Using an In Vitro-In Vivo Correlation for the ‘Bioequivalence by Design’ Development of an Immediate Release Carbamazepine Product

Douglas Steinbach

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USING AN *IN VITRO-IN VIVO* CORRELATION FOR THE
‘BIOEQUIVALENCE BY DESIGN’ DEVELOPMENT
OF AN IMMEDIATE RELEASE CARBAMAZEPINE PRODUCT

A Dissertation
Submitted to the Graduate School of Pharmaceutical Sciences

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In partial fulfillment of the requirements for
the degree of Doctor of Philosophy

By
Douglas G. Steinbach

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USING AN IN VITRO-IN VIVO CORRELATION FOR THE ‘BIOEQUIVALENCE BY DESIGN’ DEVELOPMENT OF AN IMMEDIATE RELEASE CARBAMAZEPINE PRODUCT

By

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ABSTRACT

USING AN IN VITRO-IN VIVO CORRELATION FOR THE
‘BIOEQUIVALENCE BY DESIGN’ DEVELOPMENT OF AN IMMEDIATE RELEASE
CARBAMAZEPINE PRODUCT

By

Douglas G. Steinbach

December 2018

Dissertation supervised by James K. Drennen, III, Ph.D.

The quality of a drug product may be characterized by the consistency with which its indicated clinical effect, and safety profile, is experienced by the patient. The concept that such quality should be built into a product is at the core of the United States Food and Drug Administration’s (FDA) quality by design (QbD) initiative. This vision for pharmaceutical product development emphasizes the risk-based identification of critical quality attributes (CQA) which summarize a product’s performance, the efficient refinement of critical product/process parameters (CPP) that can affect such attributes, and the systematic development of CPP limits, which assure appropriate performance of CQAs. For a tableted drug product, a cornerstone CQA is dissolution.
Often, a formulation and/or manufacturing process can change during a patient’s course of treatment, potentially jeopardizing the consistent performance of the drug product. Regulatory agencies typically require that sponsors demonstrate how the generic/post-change product is bioequivalent to the reference/pre-change product. While in vivo clinical trials are one strategy for demonstrating this, sponsors typically prefer in vitro dissolution tests as an alternative. During these in vitro test, the F2 metric is commonly used to assess dissolution profile similarity. This work sought to compare the F2 method with an alternative method, on the basis of errors in bioequivalence. The alternative method was based on the use of a physiologically based in vitro-in vivo correlation (PB-IVIVC) model that had been nested within a clinical trial simulation platform. The PB-IVIVC method provides a direct link from dissolution performance to clinical performance. Thus, when it is used to refine a CPP-vs-dissolution response surface, based on the performance of reference product, the assurance of clinically defined bioequivalence can be directly built into a model tablet system.

The model drug product for this work was an immediate release carbamazepine tablet. Carbamazepine was selected as the model active pharmaceutical ingredient because it has a narrow therapeutic index and is designated as a class II compound (i.e. high permeability, low solubility) according to the FDA’s biopharmaceutics classification system (BCS). As such, this compound is identified within the FDA’s scale-up and post-approval change guidance as possessing elevated risk for biononequivalence when changes are imposed to its formulation and/or manufacturing process.

After gathering single dose in vitro-in vivo data from the literature, the construction of the PB-IVIVC began according to a two step process. Here, the respective parameters for the rate and extent of each product’s absorption were calculated using classical pharmacokinetic modeling and then regressed against each product’s rate and extent of dissolution. Next, the classically defined clearance parameter was replaced using a physiologically based clearance model. This allowed routinely available population
pharmacokinetic data to be combined with first principles of human physiology, for the mechanistic prediction of intersubject variability via correlated Monte Carlo simulations. This PB-IVIVC was then used to not only define the CPP ranges for the model carbamazepine tablet system that would directly provide for bioequivalent performance, but to perform a post hoc assessment of the CPP ranges conferred by the use of F2 statistic. Ultimately, the results showed that when the product’s CPPs were refined using the F2 statistic the was higher risk of biononequivalence was higher when compared to a product that had been refined using the PB-IVIVC. It is intended that this work support the movement of product/process optimization practices away from methods that result in rigid factors of unknown clinical significance, and towards those that are focused on efficiently achieving specific clinical objectives.
DEDICATION

This dissertation is dedicated to my wife Christal, my parents, Richard and Judith, my grandparents, Robert Steinbach, Anne Steinbach, Mary Mueller, and Roth Mueller, my sister, Alison, wife’s family, Chris, Celia, and Clay Vuich, and most especially God.
I have pursued my PhD. in Pittsburgh PA, the “Steel City.” While many of the mills which gave rise to the city’s nickname have closed, an aspect of their daily activities still remains at Duquesne University’s Graduate School of Pharmaceutical Sciences. Many parallels can be drawn between the work hardening of steel and the pursuit of a PhD. The process of work hardening a metal is defined as strengthening through plastic deformation. Steel smiths historically have performed this by exposing the material to great heat and repeated hammer blows on an anvil. This imparts dislocations into the fundamental structure of the metal, wherein the accumulation of what would seem to be imperfections results in an increased strength of the material. Just as the strengthening of steel requires repeated effort, a skilled smith, and the support from stresses, achieving a PhD is the result of a considerable combined effort performed by numerous supporters of the candidate. I would like to take this opportunity to thank those who, during this chapter of my life, have left an indelible mark on my heart and mind.

I would like to thank Dr. James K. Drennen for all of the support he has shared with me over the years. Since working in his lab as an undergraduate researcher, Dr. Drennen has unwaveringly believed in my potential as a developing scientist. From when I first met him as a prospective pharmacy student at seventeen years old to now, he has always been an architype of poise and technical expertise. I am honored to have been associated with his professional stature and imprimatur of class. I will carry his lessons with me for the rest of my life and am grateful for his trust and guidance.

My experiences and education were also greatly shaped by Dr. Carl Anderson. Although he is identified in this document as a committee member, he indeed performed as a second adviser
to me. Despite his many appointments, he has always been a source of advice and counsel. His technical acumen and openness has always pushed me to sharpen my skills and constantly strive to be the best scientist I can be.

I would also like to thank the other committee members, Dr. Ira Buckner, Dr. Frank D’Amico, Dr. Robert Guttendorf, and Dr. David Good. The willingness of these scientists and educators to share with me their time and support has been invaluable. I am grateful to these members for their openness and the insights which have helped to shaped not only this document but my skills as a scientist.

I would like to thank the faculty and students of Duquesne University’s Graduate School of Pharmaceutical Sciences, most notably my B11 colleagues. The environment I have worked in for the past 5 years has pushed me scientifically and emotionally to be a better teammate. I will always be grateful for the lessons they have taught me, and the friendships I have made. I would specifically like to thank past and current graduate colleagues: Dr. Robert Bondi, Dr. Steven M. Short, Dr. Zhenqi (Pete) Shi, Dr. Brian M. Zacour, Dr. Benoît Igne, Jeff Katz, Sameer Talwar, Ryanne Palermo, Shikhar Mohan, Anik Alamm, Nayeem Hossain, Yi Li, Hanzhou Feng, Natasha Velez, Suyang Wu, and Adam Rish.

I would like to thank Bristol-Myers Squibb for selecting me as a research fellow and their support of this work. The interactions I have had with the company have been rewarding and enriching. The scientists of Bristol-Myers Squibb have stood as an example of professional expertise and innovative excellence.

I would most certainly not have had the opportunities that I see before me if it were not for my parents, Richard and Judith. In addition to the gift of life, they have provided me with their gift of unceasing love, support, and inspiration. For my entire life they have sacrificed and worked
for a better life for my sister, Alison, and I. Their passion for their family knows no bounds and their commitment to the field of global healthcare has been an unyielding source of inspiration to me for as long as I can remember. I can only hope and pray that I have the fortitude to display their example strength, discipline, and love.

Words cannot express the gratitude I feel towards my wife, Christal. I simply would not have made it through graduate school were it not for her steadfast love and support. Even in the years before we married, she, and her entire family, supported my PhD ambitions. They did this knowing the sacrifices it would demand. Her mother Celia, father Chris, and brother Clay have, for the past 10 years taken me in as if I were their own son and brother. The motivating love of Christal and her extended family has been an indescribable source of inspiration. She has been at the vanguard of all my life’s challenges and struggles. She has been the light at the end of every long day, conference trip, and tight deadline. As her name implies, she is as beautiful and strong as a diamond. She has been a fresh perspective and an astute advisor throughout my life. Simply stated, she is the marrow in my bones and, were not for her, I would be a shell of the man I am today. Everything I work towards is for her and the life I hope to provide for our family.

Finally, I thank God for his mercy and blessings, I hope use them to the best of my abilities.
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<tr>
<td>ADME</td>
<td>Absorption, Distribution, Metabolism, and Excretion</td>
</tr>
<tr>
<td>ANDA</td>
<td>Abbreviated New Drug Application</td>
</tr>
<tr>
<td>API</td>
<td>Active Pharmaceutical Ingredient</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>BCS</td>
<td>Biopharmaceutics Classification System</td>
</tr>
<tr>
<td>CFR</td>
<td>Code of Federal Regulations</td>
</tr>
<tr>
<td>CGMPs</td>
<td>Current Good Manufacturing Practices</td>
</tr>
<tr>
<td>CL</td>
<td>Clearance</td>
</tr>
<tr>
<td>$CL_{int}$</td>
<td>Intrinsict Clearance</td>
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<tr>
<td>Cmax</td>
<td>Maximum plasma concentration</td>
</tr>
<tr>
<td>CQAs</td>
<td>Critical Quality Attributes</td>
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<tr>
<td>Css</td>
<td>Average plasma concentration at steady state</td>
</tr>
<tr>
<td>$C_{ss,max}$</td>
<td>Maximum plasma concentration at steady state</td>
</tr>
<tr>
<td>$C_{ss,min}$</td>
<td>Minimum plasma concentration at steady state</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>$F$</td>
<td>Bioavailability (a.k.a. fraction of drug absorbed)</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>$f_u$</td>
<td>Fraction Unbound</td>
</tr>
<tr>
<td>ICH</td>
<td>International Conference on Harmonisation</td>
</tr>
<tr>
<td>IVIVC</td>
<td><em>In Vitro-In Vivo</em> Correlation</td>
</tr>
<tr>
<td>$k_a$</td>
<td>Absorption rate constant</td>
</tr>
<tr>
<td>$K_p$</td>
<td>Partition Coefficient</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>LogP</td>
<td>The Log transform of the octonal-water partition coefficient</td>
</tr>
<tr>
<td>MCC</td>
<td>Microcrystalline Cellulose</td>
</tr>
<tr>
<td>MCS</td>
<td>Monte Carlo Simulation</td>
</tr>
<tr>
<td>NDA</td>
<td>New Drug Application</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
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<tr>
<td>PB-IVIVC</td>
<td>Physiologically Based In Vitro-In Vivo Correlation</td>
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<tr>
<td>PB-IVIVC-CTS</td>
<td>Physiologically Based In Vitro-In Vivo Correlation nested within a clinical trial simulation platform</td>
</tr>
<tr>
<td>PBPK</td>
<td>Physiologically Based Pharmacokinetic</td>
</tr>
<tr>
<td>PD</td>
<td>Pharmacodynamic</td>
</tr>
<tr>
<td>PDF</td>
<td>Probability Density Function</td>
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<td>PE</td>
<td>Prediction Error</td>
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<td>Pharmacokinetic</td>
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<tr>
<td>QbD</td>
<td>Quality by Design</td>
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<td>QTPP</td>
<td>Quality Target Product Profile</td>
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<td>RMSE</td>
<td>Root-Mean-Standard Error</td>
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<td>RMSEC</td>
<td>Root-Mean-Standard Error of Calibration</td>
</tr>
<tr>
<td>RMSECV</td>
<td>Root-Mean-Standard Error of Cross Validation</td>
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<tr>
<td>R&amp;D</td>
<td>Research and Development</td>
</tr>
<tr>
<td>SUPAC</td>
<td>Scale-Up and Postapproval Changes</td>
</tr>
<tr>
<td>Tmax</td>
<td>Time at which the maximum plasma concentration occurs</td>
</tr>
<tr>
<td>UGT</td>
<td>Uridine 5'-diphospho-glucuronosyltransferase</td>
</tr>
<tr>
<td>US</td>
<td>United States</td>
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<tr>
<td>USP</td>
<td>United States Pharmacopeia</td>
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Chapter 1. Introduction

1.1 Bioequivalence Assessment: Current Practice and Associated Issues

The first pharmaceutical regulations were born from society’s need for quality drug products. A concept common to most definitions of pharmaceutical quality involves meeting or exceeding patients’ needs. For individuals on established medication regimens, quality is largely defined in terms of consistency. The expectation is that the medication will perform consistently throughout the duration of therapy. Bioequivalence studies are used by sponsors to assure consistent product performance despite the occurrence of product variations (e.g. scale up, post approval changes [SUPAC], and therapeutic conversion to generic product). Drug products are considered bioequivalent by the Food and Drug Administration (FDA) when there is no statistical difference in the rate or extent of absorption between the comparators. In vivo and in vitro methods may be used to establish bioequivalence according to the Code of Federal Regulations (CFR) Title 21.

Generic drug applications typically rely on at least one in vivo clinical trial for bioequivalence as the cornerstone of their abbreviated new drug application. Such trials have often been considered the most rigorous means of assessing bioequivalence. The statistical assessment of bioequivalence requires that inter-subject variability be accounted for during the calculation of the critical values of a hypothesis test or confidence interval. Traditionally, inter-subject variability is estimated within the treatment periods of conventional bioequivalence studies. These studies typically rely on a small cohort (n ∼ 24) of young healthy patient volunteers who generally undergo a single dose of the test and reference drug products. The reliability of inter-subject variability estimates
can be increased by i) using clinical trials in the actual patient population, and ii) performing them on the scale of trials conducted during the second and third clinical development phases. The time and overhead demands that such large scale trials place on pharmaceutical sponsors, however, is substantial. This reality, along with the ethical limitations associated with the performance of in vivo trials in a clinical population for the sole purpose of quality assessment results in the avoidance of such measures during the assessment of bioequivalence. However, this necessary compromise often results in the tenuous assertion that the inter-subject variance estimates defined by the pharmacokinetic conditions expressed in the healthy, single-dosed bioequivalence study populations are representative of those PK conditions expressed by the actual patient population during day-to-day clinical practice. The reliance upon this assertion can lead to erroneous inferences being drawn in in vivo trials aimed at the assessment of consistent product performance. Other factors that limit the reliability of bioequivalence testing include: the ethical restrictions that limit testable strengths, the study design limitation associated with narrow therapeutic index drugs, the often subjective nature of efficacy/toxicity, and the appropriateness of washout periods.34

Brand drug sponsors encounter activities related to the assurance of bioequivalence during changes to a product or manufacturing process. Unlike generic drug sponsors, the sponsors of new drugs typically avoid the clinical demonstration of pre-vs-post change bioequivalence. This avoidance is due, in part, to the minimal amount of information yielded from traditional in vivo bioequivalence trials (i.e., pass or fail), the absence of long-term safety data, and the large amount of resources required to run a clinical trial, and the expenses due to downtime. In vitro approaches are more expedient and resource sparing. Here, the
similarity of dissolution profiles is typically evaluated using an empirical USP window
criteria, or in the absence thereof, the F2 statistic. A limitation of this approach is that
inter-subject variability is no longer accounted for by these quality measurements. Such
methods do not provide for the continuous movement toward a risk-based and science-based
approach of pharmaceutical quality as articulated in the Pharmaceutical CGMPs for the 21st
Century and echoed by Dr. Janet Woodcock (the FDA’s acting director for the Center for
Drug Evaluation and Research). In a perspective article, Woodcock identified the primary
cause of most pharmaceutical quality issues to be a weakly defined link between critical
quality attribute (CQA) measurements and clinical performance citing dissolution based
biowavers as a specific example of where this can occur.

The concept that a strong link between the surrogates of in vivo performance and
clinical response is essential for the efficient assurance of quality is a foundational principle
this work. An example of the ramifications stemming from a weak link between a quality
measurement and clinical significance can be seen in the 2012 recall of 300mg extended
release generic product of Buproprion (trade name Wellbutrin XL). The generic sponsor
was attempting to bring to market two strengths of an extended release Buproprion product:
150 mg and 300mg. The 150mg generic drug product had a sufficiently wide therapeutic
plasma concentration window. Thus, it was approved for the market following a traditional
in vivo generic-vs-brand in bioequivalence trial. The 300mg dose however, was associated
with an increased risk of seizures, making clinical trials for bioequivalence prohibitive.
Therefore, after the 150mg was approved the FDA approved the 300mg form in 2006 based
on 1) the formulation of the 300mg generic product being proportionally equivalent to the
150 mg generic product, and 2) satisfactory compendial (USP) dissolution tests of the 300
mg product. Unfortunately, reports of increased side effects or reduced efficacy (subjectively defined) began soon after the generic entered the market. *In vivo* clinical trials were performed in 2012, showing a significant difference between the performance of brand and generic formulations, ultimately necessitating a product recall.

A literature review by Desmarais et al. suggests the Bupropion incident may not be isolated. The researchers examined the PubMed database to find works related to adverse events following the brand-to-generic switching of psychotropic medications. The work notably excluded pharmacokinetic studies conducted with healthy volunteers unless it contained data relevant to the clinical population. The researchers expressed significant concerns when the results indicated a large and growing body of literature reporting an increased risk of adverse events following switches. Similarly, several large scale (n > 1500) case-control studies have specifically found that changes in anticonvulsant drug formulation involving generics was a risk factor for emergency or hospital-level treatment of epilepsy (OR: 1.78 to 1.81). The combined evidence in the literature suggests that the origin of bioequivalence related failures is multifaceted, resulting, in part, from the practical limitations of conventional clinical methods and the absence of clinically relevant quality specifications.

While a strong link between the surrogates of *in vivo* performance and clinical response is critical for the market entry of generic drug products, it is also important for navigating the product/process changes that can occur over the lifecycle of a brand or generic product. The inevitable reality of change events is evident in the focus of recent ‘scale-up/post-approval change’ regulatory documents (e.g., see ICH Q12 draft guidance). A theme shared by this and other documents is an emphasis on how a sponsor’s possession of advanced product and
process knowledge can contribute to a reduction in the number of subsequent regulatory
submissions. The assurance of such flexibility comes from effectively incorporating
knowledge–driven tools and strategies within the initially approved *Pharmaceutical Quality
System*. Thus, product/process changes can be made with less need for extensive regulatory
oversight prior to implementation due to prior approval. According to the CFR, one of the
most informative and preferred methods by the FDA for the overall determination of
bioequivalence is one that correlates *in vitro* tests with human *in vivo* bioavailability data.
However, current IVIVC (*in vitro-in vivo* correlation) guidance documents and literature
focus on illustrating best practices for extended release products that are Biopharmaceutical
Classification System (BCS) class I and III. *Therefore, research on the development of
adequate surrogates for clinical performance (and the illustration of such practices) is
critical for immediate release products that are BCS class II.* This is especially critical for
therapies that are associated with a narrow therapeutic index.

The model drug in this dissertation is carbamazepine. The difference between the toxic
plasma concentrations and the minimum effective plasma concentration for this compound
is approximately three fold and as such is generally considered to have a ‘narrow therapeutic
index’. Additionally, the release performance of tableted formulations containing
carbamazepine as the active pharmaceutical ingredient (API) has been demonstrated to be
sensitive to product/process variability. These features, combined with the biochemical
properties which place it in the category of a BCS class II compound, makes a solid oral
dosage form of carbamazepine a high-risk product from a bioequivalence perspective.
Therefore, carbamazepine was considered a suitable model system for this project.
1.2. Hypothesis, Specific Aims, and Purpose

The intent of this work is to strengthen the understanding between quality assessment measurements and clinical performance according to quality by design (QbD) principles. This dissertation is based on the central hypothesis that,

for an immediate release carbamazepine product,

a physiologically-based IVIVC, coupled with a clinical trial simulation platform, provides a more accurate estimation of bioequivalence than the conventional $F_2$ similarity criterion during the comparative assessment of test vs. reference product performance.

Given this, four specific aims were performed for the assessment of the central hypothesis of this dissertation.

Specific Aim 1: Develop an IVIVC for an immediate release carbamazepine product using a classical pharmacokinetic model -●-

The ability of an IVIVC to predict an entire plasma concentration profile given a product’s dissolution performance enhances its meaningfulness. This is often achieved by constructing a hierarchical framework around a pharmacokinetic model. The goal of the first specific aim is to construct an IVIVC that can predict the entire plasma concentration profile by parameterizing its pharmacokinetic model using mean plasma concentration profiles of carbamazepine products in a normal healthy population. In using plasma data as the basis for pharmacokinetic parameterization, the approach is termed: top down pharmacokinetic modeling (i.e. pharmacokinetic parameters are solely informed by the plasma concentration profiles).
Specific Aim 2:
Account for population level pharmacokinetic variance within the pharmacokinetic model of the in vitro-in vivo correlation by appropriately integrating physiologically based inter-subject variability.

Equivalence testing requires that variance of the response be taken into consideration during the assessment. The identification of population-level pharmacokinetic variance typically relies on inefficient large-scale in vivo trials (i.e., top-down). In this work, pharmacokinetic variance will be accounted for from the bottom up by coupling the results of quantitative pharmacologic assays performed in vitro, with systemic physiologically based pharmacokinetic modeling and simulation.

Specific Aim 3:
Define a formulation for an immediate release carbamazepine tablet and model its change in dissolution performance as a function of defined critical attributes.

The mathematical equations used to model the immediate release dissolution behavior of a tablet can be manipulated to generate a host of profiles ranging from mono-exponential curves to sigmoidal shapes. Specific aim three ensures that the two bioequivalence assurance methods identified in the hypothesis are compared across a realistic spectrum of dissolution profiles.

Specific Aim 4:
Derive a distribution of clinical responses resulting from use of an F2-based equivalence criteria and compare with that derived from a physiologically based IVIVC on the basis of type I/II errors in bioequivalence.

The fourth specific aim of this work is the culmination of the first three specific aims. After defining the dissolution performance of the benchmark product, this specific aim uses the physiologically based IVIVC (developed from specific aims...
one and two) to process the interpolated F2 dissolution profiles (from specific aim three) and generate a distribution of clinical responses. This F2-distribution of clinical responses can then be compared against the reference distribution of clinical responses representing all of the responses that would be bioequivalent to the benchmark product. This will be performed through integration of the respective probably density functions.

While IVIVCs are of significant interest to regulatory agencies, and the subject of much research, their approval rate remains relatively low. This is despite the compounding costs to society and sponsors that stem from unintended delays to product development timelines due to a sponsor’s inability to assure that an observed difference in dissolution performance is inconsequential. The use of an IVIVC provides a path towards avoiding such delays as they provide a more informative interpretation of pivotal dissolution tests. The intention of this dissertation is to provide a streamlined framework to facilitate the development of a population level IVIVC via physiologically-based pharmacokinetic modeling and simulation for informing clinically-based decision making during product development.
1.3. Advancing Pharmaceutical Quality: *In vitro-In vivo Correlation Models*

According to an FDA guidance document entitled “Extended Release Oral Dosage Forms: Development, Evaluation, and Application of *In vitro*/*In vivo* Correlations” there are three *in vitro-in vivo* correlation (IVIVC) levels: A, B, and C. The differential criteria for these three levels have been generally accepted by numerous regulatory agencies and scientific communities. The levels differ primarily in the amount of detail required of both input and output data. The defining characteristic of a level A IVIVC is the ability to predict the entire *in vivo* time course from the *in vitro* data. A level B IVIVC uses the principles of statistical moment analysis (e.g., the output is defined in terms such as the mean residence time); it does not uniquely reflect the actual *in vivo* plasma level curve because a number of different *in vivo* curves will produce similar mean residence time values. Similarly, a Level C correlation does not reflect the complete shape of the plasma concentration time curve; instead, it draws a relationship between an amount dissolved at a given time and some summary pharmacokinetic parameter such as the area under the curve (AUC), maximum plasma concentration, or the time of the maximum plasma concentration.

Level A correlations are the most common type of correlation provided in NDAs submitted to the FDA according to guidance documents and other regulatory literature. This is because the level A IVIVC is considered the most informative. The specifics for establishing a level A correlation is left up to the sponsor. Examples cited in the guidance document make reference to convolution and deconvolution methods; however, numerous methods for creating a level A IVIVC exist. A
subsequent section will detail the areas of commonality and differentiation among these methods.

1.4. Theoretical Background for Project

The feasibility of this project is based on the founding principles of the Biopharmaceutical Classification System established by Amidon et al. and subsequently adopted by the FDA.\textsuperscript{14,15} This system is based on solubility and permeability and is used, in part, to guide expectations concerning the rate limiting step of absorption; i.e., dissolution rate limited, permeability limited, and solubility–permeability limited. Here, the systemic absorption of a high permeability, low solubility drug is considered dissolution rate limited. Amidon et al. justified such expectations by combining the Noyes-Whitney Equation and a first-order absorption rate model to describe the fraction of drug absorbed (see eq. 1.4.1).

\begin{equation}
F_a = 1 - \frac{k_{perm}}{k_{perm} - k_{diss}} \exp (-k_{diss}T_{abs}) \left[ \frac{k_{diss}}{k_{diss} - k_{perm}} \exp (-k_{perm}T_{abs}) \right] \end{equation}

<table>
<thead>
<tr>
<th>Eq. 1.4.1</th>
<th>$k_{perm}$ = permeation rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{diss}$ = dissolution rate</td>
<td></td>
</tr>
<tr>
<td>$T_{abs}$ = the transit time through the absorption site</td>
<td></td>
</tr>
</tbody>
</table>

The existence of a dissolution rate limited scenario was illustrated by calculating

\[ \lim_{k_{perm} \to \infty} \text{Eq. 1.4.2.} \]  

(see eq. 1.4.2 for result). If the rate of permeation into the systemic circulation is instantaneous relative to the rate of drug dissolution (i.e., solubilized drug is immediately absorbed and sink conditions are
effectively generated; \( k_{\text{perm}} \gg k_{\text{diss}} \), systemic absorption can be considered limited by the rate of dissolution.

**Eq. 1.4.2** \[
\lim_{k_{\text{perm}} \to \infty} [F_a] = [1 - \exp (-k_{\text{diss}} T_{\text{abs}})]
\]

In the seminal work, Amidon et al. describe expectations concerning possibility of successfully developing an *in vitro-in vivo* correlation for immediate release products based on solubility and permeability. Table 1.4.1 presents these expectations, which were subsequently adapted in guidance documents released by numerous regulatory agencies.

<table>
<thead>
<tr>
<th>Class</th>
<th>Solubility</th>
<th>Permeability</th>
<th>In vitro-In vivo Correlation Expectation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>High</td>
<td>High</td>
<td><em>In vitro-in vivo</em> correlation expected if dissolution rate is slower than gastric emptying rate, otherwise limited or no correlation</td>
</tr>
<tr>
<td>II</td>
<td>Low</td>
<td>High</td>
<td><em>In vitro-in vivo</em> correlation expected if <em>in vitro</em> dissolution rate is similar to <em>in vivo</em> dissolution rate, unless dose is very high</td>
</tr>
<tr>
<td>III</td>
<td>High</td>
<td>Low</td>
<td>Absorption (permeability) is rate determining and limited or else no <em>in vitro-in vivo</em> correlation with dissolution rate</td>
</tr>
<tr>
<td>IV</td>
<td>Low</td>
<td>Low</td>
<td>Limited or no <em>in vitro-in vivo</em> correlation is expected</td>
</tr>
</tbody>
</table>

A limited correlation means that the dissolution rate, while not the absolute rate controlling step, may be similar to the absorption rate; thus, the extent of correlation will depend on the relative rates.
Chapter 2. Literature Survey

The major areas of interest and implication for this work include \( i \) events that require the assessment of bioequivalence, \( ii \) the approaches used to assess bioequivalence, \( iii \) in vitro-in vivo correlation modeling, and \( iv \) physiologically based modeling. Therefore, this document will begin by reviewing these concepts using pharmaceutical science literature. Additionally, it is a reality of the pharmaceutical industry that drug product development activities are shaped by federal guidances and regulations. Therefore, this section also covers regulatory policies pertinent to the assessment of bioequivalence in the pharmaceutical industry.

2.1 The Assessment of Bioequivalence

2.1.1 Regulations, Guidances, and Examples

Title 21 of the Code of Federal Regulations (CFR) governs food and drugs within the United States by the Food and Drug Administration. Sections 200 and 300 include regulations pertaining to pharmaceuticals. The specific sections in the CFR which \( i \) identify the circumstances that require the assessment of bioequivalence, and \( ii \) discuss the methods for its evaluation, are identified in Table 2.1.1.1.
**Table 2.1.1.1. Relevant Sections of the Code of Federal Regulations Related to BA/BE**

<table>
<thead>
<tr>
<th>21CFR section</th>
<th>Type of provision/information</th>
</tr>
</thead>
<tbody>
<tr>
<td>314.94(a)(9)</td>
<td>Chemistry, manufacturing, and controls; permitted changes in inactive ingredients for parenteral, otic, opthalmic, and topical drug products</td>
</tr>
<tr>
<td>320.1</td>
<td>Definitions of bioavailability, pharmaceutical equivalents, pharmaceutical alternatives, and BE</td>
</tr>
<tr>
<td>320.21</td>
<td>Regulatory requirements related to submission of in vivo bioavailability and bioequivalence data</td>
</tr>
<tr>
<td>320.22</td>
<td>Criteria for waiver of evidence of in vivo bioavailability or bioequivalence data</td>
</tr>
<tr>
<td>320.23</td>
<td>Basis for measuring in vivo bioavailability or demonstrating bioequivalence</td>
</tr>
<tr>
<td>320.24</td>
<td>Types of evidence to measure bioavailability or establish bioequivalence</td>
</tr>
<tr>
<td>320.25</td>
<td>Guidelines for the conduct of an in vivo bioavailability study</td>
</tr>
<tr>
<td>320.26</td>
<td>Guidelines on the design of a single dose in vivo bioavailability or bioequivalence study</td>
</tr>
<tr>
<td>320.27</td>
<td>Guidelines on the design of a multiple-dose in vivo bioavailability study</td>
</tr>
<tr>
<td>320.28</td>
<td>Correlation of bioavailability with an acute pharmacological effect or clinical evidence</td>
</tr>
<tr>
<td>320.29</td>
<td>Analytical methods for an in vivo bioavailability or bioequivalence study</td>
</tr>
<tr>
<td>320.30</td>
<td>Inquiries regarding bioavailability and BE requirements and review of protocols by the FDA</td>
</tr>
<tr>
<td>320.38</td>
<td>Retention of bioavailability samples</td>
</tr>
<tr>
<td>320.63</td>
<td>Retention of bioequivalence samples</td>
</tr>
</tbody>
</table>

Adapted from the CFR and Midha et al. (2009)

Section 320.21 specifically references the role of bioequivalence studies with respect to abbreviated new drug applications (ANDAs) and supplements to new drug applications (NDAs). The regulations of this section may be summarized as in the following table.

**Table 2.1.1.2. Summary of Requirements for Submission of Bioequivalence Data cited in 21CFR320.21**

<table>
<thead>
<tr>
<th>ANDAs</th>
<th>Any sponsor submitting an abbreviated new drug application to FDA shall include in the application either:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>⊲ Evidence demonstrating that the drug product that is the subject of the abbreviated new drug application is bioequivalent to the reference listed drug</td>
</tr>
<tr>
<td></td>
<td>⊲ Information to show that the drug product is bioequivalent to the reference listed drug which would permit FDA to waive the submission of evidence demonstrating in vivo bioequivalence [i.e. a biowaiver]</td>
</tr>
<tr>
<td>Supplements to NDAs</td>
<td>Any person submitting a supplemental application to FDA shall include in the supplemental application the evidence or information set forth in the section above if the supplemental application proposes any of the following changes:</td>
</tr>
<tr>
<td></td>
<td>⊲ A change in the manufacturing site or process, including a change in product formulation or dosage strength, beyond the variations provided for in the approved application</td>
</tr>
</tbody>
</table>

For full text of CFR title 21 section 320.21 see: [17]
Events which may necessitate submission of bioequivalence data are identified in section 320.21 as “changes beyond those provided for in the approved drug application.” Such changes can often occur over the lifecycle of a product. Section 314.70 provides further detail regarding the nature of a proposed change by identifying three categories: minor, moderate, and major. The classifications are based on the “potential to have an adverse effect on the identity, strength, quality, purity, or potency of the drug product as these factors may relate to the safety or effectiveness of the drug product.” Determining the magnitude of this potential adverse effect is performed during the review process of the application for change approval. However, it can be stated that the FDA typically errs on the side of caution, often electing to require in vivo assessments of bioequivalence in the absence of rigorously supported justification(s) for the waiver of such trials (i.e., biowaiver). Examples for minor, moderate and major changes are provided in section 314.70. A selection of notable instances are contained in Table 2.1.1.3.
Table 2.1.1.3. Summary of the Severity of Changes to New Drug Applications
Cited in 21CFR314.70

<table>
<thead>
<tr>
<th>Severity of change</th>
<th>Examples</th>
</tr>
</thead>
</table>
| Minor              | • The deletion or reduction of an true colorant  
• Replacement of manufacturing equipment with that of the same design and operating principles  
• Change in the size and/or shape of a nonsterile solid dosage form container without a change in system |
| Moderate           | • Change in the container closure system  
• An increase or decrease in biopharmaceutical production scale during finishing steps that involves different equipment  
• Addition to a specification or changes in the methods or controls to provide increased assurance that the drug substance or drug product will have the characteristics of identity, strength, quality, purity, or potency that it purports or is represented to possess |
| Major              | • Changes that may affect drug substance or drug product sterility assurance  
• Changes in the synthesis or manufacture of the drug substance that may affect the impurity profile and/or the physical, chemical, or biological properties of the drug substance  
• A change in the manufacturing site or a change in the manufacturing process, including a change in product formulation or dosage strength, beyond the variations provided for in the approved application. |

For full text of CFR title 21 section 314.70 see: [18]

The CFR is considered to be federal law. Therefore, sponsors must interpret the document as legally-binding regulations. The FDA, in an effort to complement the CFR, periodically releases “Guidance for Industry” documents. Guidance documents represent the Agency’s current thinking on a particular subject. They do not create or confer any rights for or on any person and do not operate to bind FDA or the public. The primary guidance document concerning the regulatory imposition of studies for the assessment of post-approval bioequivalence is entitled, Immediate Release Solid Oral Dosage Forms; Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls, In vitro
Dissolution Testing, and In vivo Bioequivalence Documentation (henceforth referred to as the SUPAC guidance).

