Predictive Pharmacokinetic Modeling of Orally Administered Drugs

Acknowledgments

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Introduction

In the modern pharmaceutical industry, very little certainty exists about the success of any newly discovered drug until it is actually investigated in some form of clinical trail. In order to gain FDA approval to become a prescription drug, new drugs must endure a series of trials including discovery, pre-clinical testing (e.g. gaining *in vitro* and *in silico* information about the drug), phase I (e.g. basic pharmacokinetics), phase II ("proof of principle"), and phase III clinical trails (e.g. dose validation, marketing, etc.)[1] Over the course of these trials, newly discovered drugs face a nearly 13 year road and greater than 98% failure rate.[2] Beyond the over decade-long investment of time, the monetary costs of the drug development process are also astronomical.[1,3] Figure 1 below illustrates the rising costs of gaining market approval in the United States (note that the costs are in millions of dollars):[1,3]

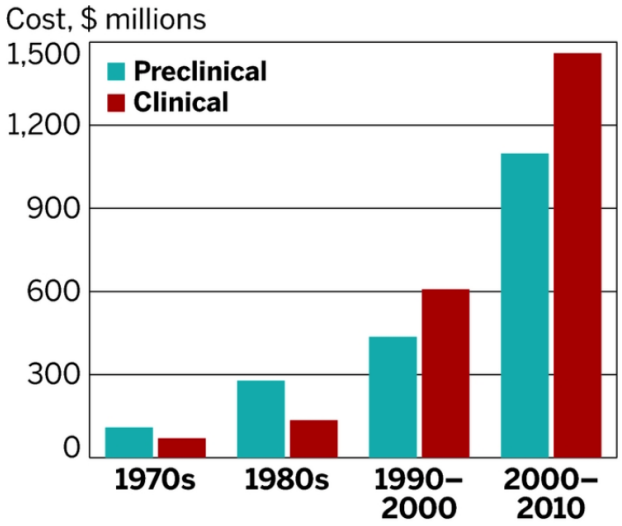

Figure 1. Graphic illustrating costs of clinical and pre-clinical process during drug discovery, 1970s-2010[3]

Despite this upward trend in spending on drug development, the success rate of drugs throughout the whole process is still incredibly low.[2,4] The time and resources devoted to these failed trials could certainly be allocated more appropriately if fewer high risk drugs were tested, or if alternative strategies could make the process more efficient. One method that pharmaceutical industries are currently exploring in order to increase the efficiency of the drug trial process involves predicting pharmacokinetic parameters during the pre-clinical stage in order to understand what to expect from the drug before moving forward.[5–7] By predicting some parameters early on, investigators can better understand

and evaluate how a drug may perform in clinical trials before actually investing the time or money. Specifically, avoiding investment in *in vivo* trials for risky drugs could save billions of dollars and decades of wasted research.

Although not necessarily in a coordinated effort, much research has been dedicated (and with varying degrees of success) to predicting nearly every major pharmacokinetic parameter. With the development of each new mechanistic equation or *in vitro* technique to estimate *in vivo* interactions, the pharmaceutical industry comes closer to understanding drugs more completely before investing in clinical trials.

In response to the limited scope and inefficiencies of the drug discovery and development process, this paper explores using a simple physiologically-based pharmacokinetic model as a predictive tool to generate pharmacokinetic profiles and simulate tissue concentrations over time, in the absence of any *in vivo* data. Instead, this function will require a handful of relatively cheap and time-inexpensive *in vitro, in silico* and other laboratory tests in order to understand the chemical and physical properties of the drug and predict the drug's absorption, distribution, metabolism, and elimination (ADME). The goal of creating such a function is to provide insight into dosing, steady state concentrations, target tissues, and other drug interactions in an entirely pre-clinical environment.

Methods: Modeling

The premise of pharmacokinetic (PK) modeling is that it allows us to model the movement of drugs through the body by describing the body's tissues as a series of compartments. Whereas many PK models use empirical data to justify the relationships of these "compartments," physiologically-based pharmacokinetic (PBPK) modeling combines known physiological properties about tissues with physiochemical properties of the drug in order to *predict* the clinical data we expect to see during *in vivo* testing.[8] The simplest PK model is the 2-compartment model, which treats the blood as a central "compartment" and the rest of the body as a "peripheral compartment," and follows the changes in the

concentrations of each.[9,10] The rate of drug movement from the central compartment to the peripheral is simply k_{12}, and movement in the opposite direction is k_{21}; elimination of drug from the system is described by a rate k_e. Given empirical data, a nonlinear regression (bi-exponential regressions are used for the 2-compartment model) proves extremely useful for finding parameters such as drug half life, absorption, and clearance.[7,9,10] Below is a diagram of the two compartment model:

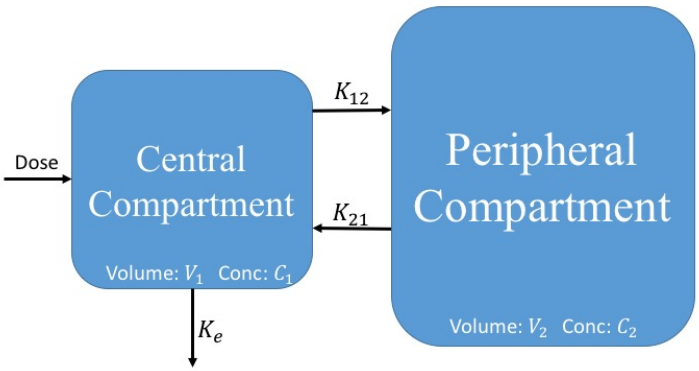

Figure 2. The 2-compartment model.[10]

As more compartments are considered the model becomes more complicated, but does not necessarily provide more information. For instance, expansions of the 2-compartment model may add new compartments in order to create an equation that fits the empirical data better, but these new compartments are actually arbitrary and do not provide any physiological insight. That is to say that when fitting data to a curve, we almost never gain knowledge as to which tissues are associated with the various compartments, aside from the central blood compartment.[11] Using a PBPK model, however, we attempt to predict the data ourselves by modeling the drug's interactions with the body as a series of tissue-specific compartments that are connected by arterial and venous blood flows.

