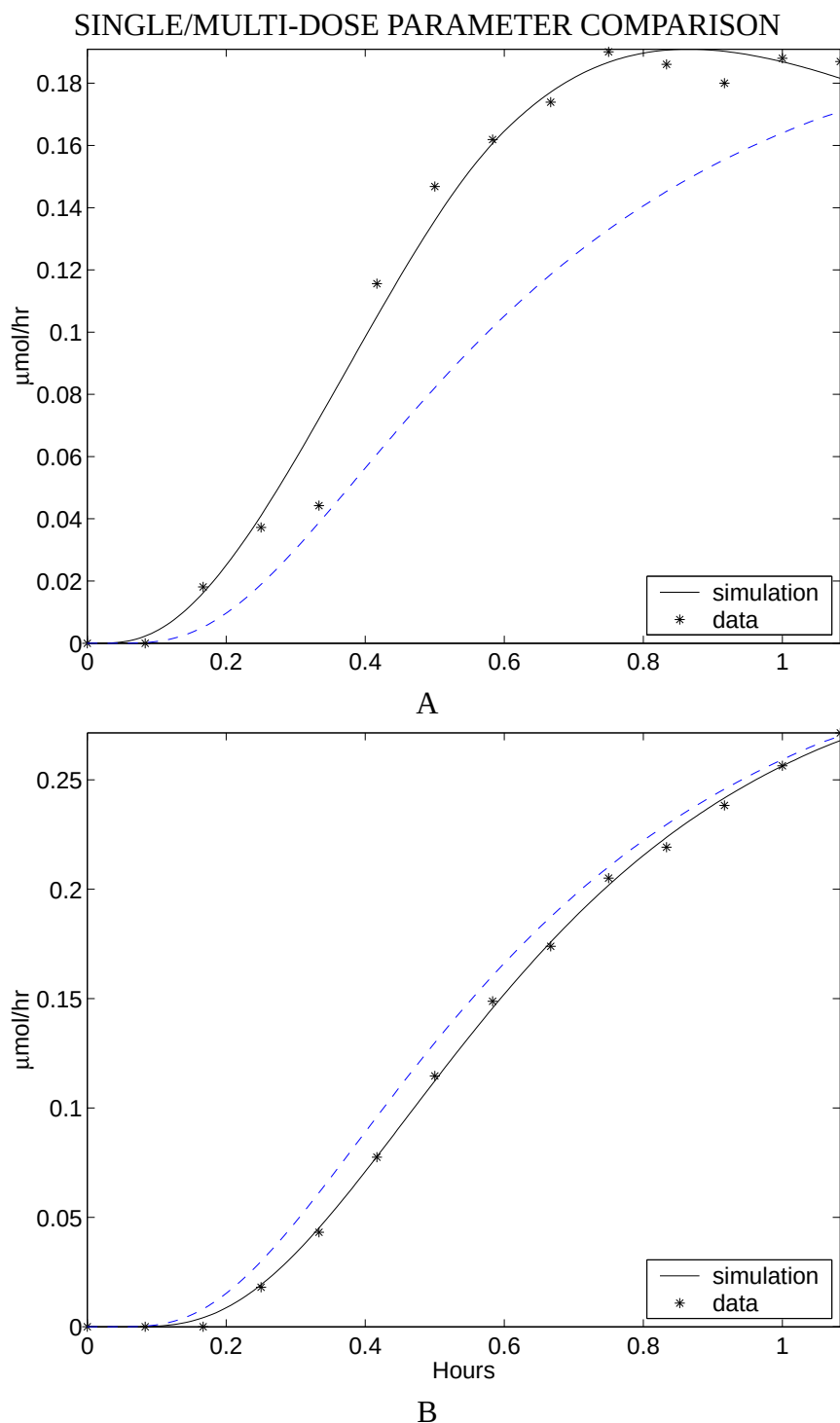


Figure 5.15: A: Biliary excretion rate of conjugates after a 1 hour dose of pure genistein, where the solid line is the model simulation using the parameter set from Dose 3 and the dashed line is the model simulation using the multi-dose parameter set. B: Biliary excretion rate of conjugates after a 1 hour dose of pure genistein, where the solid line is the model simulation using the parameter set from Dose 4 and the dashed line is the model simulation using the multi-dose parameter set.