This SUPAC guidance provides the following support to industry:

i) a framework of product lifecycle management categories within which changes occur

ii) examples of levels of change that can occur within each category

iii) suggested chemistry, manufacturing, and controls (CMC) tests for each level of change

iv) in vitro dissolution tests and/or in vivo bioequivalence tests for each level of change

v) documentation to support the change.

The four specifically described categories in which a change can occur are identified as i) changes to the components and composition of the drug product ii) site changes iii) changes in the batch size (scale-up(scale-down), and iv) changes to the manufacturing process. The differences in these categories are described in Table 2.1.1.4.
Table 2.1.1.4. Post Approval Change Categories as Defined in the SUPAC Guidance

<table>
<thead>
<tr>
<th>Category of SUPAC-defined Change</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Components and composition</td>
<td>Focuses only on [batch-wise] changes in excipients and not on changes in the amount of drug substance</td>
</tr>
<tr>
<td>Site</td>
<td>Changes in location of the site of manufacture for both company-owned and contract manufacturing facilities</td>
</tr>
<tr>
<td>Batch size (scale-up/scale-down)</td>
<td>Postapproval changes in the size of a batch from the pivotal/pilot scale biobatch material to larger or smaller production batches call for submission of additional information in the application. Scale-down below 100,000 dosage units is not covered by the SUPAC guidance</td>
</tr>
<tr>
<td>Manufacturing</td>
<td>Changes that may affect both equipment used in the manufacturing process and the process itself.</td>
</tr>
</tbody>
</table>

For full text of SUPAC Guidance see: [19]

The SUPAC guidance echoes the CFR by identifying up to three levels of change within each category (now referred to as levels 1, 2 and 3). Some of the general examples used in the SUPAC guidance broadly mirror those found in the CFR. However, the guidance moves a step beyond the qualitative criteria used in the CFR (e.g., likelihood of detectable impact vs. potential to have an adverse effect, respectively) by citing quantitative examples and invoking the concepts of therapeutic range, solubility, and permeability in definitions and examples. In each category, the levels are uniquely defined, with a guiding principle of maintaining pharmaceutical quality (i.e., consistently meeting the needs of the patient) over varying degrees of change.

Level 1 changes are defined as component and composition changes that are unlikely to have any detectable impact on quality and performance (e.g., nonfunctional colorants). An example of level 1 changes includes changes to excipients that do not effect API performance characteristics. Such changes are cited as percentage-wise (w/w) changes of
total formulation, less than or equal to the following ranges and predicated on the sum of all excipient changes being ≤ 5%:

- “filler” \( \rightarrow \) ±5
- “starch disintegrant” (or other) \( \rightarrow \) ±3 (±1)
- “binder” \( \rightarrow \) ±0.5
- Ca or Mg stearate lubricant (or other) \( \rightarrow \) ±0.25 (±1)
- talc glidants (or others) \( \rightarrow \) ±1 (±0.1)
- “film coating” \( \rightarrow \) ±1

It is minimally suggested that such changes be formally filed with the FDA for prior approval. Instead, the suggestion is to document level 1 changes as part of the sponsor’s annual report.

Level 2 changes to excipients are broadly considered as those that “could have a significant impact on formulation quality and performance.” Suggestions for the assessments of consistent performance, and the filing of the associated documentation, for a level 2 change vary depending on three prefaced factors of the API in the drug product whose excipients are undergoing some change, namely the therapeutic range (i.e., narrow or non-narrow), solubility (i.e., low or high), and permeability (i.e., high or not high). Identified examples of level 2 excipient changes can include the technical grade of an excipient. Additionally, examples of level 2 changes are once again identified as changes in the relative percent, only now the when the degree of change is double than those that what could be considered a level 1 changes. Here, specific dissolution tests are recommended for BCS class I, II (e.g., Carbamazepine), and III with the suggestion for in vivo bioequivalence documentation still being done if the situation meets the previous
BCS-based dissolution test. If the dissolution tests are not met or do not meet the requirements for level 2, then the recommendation is to refer to level 3 changes. Level 3 changes are those that are “likely to have a significant impact on formulation quality and performance.” Suggestions for the assessments of consistent performance, and the filing of the associated documentation, for a level 3 change vary depending on the therapeutic range, solubility, and permeability of the drug product’s API. Some cited examples of level 3 changes are:

- Any qualitative and quantitative excipient changes to a narrow therapeutic index drug beyond the level 1 ranges
- Level 1 and 2 changes that occur in BCS class 4 drugs (i.e. low permeability and low solubility)
- Changes in the excipient ranges of all drugs beyond those considered to be level 2

A generally defined dissolution test is recommended for all level 3 changes (i.e. multi-point dissolution profile from the application/compendial medium at 15, 30, 45, 60 and 120 minutes or until asymptote). Additionally, a full bioequivalence study is recommended. However, the bioequivalence study may be waived where an acceptable IVIVC has been established. Based on this information, it can be expected that in vivo bioequivalence assessment will be required by the FDA for the approval of excipient changes to an immediate release carbamazepine product (due to carbamazepine’s narrow therapeutic index and status as BCS Class 2) beyond those identified as level 1, unless a suitable IVIVC exists.
Changes to an approved manufacturing process are the other instance were bioequivalence trials are recommended by the SUPAC guidance. Specifically, such trials are recommended for level 3 manufacturing process changes. This level includes processes change such as a change from wet granulation to direct compression of dry powder. Here, the same recommendations for the performance of dissolution trials and bioequivalence hold true, as as previously described in the case of level 3 excipient changes.

It should be noted that the descriptions for the specific level of change are not defined in totality; the level is defined as part of the iterative review process.

2.1.2 Current practices for the Assessment of Bioequivalence

Current approaches for the demonstration of bioequivalence can be classified as being performed either *in vitro* or *in vivo*. Section 320.24 in the CFR describes types of evidence to measure bioavailability or establish bioequivalence. The following summary of *in vivo* and *in vitro* approaches, in descending order of accuracy, sensitivity, and reproducibility, are acceptable for determining the bioavailability or bioequivalence of a drug product:

1. a) An *in vivo* test in humans in which the concentration of the active ingredient in the plasma is measured as a function of time (i.e. a pharmacokinetic method)

b) An *in vitro* test that has been correlated with and is predictive of human *in vivo* bioavailability data (i.e. an IVIVC)

2. An *in vivo* test in humans in which the concentration of the active ingredient in the (urinary) excretions of subject are measured as a function of time.
3. An *in vivo* test in humans in which an appropriate acute pharmacological effect of the active moiety, and are measured as a function of time (i.e. a pharmacodynamic method)

4. Well-controlled clinical trials of safety and effectiveness, for purposes of demonstrating bioequivalence. This approach is one of the least accurate, sensitive, and reproducible of the general approaches for measuring bioavailability or demonstrating bioequivalence.

5. A compendial *in vitro* test acceptable to FDA (i.e. USP based dissolution test)

Interestingly an IVIVC is in fact the most preferred method for bioequivalence determination after pharmacokinetic methods.

2.1.2.1 *In vivo* Assessment of Bioequivalence

A detailed protocol, including study objective(s), patient inclusion criteria, dosing schedules, a study design, and statistical methods, must be identified before a human *in vivo* trial is conducted.

The objective of bioequivalence trials, as found in the pharmaceutical literature, are based, typically, on the 21CFR320.1 definition of bioequivalence and the FDA guidance documents on bioavailability and bioequivalence studies. They generally recapitulate the objective for bioequivalence studies stated in the SUPAC guidance:

“To compare the rate and extent of absorption of the drug product for which the manufacture has been changed, as defined in this guidance, to the drug product manufactured prior to the change.”
The patient inclusion criteria for *in vivo* bioequivalence trials is typically shaped by ethical constraints. It is untenable to expose a patient who is medically dependent on a certain drug product to a potentially toxic/ineffective product for the sole purpose of determining bioequivalence. As such, bioequivalence studies are typically conducted in healthy patients. Also, dosing schedules can again be ethically constrained by the side effect profiles of medications. Most bioequivalence trials therefore utilize a single-dose format as evidenced in a 2009 retrospective study conducted by several FDA officials regarding bioequivalence trials of generic drug products approved by the agency from 1996 to 2007.20

2.1.2.2 Trial design

Prospective experimental designs are used to analyze the effect of independent variables. *In vivo* bioequivalence trials typically follow the characteristics of a randomized controlled trial (RCT). According to the Cochrane Collaboration, RCTs are highly regarded because they are designed specifically to minimize bias. By randomly assigning participants to groups, for example, the likelihood that the groups will differ on important baseline characteristics that confound the assessment of an intervention (whether the investigator knows them or not) is minimized. The majority of regulatory documents recommend a two-treatment, two-period crossover study with an equal number of subjects randomly assigned to each of the two dosing sequences.21 22 These regulatory documents typically make provisions that alternative methods can be selected if appropriate. To maintain efficiencies, however, the most common design employed is the 2-by-2 crossover design. This design allows each
subject to serve as their own control, providing an increase in statistical power. This is important because the statistical power of a simple parallel trial would be extremely limited when considering that most clinical trials rely on only 24 to 36 healthy patients.\textsuperscript{20}

The 2-by-2 crossover designs means that there are two product groups (i.e. the reference product and the test product) and two treatment sequences (test product administration “crossed over” to reference product administration and vice versa; see Figure 2.1.2.2.1).

![Figure 2.1.2.2.1 Illustration of a 2-by-2 Crossover Study Design](image)

Given the absorption and elimination kinetics for carbamazepine, this type of study can take over two weeks (in addition to the setup time for such a trial).\textsuperscript{23} Other higher-order designs exist but are rarely used; such methods account for high within-subject variability. However, as recent FDA draft guidance states, carbamazepine has low within-subject variability.\textsuperscript{24}
2.1.2.3 Trial Analysis

Variability in outcomes is a hallmark trait of higher order biologic systems. It is unrealistic to expect ‘test’ and ‘reference’ responses to ever be exactly identical. Therefore, \textit{in vivo} methods utilize intervals to define acceptable degrees of difference. When analyzing the results of a clinical trial for bioequivalence, a generally stated goal is to determine whether it is reasonable for one to expect the difference between test and reference responses to exceed reasonable limits, given the observed data. In statistical terms, the \textit{expected value} for a normal distribution of responses is the mean. In frequentist (classical) statistics, the standard deviation of a normal distribution is the basis for defining the certainty with which the mean of a sample from a population (i.e. subjects in a clinical trial) is said to be known. Based on this, FDA has adopted the two one-sided test (TOST) procedure described by Schuirmann et al.\textsuperscript{25,27} The level of bioequivalence most commonly recommended is \textit{average bioequivalence}, there are however other less frequently employed levels of bioequivalence which require higher order study designs.\textsuperscript{1}

Schuirmann’s TOST procedure begins by defining the hypotheses to be tested,

\textbf{Eq. 2.1.2.3.1} \quad H_{01} = \mu_T - \mu_R \leq \theta_L \quad \text{vs} \quad H_{a1} = \mu_T - \mu_R > \theta_L

and

\textbf{Eq. 2.1.2.3.2} \quad H_{02} = \mu_T - \mu_R \geq \theta_U \quad \text{vs} \quad H_{a2} = \mu_T - \mu_R < \theta_U

Hypothesis 2.1.2.3.1 is tested to verify that the average bioavailability of the \( T \) product does not exceed a lower limit (\( \theta_L \)). Hypothesis 2.1.2.3.2 is tested to verify that the average bioavailability of the \( T \) product does not exceed an upper limit (\( \theta_U \)). In both
equations, the null hypothesis ($H_0\#$) represents bioinequivalence. This setup is the reverse of the ordinary view of hypothesis testing. During conventional hypothesis testing the null hypothesis is usually the hypothesis of interest, but the set up in the equations is more applicable for bioequivalence assessment. Here, the type I error ($\alpha$) is declaring the drugs to be bioequivalent, when they are not. By setting up the hypothesis as shown, the consumer’s risk is protected. The two hypothesis tests can performed using one sided t-tests

$$\text{Eq. 2.1.2.3.3} \quad T_L = \frac{(\bar{Y}_T - \bar{Y}_R) - \theta_L}{\hat{\sigma}_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} > t(\alpha, n_1 + n_2 - 2)$$

and

$$\text{Eq. 2.1.2.3.4} \quad T_U = \frac{(\bar{Y}_T - \bar{Y}_R) - \theta_U}{\hat{\sigma}_d \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} > t(\alpha, n_1 + n_2 - 2)$$

where $\bar{Y}_T$ is the average response of the T product, $\bar{Y}_R$ is the average response of the R product, $n_1$ is the number of observations in the T product, $n_2$ is the number of observations in the R product, and $\hat{\sigma}_d$ is the pooled sample standard deviation of period differences from both sequences (see Equations 2.1.2.3.3 and 2.1.2.3.4). It is considered an unbiased estimator of the true standard deviation ($\sigma_d$) in the absence of carryover effects.

$$\hat{\sigma}_d = \sqrt{\hat{\sigma}_d^2} \quad (2.1.2.3.5)$$

where,

$$\hat{\sigma}_d^2 = \frac{1}{n_1 + n_2 - 2} \sum_{k=1}^{2} \Sigma_{i=1}^{n_k} (d_{ik} - \bar{d}_{.k})^2 \quad \text{and} \quad d_{ik} = \frac{1}{2} (y_{i2k} - y_{i1k})$$
Thus, the TOST procedure confirms bioequivalence if, and only if, $H_{01}$ and $H_{02}$ are both rejected at a predefined $\alpha$-level; otherwise stated, when both $|T_U|$ and $|T_L|$ are greater than test statistic: $t(\alpha, n_1 + n_2 - 2)$.

2.1.2.4 *In vitro* Demonstration of Bioequivalence

2.1.2.4.1 Dissolution-based Biowaivers

The waiver of *in vivo* bioequivalence studies is federally provided under certain conditions by 21CFR320.22. This type of a waiver is referred to as a biowaiver. The circumstances stated in the CFR provides, for certain drug products, that bioequivalence may be demonstrated by evidence obtained *in vitro* in lieu of *in vivo* data is summarized in the following Table 2.1.2.4.1.1.

**Table 2.1.2.4.1.1. Highlights of Requirements of Bioavailability and Bioequivalence Assessments as Cited in 21CFR320.22**

<table>
<thead>
<tr>
<th>Bioequivalence may be demonstrated by evidence obtained <em>in vitro</em> in lieu of <em>in vivo</em> data under one of the following criteria:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) The drug product is in the same dosage form, but in a different strength, and is proportionally similar in its active and inactive ingredients to another drug product for which the same manufacturer has obtained approval and the following criteria are met:</td>
</tr>
<tr>
<td>a) The bioavailability of the other drug product has been measured</td>
</tr>
<tr>
<td>b) Both drug products meet an appropriate <em>in vitro</em> test approved by FDA, and</td>
</tr>
<tr>
<td>c) The applicant submits evidence showing that both drug products are proportionally similar in their active and inactive ingredients.</td>
</tr>
<tr>
<td>2) The drug product is, on the basis of scientific evidence submitted in the biowaiver application, shown to meet an <em>in vitro test that has been correlated with in vivo data</em>.</td>
</tr>
<tr>
<td>3) The drug product is a reformulated product that is identical, except for a different color, flavor, or preservative that could not affect the bioavailability of the reformulated product, to another drug product for which the same manufacturer has obtained approval and the following conditions are met:</td>
</tr>
<tr>
<td>a) The bioavailability of the other product has been measured, and</td>
</tr>
<tr>
<td>b) Both drug products meet an appropriate <em>in vitro</em> test approved by FDA.</td>
</tr>
</tbody>
</table>

For full text of title 21 section 320.22 of the CRF see: [28]
The critical role an IVIVC can play for the approval of a biowaiver application (as referenced in criterion 2 of the previous summary table) is another example of its value.

As before, there are FDA guidance documents to augment section 320.22 of the CFR. One such document is entitled *Waiver of In vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System Guidance*. This guidance explains how biowaivers can be requested for an IR solid oral dosage forms based on its BCS class. The guidance notes that, "when combined with the dissolution of the drug product, the BCS takes into account three major factors that govern the rate and extent of drug absorption from IR solid oral dosage forms: (1) dissolution, (2) solubility, and (3) intestinal permeability." However, this guidance is focused on BSC class I/III products and does not address BCS-based biowaivers for the narrow therapeutic index drugs. Therefore, it is of little help for drugs such as carbamazepine (i.e. BSC class II and a narrow therapeutic index).

Immediate release BCS class II products are discussed (albeit briefly) in the FDA’s 1997 guidance entitled *Dissolution Testing of Immediate Release Solid Oral Dosage Forms* with no preclusions concerning the breadth of the therapeutic index. This document specifically mentions BCS class II products in two sections: i) *Approaches for Setting Dissolution Specifications for a New Chemical Entity* and, ii) *In vivo-In vitro Correlations*. For dissolution specification setting, the guidance notes that “a two-point dissolution specification, one at 15 minutes to include a dissolution range (a dissolution window) and the other at a later point (30, 45, or 60 minutes) to ensure 85% dissolution, is recommended to characterize the quality of the [BCS class II] product.” For IVIVCs, the guidance only
states that an IVIVC may be possible with BCS class II products as opposed to BCS classes I and III.

2.1.2.4.2 F2 Analysis

It is noted in the 1997 *Dissolution Testing of Immediate Release Solid Oral Dosage Forms* guidance single-point dissolution tests and specifications have been employed in evaluating scale-up and post-approval changes. This test may be appropriate for assuring consistent product quality and performance for certain minor changes. For more major changes however it is recommended that an entire dissolution profile comparison for similarity be performed under identical conditions for the product before and after the change(s). Indeed, this guidance, the biowaiver guidance, and the SUPAC guidance all state that dissolution profiles may be compared using a similarity factor ($F_2$).

$F_2$ is a logarithmic reciprocal square root transformation of the sum of squared error. This represents a measurement of the similarity in the percent (%) dissolution between the two curves. This metric will be calculated using the mean dissolution profile of the two products (test and reference), using the same time points for both the profiles (e.g., 15, 30, 45, 60 minutes).

$$f_2 = 50 \cdot \log\{[1 + (1/n)\sum_{t=1}^{n} (R_t - T_t)^2]^{0.5} \cdot 100\}$$

In this equation, $n$ is the number of time points, $R_t$ is the dissolution value of the reference (prechange) batch at time $t$, and $T_t$ is the dissolution value of the test (postchange) sample at time $t$. Curves are identical if $F_2$ is 100. FDA guidances state that generally, $F_2$ values greater than 50 (50-100) ensure sameness or equivalence of the two curves and, thus, of the performance of the test (postchange) and reference (pre-change) products.
2.1.2.5 IVIVC Development

2.1.2.5.1 Levels of IVIVCs

There exist three levels of IVIVC: A, B, and C. A level C IVIVC establishes a relationship between an amount dissolved at a specific time and a summary pharmacokinetic parameter (e.g. AUC, \( C_{\text{max}} \), \( T_{\text{max}} \)). A level B IVIVC uses the principles of statistical moment analysis. The mean \textit{in vitro} dissolution time is compared to either the mean residence time or to the mean \textit{in vivo} dissolution time. A level A IVIVC focuses on the “the relationship between \textit{in vitro} dissolution and the \textit{in vivo} input rate.” According to regulatory documents, a level A IVIVC is the most informative. Guidance points out that “whatever the method used to establish a level A IVIVC, the model should predict the entire \textit{in vivo} time course from the \textit{in vitro} data”.

2.1.2.5.2 Existing Methods for IVIVC Development

There are numerous approaches to developing an IVIVC. They can be considered as either a one-step or a two-step procedure. The direct differential equation based method described by Buchwald is a defining example of a one-step procedure.\(^{29}\) This method, and others like it, directly relate the time-profiles of \textit{in vitro} dissolution rates and \textit{in vivo} plasma concentrations by using a one compartment pharmacokinetic models and a corresponding system of differential equations.” Here, the rate of \textit{in vivo} input is connected to the rate of \textit{in vitro} dissolution through a time scaling/shifting function that was simultaneously parameterized with the rates of clearance using fitting algorithms (e.g. Nelder-Mead). These types of methods can be advantageous because they can recover \textit{in vivo} plasma profiles from
several formulations directly from the \textit{in vitro} dissolution data in a “single [modeling] step”. However, these methods can be subject to overfitting. This can be avoided by placing constraints on the parameters of the pharmacokinetic model parameters and time scaling factors that are guided by \textit{a priori} information.

Two step methods are typically referred to as deconvolution-convolution based methods. In the first stage, the \textit{in vivo} absorption profile (fraction absorbed vs. time) is deconvoluted from the plasma concentration profile. Systematic methods for the deconvolution of the \textit{in vivo} absorption profile have been notably described by Wagner & Nelson, and Loo & Riegelman.\textsuperscript{30,31} Other methods, based on noncompartmental analysis, exist for deconvoluting of the \textit{in vivo} absorption profile exist (e.g. Wagner\textsuperscript{32}; Wang & Nedelman\textsuperscript{33}; Gibaldi & Perrier\textsuperscript{34}) however they often require many assumptions to handle the underlying pharmacokinetic behavior. Nevertheless, once the \textit{in vivo} absorption profile has been deconvoluted, the second stage is focused on regressing the \textit{in vivo} absorption profile to the \textit{in vitro} dissolution profile. This is typically accomplished by any host of regression models wherein the \textit{in vitro} dissolution profile is the independent variable and the \textit{in vivo} input profile is the dependent variable. The model most frequently desired to achieve a satisfactory recovery of the plasma concentration profile is a linear model. However, sometimes nonlinear models such as the Weibull or Hill models are necessary. The reliance upon what is essentially a regression model is why some authors prefer to call IVIVCs \textit{in vitro-\textit{invivo}} regressions (IVIVRs).\textsuperscript{35} A graphical representation of the two step IVIVC procedure and the one step IVIVC procedure can be found in the following Figure 2.1.2.5.2.1.
Figure 2.1.2.3.2.1. Illustration of Conventional Two and One Step methods for the Generation of an IVIVC

**One Step Method**

- Compare & refit all parameters simultaneously as needed

- **Formulation PK**
  - Plasma Conc (ng/mL)
  - Time (h): -6, 4, 14, 24

- **Solve PK Param.’s:**
  - $ke, V$

- **In vitro Dissolution**
  - % Dissolved
  - Time (h): 0.0, 4.0, 8.0, 12.0

- **Predict PK Profile**

- **Predict PK**
  - Plasma Conc (ng/mL)
  - Time (h): -6, 4, 14, 24

**Two Step Method**

- **Predict PK profile, compare, and refit**

- **Step I.**
  - **Deconvolute necessary in vivo absorption**
  - **In Vivo Drug Absorption**
  - % In vivo % Dissolved
  - Time (h): 0.0, 4.0, 8.0, 12.0

- **Step II.**
  - **Establish IVIVC**
  - **IVIVR Predicted in vivo Abs**
  - $y = 0.9941x$
  - $R^2 = 0.9703$
2.1.2.5.3 Regulatory Perspectives on IVIVC Development

While there are several guidance documents and sections in the CFR that make reference to the utility of IVIVCs, few specifically address the area of IVIVC development methodologies. The FDA guidance entitled *Dissolution Testing of Immediate Release Solid Oral Dosage Forms* is one such guidance that briefly discusses this area. As its title implies, IVIVC development is referred to in only in the larger context of dissolution test development. With respect to IVIVCs, this guidance focuses on how the presence or absence of clinical differences should be used to develop the dissolution test. It described how at least three batches that differ in the *in vivo* as well as the *in vitro* performance should be used to develop an IVIVC. The guidance notes how “If the batches show differences in *in vivo* performance, then *in vitro* test conditions can be modified to correspond with the *in vivo* data to achieve an *in vitro-*in vivo correlation [and likewise modified for equivalence if no *in vivo* difference is found].” This document does not detail how to evaluate an IVIVC for predictability.

The guidance does state that, very often, the *in vitro* dissolution test is found to be more sensitive and discriminating than the *in vivo* test. It goes on to note that this is not entirely undesired. This is because, from a quality assurance point of view, a more discriminative dissolution method could indicate possible changes in the quality of the product before *in vivo* performance is affected. However, this scenario is primed to raise inappropriate cause for concern when the link between the *in vitro* quality measurement and clinical significance is poorly defined.

The guidance document which does specifically address IVIVC development and evaluation is entitled *Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In vitro/In vivo Correlations* (henceforth referred to at the IVIVC guidance).
While no guidance document is rigidly prescriptive, this one is geared towards extended release products. Therefore, its recommendations must be carefully interpreted when referenced in the context of immediate release products. Nevertheless, the document is the most instructive piece of regulatory literature available on the topic of IVIVCs. The guidance divides its discussion between general discussions on “developing the correlation” and IVIVC-level specific recommendations. The first general comments state how, despite the existence of commonly used approaches, the strategy used to develop an IVIVC is largely the prerogative of the sponsor. This is stated with the understanding that any IVIVC must be reviewed before it can considered fit for sponsor-proposed purposes that are regulatorily approved. The following points summarize the key general recommendations of the guidance:

- The review of $\geq 3$ formulations with different release rates is considered optimal
- \textit{In vivo} comparison is to be made using a single crossover study
- If one or more of the formulations (highest or lowest release rate formulations) does not show the same relationship between \textit{in vitro} dissolution and \textit{in vivo} performance compared with the other formulations, the correlation may still be used within the range of release rates encompassed by the remaining formulations
- Once a discriminating system is developed, dissolution test conditions should be consistent across formulations during IVIVC development

With respect to the discussion of level A IVIVCs, the guidance specifically recommended that the release rates for each formulation studied should differ “adequately” (e.g. by 10%). Moreover, this difference should result in comparable \textit{in vivo} profile differences (e.g. a 10%
difference in the pharmacokinetic parameters of interest ($C_{max}$ or $AUC$) between each formulation).

Methodology for the evaluation of IVIVC predictability is an active area of investigation and a variety of methods are possible and potentially acceptable.\(^{36}\) The agency’s stated IVIVC evaluation approaches was developed according to the objective of a level $A$ IVIVC, i.e. to establish a predictive mathematical model describing the relationship between an *in vitro* property and a relevant *in vivo* response. As such, the proposed evaluation approaches focus on the estimation of predictive performance or, conversely, prediction error. The guidance notes that the evaluation of prediction error should be guided by intended application of an IVIVC and the therapeutic index of the drug.

Two distinct aspects of predictability are discussed by the IVIVC guidance: internal and external predictability. Evaluation of internal predictability is based on the initial data used to define the IVIVC model. Evaluation of external predictability is based on additional test data sets. Both aspects are not recommended in all instances. However, in the case of narrow therapeutic index drugs such as carbamazepine, the external predictability of the correlation should be evaluated along with internal predictability. According to the guidance, internal predictability is established when the average absolute percent prediction error (% PE) of 10% or less for $C_{max}$ and $AUC$. In addition, the % PE for each formulation should not exceed 15%. Likewise, the external predictability is established when the average absolute percent prediction error (% PE) of 10% or less for $C_{max}$ and $AUC$ establishes the predictability of the IVIVC. Non-compartmental reference methods for determining $C_{max}$ and $AUC$ are identified in guidance documents and detailed by Shargel et al. The methods include the trapezoidal approach for $AUC$ calculation, analytical integration of spline interpolations for
the plasma concentration profile, and the absorption-elimination rate derived max concentration.37

2.1.3 Emerging Trends and Alternatives in the Assessment of in vivo Bioequivalence

Equivalence testing is used to provide statistical statements about parameter estimates by relating them to intervals of effect sizes considered practically important rather than just to an effect size of zero. The tests are specific examples of applied inferential statistics. As such, there are a host of different inferential techniques that can be used to accomplish tests of equivalence. The options can largely be classified as either Bayesian or classical (i.e. frequentist) techniques. A frequentist framework considers that, for a given model parameter \( \theta \), there exists a fixed value, and the data represents a random sample from a larger population. They are based on the expected results of hypothetically repeated samples, generated by the same sampling process. Bayesian inference considers observed data as fixed and model parameters random. Bayesian inference is described probabilistically through the use of probability distributions for model parameters. Simply stated, a Bayesian approach allows for a researcher to make statements such as: “the probability that a parameter \( \theta \) lies in the range 0.8-1.2 is 95%.” By contrast, the equivalent frequentist statement is: “if the same experiment is repeated many times and intervals are computed for each experiment, then 95% of those intervals will contain the true value of the parameter.”

Alternatives to the classical TOST procedure for assessing bioequivalence includes the Anderson and Hauck test.38 This method uses a hypothesis test statistic \( T_{AH} \) to directly
test \( H_0 \) rather than testing \( H_{01} \) and \( H_{02} \) independently. Ennis and Ennis proposed an expansion of the Anderson and Hauck test that controlled type I error. In their work, they recommended adjusting the non-centrality parameter using a positive constant \( c \) to ensure that the Type I error remains at or below a nominal level. Additionally, there are nonparametric alternatives to the classical parameters procedures discussed thus far. They can be considered in a unified manner by treating them as variations of a rank transformation procedure. Of nonparametric methods, the Wilcoxon rank-sum test (\( WRS \); otherwise known as the Wilcoxon-Mann-Whitney test) is arguably the most appropriate method due to a 2x2 crossover trial being composed of a pair of sequences. Several examples exist in the literature where this method was used during bioequivalence assessment. A common critique of non-parametric methods is that they are considered to be less efficient than their parametric counterparts. For example, the rank correlation test has an efficiency rating 0.91, see Table 5.6 from Triola. That is to say, for all other things being equal, the nonparametric rank correlation test requires 100 sample observations to achieve the same results as 91 sample observations analyzed through parametric linear correlation. This assumes the requirements for the parametric method are satisfied. When extrapolated to a clinical trial, this difference could be significant. However, satisfying the assumptions of a specific distribution, such as normality, are not required for the use of nonparametric methods as they are with parametric methods.

The assessment of bioequivalence can be performed using several Bayesian approaches. In the literature, procedures have been constructed by attempting to answer one of two questions: Does the evidence offered by the data support a model
representing bioequivalence or biononequivalence? What percent of the posterior distribution for the treatment effect (i.e. AUC mean) in a random effects model of 2x2 crossover lies outside of a relevant interval? The first question has been notably addressed by Ghosh and Khattree, the second by Fluehler et al.\(^{48,49}\) Ghosh and Khattree described how hypothesis testing can be accomplished by calculating the Bayes’ factor between two competing hypotheses. Their method is based on principles established by Harrold Jefferys and Alan Turning for the Bayesian comparison of two models.\(^{50,51}\) The approach is especially useful when using uninformative prior distributions on model parameters.

The approach by Fluehler, et al. focuses on integrating a specific region of the posterior probability distribution for the treatment response, i.e. the treatment-wise AUC responses. It is essentially a Bayesian interval hypothesis test. As such the results can be easily interpreted in probabilistic terms and distributional assumptions can be customized. Bayesian interval hypothesis testing estimates the probability of a model parameter (\(\theta\)) satisfying a given inequality, e.g. \(P(H_0) = P(-\delta \leq \theta \leq \delta) = P(\theta \in [-\delta, \delta])\). Evaluation is accomplished by simulating, to convergence, a posterior distribution of model parameters and testing the rate at which \(\theta\) lies in the test interval:

\[
\hat{P}(H_0) = \frac{1}{T} \sum_{t=1}^{T} 1_{\{\theta \in [-\delta, \delta]\}}
\]

where \(1_{\{f\}}\) is an indicator function returning 1 when \(f\) is true and 0 when false.
2.2 Model Drug: Carbamazepine

2.2.1 Background

The model drug in this study is carbamazepine. Carbamazepine was discovered by chemist Walter Schindler in Switzerland (1953). It was first marketed as a drug to treat trigeminal neuralgia in 1962 and has been approved in the United States since 1974 under the trade name Tegretol® (Ciba-Geigy, today owned by Novartis International AG). According to archived ANDA approval reports the first-time generic for Tegretol was approved in August of 1986. The application was sponsored by Inwood Laborites Inc, Ltd.

Oral preparations consist of various solid dosage forms, including immediate release formulations, controlled-release formulations, and an oral suspension. The marketed formulations include tablets of 100, 200, or 400 mg, chewable tablets of 100 or 200 mg, controlled-release tablets of 100, 200, or 400 mg, and a suspension of 100 mg/5 mL. Tablet preparations with extended release profiles have been developed to reduce peak-related toxic effects and to decrease variations in plasma carbamazepine concentrations during the dosage interval. In addition to the commercially available oral preparations, an intravenous preparation has recently (October 2016) gained approval in the US under the trade name Carnexiv™ (Lundbeck A/S, Copenhagen, Denmark). Intravenous formulations prior to this point were developed and used for research purposes only.
Carbamazepine is an iminostilbene and a structural conjugate of the tricyclic antidepressant drug imipramine (Figure 2.2.1.1). Its labeled indications include specific forms of epilepsy and trigeminal neuralgia. It has been shown to be effective in the treatment of simple partial, complex partial, and generalized tonic-clonic seizures, but it is ineffective against generalized absence seizures. Carbamazepine and the anticonvulsant drug phenytoin have been shown to be effective in treatment of partial seizures and tonic-clonic seizures when they are used alone or as initial therapy, and both carbamazepine and phenytoin are drugs of first choice in the treatment of such seizure disorders. However, carbamazepine may be more effective in the treatment of complex partial seizures when complete seizure control is used as an end point. Carbamazepine is an effective anticonvulsant drug in experimental animals, and it has an anticonvulsant profile that is similar to that of phenytoin. It is effective against maximal electroshock seizures at nontoxic doses but is not active against subcutaneous metrazol-induced seizures. Carbamazepine may also be effective in the short-term and long-term treatment of manic-depressive illness, and it is the drug of choice for treatment of trigeminal neuralgia. It is administered to adults in doses of 10 to 20 mg/kg/day to achieve total plasma concentrations of $6.5 \pm 3 \mu g/mL$. The lower range of plasma concentrations are adequate to control seizures in patients with primary or secondarily generalized tonic-clonic seizures alone, but the higher plasma concentrations are often required to treat seizures in patients with partial seizures with or without tonic-clonic seizures.
2.2.2 Mechanism of Action

The mechanism of action for carbamazepine is unknown according to the package insert for Tegretol®. However, several pharmacologic mechanisms of action for carbamazepine have been independently agreed upon in the literature. They can be generally classified under two categories, including i) activity on neuronal voltage gated sodium ion channels and ii) synaptic activity on specific receptors and neurotransmitters, most notably those related to the N-methyl-D-aspartate (NMDA) system. The former mechanism of action is commonly held as the primary mechanism responsible for carbamazepine’s antiepileptic properties. This viewpoint is a result of the established relationship between voltage gated sodium ion channels, the propagation of action potentials, and the pathophysiology of electrical signals in epileptic disorders.