Basics in PBPK Modeling

Unlike the 2-compartment model, which fits data to a curve, a PBPK model attempts to predict the absorption, distribution, metabolism, and elimination (ADME) from the body using known physiological properties and a series of tissue-blood interactions.[7,8,12] Rather than using a system of arbitrary compartments, a PBPK model uses compartments to represent specific tissues, and does so using known physiological parameters such as blood flow to the tissue as well as tissue volume. The fundamental equation allowing us to describe the movement of drug through tissues is the mass-balance equation, which describes the change in the *amount* of drug in tissue i over time, $A_i(t)$:[8]

$$\frac{dA_i(t)}{dt} = Q_i \cdot C_{\text{ART}}(t) - Q_i \cdot C_{\text{VEN},i}(t) \tag{1}$$

Here, Q_i describes the volume of blood flow to tissue i, C_{ART} describes the concentration of drug in the arterial blood reaching the tissue, and $C_{\text{VEN},i}$ describes the concentration of drug in the venous blood leaving the tissue. Given that $Q_i \cdot C_{\text{ART}}$ describes the mass of drug in the arterial blood and $Q_i \cdot C_{\text{VEN},i}$ describes the mass of drug in the venous blood, we can see that the mass-balance equation states that the change in the amount of drug in tissue i is simply the difference in the amount of drug in the blood arriving to (A_{ART}) and leaving ($A_{\text{VEN},i}$) the tissue. Figure 3 below shows the interactions between the arterial and venous blood with the tissue:

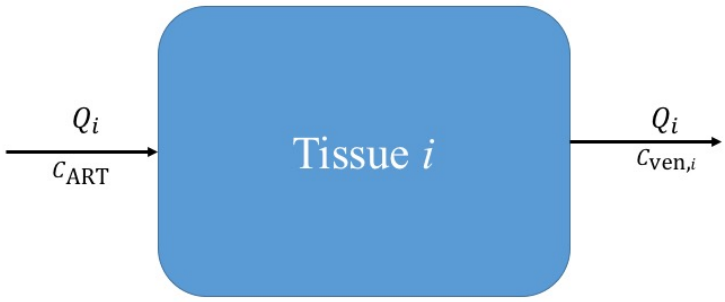

Figure 3. Blood flow through a tissue.[6,8]

Because a concentration is described by an mass/volume, we only have to divide the mass-balance equation by the volume of the tissue in order to change it into an equation for the change in the concentration of drug. Therefore, the fundamental differential equation detailing the change in the concentration of drug in tissue i (C_i) over time is simply an updated version of equation (1):

$$\frac{dC_i(t)}{dt} = \frac{Q_i[C_{\text{ART}}(t) - C_{\text{VEN},i}(t)]}{V_i} \tag{2}$$

In order to simplify the modeling process, it is assumed that the blood and tissue reach an equilibrium of relative concentrations ("venous equilibration") in the time that it takes for the blood to completely perfuse the tissue.[8] As a result, we can describe the venous concentration of drug leaving tissue i ($C_{\text{VEN},i}$) as the ratio of the tissue's concentration and the tissue:blood partition coefficient (P_i):

$$C_{\text{VEN},i}(t) = \frac{C_i(t)}{P_i} \tag{3}$$

Partition coefficients (PCs) are important parameters describing what the relative concentrations between the tissue and blood at equilibrium. For instance, if $P_i = 1$, we would expect the tissue and venous blood to have equal concentrations at equilibrium. A PC > 1 denotes a tissue in which we expect high concentrations relative to the venous blood, while a PC < 1 denotes the opposite. Due to the relationship expressed in equation (3), a substitution into equation (2) yields the following differential equation for a non-eliminating tissue (the time function of variable concentrations from this point on is implied, but no longer explicitly stated):

$$\frac{dC_i}{dt} = \frac{Q_i(C_{\text{ART}} - C_i/P_i)}{V_i} \tag{4}$$

Consider the model described by Figure 4, in which there are two non-eliminating tissues and a blood compartment. Such a model could be described by the following system of differential equations:

$$\frac{dC_b}{dt} = \frac{Q(C_{\text{VEN}} - C_{\text{ART}})}{V_b} \qquad \frac{dC_1}{dt} = \frac{Q_1(C_{\text{ART}} - C_1/P_1)}{V_1} \qquad \frac{dC_2}{dt} = \frac{Q_2(C_A - C_2/P_2)}{V_2}$$

Notice that due to the direction of blood flow through the blood compartment, the differential equation seems reversed. It is also worth noting that $C_{\text{ART}} = C_b$, meaning that the adjusted concentration in the blood compartment after a pass through the organs is then used as the arterial concentration that each tissue experiences. Also note that the total blood flow (Q) is the sum of the blood flow to each tissue, which in this case means $Q = Q_1 + Q_2$. The overall venous concentration (C_{VEN}) can then be found by dividing the total amount of drug in venous blood by the total blood flow:
$C_{\text{VEN}} = (Q_1 C_{\text{VEN},1} + Q_2 C_{\text{VEN},2})/Q$.