Table 5.2: Resulting values from parameter estimation for 4-dose optimization.

Parameter	Value
k_{bile}	13.366 hr^{-1}
V_{max}	$8.4307 \times 10^{13} \mu\text{mol}/\text{L}/\text{hr}$
k_m	$2.752 \times 10^7 \mu\text{mol}/\text{L}$
P_{GEN}	$9.6224 \text{ L}/\text{hr}$
P_{CON}	$0.0165 \text{ L}/\text{hr}$
$\mathcal{D}_l^N$	5.4864×10^5
$\mathcal{D}_b^N$	0.1934

Chapter 6

Conclusions and Future Directions

In this dissertation, two models were developed to simulate the distribution and metabolism of the phytoestrogen genistein in rats. Genistein has been shown to induce an acute, dose-dependent lag in biliary excretion, and standard PBPK modeling techniques are unable to reproduce this phenomenon. With this in mind, we developed the two models discussed in this dissertation. The DDE model was developed from a perspective of plug flow and the dispersion model is based on fluid-flow dynamics, where the concentrations of the chemical are distributed with respect to space. Each of these models have been useful on a variety of levels in understanding genistein pharmacokinetics and each model has several exciting directions for future research.

6.1 DDE Model

6.1.1 Concluding Remarks

Though the delay observed in the data is present in the DDE model simulations, the qualitative dynamics of the delay are clearly not captured with great accuracy. The failure to reproduce these dynamics limits the potential ability of the model to fit biliary excretion rate data. A possible solution is using a different numerical optimization method and/or a

different delay function. Another solution is developing a high-fidelity model with more biological consideration for biliary excretion, which was done in the later chapters of this document. However, such a model requires numerical simulation of PDEs, which is computationally much more complex. So it seemed reasonable to first explore a simpler approximation, using delay-differential equations as described in Chapter 2, to determine if that approach would be sufficient. We have shown that such a model can be successfully simulated numerically and that incorporation of a delay gives a statistically significant improvement over a model lacking that delay.

6.1.2 Future of the DDE Model

Since there are two urinary elimination rate constants in the DDE model and no urinary elimination data to compare under the experimental conditions described in Section 2.1, the optimized parameters obtained are not unique. In Table 2.4, the estimate for the rate constant governing the urinary elimination of genistein conjugates (k_{urine}^{con}) is essentially zero. This is not suggested by experimental data (21). However, if we bound k_{urine}^{con} away from zero, by adjusting the rate constant for urinary elimination of pure genistein, we can obtain similar data fits to what is seen in Figures 2.4 and 2.5. Hence, even local uniqueness of our parameter set is not possible. If urinary elimination data obtained under the same experimental conditions were available, using those data to estimate the unknown kinetic parameters governing the urinary elimination of the parent compound, metabolites, or both would hopefully yield identifiability of the parameters. For a thorough discussion on identifiability and consistency of estimators we refer the interested reader to Banks and Kunisch (28).

As we mentioned, better performance of the DDE model could possibly be obtained by rederiving the delay function based on another approach. This would require re-optimization

of the parameters, where using a different numerical optimization routine and/or cost function may result in better performance as well, such as a global optimization algorithm.

The estimated parameters and system of DDE defining our model can be incorporated in a full PBPK model capable of predicting a wide range of genistein dosimetry under various experimental conditions. If the urinary elimination data mentioned above are obtained, we can use the full PBPK model to estimate the urinary elimination parameters. Ultimately, this approach can blueprint the modeling of other chemicals that undergo enterohepatic circulation.

6.2 Dispersion Model

6.2.1 Concluding Remarks

Rather than continuing development of the DDE model, which would include the ideas described in Section 6.1.2, we pursued an approach that would inevitably yield a more physiological foundation. The dispersion modeling techniques described in (43) and enhanced in (44) were the building blocks for our dispersion model. Our modeling efforts are advanced in that our dispersion model takes the approach used in (43) and adapted in (44), and uses these techniques to model a fully functioning liver, from uptake and metabolism to excretion. Our objective in this research effort was to develop a fully functioning model for the liver that simulates the distribution, metabolism and excretion of chemicals, and is able to accommodate spatial variations in biologically- and physiologically-based parameters. These spatial variations may include enzyme activity, hepatic cell permeability, cellular transport inhibition, etc. Such a model can be used as a template for a class of chemicals that undergo biliary excretion.