Early efforts aimed at explaining a basic physiologic description of carbamazepine’s antiepileptic mechanism of action are found in the reports by Krupp (1969) and subsequently Honda and Allen (1973). Using electrical stimulation, Krupp reported that carbamazepine caused a reduction in amplitude and an increase in latency and duration of signals in the peroneal and sciatic nerves of rabbits. Honda and Allen demonstrated that carbamazepine reduced the spontaneous firing of action potentials recorded from peripheral nerves immersed in isotonic sodium oxalate or phosphate solutions. Additionally, in controlled electromyography studies performed by Heshkowitz, et al. carbamazepine was demonstrated to limit sustained high frequency tetany of muscle bundles without affecting the conduction of single action potentials. This was demonstrated at therapeutic free carbamazepine serum concentrations of >1 µmol/L (4.2 µg/mL). Ultimately, a more
Pharmacologic description of carbamazepine’s activity on sodium channels was specified through the performance of studies using voltage-clamp techniques.\textsuperscript{77,78} Notable studies are listed in Table 2.2.2.1 along with the cell systems in which they were performed.

<table>
<thead>
<tr>
<th>Cell System</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>peripheral nerve and muscle cells</td>
<td>79, 80</td>
</tr>
<tr>
<td>neuroblastoma cells in culture</td>
<td>81, 82</td>
</tr>
<tr>
<td>human NT2-N cells in culture</td>
<td>83</td>
</tr>
<tr>
<td>acutely dissociated hippocampal neurons</td>
<td>83, 84, 85</td>
</tr>
<tr>
<td>rat brain type IIA sodium channels stable expressed in CHO cells</td>
<td>86</td>
</tr>
</tbody>
</table>

Carbamazepine was shown in these studies to slow the rate of recovery from inactivation and to shift the voltage dependency of steady-state inactivation to more negative voltages by producing a frequency and voltage dependent blockade of the sodium ion channels. This pharmacologic explanation of carbamazepine’s mechanism of action agreed with the initial physiologic studies and additionally indicated that the blockade appeared to be selective for the inactive form of the closed sodium ion channel.

2.2.3 Pharmacokinetics

Carbamazepine pharmacokinetics have been qualitatively described in FDA documents to demonstrate low within subject variability.\textsuperscript{24} This section will go over the absorption, distribution, metabolism, and excretion (ADME) properties of carbamazepine which describe the absorption, distribution, metabolism and elimination characteristics of the drug.
2.2.3.1 Absorption

Oral absorption characteristics are defined by the rate and extent of absorption. The extent of absorption is typically described in terms of bioavailability, \( F \). The overall extent of oral bioavailability \( F_{\text{oral}} \) of a drug can be expressed by the following equation

\[
F_{\text{oral}} = f_a \cdot F_g \cdot F_H
\]

where the fraction of drug absorbed \( (f_a) \) is the fraction of the dose entering the cellular space of the enterocytes, \( F_g \) is the fraction of the drug entering the enterocytes that escapes first-pass gut wall metabolism (equivalent to \( 1 - E_G \), the intestinal extraction ratio) and \( F_H \) is the fraction of drug entering (or by-passing) the liver that escapes first-pass hepatic metabolism and biliary secretion (equivalent to \( 1 - E_H \), the hepatic extraction ratio).

\( F_{\text{oral}} \) has several subcategories but the most informative is the absolute bioavailability. This type of oral bioavailability is calculated by dosing a drug by both an intravenous and oral route. The area under the plasma concentration profile (AUC; area under the curve) is then calculated for both routes of administration and the dose normalized oral-to-intravenous ratio is defined as the absolute bioavailability.\(^{87}\)

Marino et al. used stable labeled carbamazepine in an intravenous formulation and commercially available tablets to calculate \( F \). Its estimated value was 78% with a reported coefficient of variation equal to 30.8%. This range was similar to the range of 75% to 85% estimated through the recovery of radio labeled carbamazepine in urine and feces after single-dose administration reported by Faigle and Feldmann.\(^{88}\) Data from different studies suggest that the oral bioavailability of carbamazepine is similar whether given as conventional tablets, solutions, suspensions, syrups, or newly developed chewable or
sustained-release formulations. Notably, Levy et al. did demonstrate a slight food effect was present in the case of carbamazepine wherein the bioavailability was increased when taken with food (approximately 20% relative increase in $F$ on average which broadly varied and was not statistically significant). This effect is generally believed to be the result of an increase in the solubilization of the drug by the presence of food and the physiologic response therein.

Interestingly, Levy et al. also examined the relative bioavailability of carbamazepine. Here, carbamazepine tablets and bulk powder were supplied by the Ciba-Geigy Corporation. Since the drug is poorly soluble in water, a propylene glycol solution at a concentration of 20 mg/ml was prepared for oral administration. Both formulations were given to six normal drug-free volunteers (3 male, 3 female) in good health. Their mean age was 25.8 ± 3.8 yr and mean weight was 69.3 ± 18.8 kg. All were Caucasian, and none had any significant medical history. Physical examinations and clinical laboratory studies conducted prior to the study and every 2 weeks during the study revealed no significant medical findings. The subjects fasted overnight, took the drug with 100 ml of water, and fasted for 3 hours. Sixteen blood samples were collected over a 72 hour period at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48, and 72 hours (see Figure 2.2.3.1). The relative bioavailability was calculated using the following equation:

$$\text{Bioavailability} = \frac{(\text{AUC})_{\text{tablet}} \times k_e \text{tablet}}{(\text{AUC})_{\text{solution}} \times k_e \text{solution}} \times 100.$$ 

The relative bioavailability of the tablet compared to the solution was 79% (p < 0.05) and ranged between 56% and 109%. This value agrees with the absolute bioavailability of
carbamazepine from intravenous dosing studies of Marino et al. Therefore, it is reasonable to assume that the contribution of metabolism from the gut is minimal.

**Figure 2.2.3.1 Relative Bioavailability Study by Levy et al.**

![Plot of average serum carbamazepine concentration vs time following the administration of 6 mg/kg propylene glycol solution (○) and Tegretol Tablet (●). Adapted from Levy (1997) 97](image)

The *fraction absorbed at a given time* in Equation 2.2.3.1 can be determined using the rate of absorption ($k_a$). For a one compartment model, the rate value is typically calculated using the *method of residuals* or using the Wagner-Nelson method. Shargel, et al. describe these methods in detail. The method of residuals separates the initial exponential phase of a biexponential plot of plasma concentration against time by extrapolation of the terminal elimination rate constant. The Wagner-Nelson method estimates the loss of drug from the gastrointestinal tract over time, whose slope is inversely proportional to $k_a$. Its methodology is based on calculating partial $AUC$s.
Carbamazepine, like many small molecules, has been demonstrated to be orally absorbed by passive diffusion. This mode of absorption is characterized by the permeability of a molecule through the apical and basolateral cell membranes of the intestinal epithelium. Permeability can be estimated by a variety of means. The permeability of carbamazepine has been estimated in Caco-II permeability assays to be $22.8 \times 10^{-6}$ cm/s. Additionally, studies performed using the Ussing chamber technique in rodents have reported the apparent permeability of carbamazepine to be $15.2 \pm 5 \times 10^{-6}$ cm/s. Such in vitro permeability methods however often require calibration to effective permeability ($P_{eff}$) of humans in vivo. This value is derived from gut perfusion experiments such the Loc-i-Gut technique. Lennernäs et al. performed such a study where ‘high permeability’ characteristics (i.e. $2-4 \times 10^{-4}$ cm/s as defined by Amidon at al. and the FDA) were verified with a $P_{eff,CBZ} \approx 4 \times 10^{-4}$ cm/s. This value serves a primary drug-dependent parameter for the physiologically based prediction of oral drug absorption described by Jamei et al.

Olling et al. used the MoR to calculate $k_a$, reporting the absorption half-lives of four different carbamazepine products including the reference product, Tegretol®, from a relative bioavailability study. A similar study including Tegretol® was performed by Meyer et al. in 1992. The details of these studies will be discussed in greater detail later. However, it is interesting to note that the absorption rates for the common drug product reported in the studies were in good agreement: 0.23 vs 0.24 hr$^{-1}$, respectively. This consistency is observed despite different subjects being used in the two studies and a seven year difference between when the trials were performed.
2.2.3.2 Metabolism

Carbamazepine has been reported to undergo >97% hepatic metabolism via three pathways: epoxidation and hydroxylation by CYP3A4, CYP3A5, and CYP2C8 and glucuronidation by UGT2B7. Carbamazepine also strongly stimulates the transcriptional upregulation of genes involved in its own metabolism through RNA analysis. The only form of the drug that has been demonstrated to be therapeutically active is its unmetabolized form, with little (<3%) carbamazepine being excreted unchanged. Mechanistic drug-drug interactions via CYP3A4 and CYP2B6 induction are well documented and can complicate the use of carbamazepine in polytherapy.

Cazali et al. performed studies to characterize the in vitro and in vivo inhibitory effect of a new anticonvulsant, saquinavir, on the metabolism of carbamazepine. The performance of their study required the performance of control studies on carbamazepine alone. Human liver microsomes (HLMs) and cDNA-expressed CYP enzymes were used for the in vitro experiments where nonspecific binding was controlled for during the calculations. Pharmacokinetic data from epileptic children and healthy adults were used for the carbamazepine and saquinavir in vivo studies, respectively. Carbamazepine biotransformation by human liver microsomes (Vmax=10.3 nmol min⁻¹ nmol⁻¹ P450, apparent Km=362 uM), cDNA expressed CYP3A4 (Vmax= 1.44 nmol min⁻¹ nmol⁻¹ P450, apparent Km= 335.5 uM), and cDNA expressed CYP2C8 (Vmax=0.669 nmol min⁻¹ nmol⁻¹ P450, apparent Km=757 uM) was reported by the authors. The findings from this study were supported by the findings from similar studies that reported the intrinsic clearance (Cl_int = V_max/K_m) of carbamazepine in both HLMs and other cDNA-expressed CYP
enzyme systems including CYP3A5 (Vmax=1.17 nmol min⁻¹nmol⁻¹P450, apparent Km=119uM). Staines et al. in 2004 reported the results of studies aimed at identifying the uridine diphosphate glucuronosyltransferase (UGT) isoform responsible for the N-glucuronidation of carbamazepine. They developed a sensitive liquid chromatography/mass spectrometry assay to quantify carbamazepine glucuronidation. There, the researchers reported that carbamazepine is specifically glucuronidated by human UGT2B7. The kinetics of carbamazepine glucuronidation in human liver, kidney, and intestine microsomes were reported in their study and shown to be consistent with those of recombinant UGT2B7, which displayed a Km value of 214 uM and Vmax value of 0.79 pmol/mg/min. This was the first example of primary amine glucuronidation by UGT2B7.

Oscarson et al. studied the global induction response of drug-metabolizing enzymes, drug transporters, and nuclear receptors using liver sampled from epileptic patients treated with carbamazepine and control subjects. They confirmed the induction of several genes previously shown to be inducible in vitro, including multiple cytochrome P450 (CYP) genes in the CYP1A, CYP2A, CYP2B, CYP2C, and CYP3A subfamilies, as well as glutathione S-transferase A1, UGT 1As, the drug transporter ABCC2, and the nuclear receptors CAR (constitutive androstane receptor) and PXR (pregnane X receptor). Additionally, the research reported the relative increase in the expression of the CYP isoforms for treated patients. These values were then used to inform induction factor calculations (i.e. max induction value; Indmax). Almond et al. specifically reported the CYP3A4 Indmax and IndC50 for carbamazepine using RNA quantification in hepatocytes across serial carbamazepine concentrations (21.9 fold and 58.7uM, respectively). The autoinduction characteristics of
CYP2C8 and CYP3A4 deconvoluted by application of the mechanistic framework for the prediction of drug-drug interactions laid were out by Almond et al.\textsuperscript{118} The approaches predicted the extent and time-course of enzyme induction \textit{in vivo} based on \textit{in vitro} experimentation using serial dosing, probe substrates, and knowledge of the percent contributed toward total clearance by each enzymatic isoform.\textsuperscript{119,120}

\subsection*{2.2.3.3 Distribution}

Carbamazepine is a neutral and lipophilic compound that easily crosses the blood–brain barrier and other biologic membranes of the body and rapidly distributes to various organs and tissues. A study by Takayasu et al. simultaneously assayed carbamazepine and its metabolites by gas chromatography-mass spectrometry (GC-MS) in body fluids and organ tissues taken from victims in five autopsy cases.\textsuperscript{121} The concentrations of carbamazepine were generally much higher in organ tissues than in blood and urine, and were higher in the liver than in the lung in three cases. Practically speaking, however, the concentration of interest for clinical purposes is the concentration in the plasma. The volume of distribution ($V_d$) represents a volume that must be considered in estimating the amount of drug in the body from the concentration of drug found in the sampling compartment i.e. the plasma. A reliable means by which this value can be calculated is through serial plasma collections following i.v. dosing. This was performed by Marino et al.\textsuperscript{122} The researchers developed an intravenous, stable-labeled (SL) formulation in order to characterize carbamazepine pharmacokinetics in patients. Ninety-two patients received a 100 mg infusion of SL-carbamazepine as part of their morning dose. Blood samples were collected up to 96 hours after drug administration. Plasma drug concentrations were
measured with LC-MS and concentration-time data were analyzed. The weight normalized volume of distribution was 1.11 L/kg with a standard deviation of 0.26. Given the study design, this value represented a Vd at steady state and was observed to be consistent across the subpopulations of the study. The value reported is in agreement with those reported by other researchers. The combined interpretation of these works is that the Vd of carbamazepine can vary from 0.8 to 2 L/kg in adults and older children.

The value of Vd can be shaped in large part by the binding of drug to plasma proteins. The two most common plasma binding proteins are human serum albumin (HSA) and α₁ acid-glycoprotein (AGP). The acidic nature of AGP means that most of the drugs that bind to it are basic ones with pK values of 8 or higher, which implies that such drugs are positively charged at physiologic pH. Indeed is it clear from binding studies, which have included carbamazepine as a model drug, that HSA accounts mainly for the binding of acidic and neutral drugs (i.e. carbamazepine) whereas AGP associates more readily with basic drugs. Indeed the studies that have been performed in vitro using equilibrium dialysis methods have shown that the unbound carbamazepine fraction for AGP is 25%, a value that agrees with the in vivo free fraction reported by Marino et al.

2.2.3.4 Elimination

Carbamazepine is eliminated by biotransformation followed by urinary and biliary excretion of the parent drug and the formed metabolites with less than 5% of the drug being excreted unchanged. After administration of a single oral dose of 14C-labeled carbamazepine, 72% of the radioactivity was excreted in the urine, and the remaining 28% was recovered in feces.
2.2.4 Carbamazepine Historical Issues with Bioequivalence

All available generic carbamazepine products are designated as AB as defined in a publication entitled *Approved Drug Products With Therapeutic Equivalence Evaluations* (commonly known as the Orange Book). It contains therapeutic equivalence evaluations for FDA approved multisource prescription drug products. Drug products are considered to be therapeutic equivalents only if they are pharmaceutical equivalents for which bioequivalence has been demonstrated, and they can be expected to have the same clinical effect and safety profile when administered to patients under the conditions specified in the labeling. Drug products are considered pharmaceutical equivalents if they contain the same active ingredients, are of the same dosage form and route of administration, and are formulated to contain the same amount of active ingredient and to meet the same or compendial or other applicable standards (i.e., strength, quality, purity, and identity). Drug products that FDA considers to be therapeutically equivalent to other pharmaceutically equivalent products include those for which:

(1) there are no known or suspected bioequivalence problems; these are designated AA

(2) actual or potential bioequivalence problems have been resolved with adequate *in vivo* and/or *in vitro* evidence supporting bioequivalence (designated as ‘AB’).

Despite the AB status of carbamazepine, the use of generic versus brand-name antiseizure medications, such as carbamazepine, have attracted much attention and debate. This is a results of studies such as that performed by Krauss et al.\textsuperscript{138} Using pharmacokinetic data submitted to the FDA, the researchers found that while most generic antiseizure drugs provide total drug delivery similar to the reference product, differences in peak concentrations were more common. Additionally, these researchers identified how switches
between generic products caused greater changes in plasma drug concentrations than generic substitution of the reference product.

In 1992 Meyer et al. performed studies to assess the *in vitro* dissolution and *in vivo* bioavailability of brand carbamazepine (Tegretol®; product 1) and three lots from a generic manufacturer (Pharmaceutical Basics Inc.; products 2, 3 and 4) which were reported to have clinical failures. This study is an important source of *in vitro-in vivo* data for carbamazepine. The *in vitro* tests were performed using the compendial dissolution method for carbamazepine: type II dissolution apparatus, 75 rpm paddle speed and 900 ml of water containing 1% sodium laurel sulfate at 37°C. The *in vivo* study was performed in twenty-four, nonsmoking males. Their ages ranged from 21 to 35 years and their weights ranged from 61 to 93 kg. All subjects had normal clinical chemistry laboratory values, including reticulocyte counts and serum iron. The subjects did not ingest any drugs for twenty-one days and avoided alcohol for forty-eight hours prior to any carbamazepine dose. The subjects were randomly divided into four groups and each group received a single 200mg dose of each of the four medications in a different sequence. There was a twenty-one day washout period between doses. After an overnight fast, each of the subjects receive one of the products with 180ml of room temperature water. No food was allowed until a standard meal was served 4 hours after dosing. Ten milliliter blood samples were obtained just before doing and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 25, 49, 73, 97, 121, and 169 hours after dosing. Acceptable accuracy and precision was indicated by the quantitative analysis of triplicate fortified plasma quality-control samples containing 0.43, 1.6, and 3.1 ug/ml of carbamazepine along with each set of unknown plasma samples. The between day and within day coefficient of variation for these assays was < 10% and < 4% respectively.
The wide range of *in vitro* results are shown in Figure 2.2.4.1. Using the USP specification for carbamazepine (i.e. between 45% and 75% dissolved at 15 minutes and that not less than 75% of the drug be dissolved within 60min) it was shown that: 

1. products 2 and 4 fail the dissolution requirements;
2. product 1 meets the dissolution requirements;
3. product 3 meets the dissolution requirements despite being faster than product 1.

The respective range of *in vivo* results are shown in Figure 2.2.4.2. An analysis of the elimination rates between the study periods demonstrated no statistical difference. However, there were some statistical differences among the clearance rates between the products. Additionally, there were some statistical differences between some of the summary pharmacokinetic metrics across the different products. These differences are reported in Table (2.2.4.1.). In the discussion of the study the authors stated that the source(s) of
difference in performance was unknown. However, they referenced how moisture storage conditions have been demonstrated to result in dissolution and bioavailability changes (likely due to dihydrate conversion) and this may been a contributing factor in their work. Additionally, the authors stated that the manufacturer had suggested the cause to be related to a change in the source of the raw carbamazepine and/or changes in particle size of the active ingredient.

Table 2.2.4.1. Summary of Meyer et al. 1992 results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Product 1</th>
<th>Product 2</th>
<th>Product 3</th>
<th>Product 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (ug/ml)</td>
<td>1.89 (20)</td>
<td>1.15 (62)</td>
<td>2.69 (18)***</td>
<td>1.40 (39)***</td>
</tr>
<tr>
<td>$T_{max}$ (hr)</td>
<td>15.9 (51)†</td>
<td>13.6 (74)†,††</td>
<td>8.3 (72)††</td>
<td>19.6 (78)†</td>
</tr>
<tr>
<td>$AUC$ (ug · hr/ml)</td>
<td>134.8 (15)*</td>
<td>80.9 (48)***</td>
<td>154.2 (18)***</td>
<td>104.5 (30)****</td>
</tr>
<tr>
<td>$k_{el}$ (hr⁻¹)</td>
<td>0.0183 (15)***</td>
<td>0.0173 (19)††</td>
<td>0.0191 (16)††</td>
<td>0.0177 (16)††</td>
</tr>
</tbody>
</table>

Number in parenthesis is the coefficient of variation; %
Differences in the number of row-wise symbols denotes a statistically significant difference (p > 0.05)
Another study that investigated biononequivalence issues specifically related to differences in the side effect profile of immediate release carbamazepine products was performed by Olling et al. This work was performed in response to several publications over the years leading up to the work describing the occurrence of side effects after changing from one carbamazepine product to another.\textsuperscript{141-145} In different countries experiments were started to find some explanations for these reports.\textsuperscript{146-148} The studies seemed to indicate that between-product differences in the rate of absorption might be responsible for the occurrence of switch-related side effects.

The Olling et al. study, like the previous Meyer et al. work (1992), represents an important source of in vitro-in vivo data for carbamazepine. In this study, the subject sample was again taken from a normal healthy volunteer population (i.e. the results of routine laboratory tests on blood and urine of the volunteers were within normal ranges). Eighteen healthy, non-smoking volunteers, ranging in age from 20 to 38 years, weighing 49 to 88 kg were enrolled. Included in the study were three 200 mg carbamazepine products with large differences in compendially measured dissolution rates (Figure 2.2.4.3.), as well as the innovator product Pharmachemie, Lot no. 92 A 21 NF (product A), 200 mg Centrafarm, Lot no. 92 E 18A (product B), Pharbita, Lot no. 920401 (product C) and Tegretol\textregistered Ciba Geigy 200 mg, Lot no. 92 F 22 (product D). All of the products were licensed and were purchased from a hospital pharmacy.

The study protocol used volunteers that were currently not receiving medication. The volunteers were to abstain from alcohol use 24 hour prior to the first study day until the end of the study. The administration of the drugs was accomplished in a four-way randomized cross-over design with 2 week washout periods. Before administration the volunteers fasted
overnight. After administration of the products the volunteers were instructed to sit in an upright position for the first 4 hours. Standardized meals were given 4 and 10 hours postdose. Blood samples were taken just before dosing and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16, 20, 24, 28, 32, 48, 56, 72, 80 and 96 hours postdose and analyzed using a suitable HPLC method (i.e. linear over the range of 0.05–6 ug/mL plasma and the variability less than 10%).

The qualitative differences in the *in vitro* dissolution rates of the four products investigated were in the same order as the *in vivo* absorption rates after administration of the products to healthy volunteers.
All of the summary pharmacokinetic metrics obtained from product A are statistically significantly different (p < 0.05) from the reference product D. Product B did not show any significant difference from product D. For product C all pharmacokinetic characteristics

Figure 2.2.4.3. Dissolution (top) and plasma (bottom) profiles of four carbamazepine 200 mg products in 1% lauryl sulphate (Paddle method): product A (▽); product B (○); product C (▲); product D (●). Adapted from: Olling (1992)
except the AUC values are significantly different (p < 0.05) from product D. The summary pharmacokinetic metrics are contained in Table 2.2.4.2.

Table 2.2.4.2. Summary of Olling et al. 1999 results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Product A</th>
<th>Product B</th>
<th>Product C</th>
<th>Product D</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ug/ml)</td>
<td>3.2 (31)</td>
<td>5.9 (27)</td>
<td>6.1 (26)</td>
<td>4.5 (18)</td>
</tr>
<tr>
<td>$AUC$ (ug - hr/ml)</td>
<td>246 (25)</td>
<td>294 (29)</td>
<td>292 (24)</td>
<td>295 (20)</td>
</tr>
<tr>
<td>$t_{1/2,\text{abs}}$ (hr)</td>
<td>16 (63)</td>
<td>3.8 (184)</td>
<td>1.9 (263)</td>
<td>13 (62)</td>
</tr>
<tr>
<td>$t_{1/2,\text{el}}$ (hr)</td>
<td>39 (18)</td>
<td>34 (18)</td>
<td>32 (16)</td>
<td>34 (15)</td>
</tr>
</tbody>
</table>

Number in parenthesis is the coefficient of variation; %

The extent of absorption (i.e. $AUC$) of products B and C were well within the range of acceptance (0.8–1.20) for bioequivalence. With respect to the $C_{\text{max}}$ values, however, none of the test products were bioequivalent with Tegretol as the 90% confidence intervals are out of the 0.75–1.35 range. When Olling et al. examined the product-wise differences in they observed the measured side effects followed the same pattern for the total adverse events as for the of the $C_{\text{max}}$ values.

Carbamazepine was one of the medications investigated in a 2010 literature review performed by Desmarais et al. focusing on the development of clinical deterioration and decreased tolerability associated with switching from brand-name to generic psychotropic medications. The results of that work specific to carbamazepine are summarized in the following Table.
Table 2.2.4.3. Literature findings concerning Carbamazepine brand-generic switches

<table>
<thead>
<tr>
<th>Finding</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased seizures after generic substitution</td>
<td>150,151-154</td>
</tr>
<tr>
<td>Decreased levels after generic substitution</td>
<td>150,152</td>
</tr>
<tr>
<td>Toxicity and increased levels after generic substitution</td>
<td>155,156</td>
</tr>
<tr>
<td>Adrenal decompensation after generic substitution in a patient on hydrocortisone</td>
<td>157</td>
</tr>
<tr>
<td>90% CI of AUC of generic not within 80–120% of original.</td>
<td>158</td>
</tr>
<tr>
<td>Shorter average time to Cmax with generic</td>
<td>159</td>
</tr>
<tr>
<td>More neurological side effects with generic</td>
<td>160</td>
</tr>
<tr>
<td>Shorter mean time to Δ of medication, more central nervous system side effects with generic</td>
<td>161</td>
</tr>
</tbody>
</table>

Source for references [149]

While these studies were focused on clinical outcomes, a Spanish pharmacoeconomic study suggested that if 9% of epilepsy patients treated with original carbamazepine were switched to generic carbamazepine, annual per-patient cost would rise 38-fold due to the accidents, deaths, emergency visits, and days off work associated with the anticipated increased number of seizures.162

The AB status of Carbamazepine products requires that the *in vitro* and/or *in vivo* bioequivalence criteria has been satisfied. How then could the studies of table 2.2.4.3 be reconciled with this status? Consider how meeting compendial USP dissolution criteria is often used to justify the waiver of *in vivo* studies. This is done on the basis that the acceptable windows for the dissolution profiles are derived for classical *in vivo* trials for bioequivalence. However, the *in vitro* tests are still limited by the same the shortcomings of *in vivo* methods previously described. This may be a possible explanation for the cited findings, the interpretation of which suggests a disconnect between the Orange Book recommendations and best clinical practices.
2.2.5 Material Characteristics and Physical Chemistry

For decades, carbamazepine has served as a model compound for groups engaged in the study of crystal polymorphism. A portion of the seminal research article published by Grzesiak et al. focused on definitively identifying each of the four anhydrous polymorphs of carbamazepine. In that work they reexamined the reported data for the anhydrous polymorphs of carbamazepine in the literature. From that work, it was evident that early on the nomenclature of polymorphs was inconsistent, leading to confusion and misidentification of forms (see Table 2.2.5.1). Since then the most common approach to classifying the carbamazepine polymorphic forms is as follows:

- form I – triclinic cell
- form II – which is trigonal
- form III – P-monoclinic cell
- form IV – C-centered monoclinic polymorph (see Figure 2.2.5.1)
### Table 2.2.5.1. Nomenclature of the four carbamazepine polymorphs

<table>
<thead>
<tr>
<th>Year</th>
<th>Reference</th>
<th>Triclinic</th>
<th>Trigonal</th>
<th>P-Monoclinic</th>
<th>C-Monoclinic</th>
<th>Method of Confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1968</td>
<td>164</td>
<td>I</td>
<td>—</td>
<td>III</td>
<td>—</td>
<td>Melting behavior</td>
</tr>
<tr>
<td>1975</td>
<td>165</td>
<td>C₃</td>
<td>C₂</td>
<td>C₁</td>
<td>—</td>
<td>PXRD, IR</td>
</tr>
<tr>
<td>1981</td>
<td>166,167</td>
<td>—</td>
<td>Monoclinic</td>
<td>—</td>
<td>—</td>
<td>Crystal Structure</td>
</tr>
<tr>
<td>1984</td>
<td>168</td>
<td>III</td>
<td>II</td>
<td>I</td>
<td>—</td>
<td>PXRD, DSC</td>
</tr>
<tr>
<td>1984</td>
<td>169</td>
<td>III</td>
<td>II</td>
<td>I</td>
<td>—</td>
<td>PXRD, DSC</td>
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<tr>
<td>1986</td>
<td>170</td>
<td>I</td>
<td>II,IV</td>
<td>III</td>
<td>—</td>
<td>PXRD, DSC</td>
</tr>
<tr>
<td>1986</td>
<td>171</td>
<td>A</td>
<td>—</td>
<td>β</td>
<td>—</td>
<td>PXRD, DSC</td>
</tr>
<tr>
<td>1987</td>
<td>172</td>
<td>I</td>
<td>—</td>
<td>III</td>
<td>IIᵇ</td>
<td>PXRD, DSC</td>
</tr>
<tr>
<td>1987</td>
<td>173</td>
<td>—</td>
<td>α, Trigonal</td>
<td>β</td>
<td>—</td>
<td>Structure, PXRD, DSC, IR</td>
</tr>
<tr>
<td>1991</td>
<td>174</td>
<td>I</td>
<td>—</td>
<td>III</td>
<td>—</td>
<td>DSC, preparation</td>
</tr>
<tr>
<td>1991</td>
<td>175</td>
<td>Γ</td>
<td>α</td>
<td>β</td>
<td>—</td>
<td>Preparation</td>
</tr>
<tr>
<td>1992</td>
<td>176</td>
<td>I</td>
<td>—</td>
<td>III</td>
<td>—</td>
<td>IR, melting behavior</td>
</tr>
<tr>
<td>1996</td>
<td>177</td>
<td>I</td>
<td>—</td>
<td>III</td>
<td>—</td>
<td>PXRD</td>
</tr>
<tr>
<td>1997</td>
<td>178</td>
<td>Triclinic</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>PXRD</td>
</tr>
<tr>
<td>2000</td>
<td>179</td>
<td>—</td>
<td>α</td>
<td>β</td>
<td>—</td>
<td>PXRD, SEM</td>
</tr>
<tr>
<td>2000</td>
<td>180</td>
<td>I</td>
<td>—</td>
<td>III</td>
<td>—</td>
<td>PXRD, DSC</td>
</tr>
<tr>
<td>2000</td>
<td>181</td>
<td>I</td>
<td>—</td>
<td>III</td>
<td>IIᵇ</td>
<td>PXRD, DSC, IR</td>
</tr>
<tr>
<td>2002</td>
<td>182</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>IV, C-Monoclinic</td>
<td>Structure</td>
</tr>
<tr>
<td>2003</td>
<td>163</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td>XRD, DSC, IR</td>
</tr>
</tbody>
</table>

ᵃ Each form was verified by the listed method as well as the method of preparation of each form.
ᵇ These forms are most similar to C-mono clinic CBZ, however, differences noted in the text exist.

PXRD – powder X-ray diffraction; IR – infrared spectroscopy; DSC – differential scanning calorimetry; SEM – scanning electron microscopy

According to Grzesiak et al., the four forms are close in energy, the stability order at room temperature is: III > I > IV > II. Thermochemical data from Upadhyay et al. indicate an enantiotropic relationship between polymorphs III and I. As shown in Figure 2.2.5.1., free energy curves of carbamazepine polymorphs intersect at 360.5K, below the melting of both the forms. This confirms the enantiotropic relationship between them.
Form III (P-monoclinic) shows the lowest free energy of all forms at and below room temperature. Among the forms, Šehić et al. established that the anhydrous form III is the most commonly encountered form. The form II (trigonal) is the least stable of all anhydrous forms. Fast transformation of the trigonal form has made it very difficult to determine its melting point, and therefore the thermodynamic relationship of this polymorph to other anhydrous forms is still unclear.

All four polymorphs of carbamazepine share a common hydrogen bonding pattern resulting in a dimer with two amide–amide hydrogen bonds according to the Cambridge Crystallographic Data Centre. Observed polymorphism results from the alternative possibilities of packing these dimers into a stable crystal structure: forms I and II have similar

Figure 2.2.5.1. Configurational phase diagram of carbamazepine polymorphs. Adapted from Grzesiak et al. (2003)
packing of dimers, with offset π–π stacking of the aromatic rings as the main interaction between neighboring dimers. In forms III and IV aromatic rings form both π–π stacking and edge-to-face contacts in an interlocked packing arrangement (see Figure 2.2.5.1).186

![Figure 2.2.5.1. Packing diagrams of carbamazepine polymorphs: (a) form I, (b) form II, (c) form III, and (d) form IV (Cambridge Crystallographic Data Centre).](image)

While there is no officially released FDA specification for the reference polymorphic form of carbamazepine, a publication by L. Yu et al. provides some regulatory insight. The article was entitled *Regulatory considerations of pharmaceutical solid polymorphism in Abbreviated New Drug Applications (ANDAs)*, in it reviewed the impact of polymorphism on drug product manufacturability, quality, and performance. Carbamazepine was a particular example in this work. It was noted that “Approved ANDAs for carbamazepine
utilize the corresponding drug substance β-form [form III], presumably due to the unique solubility and processability characteristics associated with this form.” This will be an important concept when comparing the performance of different generic carbamazepine in later sections.