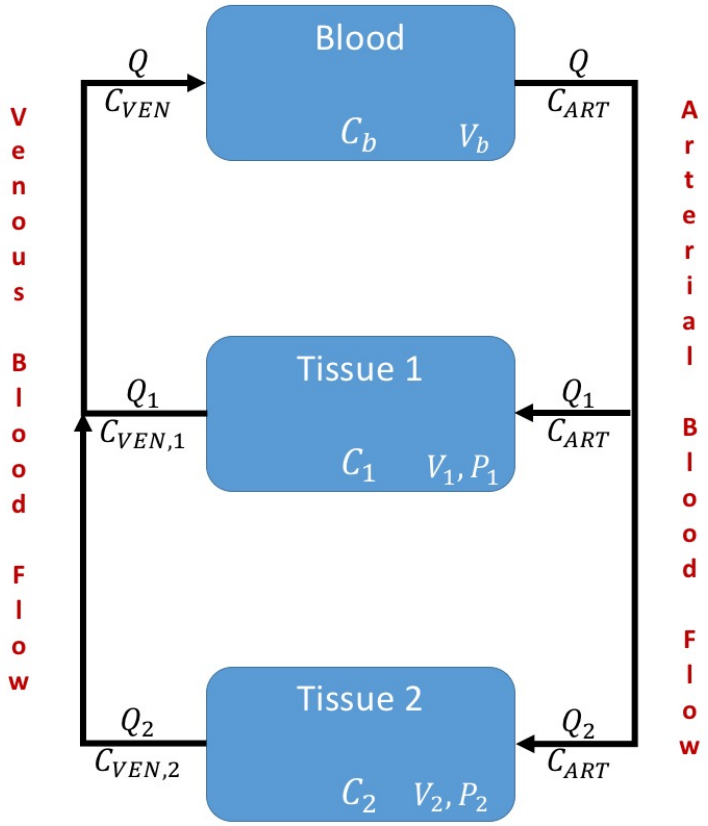

Figure 4. Model with blood compartment and two non-eliminating tissues.

Liver: Hepatic Portal System and Clearance

While the modeling method mentioned above is simple and straightforward, some physiological situations are not. In particular, the hepatic portal system presents an interesting scenario in which the liver receives more blood than its arterial flow would suggest.[6,12] Specifically, the hepatic portal system connects the liver to the gastrointestinal (GI) tract - aka *gut* - as well as the spleen and pancreas. A simplified graphic of the hepatic portal system is shown in Figure 5. The unique interactions here affect how we estimate both the liver and the venous output. While the flow through the gut, spleen, and pancreas can still be modeled with equations (2) or (4), the differential equation for the liver changes to the following when we account for its exposure to extra drug-containing blood:

$$\frac{dC_L}{dt} = \frac{(Q_L \cdot C_{\text{ART}} + Q_G \cdot C_{\text{VEN},G} + Q_S \cdot C_{\text{VEN},S} + Q_P \cdot C_{\text{VEN},P})}{V_L} - \frac{((Q_L + Q_G + Q_S + Q_P) \cdot C_{\text{VEN},L})}{V_L} \quad (5)$$

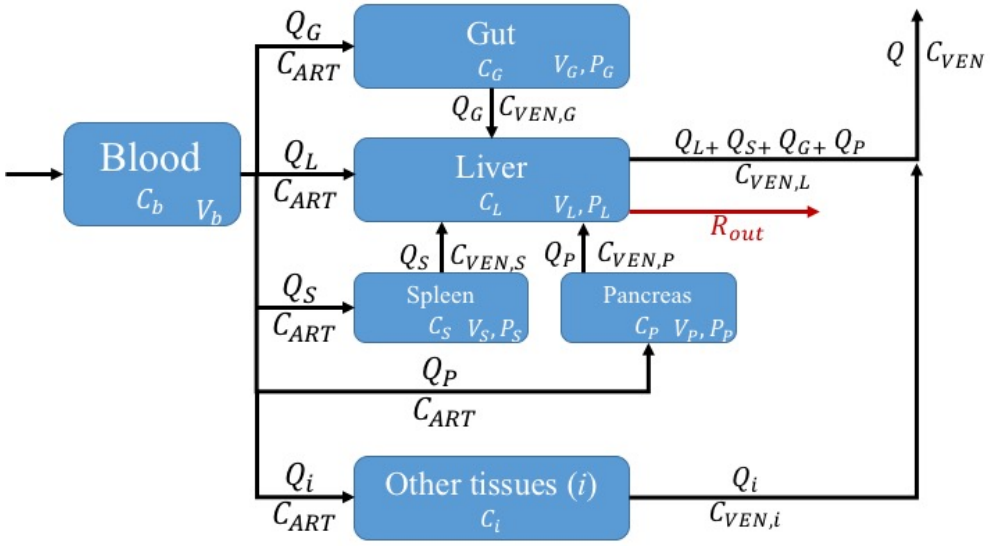

Figure 5. Hepatic portal system.