Our modeling efforts extended to a preliminary data fitting to genistein biliary excretion via parameter estimation with the goal of achieving a similar qualitative behavior of the

model simulations that is present in the experimental data. Although it is clear that model refinement is necessary (as expected), as seen from the plots in Chapter 5, this model has great promise and flexibility for an accurate description of the effect of genistein on biliary excretion. The dispersion model captures the qualitative behavior of the experimental data well, which is seen not only in the multi-dose simulations but especially in the single dose simulations. With added features of this model (see Section 6.2.2), not only does it have the potential to achieve accurate data fits, but it may be possible to gain insight into the underlying biological mechanism causing the biliary excretion lag, depending on which augmentations yield the best results. For example, if creating a dose or genistein concentration dependent parameter to govern the flow of bile in the bile duct results in a considerable increase in the model's ability to simulate the experimental data, this would point researchers in the direction of experimentally investigating genistein's effect on bile flow. To this point, the cause of this biliary excretion delay remains unknown. Such as insight could have a considerable impact on the experimental study of genistein. Clearly this model can also be used as a template for other chemicals metabolized in the liver and are excreted in the bile, and can be linked with pharmacodynamic models to aid in obtaining a dose-response assessment of toxic compounds.

6.2.2 Future of the Dispersion Model

Although partial differential equations and delay-differential equations are intimately related, we did not research the connection between the two models developed here. In order to better understand the behavior of the DDE model, this may be an advantageous direction to take.

In our preliminary inverse problem for the dispersion model, we used a simple least squares cost function. The data sets involved are correlated, and therefore the maximum

likelihood function used in the DDE model inverse problem, in theory, cannot be guaranteed to perform. However, we found that in spite of the correlated data sets, the MLF performed better than any other cost function used. Therefore, it is reasonable to presume the same may be true in the case of the dispersion model inverse problem.

As mentioned in Section 5.6, we found that using the experimental values for the free fractions of genistein did not allow for enough free genistein to be uptaken, metabolized and excreted in the bile to even remotely match the experimental data. In his paper (38), Mendell suggests that using the free fractions alone may not be biologically accurate in modeling the uptake of chemicals into cells. If the dissociation constant between the chemical and the blood protein is large enough, the chemical may, in actuality, be more available for uptake by a cell than the free fraction may suggest. In the dispersion model, using the dissociation constants (if experimentally known) to determine what values to use for the free fractions in the model may enhance the performance of the model. If the dissociation constants are not experimentally known, the values of the free fractions could, in theory, be estimated from the experimental data, however the reliability of accuracy may be an issue without a more diverse experimental data set.

Currently in the dispersion model, the volumetric flow rate of bile is constant. This is a gross simplification, since the bile duct canaliculi have a clear, physical boundary (where each canaliculi begin). The flow rate will likely increase, on average, as the bile travels down the canaliculi toward the bile duct, and ultimately in the main bile duct and into the gut lumen. We wish to make the volumetric flow rate of bile a function of space. However, a problem arises in that this will create a nonlinearity in the model equations. Nevertheless, this augmentation to the model would yield greater biological relevance.

The preliminary, multiple-dose, dispersion model simulations seem to mainly suggest two important issues. First, as noted in Section 5.6, the results may suggest that further saturable terms should be added to the model. Second, it is clear that the state-dependence of the delay is not captured. This was not expected, since nowhere in the model did we

include a state-dependent parameter or variable. However, the model is able to reproduce the data with multiple parameter sets with great accuracy, as seen in the single-dose simulations. But in order to find a single set of parameters that will allow the model to fit the data over all four doses, model augmentation will most likely be needed. Some possibilities include making the volumetric flow rate of bile a function of the concentration of genistein conjugates, as discussed above. Other possibilities range from a dose or concentration dependent inhibition of active genistein transport in the hepatocytes to concentration dependent liver sinusoid diameter constriction. Inevitably some possibilities are more or less biologically plausible, and in that lies a perfect example of the importance of cross disciplinary collaboration between mathematicians and experimental biologists.

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