The dihydrate of carbamazepine has been studied in numerous publications.\textsuperscript{187-200} Like anhydrous polymorphs, there can exist different configurations of hydrates (i.e. stoichiometric or nonstoichiometric\textsuperscript{201,202}). To assess the potential for different hydrate forms of carbamazepine dehydrate, McMahon et al. characterized materials by thermal analysis (TGA and DSC) and spectroscopic techniques, including 13C solid-state nuclear magnetic resonance (13C SSNMR) and variable-temperature Fourier transform (FT) Raman spectroscopy.\textsuperscript{203} The thermodynamic behavior of the dihydrate obtained form III was different than the one produced from form I. However, water was readily lost from dihydrates III and I in a similar manner, when maintained isothermally at 25 °C in the TGA. Additionally, the powder X-ray diffraction patterns and spectroscopic data were almost identical between all of the hydrate formations. Therefore, the observed differences were hypothesized to be the result of trace anhydrous original polymorph existing in the dihydrate phase. This was proposed to act as a seed, regenerating the original polymorph under conditions where liberated hydrate water cannot readily escape from around the sample during dehydration. Thus, they concluded that there is no evidence supporting the existence of two different dihydrate forms. This supported the findings of Khoo and Harris et al that carbamazepine exists as a channel hydrate.\textsuperscript{204,205}

In addition to understanding the polymorphic forms and hydrate forms of carbamazepine, another important feature of a drug is its ionizability and octanol-water partition-coefficient
It is known from an examination of the chemical structure that carbamazepine does not contain any ionizable groups. It can therefore be considered a neutral compound. This is an important feature as it can inform the evaluation of dissolution methods. Additionally, according to the scientific literature, it is generally agreed that the logP of carbamazepine is approximately 2.2 suggesting high lipophilicity and permeability. Such knowledge supports the findings of Lee et al., where it was reported how carbamazepine is poorly soluble and its dissolution is independent of pH.206
2.3 QbD initiatives – PBPK Modeling and Simulation

Quality by Design (QbD) is a systemic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management to meet patient needs using clinical information and safety targets in the Quality Target Product Profile (QTPP). In a September 2017 speech Dr. Scott Gottlieb, at the very start of his tenure as FDA commissioner, stated how the FDA will continue taking new steps to modernize how sponsors can evaluate clinical information, and how FDA reviews these data as part of the regulatory process for efficient assurance of QbD. He stated how this enhanced review process will involve a more widespread use of modeling and simulation, and high performance computing clusters cross FDA review programs. These efforts take aim at one of the main challenges of efficient QbD drug development: translating \textit{in vitro} observations to \textit{in vivo} performance.

The role of predictive biopharmaceutic methods in QbD has been emphasized in recent conferences and workshops sponsored or co-sponsored by the FDA. Areas of focus include the following:

- the mechanisms of \textit{in vitro} release
- physiology in relation to drug absorption, and
- \textit{in silico} models that mimic \textit{in vivo} release characteristics

Leveraging such knowledge as a tool to facilitate the implementation of QbD is a key concern. The domain of physiologically based pharmacokinetic modeling unifies these concepts in a way that greatly facilities clinical trial simulation, allowing for quantitative
predictions through the mechanistic integration of prior knowledge. The growing commitment to increase the use of PBPK modeling and simulation on the part of industry sponsors and regulatory agencies is obvious in a review of the current literature. Researchers from the FDA’s Office of Clinical Pharmacology published an article in 2013 on the utility of modeling and simulation in drug development and regulatory review. The researchers looked at the 33 INDs/NDAs received between 2008 and 2012 containing PBPK modeling approaches as part of that work. Figure 2.3.1 shows the distribution of submitted INDs and NDAs containing physiologically based pharmacokinetic modeling and/or simulation over that time period.

![Figure 2.3.1](image)

**Figure 2.3.1.** The number of PBPK applications contained in IND/NDA submissions or developed by US FDA reviewers from 2008 to 2012. (Adapted from Huang et al. (2013))

Here the increasing support in PBPK modeling is visible by the increasing trend in PBPK utilization on the part of industry sponsors. Interestingly, the authors of this data stated that the paralleling of this practice by the agency has been de novo (i.e., US FDA initiated). They note that increased use physiologically based pharmacokinetics has been a help to the
regulatory review process by aiding the characterization of pharmacokinetics in a variety of complex clinical scenarios that would be unable to test using traditional methods in vivo. The clinical applications covered by the PBPK models in Figure 2.3.1 are broken down by topic in Figure 2.3.2.

![Figure 2.3.2. Areas of applications in the 33 PBPK submissions in IND/NDA received by US FDA’s Office of Clinical Pharmacology from 2008 to 2012. (Adapted from Huang et al. (2013) 211)](image)

It is observable in this Figure that, at the time of the study, the majority of PBPK applications were related to drug-drug interactions. The authors discussed two emblematic case studies where major drug development questions were answered using PBPK modeling. The first case was concerning the combined use of in vitro inhibition data and PBPK modeling and simulation to provide for a more focused performance of clinical drug-drug interaction studies.211 The second case used a similar extrapolation approach to test unstudied drug interaction scenarios. The work ultimately allowed for labeling which read, “There is no
clinically relevant effect of moderate CYP3A inhibitors on the pharmacokinetics of [the sponsor’s active ingredient].” The language was justified using in silico simulations thus sparing a lengthy and costly clinical drug-drug interaction trial. Other questions that can be addressed using PBPK modeling and simulations are summarized in Table 2.3.1.

Table 2.3.1 Summary of general regulatory questions addressed using PBPK modeling and simulations

<table>
<thead>
<tr>
<th>Main enzymatic pathways of drug; Interacting drug/substrate relationships</th>
<th>Regulatory questions addressed related to PBPK modeling and simulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>a CYP inhibitor in vitro (I/Ki &gt; 0.1)</td>
<td>The magnitude of DDI with a CYP substrate in vivo?</td>
</tr>
<tr>
<td>a CYP substrate</td>
<td>An in vivo DDI study with a CYP inhibitor has been conducted when NME was dosed orally. Can PBPK simulation predict the magnitude of DDI when NME is given intravenously?</td>
</tr>
<tr>
<td>a CYP substrate and also renally excreted</td>
<td>Can PBPK simulations predict the magnitude of DDI in subjects with varying degrees of renal impairment (mild, moderate, or severe)?</td>
</tr>
<tr>
<td>a CYP inhibitor in vitro (I/Ki &gt; 0.1) metabolized by multiple CYPs in the liver</td>
<td>Can PBPK simulations predict the magnitude of DDI with a CYP substrate in vivo? Can PBPK simulations predict the magnitude of DDI with CYP inhibitors? Can PBPK simulations predict PK in subjects with hepatic impairments?</td>
</tr>
<tr>
<td>a substrate of a polymorphic CYP in vitro</td>
<td>Can PBPK simulations predict the PK in extensive, intermediate, or poor metabolizers of this CYP?</td>
</tr>
<tr>
<td>a CYP substrate and an in vivo DDI study using a specific inhibitor dose has been done</td>
<td>Can PBPK simulations predict the magnitude of DDI using a different inhibitor dose as recommended by the FDA?</td>
</tr>
<tr>
<td>a CYP substrate and an in vivo DDI study has been conducted with a CYP inhibitor</td>
<td>Can PBPK simulations predict the magnitude of DDI with a CYP inducer?</td>
</tr>
<tr>
<td>a TDI of a CYP metabolized in the liver</td>
<td>Its single-dose PK data are available: Can PBPK simulations predict dose- and time-dependent PK after multiple dosing? Can PBPK simulations predict TDI in vivo?</td>
</tr>
<tr>
<td>metabolized by multiple CYPs</td>
<td>In vivo data are available in hepatically impaired subjects taking lower than recommended doses of NME. Can PBPK simulations predict PK of NME in hepatic impairment patients taking recommended doses?</td>
</tr>
<tr>
<td>metabolized by multiple CYPs</td>
<td>Can PBPK simulation be used to predict fractional metabolism based on enzyme kinetic studies in vitro?</td>
</tr>
<tr>
<td>NME’s adult PK data are available</td>
<td>Can PBPK simulations help determine the optimal doses for pediatric studies?</td>
</tr>
<tr>
<td>NME and its meta. both inhibitors of a CYP</td>
<td>Can PBPK simulation predict the DDI potential of the NME?</td>
</tr>
</tbody>
</table>

CYP, cytochrome P450; DDI, drug–drug interactions; FDA, US Food and Drug Administration; NME, new molecular entity; PBPK, physiologically based pharmacokinetic; PK, pharmacokinetic; TDI, time-dependent inhibitor. (Table adapted from Zhao et al (2011))
2.4 PBPK Modeling and Simulation – Technical Components

2.4.1 Fundamental differences between PBPK modeling and classical PK modeling

A spectrum of unique approaches exist for learning the clinical pharmacology of a drug, via pharmacokinetic modeling. The classical approach to pharmacokinetic model, also known as a “top-down” model, is constructed using clinical data with model parameters and covariance estimated statistically. Typically there is a central compartment representing plasma that can be linked to one or more peripheral compartments via rate constants. When defined in terms of rate constants, the model parameters do not generally have any physiological meaning but can be used to provide more interpretable pharmacokinetic descriptors such as systemic clearance and volume of distribution. Clearance in classical pharmacokinetic modeling refers to the volume of plasma cleared of drug per unit time via metabolic or excretion processes. The volume of distribution refers to the volume required to occupy the total amount of drug in the body at the concentration observed in plasma. While useful for the concise and standardized representation of both the preclinical and clinical experimental results, classical methods have limitations. These approaches do not readily incorporate population-level covariate information from either the drug or physiology \textit{a priori}.  

Covariates are accounted for in classical population-level pharmacokinetic modeling through an observational top down process known as nonlinear mixed effects (NLME) modeling. An exhaustive explanation of NLME methods is beyond the scope of this dissertation, therefore a brief summary of the main methods will be discussed. The “mixed effects” in NLME is so called because in the modeling procedure some parameters are
assigned to vary across individuals and are considered random effects while others are constant and considered fixed effects. There are essentially three functional components of a NLME model: i) the structural model, ii) the statistical error model, and iii) the covariate model. The structural model describes the typical concentration time course within the population. The statistical model accounts for random variability (residual error) in concentration within the population. The covariate model explains variability predicted by subject characteristics (covariates). Nonlinear mixed effects modeling software (e.g. NONMEM, Phoenix NLME) brings dependent/independent data together with models and applies an estimation method for parameterization for the structural, covariate, and statistical residual models that describe the data.216

To illustrate an NLME method consider the following single-compartment intravenous-bolus plasma concentration model:

\[ C_j = \frac{D}{V_d} \exp\left[-\frac{C_l \cdot t_j}{V_d}\right] \]

where \( C_j \) is the mean plasma concentration at time \( j \) from a population, \( D \) is the dose at \( t = 0 \), \( V_d \) is the volume of distribution for the population, and \( C_l \) is the clearance for the population. If one was to assume that the \( C_l \) changes randomly between the individuals from the study population and \( V_d \) was fixed, the NLME modeling would begin by using the following equation:

\[ C_{ij} = \frac{D}{V_d} \exp\left[-(C_l + n_i) \cdot t_{ij} \right] \]

where \( n_i \) is the deviation of the \( i^{th} \) subject’s clearance from the mean \( C_l \) for the population. Accounting for the residual error of this model is accomplished by addition of the residual
model which can be expressed additively, proportionally, exponentially, and through a combination thereof in the following ways, respectively:

\[
C_{ij} = \frac{D}{V_d} \exp \left[ \frac{-(Cl + n_i) \cdot t_{ij}}{V_d} \right] + \varepsilon_{ij}
\]

\[
C_{ij} = \frac{D}{V_d} \exp \left[ \frac{-(Cl + n_i) \cdot t_{ij}}{V_d} \right] \cdot (1 + \varepsilon_{ij})
\]

\[
C_{ij} = \frac{D}{V_d} \exp \left[ \frac{-(Cl + n_i) \cdot t_{ij}}{V_d} \right] \cdot \exp(\varepsilon_{ij})
\]

\[
C_{ij} = \frac{D}{V_d} \exp \left[ \frac{-(Cl + n_i) \cdot t_{ij}}{V_d} \right] \cdot (1 + \varepsilon_{1,ij}) + \varepsilon_{2,ij}
\]

A covariate model can be added to this equation to describe various clearance-patient relationships (e.g. \(Cl = f(\text{age}, \text{weight}, \text{disease status}, \text{etc.})\)) in the following way:

\[
C_{ij} = \frac{D}{V_d} \exp \left[ \frac{-(\theta_1 + \theta_{k,i} x_{k,i} + n_{k,i}) \cdot t_{ij}}{V_d} \right] + \varepsilon_{ij}
\]

where \(Cl\) is a linear function of \(k^{th}\) clinical factor \(x\) specific to the \(i^{th}\) subject with intercept \(\theta_1\) and slope \(\theta_{k,i}\). Here, \(n_{k,i}\) now represents the deviation of subject \(i\) from the population mean of \(x_{k,i}\).

The approaches for estimating coefficients of covariates and pharmacokinetic models have undergone several evolutions since the early NLME modeling efforts. Most share the concept of parameter estimation based on minimizing an objective function value (OFV). The OFV often uses maximum likelihood estimation and is expressed as minus twice the log of the likelihood describing how closely the model predictions (given a set of parameter values) match the data (maximum likelihood = lowest OFV = best fit).
In population modeling, calculation of the likelihood comes with an analytical challenge. When fitting population data, predicted concentrations for each subject depend on the difference between each subject’s parameters ($P_i$) and the population parameters ($P_{pop}$) and the difference between each pair of observed ($C_{obs}$) and predicted ($c^*$) concentrations. Therefore, a marginal likelihood needs to be calculated based on both the influence of the fixed effect ($P_{pop}$) and the random effect ($\eta$). The challenge is that analytical solutions for the marginal likelihood do not exist. Thus, several methods have been developed for approximating the marginal likelihood while searching for the maximum likelihood. Initial methods were generally identified as first-order (FO) methods. The first was developed by Sheiner and Beal and was based on a “first order” Taylor expansion of the pharmacokinetic model under the assumption that all $n_{k,i} = 0.217$. Since then, Lindstrom and Bates developed a similar method; however, their approximation included the conditional estimates of $n_{k,i}$ and is referred to as the First Order Conditional Estimation (FOCE) method. The FOCE method is, in general, the most common method used in NLME estimation for population pharmacokinetic modeling. However, with the advent of multi-core computer processors, high computational demand methods yielding more stable parameter estimates from sparser data, such as the Quasi-Random Parametric Expectation Maximization method, are becoming more efficient. The advantages of these methods is that they follow a Bayeian framework, and, as such, can provide posterior estimates of the covariates and parameters with associated variance estimates. These variance estimates can be used in IVIVC simulations as demonstrated by Bondi, Bigora et al., O’Hara et al., Soto et al., and others.
Sound statistical practice suggests the addition of covariates should be based on some statistical criteria, such as Akaike Information Criterion (AIC), Bayes Information Criterion (BIC), p-values, log likelihood, etc.; significant covariates will lead to a reduction in the inter-subject variability (ISV) and residual variability. The error model should be selected to preserve homoscedasticity, which can be assessed based on a plot of the residuals versus the predicted plasma concentrations for the case presented. For more information on NLME and how it relates to population pharmacokinetic modeling, see the comprehensive three-part tutorial by Mould and Upton.

A fundamental limitation of NLME modeling for the classical estimation of population level covariates and pharmacokinetic parameters is that they can only be derived after the study has been performed, and critical parameters such as the elimination rate can only be resolved to units of inverse time. Thus, the ability to predict the pharmacokinetics of a similar drug, or extrapolate the pharmacokinetics to a different physiology \textit{a priori} is significantly restricted. Unlike this “top down” modeling, a physiologically-based pharmacokinetic modeling is considered “bottom-up.” This is because it uses information from \textit{i}) human physiology/pathophysiology, and \textit{ii}) detailed biological processes and interactions to mechanistically describe drug absorption, disposition, and elimination. While this type of pharmacokinetic modeling can be parameterized using the same computational methods as with the classical method, the compartments of the physiologically-based structural model are now based on different organs and tissues in the body. These compartments are then connected by tissue flow rates that are a function of cardiac output. While these models can still provide classical pharmacokinetic parameters (see previous paragraph), the means by which this is achieved is more translatable. The very units of the parameters provide a clear
way by which a dose in healthy volunteers can be extrapolated to one in a clinically relevant population, so long as the relevant physiological properties of the target population are available. Developing a pathophysiologic understanding at the level of quantitative systems pharmacology is becoming more and more the prelude to the modern drug discovery process.

The principles used for physiologically based pharmacokinetic modeling are not new. The use of differential equations parameterized using known physiological variables to represent a quantitative mechanistic framework by which the absorption, distribution, metabolism, and excretion (ADME) of drugs may be modeled can be traced back to as early as 1937. At that time, Teorell used multi-compartmental models to integrate biological and physiological components for the simulation of pharmacokinetic data. What has changed since that time is computer technology, an improved understanding of human physiology/pathophysiology, and an increased availability of highly representative *in vitro* systems which can act as surrogates for *in vivo* reactions relevant to ADME through a process known as *in vitro in vivo extrapolation* (IVIVE). The performance of such *in vitro* trials are typically termed distribution-metabolism pharmacokinetic (DMPK) studies. Advancements in the domain of DMPK studies have grown exponentially since sub-optimal DMPK properties were recognized approximately 25 years ago as a major contributor to the failure of potential new therapies in early clinical trials. Such advancements were paralleled by database innovations and evolutions in computational technology. This provided a greater ability to integrate these studies within physiologically based pharmacokinetic modeling framework for the performance of computationally demanding simulations.

Proponents of physiologically-based pharmacokinetic modeling believe that learning the clinical pharmacology of a drug, and developing a model to describe it, should not start with
the first clinical study. Rather, it is suggested to use *in vitro* tests, quantitative structure-activity relationships (QSARs), and physiologically based models to specifically define the details of what *in vivo* studies are absolutely necessary and in doing so work to eliminate studies that are doomed to fail. Leading researchers within the FDA’s Office of Clinical Pharmacology have published a perspective article on best practices when using physiologically based pharmacokinetic modeling and simulation to address clinical pharmacology regulatory questions. In the article, the discussion is based on the “predict-learn-confirm” paradigm for the physiologically-based population pharmacokinetic modeling in an optimized drug development process (see Figure 2.4.1.1). These cycles are based on screening lead compounds into candidate drugs by determining potency against the target, physicochemical properties, and basic *in vitro* data such as metabolic stability and cytochrome P450 inhibition assessment. Subsequent *in vitro* studies can be aimed at establishing, and if necessary optimizing, *in vivo* efficacy so that pivotal *in vivo* trials can be performed with better quality compounds and subsequent clinical questions can be answered with greater certainty and efficiency.
Figure 2.4.1.1 “Predict-Learn-Confirm” Paradigm for Physiologically Based Population Pharmacokinetic Modeling

- Model construction
  - Start from discovery and first in human predictions

- Model Refinement
  - Further experimental data
  - Estimation of parameters based on clinical data
  - Sensitivity analysis of uncertain parameters

- Model’s recovery of clinical data

- Model application to a specific question

Adapted from Suri et al (2015)
2.4.2 Functional Components of a PBPK Model

As stated in the previous section, physiologically based pharmacokinetic models use differential equations parameterized using known physiological data to represent a quantitative mechanistic model structure by which the ADME of drugs may be modeled. The availability of networked, open source data libraries has greatly facilitated the performance of physiologically based pharmacokinetic simulations. The data that feed into a physiologically based pharmacokinetic model can be classified as either drug-dependent or drug-independent. Drug-independent data can also be called the system components of the model. These components are defined by population level estimates of the basal physiology and pathophysiology of the in vivo study subjects. Some examples of these components include tissue flow rates, organ volumes, and gut segment transit times. Huang et al. discussed how these drug-independent components are shaped by intrinsic and extrinsic patient factors. Some examples of intrinsic patient factors included age, allometric correlations, race, organ dysfunction, disease, pregnancy, gender, and genetics. In the context of PBPK modeling, researchers have databases at their disposal that describe the drug independent parameters of populations with the following conditions: health-impaired elderly, pediatric, pregnancy, obesity, comorbid diseases such as cirrhosis and chronic kidney failure, and smoking. These data, often collected from rigorously conducted federal health interview studies (e.g., NHANES, NHIS, EHIS, etc.) have allowed the covariance between age, gender, height, weight and other anthropomorphic values to be modeled by researchers. Data libraries based on meta-analysis of these reports are maintained by many PBPK software providers (so called ‘population libraries’). These libraries provide the means by which the required drug independent parameters can be generated using correlated Monte Carlo
sampling techniques to effectively generate *in silico* patients. These databases have been integrated into modeling efforts to account for changes in hepatic blood flow, CYP abundance, liver volume, hematocrit, and liver/renal function as a function of disease or age, for the prediction of human pharmacokinetics in atypical subpopulations.\textsuperscript{238,233,234} This practice occupies the growing domain of clinical trial simulation research.\textsuperscript{239} The specifics of such methodologies in the context of this work will be introduced in subsequent sections.

While understanding the physiologic factors that influence key ADME mechanisms for a particular compound is important, the ultimate success of a prediction also requires well-defined and well-measured drug dependent parameters. The drug-dependent parameters of a physiologically based pharmacokinetic model can be broken down into pharmacologic and physical categories. The pharmacologic drug dependent parameters define values such as permeability, intrinsic clearance of drug on a per enzyme basis, protein binding, etc. For carbamazepine, some of the important drug-dependent ADME properties and methods for their determination have been discussed in section 2.2.

After conversion to relevant units, *in vitro* values can directly be used as inputs for physiologically based mechanisms, in conjunction with extrinsic factors (e.g., blood-binding data), to parameterize specific pathways such as clearance. Here, assumptions of the pathway define how the data is used. In the case of clearance from liver blood flow, for example, Poulin et al. showed how well-stirred models can be used to predict clearance based on a combination of *in vitro* drug dependent data and *a priori* knowledge of human physiology.\textsuperscript{240} This work was based on the publication of Proctor et al. In this foundational work, a model was developed for how whole organ intrinsic drug clearance may be predicted from recombinantly expressed CYPs (*r*\textsubscript{h}CYP) based on the works of Houston et al and Iwatsubo.
et al. 241-243 The specific case of predicting liver clearance can be accomplished using the following equation:

\[ \text{Eq. 2.4.2.1} \]

\[
CL_{u, \text{int (unbound)}} = \left( \sum_{p=1}^{n} \sum_{e=1}^{m} \frac{ISE_{p,e} \times V_{\text{max,p,e(vitro)}} \times Enz_{L,p,e}}{K_{m,p,e}} \times \frac{1}{f_{\text{inc}}} \right) \times MPPGL \times \text{Liver weight}
\]

where, ISEF (intersystem extrapolation factor) is a dimensionless number used as a direct scaler to convert data obtained with a \( rhCYP \) system, \( V_{\text{max,p,e(vitro)}} \) is the Michalis-Menten rate constant representing the maximum velocity of the \textit{in vitro} metabolism assay incubation for the \( p^{th} \) clearance pathway of the \( e^{th} \) enzyme isoform, \( Enz_{L,p,e} \) is the percent abundance, \( K_{m,p,e} \) is the Michalis-Menten rate constant representing the concentration that corresponds with \( 0.5 \cdot V_{\text{max,p,e(vitro)}} \), \( f_{\text{inc}} \) is the fraction unbound in the metabolism assay incubation, and MPPGL is the microsomal protein per gram of liver. The ISEF is calculated by the following equation,

\[ \text{Eq. 2.4.2.2} \]

\[
ISE_{p,e} = \frac{CL_{\text{int (HLM)}}}{CL_{u, \text{int,p,e(rhCYP)}} \times CYP \text{ abundance(HLM)}}
\]

where \( CL_{\text{int (HLM)}} \) is the intrinsic clearance in human liver microsomes, \( CL_{u, \text{int,p,e(rhCYP)}} \) is the intrinsic clearance of the recombinant enzyme system, and \( CYP \text{ abundance(HLM)} \) refers to the abundance of the \( e^{th} \) CYP in the liver sample(s) used to determine the ISEF. Proctor et al. described how the application of ISEFs in IVIVE exist along three levels, reflecting a balance between confidence in extrapolation and high-throughput compatibility. 242
Poulin et al. used the ISEF framework of Proctor et al. to model hepatic metabolic clearance of highly bound drugs. The researchers evaluated the performance of this IVIVE methodology and others using a data set of 25 compounds which including acidic, basic, and neutral model drugs. The ability to predict in vivo clearance from in vitro data is shown in Figure 2.4.2.2. Comparison between predicted and observed human clearance in this Figure returned a high correlation $R^2 = 0.95$. 

**Figure 2.4.2.1.** The incorporation of ISEFs into in vitro-in vivo extrapolation. HTS – high throughput system. Adapted from Proctor (2012)
Similar to that of Proctor et al., Jamei et al. showed how a IVIVE methodology could be used for predicting the effective permeability of the gastrointestinal tract. Jamei et al. then demonstrated how this prediction could be combined with an understanding of gastric transit times, gastrointestinal physiology/pathophysiology, and assumptions of gastrointestinal fluid dynamics to achieve a mechanistic prediction of population-level of oral drug absorption.

There is an extensive range of drug-specific and physiologic parameters which can be defined during drug development and used for the mechanistic prediction of pharmacokinetic model parameters. Although, not all parameters are needed in all circumstances depending on both the simulation mode and the models chosen. For example, the tissue:plasma partition coefficient required by a physiologically based pharmacokinetic models to describe the accumulation of drug within a specific organ would require data that is not needed for the one compartment distribution model. A list of some of the typical names, values (and units), source of the parameter values, and assumptions being made are provided in Table 2.4.2.1.

**Figure 2.4.2.2.** The solid line indicates the best fit (unity). Dashed lines on either side of unity include a factor of two and three. Cross (red), circles (green), and squares (blue) indicate bases, neutrals, and acids, respectively. CL - clearance (mL/(min kg)). Adapted from Poulin et al (2012)
### Table 2.4.2.1. Sample Input Parameters of Drug for Physiologically Based Pharmacokinetic Modeling

<table>
<thead>
<tr>
<th>Sample parameter</th>
<th>Sample assumptions and references</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound type</td>
<td>Summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>Summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>LogP</td>
<td>Measured value. Summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>pKa</td>
<td>NA (neutral compound)</td>
</tr>
<tr>
<td>Solubility (mg/ml)</td>
<td>Measured at pH 7.4. Summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>Particle size radius (mm)</td>
<td>Monodispersed. Summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>B/P ratio 1</td>
<td>Measured value from <em>in vitro</em> experiments. Summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>fp</td>
<td>Measured value from <em>in vitro</em> experiments. Summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>fu,gut</td>
<td>Assumed and tested plausible using sensitivity analysis</td>
</tr>
<tr>
<td>Fa</td>
<td>Predicted using Caco-2 Papp data; assumed first-order absorption kinetics</td>
</tr>
<tr>
<td>Ka (l/h)</td>
<td>Estimated from single-oral-dose study in young adults using compartmental analysis; summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>Qgut (l/h)</td>
<td>Predicted using the software’s built-in method</td>
</tr>
<tr>
<td>Papp</td>
<td>Measured value using Caco-2 cell lines; summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td><em>In vitro</em> transporter</td>
<td>Measured value <em>in vitro</em>; not an inhibitor of P-gp; not a substrate and inhibitor of other transporters; summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>P-glycoprotein (P-gp)</td>
<td><em>In vitro</em> CYP inhibition:</td>
</tr>
<tr>
<td>Jmax (pmol/min)</td>
<td>Measured value <em>in vitro</em>; not an inhibitor of major CYP other than CYP2D6 (see below); not a CYP inducer; summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>Km (mM)</td>
<td>Measured value from <em>in vitro</em> experiment using human liver microsomes; summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>Predicted Vss (l/kg)</td>
<td>Predicted using the software’s built-in method; observed mean value from the i.v. study in young adults = 3.5 l/kg</td>
</tr>
<tr>
<td>CLi.v. (l/h)</td>
<td>From i.v. study in young adults; summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>CLR (l/h)</td>
<td>From i.v. study in young adults; summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>CYP3A4 CLint</td>
<td>Retrospectively calculated using CLi.v. and well-stirred hepatic clearance model, assuming 80% of total hepatic metabolism; summary of clinical pharmacology studies in NDA submission</td>
</tr>
<tr>
<td>CYP1A2 CLint</td>
<td>Retrospectively calculated using CLi.v. and well-stirred hepatic clearance model, assuming 20% of total hepatic metabolism; summary of clinical pharmacology studies in NDA submission</td>
</tr>
</tbody>
</table>

B/P ratio, blood-to-plasma ratio; CLint, intrinsic clearance; CLi.v., clearance after intravenous administration; CLR, renal clearance; Fa, fraction absorbed; fp, fraction unbound in plasma; fu,gut, apparent unbound fraction in enterocytes; IC50, inhibitor concentration that causes 50% inhibition of enzyme/transporter activities; i.v., intravenous; Jmax, maximum rate of transporter-mediated efflux or uptake; Ka, first-order absorption rate constant; Ki, reversible inhibition constant; Km, Michaelis constant; LogP, logarithm of the octanol–water partition coefficient; NA, not applicable; NDA, new drug application; Papp, apparent passive permeability; pKa, logarithmic acid dissociation constant; Qgut, hypothetical blood flow term that is used to complex interplay among passive intestinal permeability, active transport, enterocyte drug binding, blood flows to enterocytes, and gut metabolism; Vss, volume of distribution at steady state.

Adapted from Zhao et al (2012) 214
The work of Zhao et al. visually summarizes the structure of data-types and equations within a PBPK modeling framework. In that regulatory review article, members of the FDA’s Office of Clinical Pharmacology used the frameworks Huang et al. and others to describe the drug-dependent and drug-independent components of physiologically based pharmacokinetic modeling applications. Sections a. and b. of Figure 2.4.2.3. represent the work’s summative adaptation of the previous two efforts.

**Figure 2.4.2.3. a and b.** Relationship between the proposed physiologically based pharmacokinetic model and extrinsic/intrinsic factors that impact drug exposure (a) Intrinsic and extrinsic patient factors to be accounted for during simulations. (b) Components of the PBPK model. Illustration modified from Huang et al (2008)
2.4.3 IVIVC-based Clinical Trial Simulation

Clinical trial simulations represent an approach to answer deterministic questions by accounting for the inherent stochastic variability of biology via random sampling algorithms. This iterative approach generates a trial population *in silico* with inter-individual differences via Monte Carlo sampling from user-defined distributions. The name for such sampling was coined by Stanislaw Ulam and John von Neumann in 1945 for the gambling capital in Europe (Ulam’s uncle apparently liked to gamble and this was his inspiration) while in Los Alamos, New Mexico working on the Manhattan Project (development of the atomic bomb) (Metropolis 1987). If the model were $Y = x + \varepsilon$ where $\varepsilon$ is a random draw from some probability density function (PDF) then the process considered is deterministic if when performed repeatedly the outcome converges to a reproducible outcome.

The use of Monte Carlo simulation can also be performed during classical NLME modeling of population pharmacokinetics. Bondi used NLME to define the coefficients of relevant patient covariates in an IVIVC model. He then resampled from their posterior levels of uncertainty to assess the performance of different delayed release products. A key advantage of physiologically based pharmacokinetic models is the ability to include sources of physiological and biochemical variability in the system parameters and to simulate the expected pharmacokinetics in a population of individuals rather than for an average subject. A virtual population can be generated from values and formulae describing demographic, anatomical, and physiological variables using a correlated Monte Carlo approach. Equations describing distributions of system parameters for the physiologically based pharmacokinetic model are derived from distributions of data based on real populations and patients. This allows prediction of variability before clinical studies in contrast to a NLME approach.
(population pharmacokinetic analysis), which requires prior clinical data to characterize variability. Being able to assess variability in a population is particularly important when considering the risk, as it is usually a few individuals with certain characteristics that are of more concern than the average individual.

In the case of physiologically simulated hepatic clearance, individualized liver weights are generated by first randomly sampling from a user defined distribution of ages and genders. Next, a sample is drawn from marginal age-vs.-gender-vs.-height-vs.-weight probability distribution. This distribution is defined by the results of rigorously conducted federal health interview studies (e.g. NHANES, NHIS, EHIS, etc.). Body surface area (BSA) is then calculated from this information using the following equation:

\[
BSA \left( m^2 \right) = \sqrt{\frac{Ht.in \; cm \times Wt.in \; kg}{3600}}.
\]

With BSA determined, liver volumes can be individualized based on the work of Johnson et al. In this work, equations were developed based on simple regression against BSA and multiple regression of liver volume with weight, height, BSA, age, gender, race, methodology, and year of publication as covariates. The equation to describe liver volume was selected according to the AIC, precision and bias and following visual inspection of residual errors and observed vs. predicted plots:

\[
Liver \; volume = 0.722 \times BSA^{1.176}
\]

These coefficients represent the best, meta-level fit of the dataset in the Johnson et al study. The simulation process would be able to create individualized estimates of these values based on the reported levels of uncertainty from that study. The fit of this liver volume vs. BSA model for the data is shown in Figure 2.4.3.1. Each individualized liver volume is then
multiplied by a sample from a probability density function describing liver densities. This parameter sampling process can then be repeated until Equation 2.4.2.1 and its components are fully parameterized for a single *in silico* patient.

![Graph](image.png)

**Figure 2.4.3.1.** The model fit for liver volume vs body surface area described by Johnson et al. Adapted from Johnson et al (2005)²⁵⁴

The number of *in silico* patients is usually defined by the objective of the simulation study and the variability in the parameters. Greater variability requires more patients to reach a stable estimation of the pharmacokinetic metric of interest.