Although a bit more complex, equation (5) still does not capture the entire function of the liver. As shown in Figure 5, the liver is also the main site of metabolism and elimination, and therefore serves as the site of clearance in our model. Here we assume

clearance to be a first-order constant, which we refer to as CL (volume/time). Since the rate of clearance is first-order and elimination depends only on the concentration of drug in the liver, the term R_{out} used in Figure 5 describes the amount of drug being metabolized and eliminated, and is simply equal to $CL \cdot C_L$.[6] Thus the completed differential equation we will use for liver function is as follows:

$$\frac{dC_L}{dt} = \frac{(Q_L \cdot C_{\text{ART}} + Q_G \cdot C_{\text{VEN},G} + Q_S \cdot C_{\text{VEN},S} + Q_P \cdot C_{\text{VEN},P})}{V_L} - \frac{((Q_L + Q_G + Q_S + Q_P) \cdot C_{\text{VEN},L} + R_{\text{out}})}{V_L} \quad (6)$$

Absorption

Given that there are many possible ways to administer drugs (e.g. bolus injection, oral, inhalant, etc.), there are even more ways to capture drug absorption in a PBPK model. Due to the relevance to our collaborators, we only consider orally administered drugs in this model. Still, orally administered drugs are not without their complications. Firstly, because they pass through the esophagus and are absorbed to the blood through the GI tract which then passes through the liver before reaching systemic circulation, oral doses are subject to "first-pass metabolism."[9] Some PBPK models may choose to model absorption through the GI tract, but this method requires an intestinal absorption constant that can be extremely difficult to estimate.[13] Instead, this model treats absorption as a first-order process from an external compartment that feeds directly to systemic circulation at a constant rate k_a (1/hr). In order to account for the effects of first-pass metabolism, we only expose the system to the fraction of the dose that is orally bioavailable (f). Figure 6 shows the interactions leading to absorption into circulation:

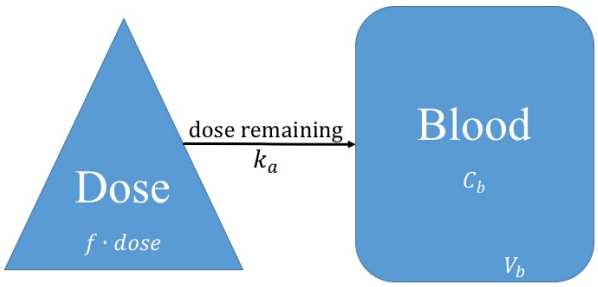

Figure 6. First-order absorption.

The interaction between the blood and external "dosage" compartment can be modeled using two simple differential equations. While the drug is removed from the dosage compartment at rate k_a, it is gained by the blood at the same rate. Therefore, without considering other interactions with the blood compartment, we see the following relationship:

$$\frac{d}{dt}\left(\text{amount of drug remaining}\right) = -k_a \cdot \text{amount of drug remaining}$$

$$\frac{dC_b}{dt} = \frac{k_a \cdot \text{amount of drug remaining}}{V_b}$$

In which the amount of drug remaining at time $t = 0$ is $dose \cdot f$, or the fraction of the dose that is bioavailable. By solving the first equation for the amount of drug remaining as a function of time, we find that it can be written according to the following equation:

$$\text{drug remaining}(t) = dose \cdot f \cdot e^{-k_a t} \tag{7}$$

Our model will account for oral absorption by simply adding the right hand side of equation (7) to the differential equation used to model the blood (as seen in the example with Figure 4). Thus the differential equation for the blood concentration becomes:

$$\frac{dC_b}{dt} = \frac{Q(C_{\text{VEN}} - C_b) + dose \cdot f \cdot e^{-k_a t}}{V_b} \tag{8}$$

Complete PBPK Model

At this time we can formally introduce the PBPK model used here. Since this model aims to work strictly from predicted parameters, we are limited to the tissues for which we can predict parameters such as P_i, k_a, f, and CL. Based on the available literature, our model is most limited by its ability to predict partition coefficients, so we have a system of 12 tissues: blood, adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, skin, and spleen. Mathematically, our model can be written as follows:

Set:

T	Tissues (abbreviation): {adipose (AD), bone (BO), brain (BR), gut (G), heart (H), kidney (K), lung (LU), muscle (M), skin (SK), spleen (S)}

Parameters:

Q_i, $i \in T$	Blood flow to Tissue i (fractional blood flow in L/kg available in Table 1).
Q_L	Blood flow to Liver (fractional blood flow in L/kg available in Table 1).
$Q = Q_L + \sum_i Q_i$	Total Blood Flow.
P_i, $i \in T$	[**Input**] Tissue:blood Partition Coefficient for Tissue i.
P_L	[**Input**] Tissue:blood Partition Coefficient for Liver.
V_b	Volume of blood (fractional volume in L/kg available in Table 1).
V_L	Volume of Liver (fractional volume in L/kg available in Table 1).
$V_i, i \in T$	Volume of Tissue i (fractional volume in L/kg available in Table 1)
k_a	[**Input**] First-order absorption rate constant into systemic circulation.
CL	[**Input**] First-order clearance rate constant.
f	[**Input**] Fraction of drug that is orally bioavailable.
dose	[**Input**] Initial dose of drug.

Parameters marked with [**Input**] must be supplied by the researcher in order to execute the PBPK model.

Variables:

C_b	Concentration of drug in blood compartment.
$C_{\text{ART}} = C_b$	Arterial blood concentration.
$C_i,\ i \in T$	Concentration of drug in Tissue i.
C_L	Concentration of drug in Liver.
$C_{\text{VEN},i} = C_i/P_i$	Concentration of drug in venous blood leaving tissue i.
$C_{\text{VEN,L}} = C_L/P_L$	Concentration of drug in venous blood leaving Liver.
$C_{\text{VEN}} = (Q_L \cdot C_{\text{VEN,L}} + \sum_i Q_i \cdot C_{\text{VEN},i})/Q$	Total Venous Concentration.
$R_{\text{out}} = CL \cdot C_L$	Amount of drug removed from liver due to clearance.

Differential Equations:

Blood:

$$\frac{dC_b}{dt} = \frac{Q(C_{\text{VEN}} - C_b) + dose \cdot f \cdot e^{-k_a t}}{V_b},$$

Liver*:

$$\frac{dC_L}{dt} = \frac{(Q_L \cdot C_{\text{ART}} + Q_G \cdot C_{\text{VEN},G} + Q_S \cdot C_{\text{VEN},S})}{V_L} - \frac{((Q_L + Q_G + Q_S) \cdot C_{\text{VEN},L} + R_{\text{out}})}{V_L},$$

Other Tissues:

$$\frac{dC_i}{dt} = \frac{Q_i(C_{\text{ART}} - C_{VEN,i})}{V_i}, i \in T$$

* Notice this is different from equation (6) because we do not have the necessary physiological data to include the pancreas.

Methods: Predicting Parameters

Calculating Tissue:Blood Partition Coefficients

As mentioned earlier, our model is most limited by our ability to predict partition coefficients (PCs) for only a certain number of tissues. From the available literature, we can predict these values for the adipose tissue, brain, bone, gut, heart, kidney, liver, lungs,

muscle, skin and spleen. This section explores the various mechanistic equations used to predict PCs for these tissues for various drugs based on their chemical properties.

Simple Model

While there are methods for estimating tissue:blood partition coefficients *in vitro* or *in vivo* via methods such as quantitative structure-property relationships (QSPR), these methods often cost a lot of money and time to perform, and can present varying results by a factor up to 3.[14] As a result, mechanistic equations that predict these values within a factor of 3 have been developed and validated in order to speed up the process and allow for cheaper development of PBPK models.[14–18]

The simplest method for predicting PCs was introduced by Poulin and Theil.[14] The theoretical background behind their mechanistic equation is as follows. Since the tissue:blood partition coefficient presents the steady state equilibrium of tissue and plasma concentrations, we see that:

$$P_i = C_{i,ss}/C_{p,ss} \tag{9}$$

Where P_i represents the tissue:blood PC for tissue i, $C_{i,ss}$ is the total concentration of drug in tissue i at steady state, and $C_{p,ss}$ is the total concentration of in the plasma. In both the tissue and blood, however, the concentrations can be divided between "free" drug and "bound" drug, or drug that is bound to proteins and macromolecules. Therefore, we can modify C_i and C_p to represent the relative concentrations of free ($C_{\text{free},i/p}$) and bound drug ($C_{\text{bound},i/p}$). Thus equation (9) becomes:

$$P_i = (C_{\text{free},i} + C_{\text{bound},i})/(C_{\text{free},p} + C_{\text{bound},p}) \tag{10}$$

The terms $C_{\text{free},i}$ and $C_{\text{free},p}$ are related to what is generally referred to as "fraction unbound," which is the ratio of the concentration of free drug to the total concentration of

drug in the tissue or plasma ($f_u = C_{\text{free}}/(C_{\text{free}} + C_{\text{bound}})$.[14] Substituting into equation (10) based on the fact that $C_{\text{free}} + C_{\text{bound}} = C_{\text{free}}/f_u$ and rearranging appropriately, we now have:

$$P_i = (C_{\text{free},i}/C_{\text{free},p})(f_{u,p}/f_{u,i}) \tag{11}$$

While the fraction unbound in plasma ($f_{u,p}$) is a standard term that can be calculated via *in vitro* methods (to be discussed in a later section), the fraction unbound in tissue ($f_{u,t}$) can be estimated from $f_{u,p}$ in the following way:[15]

$$f_{u,t} = \frac{1}{1 + (((1 - f_{u,p})/f_{u,p}) * 0.5)} \tag{12}$$

The remaining term, $(C_{\text{free},t}/C_{\text{free},p})$ is equivalent to a ratio of steady-state solubilities between tissues and plasma, and can therefore be rewritten in terms of relative solubilities (S_t/S_p).[14] This ratio can be approximated using the drug's n-octanol:buffer partition coefficient of non-ionized species at pH 7.4 ($P_{o:w}$),[14,15] which can be determined through simple lab techniques, to approximate the tissue:water (S_t/S_w) and plasma:water (S_p/S_w) solubilities of the drug:[14,15]

$$S_t/S_w = P_{o:w}(V_{n,i} + 0.3V_{ph,i}) + (V_{w,i} + 0.7V_{ph,i})$$

$$S_p/S_w = P_{o:w}(V_{n,p} + 0.3V_{ph,p}) + (V_{w,p} + 0.7V_{ph,p})$$

Where $P_{o:w}$ is the aforementioned n-octanol:buffer partition coefficient, V_n is the fraction of tissue (if succeeded by i) or plasma (if succeeded by p) volume made of neutral lipids, V_{ph} is the fraction of tissue or plasma volume made of phospholipids, and V_w is the fraction of tissue or plasma volume due to water. The values for these physiological parameters can be found in Table 1.