By its nature, a physiologically based pharmacokinetic model is based on the first principles of human physiology. This allows the developers of such models to uniquely leverage the findings of high throughput *in vitro* DMPK studies that now are the foundation of preclinical drug development efforts. More and more sponsors have been able to successfully account for population variability when considering the risk associated with drug-drug interactions through the performance of simulations over these models. It is the hope of this work to illustrate how the utility of a physiologically based pharmacokinetic modeling and simulation can and should be extended beyond the preclinical and clinical
phase of drug development, existing as a post-approval tool that iteratively refines itself to facilitate the lifecycle management process.

Chapter 3: Development of an IVIVC Framework for Immediate Release Carbamazepine Using a “Top Down” Approach

3.1 Introduction

A strong link between the surrogates of in vivo performance and clinical response is essential for the efficient assurance of quality. At the center of many definitions for pharmaceutical quality is the concept of consistently meeting the needs of the patient. The CFR states that one of the most preferred methods for the assurance of consistent performance is the use of in vitro dissolution tests that have been correlated with in vivo performance. There are three in vitro-in vivo correlation (IVIVC) levels according to a FDA guidance documents: A, B, and C. The levels differ by the amount of detail output by the correlative model. For example, a Level C IVIVC draws a relationship between an amount dissolved at a given time and summary pharmacokinetic parameters such as the area under the curve (AUC), maximum plasma concentration, or the time of the maximum plasma concentration. This level does not reflect the complete shape of the plasma concentration-time profile. Similarly, a level B IVIVC does not uniquely reflect the actual in vivo plasma level curve. It uses the principles of statistical moment analysis to regress an amount dissolved at a particular time against a pharmacokinetic metric such as the mean residence time. A level A IVIVC predicts the entire in vivo time course from the in vitro data. This level of IVIVC has been identified as the most informative by the FDA and other health authorities. In this chapter, all future
references to an IVIVC made without ‘level’ specification should be considered as level A IVIVCs.

The model drug product for this work is an immediate release Carbamazepine tablet. Carbamazepine is considered as a first line therapy drug for the treatment of partial and tonic-clonic seizures. One of the reasons for carbamazepine’s efficacy is its high permeability, allowing it to readily diffuse across the blood brain barrier. This is the product of carbamazepine’s lipophilic structure, a characteristic that also translates into poor aqueous solubility. The high permeability of carbamazepine, along with its low solubility in aqueous systems, position it within the second class (II) of the FDA’s biopharmaceutical classification system (BCS) based on the work of Amidon et al. In that work, the likelihood of an IVIVC for class II compounds was favorable. This expectation of Amidon et al. was a result of dissolution of such compounds often being the rate-limiting step for the absorption.

Given the complexity of pharmaceutical drug products, health agencies do not define a unified method for how an IVIVC should be developed. Regulators do acknowledge that in order to maximize the utility of an IVIVC, certain bespoke modeling decisions must be made. Despite the reality of such individuality, certain core IVIVC components do exist that are common between applications. Specifically, all level A IVIVCs can be considered as hierarchical in nature with the overarching framework being a pharmacokinetic model. Pharmacokinetic modeling seeks to take a complex phenomena, such as the rise and fall of a drug substance’s plasma concentration, and describe it as a function of parameters within a mathematical model. Models can be classified into many different categories. Using the nomenclature of
DiStefano and Landaw, pharmacokinetic models can generally be broken down into two types: *models of data* and *models of systems*. In a pharmacokinetic *model of data*, the model parameters are derived from, and fitted to, available clinical data. In the pharmacokinetic literature, this approach has grown to be referred to as ‘top down’ pharmacokinetic modeling. This type of pharmacokinetic modeling will be the approach used to the develop the IVIVC in this chapter.

In addition to the pharmacokinetic model, an IVIVC cannot exist without a model that transforms *in vitro* drug release data into an *in vivo* input for the defined pharmacokinetic model. Generally, the approaches for deriving this model are classified as either one- or two-step procedures. A two-step procedure was used in this work. The typical two-step procedure is based on a deconvolution-convolution process. The classical approach to this method is to derive an absorption profile that, when input to the pharmacokinetic model, recovers the observed plasma concentration for each drug product used in the development of the IVIVC. For a typical one compartment open model, this input profile may be derived using either the standard Wagner Nelson method or the method of residuals. Once the respective *in vivo* input profiles are determined, they are pooled and regressed against the *in vitro* release rates. Thus, subsequent *in vitro-in vivo* transformations can be performed using this model. This chapter will take a parallel approach to such a methodology. It will be based on constructing a model that relates the parameters that define the rate and extent of absorption within a pharmacokinetic model to those that define the rate and extent of *in vitro* dissolution model.

This chapter is focused on the first specific aim of this work: the development of an IVIVC for an immediate release carbamazepine product using a classical
pharmacokinetic model. It begins by describing the methodology behind how a level A IVIVC for immediate release Carbamazepine was developed. The chapter identifies the \textit{in vitro-in vivo} data that were ultimately used during modeling activities. It then goes on to describe the types of models that were used to represent the data and how the accuracy of the models was assessed. The chapter concludes by presenting and discussing the performance of the IVIVC.

\section*{3.2 Methods and Materials}

\subsection*{3.2.1 \textit{In vitro-In vivo} Data}

The data for this study was collected from 1992 and 1998 publications by Meyer et al. and one previous work by Olling et al. Data was digitized using an open source tracing software. All of the studies were 4-by-4 crossover studies. Table 3.2.1.1 describes the type of demographics, protocol details, type of \textit{in vitro} experiments, and the levels of \textit{in vitro} and \textit{in vivo} results. In this work, the 1992 Meyer et al and Olling et al. data was used to train the IVIVC model. The 1998 Meyer et al. data was used to test the model.
## Table 3.2.1.1 Details of in vivo studies for IVIVC model development

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subjects</strong></td>
<td>24 healthy, nonsmoking males; 21-35 y.o.; weighing 61-93kg</td>
<td>18 healthy; nonsmoking volunteers, 20-38 y.o.; weighing 49-88 kg</td>
<td>20 healthy, nonsmoking subjects; 16 males and 4 females; 22-36 y.o.; weighing 50-98 kg</td>
</tr>
<tr>
<td><strong>Protocol</strong></td>
<td>Medication / Alcohol Abstinence: 21 / 2 days Products: Tegretol® and 3 lots from a marketed generic Study Design: • Randomized 4X4 • single, overnight fasted, 200mg dose with 180 ml of room temp water • 21d washout between treatments • Standard meal 4 hrs after dose</td>
<td>Medication / Alcohol Abstinence: “no active therapies” / 1 day Products: Tegretol® and 3 marketed generics Study Design: • Randomized 4X4 • double, overnight fasted, 200mg dose with 150 ml of room temperature water • 14 day washout between treatments</td>
<td>Medication / Alcohol Abstinence: 21 / 2 days Products: Tegretol® and 3 marketed generics Study Design: • Randomized 4X4 • single, overnight fasted, 200mg dose with 180 ml of room temp water • 21d washout between treatments • Standard meal 4 hrs after dose</td>
</tr>
<tr>
<td><strong>Level of in vivo results</strong></td>
<td>Entire plasma concentration profile with variance estimates on AUC, Cmax, Tmax, elimination rate</td>
<td>Olling = over 100 hours Meyer 92’= over 168 hours</td>
<td>Meyer 98’= over 168 hours</td>
</tr>
<tr>
<td><strong>In vitro experiments</strong></td>
<td>USP Method: type II apparatus; 75 rpm; 900ml of water containing 1% sodium lauryl sulfate (SLS)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Level of in vitro results</strong></td>
<td>Meyer 92’= over 90 minutes</td>
<td>Entire Dissolution Profile Meyer 92’= over 90 minutes</td>
<td>Meyer 98’= over 90 minutes</td>
</tr>
</tbody>
</table>

All of the plasma data was collected using HPLC and the calibration was demonstrated to be linear over the following ranges: 0.05 – 4.03 ug/ml, 0.05 – 4.0 ug/ml, and 0.05 – 6.0 ug/ml for the Meyer et al 1992, Meyer et al 1998, and Olling et al. studies, respectively. The digitized in vitro-in vivo data is shown in figure 3.2.1.1.
Figure 3.2.1.1. In Vitro In Vivo Data

Meyer et al. 1992

![Graphs showing in vitro and in vivo data for Product 1 to Product 4.]

Olling et al.

![Graphs showing in vitro and in vivo data for Product A to Product D.]

Meyer et al. 1998

![Graphs showing in vitro and in vivo data for Product 1 to Product 4.]

% Dissolved vs. Time (min) and [Plasma] (μg/ml) vs. Time (hr) for each product.
3.2.2 Modeling Overview

The modeling activities of this chapter proceeded in three phases: i) “top down” pharmacokinetic modeling, ii) dissolution modeling, and iii) *in vitro-in vivo* modeling. The pharmacokinetic modeling allowed each plasma concentration profile to be expressed as a function of the elimination rate (ke), the volume of distribution (Vd), the bioavailability/fraction of drug absorbed (F), and the rate of drug absorption (ka). The dissolution modeling provided a means to reduce a product’s entire dissolution profile into several key dissolution model parameters to represent the rate and extent of dissolution. Finally, two generalized linear models were used to relate the dissolution model parameters for each product to their respective absorption terms for the ultimate recovery of the plasma concentration profiles.

3.2.2.1 Pharmacokinetic Model

With the data from the reference studies digitized, the next step was modeling the plasma concentration profile from the ‘top down’. This was accomplished with a standard single-compartment model for oral absorption (see eq. 3.2.2.1). Here, $k_e$ is the first order elimination rate constant, $k_a$ is the first order absorption rate constant, $F$ is bioavailability, and $V_d$ is the volume of distribution.

\[
\frac{dC_p}{dt} = \frac{(F \cdot k_a \cdot \text{Dose} \cdot e^{\text{ka} \cdot t})}{V} - (k_e \cdot C_p)
\]

(integrated form: $C_p = \frac{F \cdot \text{Dose} \cdot k_a}{V \cdot (k_a - k_e)} \cdot [e^{-k_e \cdot t} - e^{-k_a \cdot t}]$)

This model was used for both the 1992 and 1998 Meyer et al. studies. However, a slightly amended pharmacokinetic model was used for the Olling et al. data given that
this study used two 200 mg doses administered simultaneously. The two doses were accounted for in the differential equation describing the rate of drug change in the plasma in the following way:

\[
\text{Eq. 3.2.1.2)} \quad \frac{dc_p}{dt} = 2(F \cdot k_a \cdot Dose \cdot e^{-k \cdot t})/V - (k_e \cdot C_p)
\]

The parameterization of equations 3.2.2.1 and 3.2.2.2 commenced by assessing whether the elimination followed a first order process. This was confirmed via the presence of linearity in the log-transformed terminal phase of the plasma concentration profile. Once confirmed, the slope of this linear region is the (statistically) unbiased estimation of \(k_e\). The value of \(k_a\) was determined following the calculation of \(k_e\) using the method of residuals.\(^{256}\) \(V_d\) was defined using an aggregate of i.v. dosing trials from literature using the following equation:

\[
\text{Eq. 3.2.2.1.3)} \quad V_d = \frac{Dose}{(k_e[AUC]^{\infty})}
\]

where \([AUC]^{\infty}\) is the area under the curve from time zero to infinity. The value for this parameter has been previously defined by Marino et al. and others to be approximately 70 L (see literature survey on Carbamazepine; section 2.2). The bioavailability of a product, \(F\), was calculated using the Nelder-Mead least squares fitting algorithm within WINNONLIN (Certara – Princeton, NJ). The AUCs calculated in this study were determined using the following equation:

\[
\text{Eq. 3.2.2.1.4)} \quad AUC = \frac{Dose \cdot F}{k_eV_d}
\]
Models were compared on the basis of Akaike Information Criterion (AIC) and goodness of fit was determined by calculating the root mean squared error (RMSE), and $R^2_{\text{adjusted}}$:

\begin{align*}
\text{Eq. 3.2.2.1.5)} & \quad \text{RMSE} = \sqrt{\frac{\sum_{t=1}^{n} (y_t - \hat{y}_t)^2}{n-p}} \\
\text{Eq. 3.2.2.1.6)} & \quad R^2_{\text{adjusted}} = 1 - \frac{n-1}{n-p} (1 - R^2)
\end{align*}

where $n$ is the number of dissolution data points and $p$ is the number of parameters in the dissolution model. In addition to these diagnostic statistics, slope and bias terms were evaluated for significance by assessing the residuals for homoscedasticity around 0 and by testing the null hypothesis of a slope equal to 1 for an ‘actual vs. residual’ plot. The unbiased pharmacokinetic parameters of the model were observed for deviations when they were again calculated using the plasma concentration profiles that were regenerated via the IVIVC modeling.

3.2.2.2 Dissolution Model

Various models were investigated for the modeling of the in vitro dissolution data. Those explored included the Makoid-Banakar, Weibull, first order, Hill, Higuchi, and Hixson–Crowell (see Costa et al. for a review of the explored dissolution models).257

Makoid-Banakar:

\begin{align*}
\text{Eq. 3.2.2.2.1)} & \quad C_t = F_{\max} \cdot \left(\frac{t}{T_{\max}}\right)^{b/T_{\max}} \cdot e^{\left(\frac{b}{T_{\max}} - \frac{t}{T_{\max}}\right)}, \quad \text{for } t \leq T_{\max} \\
& \quad C_t = F_{\max}, \quad \text{for } t > T_{\max}
\end{align*}

where,

Weibull:

\begin{align*}
\text{Eq. 3.2.2.2.2)} & \quad C_t = C_{\inf} \cdot \left(1 - e^{-\left(\frac{t}{\text{MDT}}\right)^{\gamma_{\text{weibull}}/\text{MDT}}}\right)
\end{align*}
First Order:

Eq. 3.2.2.2.3) \[ \ln(C_t) = \ln(C_{sol}) + K_1 t \]

Hill:

Eq. 3.2.2.2.4) \[ C_t = (C_{inf} * t^\gamma)/(MDT^\gamma + t^\gamma) \]

Higuchi:

Eq. 3.2.2.2.5) \[ C_t = K_H \sqrt{t} \]

Hixson–Crowell:

Eq. 3.2.2.2.6) \[ \frac{W_{dose}^{1/3} - W_t^{1/3}}{W_t^{1/3}} = K_s t \]

Where \( C_t \) is the amount drug dissolved at time \( t \), \( C_{inf} \) is the amount released at time infinity, \( T_{max} \) is the time at which the dissolution profile plateaus, \( b \) is the Makoid-Banakar dissolution profile slope factor, \( MDT \) is the mean dissolution time, \( K_1 \) is the first order proportionality constant, \( \gamma \) is the Hill slope factor, \( K_H \) is the Higuchi dissolution constant, \( W_{dose} \) is the initial mass of the drug in the dosage form, \( W_t \) is the remaining amount of drug in the dosage at time \( t \), and \( K_s \) is a constant incorporating the surface–volume relation. Models were fit using the Levenberg-Marquardt algorithm (nlinfit.m) within MATLAB® (v.R2018b, MathWorks, Natick, MA). The dissolution models were compared on the basis of Akaike Information Criterion (AIC) and evaluated for goodness of fit using the RMSE, \( R^2_{adjusted} \), and the slope and bias terms from the ‘actual vs. residual’ plot. The nonsignificance was used to assess the suitability of the dissolution models. The assessment of the slope and bias terms for the residuals-vs-observed plot was based on the inclusion of 0 in the confidence intervals.
3.2.2.3 *In vitro-In vivo* Model

A generalized linear modeling (GLM) approach was used to express the rate and extent of absorption for each product from section 3.1 as a function of their respective *in vitro* dissolution model parameters. As such, two generalized linear models were generated that respectively correlated $k_a$ and $F$ with dissolution model parameters. This provided a means to regenerate the entire plasma concentration profile from the dissolution data. The procedure used to generate the GLMs was based on stepwise regression and is illustrated in the following figure 3.2.4.1.
Model dissolution data using the identified dissolution models

Makoid-Banakar  Weibull  First Order  Hill  Higuchi  Hixson-Crowell

Does the model fit the disso. data well?
($R^2 > 0.95$; Low RMSE; insig. bias)

Yes

Include Dissolution Model Parameters into stepwise regression models for the

Forward addition of variables based on
Prob to Enter: 0.25
Backward removal of variables based
Prob to Leave: 0.1

Save Final Regression Models for:
$ka, F = f(disso.params)$

Discard parameters

Allow for only 1 of each...
• slope/shape parameter
• Cmax parameter
• Tmax/scale parameter
The first step in the IVIVC modeling process illustrated by figure 3.2.4.1 was to model the dissolution profiles using the array of dissolution models identified in section 3.2.2.2. Only the dissolution model parameters from models that provided suitable fits of the dissolution profiles were carried forward as potential independent variables for the *in vitro-in vivo* GLM. The model was then built using a stepwise approach within the JMP software (SAS Institute, Cary NC). Here, the forward addition of variables was based on a maximum p-value threshold that a parameter needed have in order to be entered into the model as part of the forward addition step. The backward removal of a variable was based on a minimum p-value that an effect must have to be removed from the model during a backward step. The critical p-values for the forward and backward removal of parameters were 0.25 and 0.1 respectively. The calculation of p-values for each parameter was based on an F-test. This test required determining the F-ratio for each parameter, which was calculated by dividing the sum of squared error captured by the parameter by the mean sum of squares error after addition/removal of the parameter to the model. The hierarchical procedure that decomposed a dissolution profile into the necessary dissolution model parameters, translated those parameters into $k_a$ and $F$, and then combined the absorption parameters using a first order absorption model will henceforth be referred to as the IVIVC.

### 3.2.3 IVIVC Model Evaluation

The evaluation of the IVIVC model for this project was based on regulatory recommendations found in existing regulatory guidance literature. These guidance
documents focus on the ability to predict two summary pharmacokinetic metrics: $AUC$ and $C_{max}$. As such prediction error ($PE$) for these metrics were calculated using equation 3.2.3.1.

$$\text{Eq. 3.2.3.1)} \quad \%PE = \left( \frac{|\text{Observed Value} - \text{Predicted Value}|}{\text{Observed Value}} \right) \cdot 100$$

The acceptability of the $PE$ for $AUC$ and $C_{max}$ was based on FDA recommendations which varied depended on whether an internal or external approach to the evaluation of predictability was being performed. Internal predictability is based on the initial data used to parameterize the IVIVC model, while the evaluation of external predictability is based on additional test data. The FDA recommends that the on evaluation of the IVIVC under one or both of these approaches constitutes an evaluation of predictability. The guidance documents support internal predictability when average absolute prediction error ($PE$) for $C_{max}$ and $AUC$ is less than or equal to 10% and the percent error for each individual formulation is less than or equal to 15%.$^{13}$ The criteria for satisfactory external predictability is similarly evaluated using regulatory recommendations that the prediction errors for $C_{max}$ and $AUC$ were less than or equal to 10%. The time at which the maximum concentration was achieved ($T_{max}$) was also used for the assessment of predictability. This metric was included in the IVIVC assessment process because Olling et al. showed that, in addition to the degree of exposure (summarized by the $AUC$ and $C_{max}$ metrics), $T_{max}$ was correlated with adverse events (i.e. dizziness). Here, the same predictability criteria was used for this summary pharmacokinetic metric.
While internal predictability was based on the ability to predict the 1992 Meyer et al. and Olling et al. summary pharmacokinetic metrics, external predictability was assessed using separate in vivo-in vitro data from the 1998 Meyer et al. study. In addition to the PE calculation, the RMSE was used for the evaluation of the IVIVC. Also, a plot of the predicted vs the observed was generated to assess predictability. Using this plot, additional suitability tests confirmed that i) the confidence interval for the slope term contained 1, and ii) the intercept term was non-significant.

3.3 Results

3.3.1 In vitro Dissolution Profiles

The respective fits of the dissolution models used to fit the in vitro dissolution data are shown in figure 3.3.1.1. Several models fit the dissolution data well. The Makoid-Banakar model performed especially well with an $R^2_{adjusted}$ of 0.99 and the lowest RMSE and AIC. However, a visual inspection of the fits demonstrated that the Hixson-Crowell and Higuchi models did not fit the data well. This was demonstrated in table 3.3.1.1 by the large AIC and RMSE values as well as the lowest $R^2_{adjusted}$ values. In this table, the RMSE for the Hixson-Crowell and Higuchi models were more than triple that of the next closest comparator. Additionally, neither of the 95% confidence intervals for the slope nor the bias terms included 0 for these two models. The results of the statistical analysis indicated that neither the Higuchi model nor the Hixson Crowell model was suitable for the representation of the in vitro dissolution data.
### Table 3.3.1.1. Dissolution model fits

<table>
<thead>
<tr>
<th>Model</th>
<th>$R^2_{adj}$</th>
<th>RMSE</th>
<th>$AIC$</th>
<th>$CI_{95%}$ (Bias)*</th>
<th>$CI_{95%}$ (Slope)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Order</td>
<td>0.98</td>
<td>4.48</td>
<td>658.1</td>
<td>(-2.86, 0.66)</td>
<td>(-0.007, 0.04)</td>
</tr>
<tr>
<td>Higuchi</td>
<td>0.78</td>
<td>15.9</td>
<td>941.3</td>
<td>(-19.3, -8.19)*</td>
<td>(0.14, 0.30)*</td>
</tr>
<tr>
<td>Hill</td>
<td>0.99</td>
<td>1.27</td>
<td>374.7</td>
<td>(-0.59, 0.41)</td>
<td>(-0.006, 0.008)</td>
</tr>
<tr>
<td>Hixson-Crowell</td>
<td>0.65</td>
<td>20.1</td>
<td>994.3</td>
<td>(-28.5, -15.7)*</td>
<td>(0.26, 0.44)*</td>
</tr>
<tr>
<td>Makoid-Banakar</td>
<td>0.99</td>
<td>1.21</td>
<td>365.5</td>
<td>(-0.54, 0.41)</td>
<td>(-0.005, 0.008)</td>
</tr>
<tr>
<td>Weibull</td>
<td>0.99</td>
<td>2.26</td>
<td>528.7</td>
<td>(-1.42, 0.68)</td>
<td>(-0.007, 0.021)</td>
</tr>
</tbody>
</table>

* percentage dissolved

**Figure. 3.3.1.1** Dissolution profile fitting  
(*markers* represent observed data; *lines* represent model fits)
3.3.2 *In vivo* Plasma Profiles

The observed plasma concentration profiles of the Olling et al. and the Meyer et al. studies and their respective fits using the single compartmental model for oral absorption are shown in Figure 3.3.2.1. The markers in this figure represent the observed data digitized from the original publications. The solid lines represent the fit of the pharmacokinetic model. The profiles followed a classical single peak plasma concentration profile in all the cases. A basic visual predictive check of these models figure demonstrated suitable fits. The fitting accuracy was also supported by high $R^2_{\text{adjusted}}$ in every case ($\geq 0.95$), with the ratio of the RMSE to the summary pharmacokinetic metrics being on the order of, or below, the analytical sensitivity of methods originally used to quantify the plasma concentrations.
Table 3.3.2.1 Diagnostic Metrics for Pharmacokinetic Model Fits

<table>
<thead>
<tr>
<th>Study</th>
<th>Meyer et al. 1992</th>
<th>Meyer et al. 1998</th>
<th>Olling et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Product ID</strong></td>
<td>1     2    3    4</td>
<td>1     2    3    4</td>
<td>1     2    3    4</td>
</tr>
<tr>
<td>$R^2_{adjusted}$</td>
<td>0.98  0.99  0.99  0.99</td>
<td>0.99  0.98  0.99  0.99</td>
<td>0.96  0.97  0.98  0.95</td>
</tr>
<tr>
<td>$RMSE^*$</td>
<td>0.06  0.05  0.12  0.06</td>
<td>0.06  0.13  0.05  0.06</td>
<td>0.18  0.28  0.21  0.28</td>
</tr>
<tr>
<td>$AIC$</td>
<td>-33.7 -41.9 -14.6 -35.1</td>
<td>-24.4 -41.5 -30.9 -26.3</td>
<td>-5.68 11.3 -0.64 10.4</td>
</tr>
<tr>
<td>$CI_{95%} (Bias)^*$</td>
<td>(-0.08, 0.06) (-0.06, 0.04) (-0.15, 0.11) (-0.07, 0.05)</td>
<td>(-0.26, 0.08) (-0.09, 0.08) (-0.11, 0.09) (-0.30, 0.13)</td>
<td>(-0.30, -0.31, 0.21) (-0.44, 0.21) (-0.31, 0.19) (-0.50, 0.21)</td>
</tr>
<tr>
<td>$CI_{95%} (Slope)$</td>
<td>(-0.05, 0.07) (-0.06, 0.09) (-0.06, 0.09) (-0.06, 0.07)</td>
<td>(-0.10, 0.15) (-0.04, 0.05) (-0.05, 0.06) (-0.05, 0.13)</td>
<td>(-0.05, 0.05, 0.08) (-0.06, 0.15)</td>
</tr>
</tbody>
</table>

* units: μg/ml
All of the pharmacokinetic studies used in this chapter collected data until at least 169 hours post dose. This provided each of the original researchers the ability to capture the terminal phase of the plasma concentration profile. This was important as the log transformed slope of this region is the elimination rate which was in turn is used to calculate the absorption rate and bioavailability.

The 1992 Meyer et al. data in figure 3.3.2.1 shows how the entire mean plasma concentration profile, including the terminal phase, was provided in the original publication (reported interval: 0 to 169 hrs). With this interval the elimination rate could be solved without any reference to the results section of the original document. This scenario was also the case for the Olling et al. data. However, the 1998 Meter et al. study only included plasma concentration data up to 50 hours post-dose in their figure despite collecting data until 169 hours. Fortunately, the researchers of the 1998 Meyer et al. study reported the mean elimination rates for each product in their study using the Stella II software which utilizes classical methods for the determination of rate constants (i.e., slope of the log transformed terminal phase). These reported elimination rates were then used as the starting point for subsequent modeling efforts aimed at deriving the absorption rate and bioavailability for each product.

The ranges of $k_a$ and $F$ values for the Olling et al. and 1992 Meyer et al. studies, calculated using the method of residuals and Nelder Mead least squares fitting algorithm, were [0.23 – 1.08] and [0.44 – 1.00], respectively. The ranges of $k_a$ and $F$ values from the 1998 Meyer et al. used to evaluate the in vitro-in vivo model were [0.9-0.27] and [0.77-0.90], respectively. The $k_a$ term scaled well with measurements of
$T_{max}$, while the $F$ term scaled well with measurements of $C_{max}$ and $AUC$ (see tables 3.3.2.2 and 3.3.2.3).

### Table 3.3.2. Pharmacokinetic Model Parameters

<table>
<thead>
<tr>
<th>Study</th>
<th>Meyer et al. 1992</th>
<th>Meyer et al. 1998</th>
<th>Olling et al. 1999</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product ID</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>$ka$ (hr$^{-1}$)</td>
<td>0.26 (0.0190)</td>
<td>0.40 (0.0349)</td>
<td>0.50 (0.0507)</td>
</tr>
<tr>
<td></td>
<td>0.27 (0.0247)</td>
<td>0.58 (0.0249)</td>
<td>0.90 (0.0799)</td>
</tr>
<tr>
<td></td>
<td>0.47 (0.0265)</td>
<td>0.64 (0.0265)</td>
<td>1.08 (0.059)</td>
</tr>
<tr>
<td>$F$ (%)</td>
<td>0.76 (0.0234)</td>
<td>0.44 (0.0137)</td>
<td>1.00 (0.0233)</td>
</tr>
<tr>
<td></td>
<td>0.57 (0.0239)</td>
<td>0.77 (0.0295)</td>
<td>0.84 (0.0453)</td>
</tr>
<tr>
<td></td>
<td>0.84 (0.0116)</td>
<td>0.90 (0.019)</td>
<td>1.00 (0.023)</td>
</tr>
<tr>
<td></td>
<td>0.64 (0.023)</td>
<td>1.00 (0.023)</td>
<td>0.86 (0.0340)</td>
</tr>
<tr>
<td>$ke$ (hr$^{-1}$)</td>
<td>0.015 (0.0009)</td>
<td>0.014 (0.0010)</td>
<td>0.017 (0.0014)</td>
</tr>
<tr>
<td></td>
<td>0.014 (0.0011)</td>
<td>0.012 (0.0018)</td>
<td>0.014 (0.0029)</td>
</tr>
<tr>
<td></td>
<td>0.013 (0.0009)</td>
<td>0.015 (0.0013)</td>
<td>0.015 (0.0013)</td>
</tr>
<tr>
<td></td>
<td>0.015 (0.0011)</td>
<td>0.015 (0.0012)</td>
<td>0.012 (0.0008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The values in parenthesis represent the standard error of the parameter.

### Table 3.3.3. Summary Pharmacokinetic Metrics

| Study               | Meyer et al. 1992 | Meyer et al. 1998 | Olling et al.  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Product ID</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
<td>1 2 3 4</td>
</tr>
<tr>
<td>$C_{max}$ (µg/ml)</td>
<td>1.89 1.15</td>
<td>2.69 1.40</td>
<td>3.17 1.95</td>
</tr>
<tr>
<td>$AUC$ (µg * hr/ml)</td>
<td>143 86.0</td>
<td>162 111</td>
<td>246 157</td>
</tr>
<tr>
<td>$T_{max}$ (hr)</td>
<td>11.7 9.04</td>
<td>7.19 12.8</td>
<td>13.3 6.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.10 7.67</td>
</tr>
</tbody>
</table>

The results shown in Figure 3.3.2.1 and Table 3.3.2.1 provide a basis from which it can be stated that the first-order pharmacokinetic models in equations 3.2.2.1.1 and 3.2.2.1.2 adequately described the data. Thus it was concluded that if the $in$ vitro dissolution model parameters can be accurately transformed into the $in$ vivo absorption
terms via the GLM-based IVIVC model, then the entire observed plasma concentration can be recreated in the fulfillment of a level A IVIVC.

3.3.3 In vitro-In vivo Model

The final step in this chapter was to model the rate and extent of in vivo pharmacokinetic input as a function of the rate and extent of in vitro drug release. The method selected for this objective was generalized linear modeling.

The first step was to examine these model parameters for correlations. Paired univariate regressions were performed for this step and the $R^2$ values are shown in table 3.3.4.1. The most important regions of this table are a) the column representing the correlations with the bioavailability term and, b) the last row representing correlations with the first order absorption term.

| Table 3.3.4.1. Correlation Table of Dissolution Model Parameters with $ka$ and $F$ |
|---------------------------------|---------------------------------|
| First Order                     | Hill                            |
| Cinf                            | 0.62                            |
| $K_1$                           | 0.82                            |
| $t_{scale}$                     | 0.92                            |
| Gamma                           | 0.55                            |
| Cinf                            | 0.59                            |
| MDT                             | 0.82                            |
| $b$                             | 0.89                            |
| $t_{max}$                       | 0.94                            |
| Cinf                            | 0.58                            |
| MDT                             | 0.33                            |
| $ka$                            | 0.61                            |
| F                               | Cinf                            |
| Cinf                            | 0.37                            |
| $K_1$                           | 0.17                            |
| $t_{scale}$                     | 0.01                            |
| Gamma                           | 0.20                            |
| Cinf                            | 0.34                            |
| MDT                             | 0.33                            |
It is observed in table 3.3.4.1. that some of the dissolution model parameters were more highly correlated than others with $F$ and $k_a$, respectively. Dissolution parameters were added to the respective GLMs for the prediction $F$ and $k_a$ according to the previously specified criteria and without any other supervision. The modeling process for prediction of both $k_a$ and $F$ ultimately resulted in the inclusion of three terms. The included terms ($F_{\text{max}}$, $T_{\text{max}}$, and $b$) were all from the Makoid Banakar model. The performance of the model derived for the prediction of $k_a$ and $F$ is illustrated in Figures 3.3.4.1 and 3.3.4.2. The F-statistics in tables 3.3.4.2.a and 3.3.4.3.a demonstrated that the parameterized models significantly predicted $k_a$ and $F$. The type III sum of squares test in tables 3.3.4.2.b and 3.3.4.3.b showed that when each parameter is added last, the resulting reduction in the sum of squares error was significant in all of the cases. Tables 3.3.4.2.c and 3.3.4.3.c show the final parameter estimates and the associated standard error of the two models.
Table 3.3.4.2.a Sum of Squares Analysis for the Prediction of $k_a$

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Ratio</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>0.16088118</td>
<td>0.053627</td>
<td>303.7001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Error</td>
<td>4</td>
<td>0.00070632</td>
<td>0.000177</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. Total</td>
<td>7</td>
<td>0.16158750</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3.4.2.b Tests for Type III SS Error for the Prediction of $k_a$

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{\text{max(Makoid Banakar)}}$</td>
<td>0.02345937</td>
<td>132.8548</td>
<td>0.0003</td>
</tr>
<tr>
<td>$T_{\text{max}}$</td>
<td>0.04285786</td>
<td>242.7121</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>$b$</td>
<td>0.12314352</td>
<td>697.3848</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Table 3.3.4.2.c Parameter Estimates for the Prediction of $k_a$

| Term                       | Estimate     | Std Error         | Prob>|t| |
|----------------------------|--------------|-------------------|------|
| $\text{Intercept}$         | 0.4644768517 | 0.0292334485      | <.0001|
| $F_{\text{max(Makoid Banakar)}}$ | -0.004057305 | 0.0003520052      | 0.0003|
| $T_{\text{max}}$           | 0.0714231734 | 0.0045845158      | <.0001|
| $b$                        | 0.0543586598 | 0.0020584129      | <.0001|
Table 3.3.4.3.a Sum of Squares Analysis for the Prediction of $F$

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Ratio</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>0.32119351</td>
<td>0.107065</td>
<td>2074.0135</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Error</td>
<td>4</td>
<td>0.00020649</td>
<td>0.000052</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. Total</td>
<td>7</td>
<td>0.3214</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3.4.3.b Tests for Type III SS Error for the Prediction of $F$

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>$b$</td>
<td>0.03080006</td>
<td>596.6473</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>$F_{max}(Makoid Banakar)$</td>
<td>0.01400927</td>
<td>271.3824</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>$T_{max}$</td>
<td>0.01963117</td>
<td>380.2877</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Table 3.3.4.3.c Parameter Estimates for the Prediction of $F$

| Term               | Estimate  | Std Error | Prob>|t| |
|--------------------|-----------|-----------|------|
| $Intercept$        | 0.261519  | 0.009931  | < 0.0001 |
| $b$                | 0.103141  | 0.004223  | < 0.0001 |
| $F_{max}(Makoid Banakar)$ | 0.001417 | 8.6E-05   | < 0.0001 |
| $T_{max}$          | 0.003271  | 0.000168  | < 0.0001 |
The final error statistics for $AUC$ and $C_{max}$ are reported in table 3.3.4.4. The mean internal percent error for $AUC$ was observed to be 35% lower than the external data, while the mean percent error for $C_{max}$ in the external data was 34% lower as compared to the internal data. However, the observed differences in the means were not statistically significant in any of the cases. The model was also within the % PE criteria defined by the FDA for both internal and external $AUC$ and $C_{max}$ predictions. Ultimately, the predicted vs. observed values for the $AUC$ and $C_{max}$ values were well correlated with low residuals (see table 3.3.4.4.).