Since the quantity $(S_t/S_w)/(S_p/S_w)$ is simply S_t/S_p, we can now put together the simplest approximation of partition coefficients:

$$P_i = \frac{P_{o:w}(V_{n,i} + 0.3V_{ph,i}) + (V_{w,i} + 0.7V_{ph,i})}{P_{o:w}(V_{n,p} + 0.3V_{ph,p}) + (V_{w,p} + 0.7V_{ph,p})} \cdot \frac{f_{u,p}}{f_{u,t}} \tag{13}$$

Due to non-polar nature of adipose tissue, better results are generated using an olive oil:buffer partition coefficient of both non-ionized *and* ionized species at pH 7.4 ($P^*_{vo:w}$) in place of $P_{o:w}$, and neglecting macromolecular binding in the tissue (i.e. $f_{u,\mathrm{AD}} = 1$).[15,16] This yields the following equation for adipose tissue partition coefficients (P_{AD}):

$$P_{\mathrm{AD}} = \frac{P^*_{vo:w}(V_{n,\mathrm{AD}} + 0.3V_{ph,\mathrm{AD}}) + (V_{w,\mathrm{AD}} + 0.7V_{ph,\mathrm{AD}})}{P^*_{vo:w}(V_{n,p} + 0.3V_{ph,p}) + (V_{w,p} + 0.7V_{ph,p})} \cdot \frac{f_{u,p}}{1} \tag{14}$$

More Complex Model: Moderate-to-Strong Bases

While Poulin and Theil's mechanistic equations provide a simple and straightforward way to estimate partition coefficients, their successful results were mainly limited to small neutral molecules and lost significant accuracy when applied to moderate-to-strong bases, zwitterions, and acids.[17,18] In order to expand on the simple model, a more complicated way to predict P_i does so by predicting tissue:plasma water partition coefficients based on the drug's chemical properties.[17,18]

For the remainder of this prediction model, we will consider a moderate-to-strong base to be a basic compound with at least one pKa value ≥ 7, meaning it will be at least 50% ionized at pH 7 (intracellular pH).[17,18] Next, we define two categories of zwitterions (compounds that are neutral, but have both positive and negative charge): group 1 zwitterions have at least one pKa ≥ 7 and are expected to behave more like moderate-to-strong bases, while group 2 zwitterions (no pKa ≥ 7) are expected to behave more like acids or very weak bases.[18] We will use conventional definitions for acids and neutrals.[18] This section will focus on predicting PCs for moderate-to-strong bases and group 1 zwitterions, while the following section will consider acids, weak bases, neutrals, and group 2 zwitterions. Figure 7 below illustrates the various states of ionization and binding that lead to the distribution of different types of drugs:

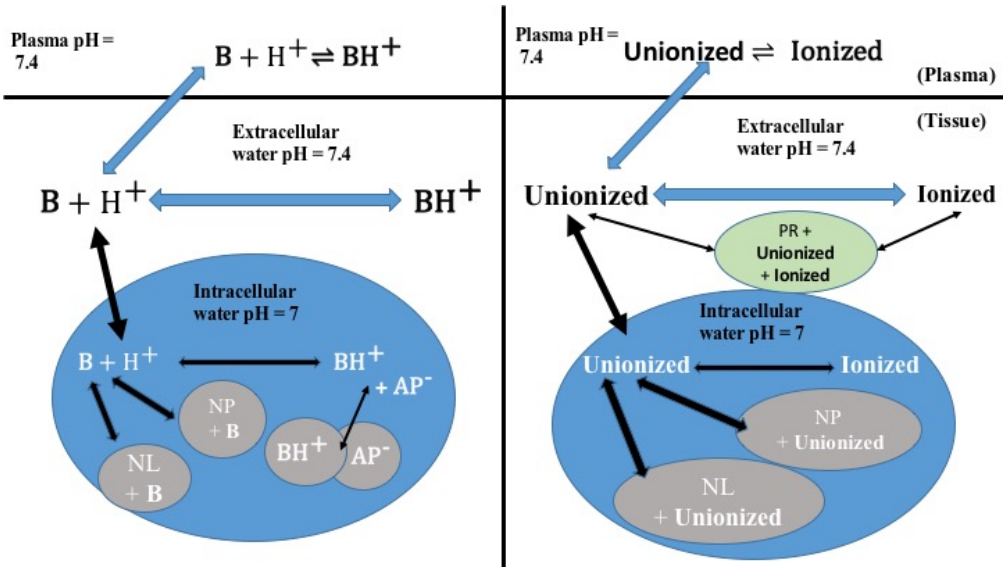

Figure 7. Left: processes involved in the distribution of moderate-to-strong bases between tissue and plasma.[17] Right: processes involved in the distribution of weak bases, acids, neutrals, and zwitterions between tissue and plasma.[18] See text for explanation of abbreviations.

One immediate departure from the earlier model is that in this case we are estimating PCs by solving for the tissue:plasma water partition coefficient at steady state, so $P_i = C_i/P_{\text{free},p}$.[17] As shown in Figure 7, there are several interactions to account for when calculating $C_i/C_{\text{free},p}$ at steady state. First, consider the tissue concentration. For a moderate-to-strong base, the components making up C_i include interactions with intracellular water (IW), extracellular water (EW), neutral lipids (NL), neutral phospholipids (NP), and acidic phospholipids (AP) within tissue cells.[17] As such, we can describe the tissue concentration as the sum of the amount of drug that is (1) free in the intracellular and extracellular water, and (2) bound to neutral lipids, neutral phospholipids, and other molecules (such as acidic phospholipids):[17]

$$C_i = \frac{A_{\text{free,IW},i} + A_{\text{free,EW},i} + A_{\text{bound,NP},i} + A_{\text{bound,NL},i} + A_{\text{bound,REM},i}}{V_i} \tag{15}$$