### Table 3.3.4.4. Parameter Estimates for the Prediction of AUC, Cmax and Tmax

<table>
<thead>
<tr>
<th>Study</th>
<th>Product ID</th>
<th>PE: AUC</th>
<th>PE: Cmax</th>
<th>PE: Tmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Internal Data Set</td>
<td>Meyer et al. 1992</td>
<td>1 4.20</td>
<td>1.06</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 2.79</td>
<td>2.61</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 7.41</td>
<td>4.09</td>
<td>7.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 5.41</td>
<td>7.14</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>Olling et al.</td>
<td>1 1.22</td>
<td>2.19</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 6.09</td>
<td>1.02</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 4.36</td>
<td>4.26</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 1.69</td>
<td>2.39</td>
<td>2.3</td>
</tr>
<tr>
<td>External Data Set</td>
<td>Meyer et al. 1998</td>
<td>1 3.18</td>
<td>5.13</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 6.13</td>
<td>0.43</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 7.55</td>
<td>0.43</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 5.56</td>
<td>2.14</td>
<td>6.7</td>
</tr>
</tbody>
</table>

PE = percent error

### Table 3.3.4.5 Summary Statistics for the Prediction of AUC and Cmax

<table>
<thead>
<tr>
<th>Summary PK Metric</th>
<th>Summary Statistics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC$ (ug/ml/hr)</td>
<td>Predicted vs. Observed: $R^2$</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Predicted vs. Observed: RMSE</td>
<td>5.97</td>
</tr>
<tr>
<td>$C_{max}$ (ug/ml)</td>
<td>Predicted vs. Observed: $R^2$</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Predicted vs. Observed: RMSE</td>
<td>0.099</td>
</tr>
<tr>
<td>$T_{max}$ (hrs)</td>
<td>Predicted vs. Observed: $R^2$</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Predicted vs. Observed: RMSE</td>
<td>0.21</td>
</tr>
</tbody>
</table>
3.4 Discussion and Conclusion

The specific aim of this chapter was to generate an IVIVC framework that could accurately predict mean *in vivo* exposure. Under an ideal scenario, this would be performed using a prospective clinical trial. Here, plasma concentration profiles would be generated within a consistent study population by deliberately altering features of the drug product in a risk-based fashion to achieve an appropriate range of *in vitro-in vivo* performance. While the performance of a prospective clinical trial could not be performed for this work due to logistic reasons, actions were taken to overcome this limitation using the wealth of publically available paired *in vitro-in vivo* data (i.e. dissolution data and its corresponding plasma concentration data) collected on carbamazepine from a variety of drug product manufacturers. The internal data used to calibrate the IVIVC described in this chapter was generated using eight products. Each product came from a unique lot that spanned across five different drug product manufacturers. The data used to evaluate the external predictability of the IVIVC was generated using four drug products, including three drug products from unique manufacturers and one new lot of the reference product. In the end, the accuracy of the generated model met regulatory, as well as in-house, criteria for internal and external predictability. The ability to accurately generate an IVIVC using such a diverse drug product data set supports the generalizability of IVIVC for future applications despite the practical study limitations. The reliability of this IVIVC will be assumed for the activities of subsequent chapters.
Chapter 4: “Bottom-Up” Convolution of IVIVC with Population-level PK Variance

4.1 Introduction

The previous chapter was built upon the concept that the rate and extent of per oral carbamazepine absorption from a tablet was primarily a function of its dissolution behavior. Chapter 4 is focused on mechanistically modeling pharmacokinetic pathways thus allowing the observed inter-subject variability to be recovered by simulations. The specific aim is to account for population level pharmacokinetic variance within the previously developed pharmacokinetic model of the IVIVC in a physiologically-based manner (see section 2.4 for a discussion on physiologically-based pharmacokinetic modeling).

The IVIVC developed in the previous chapter predicted a mean plasma concentration profile. While this was informative, the statistical assessment of bioequivalence ultimately requires that intersubject variability be considered. Chapter 4 demonstrates how this requirement was fulfilled by first embedding and refining physiologically-based clearance pathways within the previously developed IVIVC, and then performing simulations wherein the covariation of physiological parameters was propagated across a population in a rational manner. The first step used the extrapolated results of \textit{in vitro} studies using models of physiologic systems to predict systemic \textit{in vivo} performance.\textsuperscript{258} The \textit{in vitro-in vivo} extrapolation of population-based performance is considered a “bottom-up” approach to pharmacokinetic modelling. This is because it predicts \textit{in vivo} pharmacokinetic outcomes using mechanistic information that has been independently determined \textit{a priori}. This approach models the concentration profile of a
compound using 1) first principles of human physiology, 2) the results of rigorously performed federal health interview surveys, and 3) meta-analyses focused on the abundance of metabolizing pathways. The integration in vitro assays, in vivo data, and in silico modeling was performed to support the movement towards a more efficient means of estimating the pharmacokinetic conditions expressed in therapeutic populations during actual clinical practice.

4.2 Methods and a priori Data

4.2.1 Modeling Overview

A five-step strategy was used in this chapter to model the systemic exposure of carbamazepine. The first step was to refine the physiologically-based clearance pathways for carbamazepine. This required the definition of an intravenous physiologically-based pharmacokinetic model wherein intravenous dosing data was ultimately used to confirm intrinsic clearance parameters. The use of intravenous dosing for the evaluation and refinement of clearance pathways eliminates sources of bias related to absorption. The second step was to model intrinsic clearance using data collected from in vitro systems. Here, intrinsic clearance was defined as the rate of drug removal absent any constrains from limiting effects (e.g. perfusion rate of the liver). The third step was to extrapolate the in vitro intrinsic clearance to whole organ clearance. This was performed using the established relationship between height and weight as a function of age and gender for the calculation of body surface area (BSA). The relationship between BSA and liver volume was combined with an understanding of liver density to derive a value for the mass of microsomal protein. Using the microsomal weight of the tissue and blood flow to the tissue, the last step for
extrapolating whole organ clearance was to scale in vitro clearance by applying the percent abundance of the specific metabolizing enzymes within the liver as reported by pharmacogenomic meta-analyses. The fourth step for mechanistically modeling the systemic exposure of carbamazepine was to confirm the accuracy of the extrapolation process using intravenous data. The fifth step was to recover the observed variability in the summary pharmacokinetic parameters $AUC$, $C_{max}$ and $T_{max}$. This was performed updating a priori distributions placed overtop pharmacokinetic model parameters. The combination of these steps facilitated the testing of more relevant clinical scenarios via the use of correlated Monte Carlo resampling.

4.2.2 PBPK Modeling Software

Physiologically-based pharmacokinetic modeling was performed using SimCYP Version 17 (Certara Ltd. Inc., Princeton NJ). The Simcyp® population-based simulator is a platform and database that has been used for mechanistic modelling and simulation of physiologic processes involved with the oral absorption, distribution, metabolism, metabolically-based drug–drug interaction, and excretion of drugs in healthy and disease populations (e.g. populations categorized by age, disease, race). Optimization algorithms within the platform, specifically the Nelder-Mead algorithm, were used to recover in vivo data via targeted parameter estimation.

4.2.3 Intravenous Model

The works of Rowland and Jones served as the foundation for the physiologically-based pharmacokinetic modeling of this chapter. The modeling assumptions were as
follows: *i*) intercompartmental transport occurs via the blood, *ii*) drug concentrations in efferent blood, and blood within tissues, are equal, *iii*) there is instantaneous equilibrium of drug between tissue and blood within the tissue (i.e. perfusion rate limited clearance kinetics), and (d) only unbound drug is eliminated. A structural representation of the model is provided in Figure 4.2.3.1.

**Figure. 4.2.3.1** Structural form of the physiologically based pharmacokinetic model

Two sites of elimination will be considered in the model: the gut and liver. The differential equations that have been selected to define the physiologically-based pharmacokinetic model within SimCYP® following intravenous dosing are listed in Table 4.2.3.1. From the first equation in this Table (4.2.3.1), is it clear that the concentration of drug in the liver, $C_L$, is an important factor for describing the change in drug concentration in the central compartment. As described by Equation 4.2.3.3, the amount of drug in the
liver, $A_L$, is in part a function of the drug in the portal vein, $A_{PV}$. In Equation 4.2.3.2 it is seen how fraction of drug in the villous blood from that escapes gut metabolism, $F_G$, shapes $A_{PV}$. The fraction of drug that escapes gut metabolism term is described by Yang et al.\textsuperscript{263,279} Not only will this term differentially account for metabolic clearance from gut enzymes, but it will also account for permeability based clearance of drug into the gut lumen ($C_{l_{perm}}$). The impact of the fraction of drug metabolized in enterocytes ($1 - F_G$) is minimal for the intravenous model.
Table 4.2.3.1. Foundational Equations for Intravenous Physiologically Based Pharmacokinetic Model

Eq. 4.2.3.(see below)

1) \[
\frac{d}{dt} A_{\text{cent}} = i.v. \text{infusion rate} + (Q_{HV} * C_L) - (Q_{HA} * \frac{C_L}{K_P/[B:P]}) - (Q_{GI,\text{vas}} * C_{\text{cent}})
\]

2) \[
\frac{d}{dt} A_{PV} = [(Q_{PV} - \sum Q_{\text{villi},n} * C_{\text{cent}}) + \sum F_{G,n}(Q_{\text{villi},n} * C_{\text{cent}}) - (Q_{PV} * C_{PV})
\]
   where,
   \[
   F_G = \frac{Q_{\text{gut}}}{Q_{\text{gut}} + f_{\text{gut}} * C_{\text{L,T,unbound}}}
   \]
   \[
   Q_{\text{gut}} = (C_{\text{perm}} * Q_{\text{villi},n})/(C_{\text{perm}} + Q_{\text{villi},n})
   \]
   \[
   C_{\text{perm}} = S \times P_{\text{eff},n}
   \]

3) \[
\frac{d}{dt} A_L = (Q_{HA} * C_{\text{cent}}) + (Q_{PV} * C_{PV}) - (Q_{HV} * f_{uB} \frac{C_L}{K_P/[B:P]}) - (C_{L,T} * f_{uB} \frac{C_L}{K_P/[B:P]})
\]

4) \[
C_{L,T} = (Q_T \times f_u \times C_{L,T,\text{unbound}})/(Q_T + f_u \times C_{L,T,\text{unbound}});
\]
   where,
   \[
   T = [\text{liver}, \text{intestinal segment ‘n’}], \quad f_u = ‘f_{uB}’;
   \]
   \[
   T = \text{‘liver’};
   \]
   \[
   T = \text{‘intestinal segment ‘n’};
   \]

**Terms and Subscripts**

- **Q** = blood flow (L/hr)
- **C** = concentration (mg/L)
- **CL** = whole tissue clearance (L/hr)
- **A** = amount (mg)
- **CL_{T,unbound}** = unbound intrinsic clearance in tissue
- **cent** = central compartment
- **T** = tissue
- **G** = gut tissue
- **L** = liver tissue
- **villi** = villous vasculature
- **HV** = hepatic vein
- **[B: P]** = blood:plasma partition ratio
- **KP** = tissue to plasma partition coefficient
- **fu** = fraction unbound in plasma
- **fub** = fraction unbound in blood,
  \[
  = fu \times C_{P,\text{total}}/C_{B,\text{total}}
  \]
- **fu_{gut}** = fraction unbound in gut
- **P_{eff}** = effective permeability
- **FG** = fraction of drug that escapes gut metabolism
- **S** = available surface area
- **ν** = venous
- **u** = unbound
- **n** = nth region in the GI tract

fu_{gut} = 1; assumes insufficient time for plasma protein binding equilibrium or erythrocyte uptake before the drug is removed from the basolateral side of the enterocyte²⁶³

For references used to identify the center and spread of anthropomorphic inputs (i.e. drug-independent, physiological factors) of the ADAM model see Jamei et al.
The tissue to plasma partition coefficient ($K_p$) in Equations 4.2.3.1 and 4.2.3.3 was calculated using the set of equations derived by Rodgers et al. based on the ionization for a given molecule of interest. The derived equation for a neutral compound (such as carbamazepine) is:

Eq. 4.2.3.5) \[ K_p = K_{pu} \times f_u \]

where

\[ K_{pu} = f_{EW} + f_{IW} + (P \cdot f_{NL} + (0.3P + 0.7)f_{NP}) \]

\[ + (K_{aPR} [PR]_T) \]

The three major assumptions in Equation 4.2.3.5 are: i) Non-saturating conditions exist for all binding processes ii) Drug transport is a passive process, and iii) Each tissue has a well-stirred distribution model limited by blood perfusion. Equation 4.2.3.5 required the determination of a compound’s affinity constant ($K_{aPR}$) for tissue binding protein where $[PR]_T$ is the concentration of protein in tissue $T$. The binding protein for carbamazepine is albumin ($[PR]_T$ has been consistently reported for carbamazepine using in vitro techniques that include equilibrium dialysis, ultrafiltration, surface plasmon resonance, and various chromatographic methods $^{265-268}$). The plasma-to-liver and plasma-to-gut ratio for albumin has been experimentally determined to be 11.6 and 6.33, respectively. $^{264, 269-272}$ Lastly, Equation 4.5.3.5 was parametrized with the octanol to water partition coefficient for unionised species ($P$), and the fractional volume of tissue components ($f$, where $EW$ refers to extracellular water, $IW$ refers to intracellular water, $NL$ refers to neutral lipids and $NP$ refers to neutral phospholipids; see Rodgers and Rowland [2007] and Poulin and Theil [2002] for experimentally determined reference values$^{273-274}$). The interstitial fluid-to-plasma
concentration ratio will be considered as a whole organ-to-plasma ratio, an assumption that aligns with the well-stirred model of the liver and gut segment. The values and references for the drug specific parameters are reported in Table 4.3.1.1.

4.2.4 Parameterization, Extrapolation, and Refinement of \textit{in vivo} Intrinsic Clearance

The intrinsic clearance of a tissue $T$ ($\text{Cl}_{T,int}$, where the intrinsic clearance of the gut or liver $T$ equals $G$ or $L$, respectively), represents its ability to deplete the concentration of a compound absent rate limiting factors such as blood flow or tissue partitioning. The major clearance pathways for carbamzepine include metabolism cytochrome P450 (CYP) and the Uridine 5'-diphospho-glucuronosyltransferase (UGT). The specific isozymes of these pathways (discussed in section 2.2.3) have been identified as CYP3A5, CYP3A4, CYP2B6, and UGT2B7.

The methodologies used for predicting organ clearance from \textit{in vitro} systems (e.g., hepatocytes and microsomes) have been described in detail by Houston and others, and have been validated extensively.\textsuperscript{275-278} Briefly, the \textit{in vitro} data in this work was collected using substrate depletion assays. The key inclusion criteria for \textit{in vitro} clearance studies was as follows:

1) The use of validated \textit{in vitro} systems, which included but was not limited to harvested liver cells and recombinant enzymes systems

2) The reporting of unbound \textit{in vitro} intrinsic clearances by accounting for free fraction of drug in the \textit{in vitro} incubation, $f_{u,inc}$
3) The normalization of clearance parameters on a ‘per milligram of metabolizing
enzyme’ basis (or per cell basis if hepatocytes are being used) as defined by the
incubation media

The general procedure employed by the studies was to incubate various concentrations
of carbamazepine with a fixed concentration of commercially sourced, recombinantly
expressed, monoclonal isozymes. The carbamazepine was quantified for each
incubation design point using a suitable analytical method (in most cases liquid
chromatography coupled mass spectroscopy). The slope for the concentration vs time
was normalized for nonspecific binding and then recorded as the clearance of
carbamazepine. The process was then repeated at a subsequent carbamazepine
concentration. Once the process was performed at all of the carbamazepine
concentrations for each of the isozymes the ‘clearance-vs-carbamazepine
concentration’ profiles were modeled according to typical Michaelis–Menten kinetics.

This type of kinetic behavior is defined by the following
equation:

$$\text{rate of reaction} = \frac{V_{\text{max}}[\text{API}]}{K_m + [\text{API}]}.$$

Here, $V_{\text{max}}$ represents the maximum rate achieved by the system at the saturating
substrate concentration. This plateau was observed/reported in all of the studies. The
Michaelis-Menten constant $K_m$ is the substrate concentration at which the reaction rate
is half of $V_{\text{max}}$. In the context of pharmacokinetic modeling, the intrinsic clearance of
an API that follows Michaelis-Menten is defined in the following manner:

$$C_{\text{int}} = \frac{V_{\text{max}}}{K_m}.$$
The unbound intrinsic clearance (i.e. $CL_{L,\text{int (unbound)}}$) in the liver and gut was defined by Equations 4.2.4.1 and 4.2.4.2. These equations follow works reported by Rowland et al. (2010,2011) and Almond et al. (2009,2016).262,279 Here, a standardized scaling procedure was used to determine the intrinsic clearance of whole organs/tissues (i.e. $CL_L$ and $CL_G$) from in vitro intrinsic clearances (see Houston et al. for reviews282,286).

Table 4.2.4.1. Foundational Equations for Unbound Intrinsic Clearance in the Physiologically Based Pharmacokinetic Model

<table>
<thead>
<tr>
<th>Eq. 4.2.4.(see below)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CL_{u,G,\text{int (unbound)}} = \sum_{s=1}^{9} \left( \sum_{p=1}^{n} \sum_{e=1}^{m} \frac{V_{\text{max,}p,e(\text{gut})}}{K_{m,p,e} + f u_{\text{gut}} K_{p}/[B:P]} * 1/ f u_{\text{inc}} \right)$</td>
</tr>
<tr>
<td>where, $V_{\text{max,}p,e,\text{gut}} = ISEF_{p,e} \times V_{\text{max,}p,e(\text{vitro})} \times Enz_{G,p,e}$</td>
</tr>
</tbody>
</table>

| $CL_{u,L,\text{int (unbound)}} = \left( \sum_{p=1}^{n} \sum_{e=1}^{m} \frac{V_{\text{max,}p,e(\text{liver})}}{K_{m,p,e} + f u_{\text{gut}} K_{p}/[B:P]} * 1/ f u_{\text{inc}} \right)$ |
| where, $V_{\text{max,}p,e,\text{liver}} = ISEF_{p,e} \times V_{\text{max,}p,e(\text{vitro})} \times Enz_{L,p,e}$ |

Terms: L= Liver; G= Gut; p = clearance pathway (e.g. CYP, UGT); e = isoform (e.g. CYP2C9, CYP3A4); $f u_{\text{inc}}$ = in vitro fraction unbound in the incubation

The clearance parameters derived from recombinant in vitro systems were scaled to human-derived in vitro systems using the respective inter-system extrapolation factor (ISEF). These values are reported by the suppliers of recombinant metabolizing enzyme systems and are based on methods described by Proctor et al. This allowed whole organ clearances to be calculated using the abundance of the $e^{th}$ isoform ($Enz_{L,p,e}$) and the amount of microsomal protein per gram of human liver (MPPGL) or length of intestinal segment (s) (MPPL) multiplied by the liver mass or intestinal segment length.289,293
The time dependent amount of enzyme in the metabolizing tissue, $E_n$, in Equations 4.2.4.1 and 4.2.4.2, will be calculated using Equations 4.2.4.3 and 4.2.4.4. Equation 4.2.4.3 includes the induction terms: $E_{max}$ and $EC_{50}$. The maximum fold-wise induction to the synthesis rate of enzyme generation is $E_{max}$, while the concentration of inducer that results in half max induction is $EC_{50}$. These terms account for the auto-inductive effects of carbamazepine and any other concomitantly administered inducers. These values were informed by quantitative transcriptomics studies reported by Almond et al. in the relevant clearance pathways (see Chapter 2). Verification of these values were based on the in silico confirmation that a mean three-fold autoinduction would be complete within a 1-2 week period as reported in the package insert for Tegretol.

The liver weight in Equation 4.2.4.4. was calculated by multiplying liver density (1.051 g/L) by individualized liver volumes. Liver volumes and total intestinal lengths were individualized using the correlated Monte Carlo methods described in section 2.4.3. As previously detailed, the statistical sampling method randomly drew from prior distributions
of covarying anthropomorphic values for height and weight. The algorithm began by defining upfront the baseline patient demographics from which subsequent samples would be drawn. The demographics specifically included the age range and percent of female subjects within the trial. Samples were drawn from height and weight crosstabs based on the synthetic patient’s age and gender. BSA was calculated from these values using the previously discussed Du Bois Equation and individualized liver volumes were simulated using the meta-analysis of Johnson et al. which correlated BSA with liver volume. Liver weight was then calculated using the liver density reported by Heinemann et al. Similarly, total intestinal lengths (where each segment, i.e. duodenum, jejunum, or ileum, is a proportion of the total length) were generated using the correlation between BSA and total intestinal length as reported by Valentin.

It is important to note that the results of rigorously conducted federal health interview studies (i.e. NHANES, NHIS, EHIS, etc.) have allowed the covariance structures between age, gender, height, weight and other anthropomorphic values to be modeled by researchers. Data libraries based on meta-analysis of such reports are provided with the SimCYP® software and regularly updated by a consortium of industry, academic, regulatory, and SimCYP® scientists to describe healthy and diseased populations (so called “population libraries”). The information in these libraries, coupled with the physiologically based pharmacokinetic models, were used to account for the physiologic characteristics that shape drug exposure. This enabled the representative simulation of inter-individual drug exposure variability. The remaining drug specific data necessary to perform the PBPK modeling of carbamazepine, as per Equations 4.2.3.1 - 4.2.4.4, is provided in Table 4.3.1.1. (see results section).
4.2.5 Intravenous Dosing Studies

Data on plasma concentration profiles and pharmacokinetic parameters following intravenous dosing was collected from the 2012 intravenous dosing study by Marino et al. The details of this study are covered in chapter 2 section 2.2.3. Briefly, the study used liquid chromatography-coupled mass spectroscopy to quantify the elimination of a single 100mg intravenous dose of radio-labeled carbamazepine.

4.2.6 Refine and confirm clearance parameters using intravenous dosing studies

Evaluation of the intravenous physiologically based pharmacokinetic model accuracy was performed relative to the mean responses observed in the Marino et al. study. The summary pharmacokinetic metric used for this activity was apparent systemic plasma clearance ($CL_{sys}$). Each $CL_{sys}$ was calculated by $CL_{sys} = k_e \cdot V_d$, where $k_e$ is terminal rate constant of each simulated profile and $V_d = Dose / (k_e [AUC]_{\infty})$. Here, a mean response was collected from simulations run using the age range and the proportion of females reported in the Marino et al. study as the starting point for the previously described correlated Monte Carlo algorithm. These basis values were 18-61 years (similar to those reported in the per orally dosed in vitro-in vivo studies by Meyer et al. and Olling et al.) and 51%, respectively. The $CL_{sys}$ generated from the simulations were compared to those observed in the study on the basis of the percent error. Ultimately, a test for zero slope of residuals vs. observed plot was used as the test for model fit of the intravenous data and to justify successful physiologically-based modeling of the clearance pathways.
After verifying the accuracy of the clearance model, it was then inserted into the pharmacokinetic model for per oral absorption. The accuracy assessment was again undertaken. This time the evaluation was performed using the mean results from the per orally dosed Meyer et al. and Olling et al. studies to confirm Equations 4.2.4.1 and 4.2.4.2 that had been appropriated, parameterized and integrated within a physiologically based pharmacokinetic model now based on a per oral route of administration (see Equations 4.2.6.1 and 4.2.6.2).

\begin{table}
\centering
\begin{tabular}{|l|l|}
\hline
\textbf{Eq 4.2.6. (see below)} & \\
\hline
1) & \[ \frac{dA_{\text{cent}}}{dt} = (Q_{HV} \times C_L) - \left( Q_{HA} \times \frac{C_L}{K_p/[H:P]} \right) - (Q_{Gi,vas.} \times C_{\text{cent}}) \]
\hline
2) & \[ \frac{dA_{PV}}{dt} = \left[ (Q_{PV} - \sum Q_{\text{villi},n} \times C_{\text{cent}}) + \sum F_G,n \left( Q_{\text{villi},n} \times C_{\text{cent}} \right) + (F \times k_d \times Dose) \right] - (Q_{PV} \times C_{PV}) \]
where, \\
& \[ F_G = \frac{Q_{gut}}{Q_{gut} + f_{gut} Cl_{gut,int} (\text{unbound})}; \]
& \[ Q_{gut} = (Cl_{perm} \times Q_{\text{villi},n})/(Cl_{perm} + Q_{\text{villi},n}); \]
& \[ Cl_{perm} = S \times P_{\text{eff},n}; \]
\hline
\end{tabular}
\end{table}

Once the per oral model was demonstrated to be appropriately centered on the mean responses from small, strategically selected \textit{in vivo} trials, the goal of recovering intersubject response variance was pursued.
4.2.7 Perform Simulation to Recover Observed Levels of Pharmacokinetic Variance

Having the ability to simulate inter-subject variance within an IVIVC via Monte Carlo resampling algorithms is important. Such capabilities allow stochastic variability to be accounted for during the prediction of plasma concentration profiles thus providing a means by which traditional variance-based biostatistical assessments can be performed. However, it is critical that variance estimates concerning the respective system/drug dependent components of the physiologically based pharmacokinetic model be informed by data-driven means. The plasma concentration-vs-time profile following oral administration of carbamazepine is largely a function of three components: the rate of elimination (i.e. apparent systemic plasma clearance, $CL_{sys}$), the rate of absorption (i.e. first order absorption rate constant, $k_a$), and the extent of absorption (i.e. bioavailability, $F$). The accurate incorporation of inter-subject variance began with running simulations until convergence. This was defined as the mean and standard deviation of the distributions of pharmacokinetic metrics being $< 1\%$ upon the addition of an additional in silico trial. For the pharmacokinetic parameters of interest, reported vs. simulated comparisons of variance were performed using Levene’s test.\textsuperscript{305}
The Levene test is defined as:

\[ H_0: \sigma_1^2 = \sigma_2^2 = \cdots = \sigma_k^2 \]

\[ H_a: \sigma_i^2 \neq \sigma_j^2 \quad \text{for at least one pair } (i,j) \]

**Test Statistic:** Given a group of responses \( Y \) with sample of size \( N \) divided into \( k \) subgroups, where \( N_i \) is the sample size of the \( i^{th} \) subgroup, the Levene test statistic is defined as:

\[
W = \frac{n-k}{k-1} \cdot \frac{\sum_{i=1}^{k} n_i (\bar{Z}_{i} - \bar{Z})^2}{\sum_{i=1}^{k} \sum_{j=1}^{n_i} (Z_{ij} - \bar{Z})^2}
\]

where \( Z_{ij} \) has the following definition:

\[
Z_{ij} = |Y_{ij} - \bar{Y}_i|
\]

where \( \bar{Y}_i \) is the mean of the \( i^{th} \) subgroup.

\( \bar{Z}_i \) are the group means of the \( Z_{ij} \) and \( \bar{Z} \) is the overall mean of the \( Z_{ij} \).

**Critical Region:** The Levene test rejects the null hypothesis if:

\[ W > F_{\alpha,k-1,N-k} \]

where \( F_{\alpha,k-1,N-k} \) is the upper critical value of the \( F \) distribution with \( k - 1 \) and \( N - k \) degrees of freedom at a significance level of \( \alpha = 0.05 \).
The approach used to recover the coefficient of variation in clearance reported for each product in the Meyer et al and Olling et al. studies followed the work of Ke et al. Specifically, the process began by modifying the Simcyp® default value for the liver volume coefficient of variation, $CV_{liver\, volume}$. The modification was based on the distribution of values for liver weight as a percentage of body weight (simulated using the patient demographics reported by Meyer et al and Olling et al. and the liver weight individualization routine described in chapter 4 section 4.2.4). The range of liver weight as a percentage of body weight reported by Johnson et al. served as the target during the $CV_{liver\, volume}$ optimization process. In a similar manner, the $CV$ for microsomal protein per gram of liver (MPPGL) as a function of age was modified to recover the range reported by Barter et al. in the age range studied by Meyer et al and Olling et al. Lastly, default values for the $CV$ of the isozyme abundance were modified equally on a percent of default-basis for the recovery of the $CV$ in clearance reported for each product in the Meyer et al and Olling et al. studies.

Neither $CV_F$ nor $F$ was directly reported by Meyer et al or Olling et al. Therefore, after $F$ was calculated in-house using digitization software and noncompartmental analysis (see chapter 3). The objective following the determination of $F$ was i) the determination $CV_F$ for each product and, ii) the derivation of a pair-wise model relating $F$ and $CV_F$. This relationship would provide the ability to build upon the IVIVC’s dissolution-based prediction of $F$ by assigning a $CV_F$ for a given $F$ value. The basis for this model was observed clinical data, specifically the $CV$’s for $AUC$ and $C_{max}$ reported by Meyer et al. or Olling et al. for each product. This was because sensitivity analysis demonstrated a strong correlation between these summary pharmacokinetic metrics and $F$ which was independent of $ka$ in the ranges observed in the studies ($R^2$ for $AUC$ or $C_{max}$ vs $F$ was $> 0.95$ in both cases and $< 0.20$.)
when \( k_a \) was the regressor for \( AUC \) or \( C_{max} \). Therefore, \( CV_F \) was assigned for a known value of \( F \) and the relationship was modeled according to the following procedure.

1. Determine \( F_{average} \) for each product in the Meyer et al. and Olling et al. studies
   a. Performed by digitizing the plasma concentration profiles and performing noncompartmental analysis with a priori information on the volume of distribution to derive the critical pharmacokinetic parameters including \( F \) for the recovery of the observed plasma concentration profile and thereby \( AUC \) and \( C_{max} \) (see chapter 3)

2. Run simulations where each individual product’s \( F \) value is sampled from a normal distribution centered on its respective \( F_{average} \) where the spread is defined by an iteratively varied \( CV_F \)
   a. Simulations were defined by the number of trials and the number of \textit{in silico} patients within each trial
   b. The number of \textit{in silico} patients was representative of that used in the Meyer et al. and Olling et al. studies (\( n=25 \))
   c. The number of trials was dictated by the convergence upon stable pooled mean of \( CV_{AUC} \) and \( CV_{C_{max}} \), respectively.

3. For each simulation performed at a defined \( CV_F \) the distribution of outputs (i.e. plasma concentration profiles) were recorded and the \( CV_{AUC} \) and \( CV_{C_{max}} \) was recorded.

4. The \( CV_{AUC} \) and \( CV_{C_{max}} \) was plotted as a function of \( CV_F \)
5. A $CV_F$ was individually assigned, based on this relationship, to each product’s $F_{average}$ on the basis of recovering the reported product’s respective $CV_{AUC}$ and $CV_{cmax}$

   a. Recovery is based on the Levene test for equal variance

The last correlation to be modeled was between $k_a$ and the $CV$ for $k_a, CV_{k_a}$. Sensitivity analysis shows that $T_{max}$ following a one-time dose is almost exclusively a function of $k_a$ when clearance pathways are held constant. Meyer et al. and Olling et al. calculated $T_{max}$ as the time at which $C_{max}$ occurs. They reported mean $T_{max}$ values for each product and the respective $CV$ for $T_{max}, CV_{T_{max}}$. Thus, the $k_a$ vs $CV_{k_a}$ relationship was established using a similar procedure for the refinement of the $F$ vs $CV_F$, this time $CV_{T_{max}}$ took the place of $CV_{AUC}$ and $CV_{cmax}$ and $k_a/CV_{k_a}$ took the place of $F/CV_F$.

4.3 Results and Discussion

4.3.1 Recovery of Systemic Clearance: Mean and Variance

The Simcyp® platform was able to successfully integrate:

i) data generated using *in vitro* enzyme and cellular systems (as part of typical preclinical drug discovery activities), with

ii) the relevant physicochemical attributes of carbamazepine (i.e. LogP value) and,

iii) demographic, physiological and genetic information of patients for the prediction *in vivo* pharmacokinetic parameters and profiles.