As we saw in the modeling section, an amount may be rewritten as a concentration times volume, so we can replace each A_n with $C_n \cdot V_n$ where n is the phase of drug (e.g. "free, IW", "free,EW", etc.). Since each of these terms is also divided by the total volume of the

tissue (V_i), we can rewrite equation (15) as the sum of each concentration phase n multiplied by the fractional tissue volume in that phase. That is to say:

$$C_i = C_{\text{free,IW}} \cdot f_{\text{IW},i} + C_{\text{free,EW}} \cdot f_{\text{EW},i} + C_{\text{bound,NP}} \cdot f_{\text{NP},i} + C_{\text{bound,NL}} \cdot f_{\text{NL},i} + C_{\text{bound,AP}} \cdot f_{\text{REM}} \quad (16)$$

Where f_n is the fractional tissue volume in phase n, and f_{REM} is equal to $1 - f_{\text{IW},i} - f_{\text{EW},i} - f_{\text{NP},i} - f_{\text{NL},i}$. All of these values except for f_{REM} can be found in Table 2 below. By manipulating chemical equations, we can then solve for each of these concentrations in order to estimate the partition coefficient. Below are the resulting equations for each state of drug in the tissue:[17]

$$C_{\text{free,IW}} = C_{\text{free},p} \cdot \frac{1 + 10^{\text{pKa}-pH_{\text{IW}}}}{1 + 10^{\text{pKa}-pH_p}}$$

$$C_{\text{free,EW}} = C_{\text{free},p}$$

$$C_{\text{bound,NP}} = \frac{C_{\text{free},p}}{1 + 10^{\text{pKa}-pH_p}} \cdot (0.3P_{o:w} + 0.7)$$

$$C_{\text{bound,NL}} = \frac{P_{o:w} \cdot C_{\text{free},p}}{1 + 10^{\text{pKa}-pH_p}}$$

$$C_{\text{bound,AP}} = \frac{K_a \cdot [\text{AP}^-]_{\text{REM}} \cdot C_{\text{free},p} \cdot 10^{\text{pKa}-pH_{\text{IW}}}}{1 + 10^{\text{pKa}-pH_p}}$$

The new parameters in the above equations are: pKa is the pKa of the most basic part of the drug (for polyprotic bases, this can be adjusted via the Henderson-Hasselbalch equations), pH_{IW} is intracellular pH, which is 7, pH_p is the pH of plasma water, which is 7.4, $P_{o:w}$ is the same n-octanol:buffer partition coefficient used in the earlier model, K_a is the association constant of basic compounds with acidic phospholipids, and $[\text{AP}^-]_{\text{REM}}$ is the concentration of available binding sites on the acidic phospholipids.[17] Fortunately, $[\text{AP}^-]_{\text{REM}}$ is found by dividing $[\text{AP}^-]_i$ by the fractional tissue volume remaining (f_{REM}), so

when insert $C_{\text{bound,AP}}$ to equation (16), the terms f_{REM} cancel out and only $[\text{AP}^-]_i$ remains. The tissue-specific values for $[\text{AP}^-]_i$ are listed in Table 2.

Also, according to the literature, K_a can be approximated using the drug's association constant for blood cells ($K_{a,\text{BC}}$), because of their concentration of acidic phospholipids;[17] the methods for this approximation can be found in the appendix under section A. Note that in order to approximate K_a, an additional parameter deemed the drug's blood:plasma ($B:P$) ratio is necessary. Bringing all of these equations together into equation (16) yields:

$$C_i = C_{\text{free},p}[f_{\text{EW},i} + (\frac{1+10^{\text{pKa}-pH_{\text{IW}}}}{1+10^{\text{pKa}-pH_p}} \cdot f_{\text{IW},i}) + (\frac{K_a \cdot [\text{AP}^-]_i \cdot 10^{\text{pKa}-pH_{\text{IW}}}}{1+10^{\text{pKa}-pH_p}}) + (\frac{P_{o:w} \cdot f_{\text{NL},i} + (0.3P_{o:w}+0.7) \cdot f_{\text{NP},i}}{1+10^{\text{pKa}-pH_p}})]$$

Remembering that we are solving for P_i, we must apply this approximation of steady-state C_i to the equation $P_i = C_i/C_{\text{free},p}$. Then we are left with the following approximation for tissue:blood PCs for moderate-to-strong bases:

$$P_i = f_{\text{EW},i} + (\frac{1+10^{\text{pKa}-pH_{\text{IW}}}}{1+10^{\text{pKa}-pH_p}} \cdot f_{\text{IW},i}) + (\frac{K_a \cdot [\text{AP}^-]_i \cdot 10^{\text{pKa}-pH_{\text{IW}}}}{1+10^{\text{pKa}-pH_p}}) + (\frac{P_{o:w} \cdot f_{\text{NL},i} + (0.3P_{o:w}+0.7) \cdot f_{\text{NP},i}}{1+10^{\text{pKa}-pH_p}}) \quad (17)$$

Table 1. Rat and Human Physiological Parameters from Poulin and Theil[15]