An intrinsic functionality of the Simcyp® platform is the inclusion of population variability that, with proper consideration of the covariation of parameters, allows for the
performance of virtual clinical trials and capturing of inter-individual differences in drug exposure where they occur. Using robust data the software provided a framework to model the covariation of physiological parameters and propagation of the respective variability across an in silico population in a rational manner using correlated Monte Carlo sampling to preserve the observed gender-age-weight-height-BSA-liver volume relationship (see chapter 2 section 2.4). Thus, the fidelity of virtual individuals to real people was maximized by preventing the impossible combination of physiologic parameters. For further reading on the history, development, computer science, and application examples of the software see Jamei et al.307

Devising a physiologically based clearance model within the Simcyp® platform that would accurately simulate plasma concentration profiles following intravenous dosing was the first step pursuant to the goal of this chapter (i.e. the incorporation of physiologically based clearance pathways within the previously developed IVIVC for the mechanistic recovery of intersubject variance using correlated Monte Carlo simulations). The results of the literature search of drug specific data necessary to perform the physiologically based pharmacokinetic modeling of carbamazepine, as per Equations. 4.2.4.1 - 4.2.6.2, is provided in Table 4.3.1.1.
### Table 4.3.1.1. Drug Specific Parameters of Carbamazepine in Physiologically Based Pharmacokinetic Model

<table>
<thead>
<tr>
<th>PK Property</th>
<th>In vitro System</th>
<th>Parameter</th>
<th>Value</th>
<th>Comments and References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drug Specific Parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A4 (Enzyme Kinetics)</td>
<td>HLM &amp; Recombinant CYP (Baculovirus-Insect-Cell-expressed)</td>
<td>$V_{\text{max}}$</td>
<td>1.2</td>
<td>Formation of Epoxide (Major metabolite)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$K_{\text{m}}$</td>
<td>120</td>
<td>ISEF $\approx$ 1</td>
</tr>
<tr>
<td>3A5 (Enzyme Kinetics)</td>
<td>Recombiant CYP (Baculovirus-Insect-Cell-expressed)</td>
<td>$V_{\text{max}}$</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$K_{\text{m}}$</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>2C8 (Enzyme Kinetics)</td>
<td>Recombinant CYP (Baculovirus-Insect-Cell-expressed)</td>
<td>$V_{\text{max}}$</td>
<td>0.67</td>
<td>Formation of Hydroxylated Metabolites</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$K_{\text{m}}$</td>
<td>760</td>
<td>ISEF $\approx$ 1</td>
</tr>
<tr>
<td></td>
<td>UGT (Enzyme Kinetics)</td>
<td>$V_{\text{max}}$</td>
<td>0.79</td>
<td>Also measured Vmax/Km in HLM, HIM and HKM. Allowed calculation of UGT tissue scalars for Liver, Intestine and Kidney (no need for ISEF)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$K_{\text{m}}$</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Free fraction of CBZ in hepatocytes</td>
<td>HLM</td>
<td></td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Unbound percent in plasma</td>
<td>plasma samples</td>
<td>fu_p</td>
<td>25%</td>
<td>Association constant $(K_a) = 5.3 \times 10^3$ M$^{-1}$</td>
</tr>
<tr>
<td>Volume of Distribution in Central Compartment</td>
<td>In vivo studies using IV dosing (L/kg)</td>
<td>V</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Blood to plasma partition ratio</td>
<td>Whole blood samples</td>
<td>BP</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>Log P</td>
<td></td>
<td></td>
<td>2.42</td>
<td>Octanol in water partition coefficient</td>
</tr>
<tr>
<td>Fraction unbound in incubations</td>
<td>ultrafiltration following incubation</td>
<td>fu_inc</td>
<td>1</td>
<td>Reference inclusion criteria: a) unbound intrinsic clearances be reported, or b) fu-inc be reported (see Pearce et al.). The majority reported unbound Cl_int.</td>
</tr>
<tr>
<td>Fraction unbound in liver</td>
<td>$\frac{\text{PLR} \times \text{fu}<em>\text{p}}{1 + (\text{PLR} - 1) \times \text{fu}</em>\text{p}} = \text{fu}_\text{liver}$</td>
<td></td>
<td></td>
<td>PLR: plasma-to-liver ratio of binding proteins; equal to 11.6 according to a unified algorithm by Peyret et al. for Poulin et al. predicting partition coefficients in PBPK modeling studies</td>
</tr>
<tr>
<td>Fraction unbound in gut</td>
<td>Assumed to equilibrate with free concentration in blood</td>
<td>fu_gut</td>
<td>1</td>
<td>Albumin is the binding protein for CBZ, it is made by hepatocytes, is contained mainly in the central compartment</td>
</tr>
</tbody>
</table>

After including the data from Table 4.3.1.1 into Equations 4.2.4.1 - 4.2.6.2, predictions were made using the 100mg i.v. dosing strategy of Marino et al. Figure 4.3.1.1.b demonstrates good agreement between the mean plasma concentration profile observed from the intravenous data in the Marino study and the profile generated from the simulations. The Marino data used a clinical population. This means that the population was already induced. The full profile of the data from Figure 4.3.1.1.b (data not shown) demonstrated that mean autoinduction ceased at 235.2 hours during chronic administration. The accurate recovery of a the 100 mg intravenous dose under autoinduced conditions was apparent by a visual predictive check of Figure 4.3.1.1.b. These results confirmed the accuracy of the $E_{max}$ and $EC_{50}$ of 2.1 and 211 uM used in this study.

The $R^2$ for a plot of the observed vs simulated was 0.98 and the 95% confidence interval for the slope and bias terms for the data contained in Figure 4.3.1.1.b contained 1 and 0 respectively (see Figure 4.3.1.1.c). Additionally, the percent error for the simulated $Cl_{sys}$ was 2.3% when compared to the reported value (see Figure 4.3.1.1.a). This demonstrated the initial suitability of the physiologically based model parameters for clearance. The results from the modeling effort is graphically reported in Figure 4.3.1.1.c.

Terms:  
HLM: human liver microsomes; $V_{max}$: Michaelis-Menten maximum rate of metabolite formation (pmol/min/mg of rCYP); $K_m$: Michaelis-Menten constant (µM of substrate concentration); $V$: L kg$^{-1}$
**Figure 4.3.1.1.a Observed vs. Simulated Clearances**

![Observed vs. Simulated Clearances]

- 95% CI for Marino et al.
- Mean Marino et al.
- Mean Sim Clearance

**Figure 4.3.1.1.b Recovered Marino et al. (2012) Data**

![Recovered Marino et al. (2012) Data]

- Marino et al. (2014)
- Mean Sim

**Figure 4.3.1.1.c Observed vs. Simulated**

![Observed vs. Simulated]

\[
y = 1.1208x - 0.0109 \quad R^2 = 0.9794
\]
The next step was to insert the physiologically based clearance model into the model for per oral carbamazepine administration used by the IVIVC of the previous chapter. This activity had two requirements: 

1. The accurate mean elimination rate prediction for each of the formulations reported by the formative per oral studies.

2. An accurate recovery of the variance reported in these studies.

**Figure 4.3.1.2. Observed and Simulated $k_e$ Values**

Horizontal axis: product ID - Vertical axis: $k_e$ (hr$^{-1}$) - Whiskers: 95% CI

Light bar: simulated results - Dark bar: observed results
The Figure 4.3.1.2 visually demonstrates how the simulated elimination rates aligned well with those observed. The variance in the outcomes were refined by decreasing the default $CV$ for *MMPGL*, liver volume, and reported isozyme abundances (i.e. 3A4, 3A5, 2C8, and UGT 2B7) by 75%. The final distributions for the liver weights are represented in Figure 4.3.1.3. as a percentage of body weight.
Table 4.3.1.2. presents the data used to construct Figure 4.3.1.2. The ranges spanned by the 95% confidence intervals were all observed to overlap. This signified that the differences between the mean $k_e$ reported for each product in the studies and those $k_e$ generated by the respective simulations were statistically insignificant. Furthermore, the Levene test for equal variance was used to test for the equality of variances. Here, the $n$ of the test statistic was defined by the average number of patients in the Olling and Meyer studies (N=24). It was observed that none of the variances were statistically significant ($p > 0.05$ for all of the cases).
Table 4.3.1.2. Observed and Predicted Values for $k_e$

<table>
<thead>
<tr>
<th>Study</th>
<th>Product</th>
<th>Observed</th>
<th></th>
<th>Simulated</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$k_e$</td>
<td>CV</td>
<td>$k_e$</td>
<td>CV</td>
</tr>
<tr>
<td>Meyer 1998</td>
<td>1</td>
<td>0.0178</td>
<td>0.19</td>
<td>0.017</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.018</td>
<td>0.21</td>
<td>0.018</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.0178</td>
<td>0.21</td>
<td>0.017</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.0181</td>
<td>0.22</td>
<td>0.017</td>
<td>0.17</td>
</tr>
<tr>
<td>Meyer 1992</td>
<td>1</td>
<td>0.01725</td>
<td>0.15</td>
<td>0.017</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.01818</td>
<td>0.19</td>
<td>0.018</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.01721</td>
<td>0.16</td>
<td>0.017</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.0163</td>
<td>0.16</td>
<td>0.016</td>
<td>0.17</td>
</tr>
<tr>
<td>Olling</td>
<td>1</td>
<td>0.0155</td>
<td>0.18</td>
<td>0.015</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.017</td>
<td>0.18</td>
<td>0.016</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.016</td>
<td>0.16</td>
<td>0.015</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.0153</td>
<td>0.15</td>
<td>0.015</td>
<td>0.16</td>
</tr>
</tbody>
</table>

It should be noted that the Olling et al. study did not directly report elimination rates. However, the study did report elimination half lives allowing $k_e$ to be calculated using the equation: $k_e = \ln(2) / t_{1/2}$. Additionally, the 1998 Meyer et al. data was held back to be used for evaluating external predictability after the refinement process. Despite this, the model developed using the 1992 Meyer et al study and the Olling et al. could still accurately predict the center and spread of the clearances from the 1992 Meyer et al. study.

Once the clearances parameters, and their respective $CV$ converged on values that satisfied the desired accuracy criteria, the final step was to refine the model that would accurately provide a $CV_F$ and $CV_{k_a}$ based on the predicted mean of $F$ and $k_a$. As a review, this was achieved by:

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i) varying the $CV$ for $F$ and $k_a$ in a full factorial scheme from 0% to 150% for each of the four products reported by Meyer and Olling (total = 8)

ii) recording the post-simulation $CV_{AUC}$, $CV_{Cmax}$, and $CV_{Tmax}$

iii) identify the $CV_F$ that returned the observed $CV_{AUC}$ and $CV_{Cmax}$

iv) identify the $CV$ to each $k_a$ that returned the observed $CV_{Tmax}$

v) constructing models that captured the respective $k_a$-vs.-$CV_{k_a}$ and $F$-vs.-$CV_F$

relationships

It is worth noting that one feature of the plasma profile simulation process was to preserve the analytical features of each study. Specifically, the process used the same plasma concentration time points as those reported in the studies. The analytical error reported for each respective study was also accounted for within the simulations. This was accomplished by randomly adding the noise to the profile which was on the order of the observed analytical precision reported in the quality control sections for each study.

The importance of using simulation time points that were consistent with those reported in the respective studies was to maximize the fidelity of the simulation. Without this the $CV$ for the pharmacokinetic parameters would be conflated with the analytical error. This is particularly important when considering the recovery of the $CV$ for $C_{max}$ and $T_{max}$. To illustrate this, consider Figure 4.3.1.5. which was taken from the original 1992 Meyer et al. study.

Figure 4.3.1.5. Plasma Concentration Profiles from 1992 Meyer et al.
The square generated by the intersection between the red lines in Figure 4.3.1.5 indicates the region of $C_{max}$, and by extension $T_{max}$, that occurs in product 4. Given that the authors of this study directly defined $C_{max}$ as the largest concentration determined during drug-plasma quantification, noise in the analytical procedure method directly propagates to the reported variance in the $C_{max}$ metric. Furthermore, it can be seen in the peak plasma concentration profile for product 4 is relatively broad. While this type of performance makes capturing the true $C_{max}$ less of an issue, it can result in estimations of $T_{max}$ which are sensitive to the sampling interval. Therefore, it was important that the simulations in this chapter accounted for the procedural contributions to variance that were present in the CVs for $T_{max}$ and $C_{max}$ reported by the formative studies.

The resulting models are reported in Figure 4.3.1.6. a. and b. The accuracy with which the variance in $AUC$, $T_{max}$, and $C_{max}$ was predicted is demonstrated in Table 4.3.1.3. The
ability to predict the mean $AUC$, $T_{max}$, and $C_{max}$ was unchanged from the previous chapter since the center of the $k_a$ and $F$ were not altered.
The primary indicator for $ka$ variance was the variance in $T_{max}$. This was due to the strong correlation between $ka$ and $T_{max}$ across the range of bioavailabilities and clearance rates observed in this work ($R^2 > 0.95$, individual simulation studies not shown). From Figure 4.3.1.6.a it was observed that as $ka$ decreased, $CV_{ka}$ decreased. Thus, it followed that quicker releasing carbamazepine products had earlier $T_{max}$ values and were of higher variability. This increased variability at faster release rates could have been driven by a host of factors, including those related to the scenario of higher concentrations being reached in the gut by faster dissolving products. These higher concentrations could have lead to transient deviations from the sink conditions typically associated with drugs of higher permeability as is the case with carbamazepine. Thus the higher variability in $T_{max}$ could have been driven by intersubject differences in permeability and gastrointestinal tract transit transiently defining the rate of absorption in such cases. However, when the drug was released as slower rates, the saturated concentrations required to cause a temporary loss of sink conditions would not have been achieved. Thus, the rate of systemic absorption would follow classical BCS class II behavior, i.e. dissolution existing as the rate-limiting step in absorption. Given that the products were all marketed drug products, it is conceivable that they were manufactured under consistent conditions resulting in minimal within batch differences in performance. Thus, the decreased variability of $in vivo$ performance for products with a slower release was expected.
The observed trend in Figure 4.3.1.6.b was that as $F$ increased the $CV_F$ bioavailability decreased. To explain this relationship, consider how, similar to the $T_{max}$-vs.-$ka$ relationship, a strong correlation existed between $F$ and $AUC$, $F$ and $C_{max}$ ($R^2 > 0.95$, individual simulation studies not shown). Thus, variability in $AUC$ and $C_{max}$ could be used as surrogates of $CV_F$. Thus, it was observed that as $F$ increased, the $CV_F$ required to recover the observed variabilities in $AUC$ and $C_{max}$, decreased. This was likely due to the logarithmically increasing relationship of $F$ as a function of $ka$. This is to say that as $ka$ increases, $F$ increases and approaches 1. Thus, significant variation in large $ka$ values result in only minimal changes to $F$. By extension, if an immediate release drug that is highly permeable quickly enters into solution (as was the case for drug with a high $ka$) the probability of it not being thoroughly absorbed over its residence time in the gastro intestinal tract is minimal. Since, carbamazepine undergoes little gut metabolism intersubject differences in permeability and GI transit do not substantially impact $AUC$. It is only at slow dissolution rates (i.e. low values for $ka$) where the drug has difficulty escaping the dosage form and thus variability in transit times and other physiologic factors begin to more substantially vary the value of $F$.

Table 4.3.1.3. Observed vs Simulated CVs for Summary Pharmacokinetic Metrics

<table>
<thead>
<tr>
<th>Study</th>
<th>Product</th>
<th>AUC</th>
<th>Cmax</th>
<th>Tmax</th>
<th>AUC</th>
<th>Cmax</th>
<th>Tmax</th>
</tr>
</thead>
</table>

145
| Olling | 1   | 0.24 | 0.31 | 0.41 | 0.21 | 0.21 | 0.53 |
|       | 2   | 0.31 | 0.29 | 1.05 | 0.28 | 0.25 | 0.72 |
|       | 3   | 0.28 | 0.27 | 1.13 | 0.26 | 0.24 | 1.18 |
|       | 4   | 0.20 | 0.16 | 0.47 | 0.21 | 0.21 | 0.53 |
| Meyer 1992 | 1   | 0.15 | 0.20 | 0.51 | 0.22 | 0.22 | 0.53 |
|       | 2   | 0.47 | 0.62 | 0.74 | 0.47 | 0.63 | 0.53 |
|       | 3   | 0.2  | 0.18 | 0.72 | 0.21 | 0.21 | 0.57 |
|       | 4   | 0.29 | 0.39 | 0.78 | 0.26 | 0.35 | 0.53 |
| Meyer 1998 | 1   | 0.18 | 0.16 | 0.45 | 0.21 | 0.20 | 0.53 |
|       | 2   | 0.19 | 0.15 | 0.43 | 0.21 | 0.19 | 0.61 |
|       | 3   | 0.19 | 0.21 | 0.42 | 0.21 | 0.21 | 0.58 |
|       | 4   | 0.17 | 0.15 | 0.31 | 0.21 | 0.19 | 0.50 |

On average, the typical behavior of quicker dissolving drug products being absorbed at faster rates and to a consistently larger extent of absorption was observed. While slower releasing products were possibly less sensitive to physiologic differences, the risk of incomplete absorption became higher. Ultimately, the $CV$ reported by Meyer (1992) and Olling differed insignificantly ($p > 0.05$) from the $CV$ for $AUC$, $C_{max}$, and $T_{max}$ that were respectively simulated. The products and reported $CV$ for $AUC$, $C_{max}$, and $T_{max}$ from the 1998 Meyer study were used as a test for external predictability. Here the differences were again insignificant ($p > 0.05$)

### 4.4 Conclusion

This work leveraged the results of *in vitro* DMPK studies that are commonly performed during preclinical development to mechanistically identify metabolism pathways. The product of these activities augmented the results of conventional clinical pharmacokinetic studies performed *in vivo*. The combined product allowed for the systematic development of a physiologically based IVIVC. While several researchers have attempted to construct
IVIVCs for Carbamazepine, this work is unique in that it i) achieved an acceptable level A prediction ii) while accounting for mechanistic metabolism pathways and iii) recovering the observed population level variance. This work also accounted for the analytical error reported by the conventional clinical pharmacokinetic studies in the clinical trial simulation process. Here, the analytical error reported in the referenced clinical trials was added to the \textit{in silico} clinical trials to maximize the fidelity of the simulation. Following the successful development of this level of IVIVC it will be used as a tool to support the establishment and assurance of bioequivalence in a more clinically relevant manner by the mechanistic description of inter-subject variability.

Chapter 5: Assuring Consistent Performance: F2 vs. Physiologically Based IVIVC – Associated Differences in Bioequivalence Errors and Design Spaces

5.1 Introduction

Chapter five represents the zenith of this project. The quality attribute of interest discussed throughout this document has been \textit{consistent drug product performance} wherein
dissolution tests are used as a means of assessment. In this final chapter, a comparison was made between two methods for defining specifications of the dissolution performance. The methods were based on the use of the conventional F2 statistic and the outputs of a physiologically based IVIVC nested within a clinical trial simulation (PB-IVIVC-CTS) platform. As such, three core assessments were performed. First, the difference between the respective dissolution specifications was assessed. Second, the difference between the resulting product/process parameters design spaces was assessed. Finally, a post hoc assessment of F2-based dissolution profiles using the PB-IVIVC-CTS platform for type I (α) and type II (β) errors in bioequivalence was performed pursuant to the central hypothesis of this work:

For an immediate release carbamazepine product,

when a physiologically based IVIVC is coupled with a clinical trial simulation platform and used to assure the bioequivalence of an immediate release carbamazepine tablet, fewer errors for bioequivalence will be observed when compared to an approach that relies on the F2 criteria.

Many of the current tenants of pharmaceutical quality were inspired by the work of Joseph Juran. He was a luminaire of quantity management theory during the transitional period between the 20th and 21st centuries. Juran’s definition of product quality (and indeed many other’s) can be distilled into two principles: first the presence of attributes within a product which confer user satisfaction following exposure/interaction, and second the reliability of such critical attributes. The FDA rarely defines pharmaceutical quality explicitly. At most, the FDA references a product’s quality in the context of its “suitability for an intended use”. However, a survey of FDA guidances related to pharmaceutical quality provides further resolution of this definition. Here, the “intended use” of a drug product is defined by the approved “label claims”. The context of these claims are based, to a great
extent, on the clinical studies submitted by the product manufacturer and verified by FDA review. Thus, the first aspect of a more integrated and resolved definition for pharmaceutical quality includes the product’s ability to meet the dosing, safety, and efficacy criteria identified in the labeling; and, by extension, used in the investigational batches used during clinical development. Furthermore, the statutory requirement of drug products and substances to comply with the Federal Food, Drug and Cosmetic Act allows Juran’s second concept of quality, consistency, to be considered within an operational definition of pharmaceutical quality. This act states that a drug not made in accordance with current good manufacturing processes (cGMP) is deemed "adulterated." CGMP regulations assure that drugs meet the safety, identity, and strength requirements of the Federal Food, Drug, and Cosmetic Act as well as the quality and purity characteristics that the product purports to possess. As part of cGMP, firms must perform validation procedures to demonstrate that their manufacturing processes can consistently produce a product that meets established quality attributes.

In an op-ed article, Dr. Janet Woodcock (director of the FDA’s Center for Drug Evaluation and Review) stated how conventional validation procedures are often empirical in nature. Assessing the suitability of this practice is an aim of this work. Dr. Woodcock additionally discussed how the Act viewed from the context of overall quality regulation, is a statutory CGMP requirement that can be viewed as a legal representation of the frequent statement that quality must be built-in. This concept is the basis for the pharmaceutical industry’s adaptation of another of Juran’s concepts, i.e. quality by design.

The concept of quality by design, along with the utilization of design spaces and the probabilistic assessment of risk (of propagating excessively wide or unnecessarily
narrow specifications) are key aspects of this project. The term quality by design (QbD) was first coined by Juran in his 1991 publication entitled Juran on Quality by Design and has since been invoked by a variety of fields. According to Juran, achieving quality by design during industrial manufacturing is the result of three central activities (i.e. Juran’s Trilogy) which make up Juran’s philosophy for quality management. These activities include quality planning, quality control, and quality improvement. Paralleling the previous axiom articulated by Dr. Woodcock, the FDA contextualizes Juran’s concept of quality by design within the field of pharmaceutical manufacturing by stating that QbD is the process of building quality into the product. The FDA expands on the importance of achieving quality by design by emphasizing the recognition on the part of sponsors that quality “cannot be tested into products”. While this position may seem counter intuitive, its reasoning is related to the implication that such strategies (i.e. those based on “testing of quality into a product”) represent a reactive, rather than proactive, position towards the assurance of quality and that reliance upon which is a result of limited product/process understanding. This type of a strategy is not in pursuit of maximizing efficiency. Thus, it is considered to increase the risk of unnecessary waste, increased hold times, and higher cost. For these and other reasons, the more holistic perspective on quality assurance is encouraged by the FDA. The administration specifically stresses the objectives for quality measurements be risk-based and predefined. In this way, the assessment of critical product/process parameters provides a path towards improved product understanding and more appropriate process controls.

The process of modeling the quality attributes of a drug product or substance as a function of its critical product/process parameters is known as design space development. The ICH Q8 definition of design space used by the FDA is “the multidimensional
combination and interaction of input variables (e.g., material attributes) and process parameters that have been demonstrated to provide assurance of quality.” Working within an FDA approved design space is not considered as a change. Thus, the utilization of design spaces can confer greater regulatory flexibility and confer improvements to efficiency. Movement out of the design space, however, is considered to be a change and normally initiates a regulatory post approval change process. The post approval change process can be extremely costly to a sponsor and to patients. Therefore, the criteria used to define the limits of a CQA is of great importance.

As introduced at the beginning of this chapter, dissolution performance was the CQA of interest and two competing methodologies were used to define the upper and lower acceptability limits of dissolution. Specifically, the methods used were the F2 criteria and the previously developed IVIVC. Upper and lower limits on dissolution were made with respect to the dissolution performance of the reference labeled product (RLP). The focus on dissolution performance was motivated by the considerable regulatory significance placed on this measurement of quality. This high degree of importance is a result of dissolution being considered the rate limiting step for the absorption of BCS class II substances like carbamazepine. While the F2 metric was used for the sole purpose of defining an empirical region of dissolution profile similarity, the IVIVC usage illustrated in this chapter was twofold. First the IVIVC was used to propagate a distribution of clinical responses that, based on the range of F2 defined dissolution performance. Additionally, the IVIVC was used to directly derive specifications on dissolution.

The final step of this work was to illustrate the implications of selecting one CQA refinement methodology over the other in terms of the i) resulting dissolution specifications
and design spaces, and ii) the associated risk to bioequivalence with either method. Design space development began with the development of a knowledge space. Here the term *knowledge space* means the intervals over which critical product/process attributes have been systematically investigated and the dependent performance of the critical quality attribute is known (also referred to as the *calibrated range*). A design space exists within a knowledge space. The critical product attributes which defined the design and knowledge spaces used in this work were: binder concentration, disintegrant concentration, and porosity. These three factors were considered high risk and are known to significantly affect the dissolution of a solid oral dosage form which could ultimately impact clinical performance.

In statistics, *risk* is a measure of the association between a binary occurrence and set of continuous and/or binary predictors. The concept of risk is directly related to probability. The probability of event $a$ occurring out of $a$ and $b$ possible events is defined as $a/(a + b)$. This is the same calculation for the (absolute) risk of event $a$ occurring. *Monte Carlo*-based modeling techniques, such as that employed by the clinical trial simulation platform in this work, have been widely used for the purpose of probabilistic risk assessment. This is due to their ability to propagate the *a priori* variability associated with model parameters through the model resulting in a distribution of outputs. In the context of a physiologically-based IVIVC, such methods provide the ability to propagate the variability of physiologic systems over an array of product performance inputs and generate a posterior probability distribution of clinical effects. In this way, the risk of errors in bioequivalence can be compared between the two identified CQA specification methodologies using a predefined objective of what constitutes a clinically significant difference. This required estimating a probability density function for the distribution of clinical responses resulting from an F2 defined range of
dissolution profiles, and comparing the function with a PDF that represents an estimated distribution of all possible bioequivalent responses.

5.2 Methods and Materials

5.2.1 Summary PK Metrics of Interest

Cmax and AUC at time 170 (AUC<sub>∞</sub>) were initially selected as the summary pharmacokinetic metrics of interest. This was because of their widely accepted use as markers for the extent of drug exposure. The importance of these characteristic was demonstrated by works of Tothfalusi et al. and Olling et al. who reported how a predictor of toxic events was the magnitude of drug absorption. Olling et al. additionally showed that the differences in absorption rate evidenced by deviations in T<sub>max</sub> were also used. Lastly, after considering that carbamazepine is administered in a chronic fashion, the concentration at steady state (C<sub>ss</sub>) was assessed along with the minimum and maximum (C<sub>ss,Min</sub>, and C<sub>ss,Max</sub>) concentration at steady state. The concentrations at steady state was defined in this work as the average concentration over the last 5 days of a 30 day dosing period.

5.2.2 F2-based Dissolution Limits and the resulting PDF of Clinical Responses

Identifying dissolution model parameter limits using the F2 statistic and transforming the resulting range of in vitro performance into a distribution of clinical responses was accomplished according to Figure 5.2.2.1.
Defining the range of F2 defined dissolution began by nesting the Makoid Banakar dissolution model within the F2 Equation.

\[
f_2 = 50 \cdot \log \left\{ \left[ 1 + \left( \frac{1}{n} \right) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{0.5} \cdot 100 \right\}
\]

**Eq. 5.2.2.1**

where
\[ T_t = F_{max} \cdot \left( \frac{t}{T_{max}} \right)^{b_{MB}} \cdot e^{\left( \frac{b_{MB}}{T_{max}} \right) t}, \quad \text{for} \quad t \leq T_{max} \]

and where for \( t > T_{max} \), \( T_t = F_{max} \)

This allowed limits to be defined for the dissolution model parameters to achieve an F2 > 50 dissolution profile given the performance of a reference drug product. The reference labeled drug product for carbamazepine is Tegretol. Its dissolution performance served as a benchmark for the comparisons performed in this study and was based on the 1992 Meyer et al. study (see Figure 5.2.2.2). The dissolution test in that study used the compendial dissolution test for carbamazepine (see Chapter 3 section 3.2.1).

Using the referenced performance of Tegretol, the F2 metric was calculated for any set of dissolution parameters. Dissolution profiles are identical if F2 is 100. FDA guidance states that generally, F2 values greater than 50 (50-100) ensure sameness or equivalence of the two curves and, thus, of the performance of the test (e.g. postchange).
and reference (e.g. prechange) products. The range of acceptable dissolution profiles was thus defined as those with an F2 value $\geq 50$. This allowed any dissolution profile of a simulated test product to be identified as either equivalent or nonequivalent.

The combination of dissolution parameters that resulted in an $F2 \geq 50$ provided an asymmetric shape and volume. To process the infinite number of dissolution parameter combinations using an IVIVC within the Simcyp® platform, a sampling strategy was developed. The sampling routine was performed in two steps and was based on the assumption that all possible $F2 \geq 50$ dissolution parameter combinations were equally likely. From this assumption, the first step was guided by the principle that points on the surface of the shape were of particular importance. This is because those points represent dissolution profiles that were maximally different from the profile of the reference product and when processed using the IVIVC would define the tails of the distribution of clinical responses. Therefore, the surface of the shape (which describes all of the possible combinations of $b$, $F_{max}$, and $T_{max}$ that resulted in an $F2 > 50$) was sampled until the volume occupied by the samples was within 5% of the maximum possible volume. The volume of the shape was defined using the alphaShape.m and volume.m functions in MATLAB. To determine the maximum volume, ranges of $[60 : 100\%]$ , $[13 : 24000 \text{ sec}]$, and $[0.18 : 2.5]$ were respectively assigned to $F_{max}$, $T_{max}$, and $b$. Sampling commenced and proceeded by increments of 50 samples until the change in the volume occupied by the $F2 > 50$ region was $\leq 1.0\%$. The surface of the resulting shape was then sampled by rank ordering the respective F2 values (from smallest to largest, i.e. 50 to 100). Beginning with the first 10 samples (i.e. single series of $F_{max}$, $T_{max}$, and $b$) whose F2 value closest to 50, subsequent samples were added
until the volume of the subsamples converged to the max volume according to the previous criteria (i.e. 5%).

The second step of the sampling algorithm was to sample the interior of the shape. This was performed as per the assumed ability of dissolution parameters to exist anywhere within the specification limits. The number of samples randomly drawn from the interior was set equal to the number needed to represent the surface.

With the surface and interior samples drawn, each set of dissolution model parameters was processed using the IVIVC and transformed into a rate and extent of absorption. Using the outputs the IVIVC, the clinical trial simulation platform was then used to generate distributions plasma concentration profiles. These distributions were then pooled into a single probably density distribution and saved for the subsequent assessment bioequivalence errors.

5.2.3 Deriving an encompassing PDF of Bioequivalent Observations

Before bioequivalence errors associated with the use of the F2 statistic could be assessed two activities needed to performed. First, the criteria for a clinically significant difference first needed to be defined. Next, an encompassing PDF of bioequivalent observations needed to be derived based on this criteria.

5.2.3.1 Defining the Criteria for a Clinically Significant Difference

It is extremely unlikely for a test and reference product to perform identically. Therefore, the determination of bioequivalence requires that a threshold be defined for what constitutes a clinically significant difference for metrics of interest. There are a
variety of ways to assess the significance of an observed difference in test-vs-reference responses for $AUC$, $C_{max}$, $T_{max}$, $C_{ss,Max}$, $C_{ss,Min}$, and $C_{ss}$. The approach taken in this work was to base the threshold on the width of the therapeutic window for carbamazepine as reported by Shargel et al. and supported by others. Here, the authors stated how the effective concentration for carbamazepine was $9 \pm 3$ ng/ml wherein concentrations below 6 ng/ml and above 12 ng/ml would be subtherapeutic and toxic, respectively. To translate this into a threshold for bioequivalence, the range was normalized by the target concentration. This was performed according to reports which discuss how while a target concentration can vary between subjects, the normalized therapeutic window remains relatively constant. The resulting window was $T_{egretol \ response} \pm 33\%$. Additionally, the within subject variation has been reported to be approximately 10%. While this value is considered low by the FDA, it was nevertheless accounted for within the acceptance window by subtracting it from the normalized therapeutic index to ensure consistent clinical performance despite a patient’s inherent variability. Thus, the threshold for considering a difference to be clinically significant in mean responses of $AUC$, $C_{max}$, $T_{max}$, $C_{ss,Max}$, $C_{ss,Min}$, and $C_{ss}$ was $\geq \pm 23\%$. This window for the accepted difference in the median is illustrated in Figure 5.2.3.1.1.

Figure 5.2.3.1.1. Introduction of the interval for bioequivalence
5.2.3.2 Deriving the Encompassing PDF of Bioequivalent Observations

Figure 5.2.3.1.1. makes reference to a region representing 95% of the observations. When placed in the context of frequentist statistics this interval is the functional equivalent of the prediction interval for a single observation. In the context of Bayesian statistics, this interval is the credible interval for a single observation. Deriving the probability density function along this interval was accomplished according to the procedure illustrated in Figure 5.2.3.2.1. This process ultimately allowed the primary research question of this work to be assessed.

For carbamazepine, (and indeed many other drug substances) there is a dynamic relationship between the mean drug exposure and the observed variability in drug exposure given this metric of central tendency. With this understanding, the procedure for deriving the probability density function of bioequivalent observations was guided by three underlying principles. First, the pharmacokinetic parameters concerning absorption, i.e. $F_a$ and $k_a$, are largely dependent upon the dissolution performance of the final drug product. Additionally, the coefficient of variation for these parameters are a function of their central tendency. Finally, the elimination kinetics of carbamazepine are not zero-order, and, therefore, the clearance, and thus the variation in clearance, is also a function of the exposure.
Figure 5.2.3.2.1. Derivation of the PDF describing individual bioequivalent responses

- Sample from a uniform distribution over $F_a$ and $k_a$ respectively
- Predict $CV_{F_a}$ and $CV_{k_a}$ given $F_a$ and $k_a$, respectively (see chapter 4)
- Process each set of $F_a$, $CV_{F_a}$, $k_a$, and $CV_{k_a}$ using the CTS platform

Calibrated Clinical Trial Simulation Platform

Predict $CV_{F_a} = f(F_a)$ and $CV_{k_a} = f(k_a)$

Does the expected value for the PDF of $F_a$, $AUC$, and $C$ Is $E[PDF] = \text{within } \pm 45\%$ of $N$?
The first step in the process outlined by Figure 5.2.3.2.1 was to assign uniform distributions to the values of $F_a$ and $k_a$. Samples from this distribution were referred to as $F_{a,target}$ and $k_{a,target}$. The goal was that after samples were drawn and processed using the clinical trial simulation platform, the distributions of clinical responses would cover the interval responses bioequivalent to $F_a = 0.78$ and $k_a = 0.28 \text{ hr}^{-1}$ (i.e. the values for the reference product Tegretol; see chapter 3). The range of possible $F_{a,target}$ values was $[58\% : 98\%]$ while the range for $k_{a,target}$ was $[0.18 \text{ hr}^{-1} : 0.37 \text{ hr}^{-1}]$.

With the ranges of $F_{a,target}$ and $k_{a,target}$ defined, the next step was to draw a random sample from each uniform distribution. Once the $F_{a,target}$ and $k_{a,target}$ values
were sampled, the corresponding intersubject variability for a given \( F_{a,\text{target}} \) and \( k_{a,\text{target}} \) (i.e. \( CV_{F_{a,\text{target}}} \) and \( CV_{k_{a,\text{target}}} \)) were determined using the models derived in chapter 4. The set of \( CV_{F_{a,\text{target}}} \), \( CV_{k_{a,\text{target}}} \), \( F_{a,\text{target}} \) and \( k_{a,\text{target}} \) were then used as inputs to the clinical trial simulation platform. Here, in silico patients were generated following the procedure described in Chapter 4. Individual values for \( F_a \) and \( k_a \) being assigned to each generated patient based on a random draw for a normal distribution defined by a mean of \( F_{a,\text{target}} \) and \( k_{a,\text{target}} \) respectively, and a variance equal to the respective coefficients of intersubject variation as per Chapter 4. This method allowed the absorption parameter-vs-CV\(_{\text{absorption parameter}}\) relationship to be preserved across all the possible \( F_{a,\text{target}} \) and \( k_{a,\text{target}} \) values.

Two types of in silico clinical trials were run. The first was similar to a traditional bioequivalence trial in that it was based on a single 200mg dose. The average \( C_{\text{max}} \) and AUC\(_{\infty}\) values were assigned to a given set of \( F_{a,\text{target}} \) and \( k_{a,\text{target}} \) values using the results of these simulations. The second study was based on dosing of the 200mg strength three times a day for 30 days. The results of this trial were used to assign a Css value to a given set of \( F_{a,\text{target}} \) and \( k_{a,\text{target}} \) values. Both simulations were performed using 50 trials of 24 patients each drawn from the calibrated population described in Chapter 4. The number of patients per trial was based on the number of patients included in the foundational studies performed by Meyer et al and Olling et al. The number of trials was selected to ensure convergence to a stable distribution of pooled clinical responses. The resulting distribution of clinical responses for each \( F_{a,\text{target}} \) and \( k_{a,\text{target}} \) sampled in the first step of this process was saved for subsequent pooling if it satisfied the criteria described in section 5.2.3.1.
5.2.4 Comparison of F2-based Clinical Responses to the Encompassing PDF of BE responses

The product of the clinical trial simulations performed according to sections 5.2.2 and 5.2.3.2 were two distributions of clinical responses. The first distribution represented the clinical responses originating from the processing of F2 ≥ 50 dissolution profiles using the IVIVC nested within a physiologically based clinical trial simulation platform. This was considered the F2 distribution. The second distribution was the distribution of responses bioequivalent to the performance of Tegretol. This was considered the reference distribution. The distributions were fit using the fitdist.m function within Matlab (2016)). Within this function, a kernel function was used to fit the distributions. For any real values of \( x \), the kernel density estimator’s formula is given by,

\[
\hat{f}_h(x) = \frac{1}{nh} \sum_{i=1}^{n} K \left( \frac{x - x_i}{h} \right)
\]

were, \( x_1, x_2, \ldots, x_n \) are random samples from an unknown distribution, \( n \) is the sample size, \( K(\cdot) \) is the kernel smoothing function, and \( h \) is the bandwidth. The kernel estimate for the cumulative distribution function (CDF) of the results was given by,

\[
\hat{F}_h(x) = \int_{-\infty}^{x} \hat{f}_h(t) dt = \frac{1}{n} \sum_{i=1}^{n} G \left( \frac{x - x_i}{h} \right)
\]

where

\[
G(x) = \int_{-\infty}^{x} K(t) dt
\]
Here, a non-parametric kernel smoothing function was selected for $K(\cdot)$ to minimize bias. The bandwidth was selected based on assessing the root mean squared error in cross validation (RMSECV). Using a random 25% hold back of the data, the error between the predicted CDF value and an observed CDF value was assessed across varying bandwidth window sizes. The window size ultimately decided upon was 150.

With the $F_2$ and reference probability densities defined, the probability of an observation over a specific interval could be calculated and the differences between the two distributions could be assessed. The probability over an interval for a given distribution was assessed by,

$$\frac{\text{area under the interval for a PDF}}{\text{total area under PDF}}.$$

The area under the PDF was calculated using the Runge-Kutta algorithm within the ode45.m function in MATLAB. The interpretation of probabilistic differences between two distributions supported the use of Bayesian methodologies. In Figure 5.2.4.1, the green PDF represents the encompassing (i.e. maximum) distribution of bioequivalent responses. It was defined as the reference probability distribution of equivalent responses centered on the performance of a reference product. The blue PDF represents the clinical responses (propagated via a clinical trial simulation) stemming from the use alternative, dissolution based, criteria for equivalence, e.g. the $F_2$ metric. In this Figure there are three different scenarios examined to identify exactly how differences will be categorized. All differences were made with respect to the reference distribution as it was explicitly derived according to observed degrees of intersubject variability and clinically significant differences in clinical performance (see sections 5.2.3.1 and 5.2.3.2). The categories used were type I or type II errors.
(failures) for bioequivalence. The Bayesian concept of credible intervals was used for the assessment of these errors. The intervals for the distributions were defined using their respective CDF. The upper and lower 2.5% were excluded resulting in what was defined as the 95% credible interval for the distribution. These intervals were defined along the x-axis with values of $C_{I_{\text{lower},i}}$ and $C_{I_{\text{upper},i}}$ where $i = \{\text{reference (ref)}, \text{alternative (alt)}\}$. Errors were assessed at both ends of the intervals. Scenarios when either $(C_{I_{\text{lower},\text{ref}}}-C_{I_{\text{lower},\text{alt}}})$ or $(C_{I_{\text{upper},\text{ref}}}-C_{I_{\text{upper},\text{alt}}})$ was positive or negative, respectively, type I errors were said to exist. The probability of type I errors in these cases were $\int_{C_{I_{\text{lower},\text{ref}}}}^{C_{I_{\text{lower},\text{alt}}}} PDF_{\text{ref}}$ and $\int_{C_{I_{\text{upper},\text{ref}}}}^{C_{I_{\text{upper},\text{alt}}}} PDF_{\text{ref}}$, respectively. Type I errors represent the false rejection of bioequivalence for a given clinical response on the basis of excessively tight alternative equivalence criteria at a given boundary condition (i.e. upper or lower). Type II errors were identified when either $(C_{I_{\text{lower},\text{ref}}}-C_{I_{\text{lower},\text{alt}}})$ or $(C_{I_{\text{upper},\text{ref}}}-C_{I_{\text{upper},\text{alt}}})$ was negative or positive, respectively. The probability of type II errors in these cases were $\int_{C_{I_{\text{lower},\text{ref}}}}^{C_{I_{\text{lower},\text{alt}}}} PDF_{\text{alt}}$ and $\int_{C_{I_{\text{upper},\text{ref}}}}^{C_{I_{\text{upper},\text{alt}}}} PDF_{\text{alt}}$, respectively. Type I errors represent the false acceptance of bioequivalence for a given clinical response on the basis of excessively wide alternative equivalence criteria at a given boundary condition (i.e. upper or lower). In a scenario where when both $(C_{I_{\text{lower},\text{ref}}}-C_{I_{\text{lower},\text{alt}}})$ or $(C_{I_{\text{upper},\text{ref}}}-C_{I_{\text{upper},\text{alt}}})$ was negative, the $\int_{C_{I_{\text{lower},\text{ref}}}}^{C_{I_{\text{lower},\text{alt}}}} PDF_{\text{ref}}$ would represent the probability of type I errors while the $\int_{C_{I_{\text{lower},\text{ref}}}}^{C_{I_{\text{lower},\text{alt}}}} PDF_{\text{alt}}$ would represent the probability of type II errors.

**Figure 5.2.4.1.** Illustration for the interpretation of differences in distributions of clinical responses
5.2.5 Comparison of F2 Dissolution Specification with IVIVC-based Specification

The comparison of dissolution profile specifications was performed on the basis of the area occupied by the respective specifications. The F2 area was calculated by simulating to convergence a stable population of dissolution profiles whose F2 value was greater than or equal to 50 when compared to the dissolution profile of Tegretol as per section 5.2.2. The simulated dissolution profiles were made on a minute-wise basis up until 2 hours. The area under the minimum amount dissolved for each time point in the population of simulated responses was calculated using the trapz.m function in MATLAB. This function uses the trapezoidal method for calculating the area under the curve. This value was then subtracted from the area under a curve defined by the minimum value for each time point in the population of responses.

\[
\text{CI}_{\text{n,alt}} \text{ is narrower than CI}_{\text{n,ref}}
\]

Therefore, \( P(\text{CI}_{\text{n,ref}}) - P(\text{CI}_{\text{n,alt}}) = \) probability of falsely rejecting BE based on the “test” equivalence criteria (i.e. \( P(\text{type I error}) \)).

\[
\text{CI}_{\text{n,alt}} \text{ wider than CI}_{\text{n,ref}}
\]

Therefore, \( P(\text{CI}_{\text{n,alt}}) - P(\text{CI}_{\text{n,ref}}) = \) probability of falsely accepting BE based on the “test” equivalence criteria (i.e. \( P(\text{type II error}) \)).

At the upper limit of \( \text{CI}_{\text{n,ref}} \):

\( \text{CI}_{\text{n,alt}} > \text{CI}_{\text{n,ref}} \) resulting in the occurrence of type II error

At the lower limit of \( \text{CI}_{\text{n,ref}} \):

\( \text{CI}_{\text{n,alt}} < \text{CI}_{\text{n,ref}} \) resulting in the occurrence of type I error
To calculate the area of a dissolution profile specification defined using the IVIVC, the range of \( k_a \) and \( F_a \) values that resulted in performances that were bioequivalent to Tegretol as per section 5.2.3.2 were recalled. With these ranges of the acceptable absorption parameters, the IVIVC of Chapter 2 could then be used to solve for the of dissolution parameters that would equal the upper and lower limits of \( k_a \) and \( F_a \). Simulations were then run across the range of acceptable dissolution parameters to generate a population of dissolution responses just as before. The area occupied by this population of dissolution profiles was calculated in the same way as with the \( F_2 \geq 50 \) dissolution profiles.

5.2.6 Comparison of F2 Dissolution Design Space with IVIVC-based Space

The comparison of design spaces derived from the dissolution specifications based on F2 and IVIVC required the development of a knowledge space. The knowledge space was defined by the binder concentration, disintegrant concentration, and porosity of a four component tableted carbamazepine drug product. The binder used was hydroxypropyl methylcellulose (HPC; Nippon Soda Co., Ltd. Tokyo, Japan). The disintegrant used was crospovidone (CP; BASF Corporation, Ludwigshafen, Germany). The formulation also included microcrystalline cellulose (MCC; Sigma Aldrich, St. Louis, MO). Lastly, the carbamazepine was sourced from Cayman Chemical (Ann Arbor, MI). Only a single lot of each product was used in the study.

In this study the carbamazepine concentration was held constant at 50%. A 3 x 3 full factorial design was used to define the binder and disintegrant concentrations of
tableted products used for the dissolution experiments in this study (see Table 5.2.6.1). Any remaining concentration to reach a total of 100% was made up of MCC.

**Table 5.2.6.1. Design of Dissolution experiments**

<table>
<thead>
<tr>
<th>Disintegran (w/w %)</th>
<th>6.25</th>
<th>3.75</th>
<th>1.25</th>
</tr>
</thead>
</table>

Each design point was weighed directly into 100 ml plastic jars to reach a sufficient quantity of 80 grams. The materials were agitated using a benchtop vortex mixer for 30 seconds and then mixed for 20 minutes using a retrofitted bin blender. To ensure homogeneity, this process was performed in triplicate.

A direct compaction method was selected to prepare tablet formulations. Tablets of 400 mg (200 mg of carbamazepine) were prepared by a compaction simulator (Presster®, Metropolitan Computing Co., USA) using a 10-mm flat-faced punch. The rotary press Korsch 336 with 36 stations was simulated at a speed of 0.5 m/s. Compaction force was between 6 and 10 kN for all tablets.

The porosity was varied across three levels: 8%, 11% and 14%. This was achieved by altering the punch distances in the press. Porosity ($\varepsilon$) was calculated according to the following equation based on volume.
\[
epsilon = \left(1 - \frac{m}{V_t \cdot \rho_t}\right) \times 100
\]

In the porosity equation, \( m \) is the tablet mass and \( V_t \) the tablet volume, and \( \rho_t \) is the true density of the powder blends. Density of the powder blends was calculated as the weighted mean from the true density of each component. True density was assessed by a gas displacement pycnometer (AccuPyc 1330, Micromeritics, USA).

Tablets were allowed to completely relax (approximately 1 week) before dissolution tests were performed. Relaxation was verified by measuring the tablet dimensions over a series of several days until values became stable. All tablets used for dissolution tests were within ± 10% of the target porosity. Additionally, dissolution tests were performed in triplicate and the mean was used as the response.

The dissolution test was performed with a USP Apparatus II (Distek, Inc., North Brunswick, NJ). The conditions were set according to the USP monograph for carbamazepine immediate release tablets. The paddle speed was 75 rpm and media was 900 mL water containing 1% sodium lauryl sulfate (w/v) at 37°C. Samples were analyzed every 3 minutes by UV-VIS Spectrophotometry (Agilent, Santa Clara, CA) at 287 nm via an autosampler and flow through cell.

JMP (SAS Institute Inc., Cary, NC) was used to model the percent dissolved as a function of time, binder concentration, disintegrant concentration, and porosity. Here, the neural platform was used due to its inherent flexibility (see Hertz et al neural network theory). Cross validation of the model was performed by randomly dividing the original data into the training and cross validation sets. The proportion of the original data used for cross validation was 20%. The use of up to two layers was provided by the software with an unlimited number of nodes to either layer. The
activation functions available to be applied at the nodes of the hidden layers were the hyperbolic tangent function, a linear function, and the Gaussian function (see Table 5.2.6.2). The models selection was made on the basis of minimizing the negative log-likelihood.

<table>
<thead>
<tr>
<th>Title</th>
<th>Formula</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperbolic tangent function</td>
<td>( e^{2x} - 1 )</td>
<td>Sigmoid function that transforms values to be between -1 and 1, and is the centered and scaled version of the logistic function.</td>
</tr>
<tr>
<td>Linear identity function</td>
<td>( e^{2x} + 1 )</td>
<td>The linear combination of the X variables is not transformed.</td>
</tr>
<tr>
<td>Gaussian function</td>
<td>( e^{-x^2} )</td>
<td>Helpful when the response surface is Gaussian in shape.</td>
</tr>
</tbody>
</table>

An important step of the modeling process was to assess not only the accuracy, but also the generalizability of the model. Model generalizability is a concept frequently invoked in the neural modeling domain. This concept is referred to as the avoidance of overfitting the model to the data. It was assessed by means of a visual predictive check as described by Jann et al. using the “prediction profiler” function within JMP. The visual predictive check is made not only using the inputs and outputs of the calibration and cross validation samples, but also using interpolated inputs wherein the outputs are observed for adherence to trends known a priori.

Accuracy of the final model was accessed on the basis of RMSEC, RMSECV and \( R^2 \). Overfitting of the data was assessed by first selecting binder, disintegrant, and porosity values that existed between the design points of Table 5.2.6.1. These interpolated values were then processed using the model. The generalizability of the
model was supported if the resulting output of dissolution profiles were similarly interposed in a consistent manner that followed the classically observed trends (i.e. porosity, disintegrant $\propto$ dissolution rate; binder $\propto$ $1$/dissolution rate). Additionally, overfitting was assessed by profiling the marginal effects of binder, disintegrant and porosity and examining them across their respective ranges for deviations from smooth, predictable behavior.

With the generalizability of the model supported, the development of the respective product parameter design spaces was performed. The use of the three product parameters in this work resulted in three dimensional design spaces. Thus, comparisons between the design spaces were on the basis of volume. Additionally, a comparison of the binder vs disintegrant design space area at porosity values of 8, 11, and 14% was performed.

The F2 design space was refined by nesting the $\%$ dissolved $= f(time, [HPC], [CP], \text{porosity})$ model within the F2 equation. Values for HPC, CP, and porosity were then randomly sampled and F2 values were calculated. The points that resulted in F2 $\geq$ 50 were saved until a stable design space was converged upon as per section 5.2.2.

The IVIVC based design space was defined using a MATLAB function developed in-house that a randomly defined a set of binder, disintegrant, and porosity values and translated them into $ka$ and $F$ values. These absorption parameters were then compared to the bioequivalent range of $ka$ and $F$ defined after the activities of section 5.2.3.2. Those sets of binder, disintegrant, and porosity values that resulted in bioequivalent
performance were then saved and the processes was repeated until a stable design space was converged upon. The specifics of the routine are described in Figure 5.2.6.1.

**Figure 5.2.6.1.** Generating a Product Parameter Design Space using IVIVC
5.3 Results and Discussion

5.3.1 Comparison of Dissolution Specifications: F2 vs IVIVC

The combinations of Makoid Banakar dissolution model parameters that resulted in an F2 of $\geq 50$ are demonstrated in Figure 5.3.1.1. The a. pane in the Figure is a representation of the approximately 8k points satisfying the F2 criteria. The b. pane is the three dimensional shape that captures these points. The volume of this shape was 9.6716e+04 arbitrary units (A.U.).
A distribution of dissolution profiles was generated by inputting each set of points from Figure 5.3.1.1 into the Makoid Banakar dissolution model. The resulting profiles are shown in Figure 5.3.1.2. In this figure, the red dots represent the dissolution performance of the reference product. The blue region is the product of each dissolution profile being plotted on the same graph.

Figure 5.3.1.2. Dissolution Profiles with $F_2 \geq 50$
The area occupied by the blue region of Figure 5.3.1.2 was 3.2612e+03 (%*min). It is important that the y-range not be interpreted as the acceptable range of values. To demonstrate why, consider two dissolution profiles where one is defined by the minimum y-values for each timepoint and the other is defined by the maximum values. In either case the, the F2 metric is far less than 50. This is because the conventional F2 ≥ 50 criteria assured that the average difference across all the time points is ≤ 10%. Thus the upper and lower limits of the blue region in Figure 5.3.1.2 are define by dissolution profiles that may have high error at certain time points but very low error at the others. While the area of the blue region is useful for comparison is it important to keep in mind that the interpretation that any dissolution profile falling within the blue region will have an F2 ≥ 50 is incorrect. It is acceptable however, to consider that any combination of dissolution model parameters that falls within the shape of Figure 5.3.1.1.b. will result in a F2 ≥ 50. For this reason, the more representative measure of the acceptance space is the volume metric calculated in Figure 5.3.1.1.b.

To refine dissolution parameter limits using the IVIVC, the first step was to recall the $k_a$ and $F$ terms for Tegretol: $F_a = 0.78$, $k_a = 0.28$. These terms were then incrementally varied across the respective ranges of [0.2 : 1.0] and [0.01 : 2.0]. The median response for $AUC$, $C_{max}$, $T_{max}$, $C_{ss}$, $C_{ss max}$, and $C_{ss min}$ were assessed using the ± 33% window described in section 5.2.3.1.
Identifying Makoid Banakar dissolution model parameters that were bioequivalent began with defining the range of F and ka values that would provide \( AUC, C_{max}, T_{max}, C_{ssmax}, C_{ssmin}, \) and \( C_{ss} \) values that were bioequivalent to that of Tegretol. The combination of absorption parameter values is represented by Figure 5.3.1.2.a. In this figure, the red region represents the bioequivalent values for ka and F. The dissolution parameters, that (when processed using the IVIVC) returned F and ka values that were within the bioequivalent region of Figure 5.3.1.2.a are shown in Figure 5.3.1.2.b. The resulting shape was not tractable in every dimension. However, when \( F_{max}/b, F_{max}, \) and \( F_{max,Tmax} \) were plotted against one another a tractable shape emerged (see Figure 5.3.1.2.c.). This allowed a discrete encompassing volume to be calculated. The bounds were based on the max and min parameter values of the F2 based shape. That decision was made in order to normalize the volume comparison. As such, the volume of the IVIVC dissolution parameter acceptance space was \( 1.2666e+09 \) A.U. and the volume occupied by the associated dissolution profiles was \( 4.6100e+08 \) A.U. (see Figure 5.3.1.2.d.). From this data it is evident that the use of the IVIVC provides the opportunity to have a wider dissolution specification (see Figure 5.3.1.2.e.).

**Figure 5.3.1.2. Combinations of IVIVC defined Dissolution Parameters**
5.3.2 Comparison of Product Parameter Design Spaces: F2 vs IVIVC

The IVIVC-vs-F2 comparison of product parameter design spaces began by establishing a model that predicted the amount dissolved based on binder concentration, disintegrant concentration, and porosity. The results of the dissolution tests performed according to the design of experiments in Table 5.2.6.1. are shown in
Figure 5.3.2.1. In this Figure, the model fits are also superimposed over the observed dissolution profiles.

The number of dissolution profiles, and time points per profile, was 27 and 11 respectively. Therefore, using 20% random subset cross validation the number of time points in the cross validation set was 60. The summary statistics for the product parameter dissolution model demonstrated suitable accuracy. The $R^2$ for the calibration and cross validation were both $> 0.99$. The RMSEC and RMSECV was 0.88% and 1.47% respectively. The final model was built with two hidden layers each consisting of 3 TanH nodes, 2 linear nodes, and 3 Gaussian nodes. The generalizability of the model was demonstrated by the ability to predictably interpolate between time points and design points as demonstrated by Figure 5.3.2.3.

![Figure 5.3.2.1. Combinations of IVIVC defined Dissolution Parameters](image)
Figure 5.3.2.2. Calibration and Cross Validation Models

Calibration

Cross Validation
Table 5.3.2.3. Neural Model for the Prediction of % Dissolved

<table>
<thead>
<tr>
<th>Measures</th>
<th>Calibration</th>
<th>Cross Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R^2$</td>
<td>0.999</td>
<td>0.998</td>
</tr>
<tr>
<td>RMSE</td>
<td>0.88 %</td>
<td>1.47 %</td>
</tr>
<tr>
<td>-Log Likelihood</td>
<td>307.6</td>
<td>108.4</td>
</tr>
<tr>
<td>N</td>
<td>237</td>
<td>60</td>
</tr>
</tbody>
</table>

With the demonstration of acceptable accuracy and generalizability of the product parameter dissolution model, the next step in was to generate product parameter F2 metric and IVIVC design spaces. After nesting the developed dissolution model inside of the F2 calculation, the design space that resulted is presented in Figure 5.3.2.4. The volume of this shape was 49.4013 A.U.

Figure 5.3.2.4. Example Response Surface Interpolated using Product Parameter Dissolution Model

[Disintegrant] = 1.355%; Porosity = 8.5%
(Black points are observed dissolution time points)

With the demonstration of acceptable accuracy and generalizability of the product parameter dissolution model, the next step in was to generate product parameter F2 metric and IVIVC design spaces. After nesting the developed dissolution model inside of the F2 calculation, the design space that resulted is presented in Figure 5.3.2.4. The volume of this shape was 49.4013 A.U.

Figure 5.3.2.4. Product Parameter design spaced based on $F2 \geq 50$
When the product parameter dissolution model was nested within a bioequivalence (IVIVC-based) loss function, the volume of the acceptable product parameter shape was 40.8200 A.U. (see Figure 5.3.2.5).

**Figure 5.3.2.5.** Product Parameter design spaced based on IVIVC

The bioequivalence loss function was constructed by:

i. Modeling the dissolution profiles generated by the product parameter dissolution model reported in Figures 5.3.2.2 through 5.3.2.4 using the Makoid Banakar dissolution model
ii. Transforming the dissolution model parameters into absorption rate and bioavailability terms using the IVIVC from chapter 3

iii. Identifying whether or not the paired absorption rate and bioavailability terms existed within the bioequivalence region identified in Figure 5.3.1.2.a. and saving the product parameter values if true

To understand why the IVIVC design space was smaller than the F2 design space despite the former having a wider dissolution specification, one must evaluate the dissolution profiles resulting from each model. Figure 5.3.2.6. illustrates the respective dissolution profile ranges. The red range in this Figure corresponds to the F2 product parameter design space and the blue range corresponds to the IVIVC design space. Within Figure 5.3.2.6 the most apparent difference was that the F2 criteria allowed for release rates that were faster than those provided with the IVIVC. By combining this observation with the information contained within Figure 5.3.2.1. the reason for the differences between the product parameter design spaces becomes clearer. The earlier Figure demonstrates how faster dissolution rates can be achieved by a wider variety of product parameter combinations. This allows the larger F2 design space to align with the observed results of the faster releasing F2 dissolution profiles.

Figure 5.3.2.6. Dissolution Profile Ranges for the F2 and IVIVC Design Spaces
An additional feature of the profiles that made up Figure 5.3.2.6 was that when each profile was fit using the Makoid Banakar dissolution model, the slope parameter ($b$) was $\leq$ 1 in all cases. This scenario meant that the profiles were all mono-exponential as opposed to a combination of mono-exponential and sigmoidal profiles as was the case in Figure 5.3.1.2.e. This was interpreted to mean that, while sigmoidal release rate can occur (as evidenced by the slowest releasing products of Figure 5.3.1.2.e.) in the ranges of interest the release characteristics are most realistically represented by Makoid Banakar slope terms of $\leq$ 1. This characteristic of the model drug product was carried forward to the simulations of the next section.

5.3.3 Post hoc Assessment of F2 Dissolution Spec using the PB-IVIVC-CTS Platform

The population of F2 > 50 dissolution profiles from Figure 5.3.2.6. were processed using the IVIVC of Chapter 3 coupled with the clinical trial platform of Chapter 4 to create distributions of pharmacokinetic profiles. The summary metrics for these profiles were individually calculated and the results are shown in Figures 5.3.3.1.

**Figure 5.3.3.1.** Bioequivalent Pharmacokinetic Responses and Responses Resulting from F2 $\geq$ 50 Dissolution Profiles
Interpreting the calculated F2 distributions shown Figures 5.3.3.1. was accomplished by recalling several concepts:

i) The observations which made up the distributions were paired

ii) Errors for bioequivalence were assess at both the upper and lower limits of the distribution of bioequivalent responses (upper and lower green lines)
F2 associated type I errors for bioequivalence could only occur at the upper or lower limits if there were no type II errors at those limits for any of the summary pharmacokinetic metrics.

F2 associated type II errors were defined as red observations made outside upper and lower boundaries identified by the green lines.

If an \textit{in silico} subject from the red distribution had a single pharmacokinetic metric that was outside the upper and lower boundaries for what constituted a bioequivalent response, the subject’s experience was recorded as type II error for bioequivalence.

In Figure 5.3.3.1.e it is observed that the lower limit for the 95\% credible interval associated F2 ($LL_{95\% CI,F2}$) distribution is below that of the lower limit for the 95\% CI defined by the distribution of truly bioequivalent responses ($LL_{95\% CI, BE}$). The red area occupied by the region between the $LL_{95\% CI,F2}$ and $LL_{95\% CI, BE}$ thus constitutes the probability of type two errors at the lower limit of bioequivalence ($P(\beta_{LL}) = 5.8\%$).

In graphs a., b., c., d., and f. of Figure 5.3.3.1. it is observed that the upper limit for the 95\% credible interval associated F2 ($UL_{95\% CI,F2}$) distribution is above that of the upper limit for the 95\% CI defined by the distribution of truly bioequivalent responses ($UL_{95\% CI, BE}$). The red area occupied by the region between the $UL_{95\% CI,F2}$ and $UL_{95\% CI, BE}$ thus constitutes the probability of type two errors in bioequivalence at the upper limit ($P(\beta_{UL})$). Internal assessment of the paired responses showed that the greatest occurrence of unique type II errors at the upper limit of bioequivalence
occurred for the $C_{max}$ metric. The $P(\beta_{UL})$ for this term was 6.6%. With such errors for bioequivalence occurring at both the upper and lower limits, it can be stated that the results of this work indicate that only type II errors for bioequivalence are associated with the use of the F2 statistic. Furthermore, the probability of biononequivalence occurring was calculated to be 12.3% ($P(\beta_{total}) = P(\beta_{UL}) + P(\beta_{LL})$). The significance of $P(\beta_{total})$ associated with the use of the F2 statistic can be placed into context by considering that approximately 3.4 million Americans suffer from epilepsy, approximately 280k+ adults suffer from trigeminal neuralgia, and approximately 31 million Americans suffer from mood disorders – all of which can be treated using carbamazepine. With an estimated 12.3% of patients being put at risk for biononequivalence when the F2 statistic is selected to justify similar product performance, over 400,000 patients could be affected by the strategic decision. However, if the IVIVC is used to justify similar product performance, the risk of biononequivalence can be directly controlled.

### 5.4 Conclusion

The activities of this chapter focused on comparing design spaces and specifications derived from the use of the F2 statistic with those defined using a physiologically based IVIVC. This was performed in three steps:

1. Dissolution specifications were defined using the F2 and IVIVC methods respectively without constraining the possible parameters for the Makorid Banakar dissolution model,
2\textsuperscript{nd}) A product parameter design space was constructed using dissolution profiles expressed by the product parameter dissolution model, and

3\textsuperscript{rd}) The F2 product parameter design space was processed using the IVIVC and the distribution of clinical responses were compared against the distribution of clinical responses explicitly controlled by the use of the IVIVC from the onset of design space refinement.

The first step showed that IVIVC allowed for a larger range of Makoid Banakar dissolution model parameters when compared to the F2 range. This lead to an initial expectation that the IVIVC would similarly allow for a larger space of acceptable product parameter values. However, the second step exposed that upon using dissolution profiles that were based on the performance of a real world tablet system, the opposite was observed to be true: the IVIVC product parameter design space was smaller than the F2 design space. This observation was due to the effect of the binder, disintegrant, and porosity plateauing within the ranges examined by the design of dissolution experiments. Steps one and two highlighted the importance of using the principles of experimental design to develop experimentally informed knowledge spaces for a pharmaceutical tablet.

The final step of this chapter illustrated how in order to minimize the probability of setting specifications for quality measurements that are excessively wide or unnecessarily narrow development tools must be linked back to clinical performance. The F2 statistic did not provide a connection back to clinical performance and resulted in an expected 12.3\% occurrence of biononequivalent events. This expectation could be avoided, however, through the use of a physiologically based IVIVC as it allows
the risk of biononequivalence to be directly controlled for through the refinement of product parameters with the knowledge of their impact on dissolution.

Chapter 6: Summary and Conclusion

Heraclitus (Greek philosopher c. 500 BCE) wrote, “The only thing constant is change.” This concept is especially true for the development, and lifecycle management, of pharmaceutical products. Here, change can result from modification to facilities, utilities, equipment, computer systems, formulations, analytical methods, specifications, manufacturing and cleaning processes, vendors and components, and documentation. With the pharmaceutical industry being one of the most tightly regulated industries in the world, managing change is often a challenging part of a sponsor’s 

pharmaceutical quality system.337
Modeling and computer simulation offer significant opportunities for enhancing both quality and efficiency in our industry. The physiologically-based IVIVC and the F2 method are mathematical modeling approaches used to bridge product development activities over changes that can occur to a pharmaceutical product’s formulation or manufacturing process. It was hypothesized in this work that the use of an IVIVC provided superior estimation of bioequivalence relative to the F2 statistic. This superiority was posited despite reliance upon the F2 statistic being more efficient and not requiring a paradigm shift away from conventional biowaiver strategies. Regardless of the industry’s conventional use of the F2 statistic, and its advantage in efficiency, this work demonstrated a clear performance advantage for the IVIVC approach.

Since 1997, when the FDA adopted the F2 test proposed by Moore and Flanner, the F2 metric has conventionally been used to assess the test-vs.-reference dissolution performance similarity. Currently, sponsors rely on the historical track record of the F2 metric. Users of this metric benefit from its efficiency. When a formulation or manufacturing process change occurs, the F2 metric allows a sponsor to move through the resulting decision point via a method that is straightforward and thoroughly familiar to regulatory agencies. However, such practice does not provide a clear link back to clinical significance; thus, the criticality of such a change cannot be accurately assessed using the F2 metric. The major limitation of the F2 statistic is that it does not account for the inter-subject variance required for the effective in vivo assessment of similarity for a test vs. reference comparison.

An IVIVC allows for the direct linkage between a dissolution change and its clinical effects (see Specific Aim I - Chapter 3). Indeed, by nesting an IVIVC within a clinical trial simulation platform, as shown by the work herein, the prediction of inter-subject variability
can also be accomplished. The performance of such simulations has historically required that a large-scale clinical trial be conducted so that population pharmacokinetic analysis could be carried out on the results (see discussion on NLME modeling in Chapter 2 section 2.4.1). However, such trials are demanding of resources and require extended timelines. The work presented demonstrates how the consideration of inter-subject variability was achieved without the need for such population level clinical trials. Here, physiologically based pharmacokinetic modeling and DMPK assays were relied upon for the mechanistic prediction of such variability (Specific Aim II – See Chapter 4). Ultimately, this work supports the justification of the increased cost associated with IVIVC development by demonstrating a scenario wherein the use of the F2 was associated with a higher risk of bioequivalence failures.

The IVIVC method confers equivalence by directly controlling errors for bioequivalence. Alternatively, the F2 method confers similarity when its value is > 50. This assures that the average test vs. reference difference between dissolution profiles is < 10%. However, using the F2 criterion fails to emphasize the link between a quality measurement and clinical significance. Additionally, it does not directly control the risk of bioequivalence, nor does it provide sponsors an opportunity to assess the risk of setting excessively narrow quality measurement specifications. This work demonstrated how bioequivalence could be directly built into the design space development process (see Specific Aim III - Chapter 5). The project illustrated the superiority of a carbamazepine product development strategy based on IVIVC methods, over F2 methods, by quantifying the increased risk associated with the use of the F2 (see Specific Aim IV - Chapter 5). Future efforts should be conducted on
incorporating physiologically based pharmacodynamic models within the PB-IVIVC for a further, more mechanistic, refinement of clinically significant deviations in performance. It is intended that this work support the movement of product/process optimization practices away from methods that result in rigid factors of unknown clinical significance, and towards those that are focused on efficiently achieving specific clinical objectives.

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