	Tissue Volume		Tissue Composition(Volume Fraction of Wet Tissue Weight)						
	Fraction of Body Weight (L/kg)		Water(V_w)		Neutral Lipids (V_{nl})		Phospholipids (V_{ph})		Physiological Data[19]
Tissues	Rat	Human	Rat	Human	Rat	Human	Rat	Human	Blood Flow Rates (Q_i)*
Adipose	0.0761	0.11957	0.12	0.18	0.853	0.79	0.002	0.002	0.07
Bone	0.041476	0.085629	0.446	0.439	0.0273	0.074	0.0027	0.0011	0.122
Brain	0.0057	0.02	0.788	0.77	0.0392	0.051	0.0533	0.0565	0.02
Gut	0.027	0.0171	0.749	0.718	0.0292	0.0487	0.0138	0.0163	0.131
Heart	0.0033	0.0047	0.779	0.758	0.014	0.0115	0.0118	0.0166	0.049
Kidney	0.0073	0.0044	0.771	0.783	0.0123	0.0207	0.0284	0.0162	0.141
Liver	0.0366	0.026	0.705	0.751	0.0138	0.0348	0.0303	0.0252	0.175
Lung	0.005	0.0076	0.79	0.811	0.0219	0.003	0.014	0.009	**
Muscle	0.404	0.40	0.756	0.76	0.01	0.0238	0.009	0.0072	0.278
Skin	0.190	0.0371	0.651	0.718	0.0239	0.0284	0.018	0.0111	0.058
Spleen	0.002	0.0026	0.771	0.788	0.0077	0.0201	0.0136	0.0198	0.02
Plasma	0.0449	0.0424	0.96	0.945	0.00147	0.0035	0.00083	0.00225	-
Erythrocytes	0.0367	0.0347	-	-	-	-	-	-	-

* fraction of cardiac output in L/min. ** calculated as 0.235*BW (kg)

Table 2. Parameters from Rodgers et al., Rodgers and Rowland, and Poulin and Theil

Tissues	Fractional Tissue Volume[17, 18]				Tissue-to-Plasma ($[PR]_i$)[18]		Tissue Concentration of Acidic Phospholipids in mg/g ($[AP^-]_i$)[17]
	Extracellular Water ($f_{EW,i}$)	Intracellular Water ($f_{IW,i}$)	Neutral Lipid ($f_{NL,i}$)	Neutral Phospholipid ($f_{NP,i}$)	Albumin Ratio	Lipoprotein Ratio	
Blood cells	-	0.603	0.0017	0.0029	-	-	0.50
Adipose	0.135	0.017	0.853	0.0016	0.049	0.068	0.40
Bone	0.100	0.346	0.0174	0.0016	0.100	0.050	0.67
Brain	0.162	0.620	0.0391	0.0015	0.048	0.041	0.40
Gut	0.282	0.475	0.0375	0.0124	0.158	0.141	2.41
Heart	0.320	0.456	0.0135	0.0106	0.157	0.160	2.25
Kidney	0.273	0.483	0.0121	0.0240	0.130	0.137	5.03
Liver	0.161	0.573	0.0135	0.0238	0.086	0.161	4.56
Lung	0.336	0.446	0.0215	0.0123	0.212	0.168	3.91
Muscle	0.118	0.630	0.0100	0.0072	0.064	0.059	1.53
Skin	0.382	0.291	0.0603	0.0044	0.277	0.096	1.32
Spleen	0.207	0.579	0.0071	0.0107	0.097	0.207	3.18

More Complex Model: Acids, weak bases, Zwitterions

Revisiting Figure 7, we see that acids, weak bases, and type 2 zwitterions undergo interactions that are relatively similar to moderate-to-strong bases.[18] As such, we can represent these interactions with a modified version of equation (16) that removes $C_{\text{bound,AP}}$ and incorporates the concentration of drug bound to tissue proteins in the extracellular water ($C_{\text{PR,EW}}$):

$$C_i = C_{\text{free,IW}} \cdot f_{\text{IW},i} + (C_{\text{free,EW}} + C_{\text{PR,EW}}) \cdot f_{\text{EW},i} + C_{\text{bound,NP}} \cdot f_{\text{NP},i} + C_{\text{bound,NL}} \cdot f_{\text{NL},i} + C_{\text{REM}} \cdot f_{\text{REM}} \tag{18}$$

Once again, the abbreviations IW, EW, PR, NP, NL, and REM refer to intracellular water, extracellular water, binding proteins, neutral phospholipids, neutral lipids, and remaining interactions, respectively.[18] Also, consistent with the earlier section, $f_{\text{REM}} = 1 - f_{\text{IW},i} - f_{\text{EW},i} - f_{\text{NP},i} - f_{\text{NL},i}$. Solving for f_{REM} is unimportant, however, because this model assumes that residual interactions for acids, neutrals, and very weak bases are negligible (so $C_{\text{REM}} = 0$).[18]

In order to simplify the notation in this section, we will introduce variables X and Y to represent ionization factors for different types of molecules.[18] Table 3 summarizes how

to solve for X and Y for the various drugs that will be used in this section:

Table 3: Summary of X and Y for estimating PCs[18]

Type of Molecule	X	Y
Monoprotic Bases	$1 + 10^{\text{pKa}-pH_{\text{IW}}}$	$1 + 10^{\text{pKa}-pH_p}$
Monoprotic Acids	$1 + 10^{pH_{\text{IW}}-\text{pKa}}$	$1 + 10^{pH_p-\text{pKa}}$
Neutral	1	1
Zwitterions*	$1 + 10^{\text{pKa}_{\text{base}}-pH_{\text{IW}}} + 10^{pH_{\text{IW}}-\text{pKa}_{\text{acid}}}$	$1 + 10^{\text{pKa}_{\text{base}}-pH_p} + 10^{pH_p-\text{pKa}_{\text{acid}}}$

* For polyprotic acids, bases, and zwitterions, X and Y should be adjusted via the Henderson Hasselbalch equation. $pH_{\text{IW}} = 7$. $pH_p = 7.4$.

Since nothing has changed about the interactions determining $C_{\text{free,EW}}$, $C_{\text{free,IW}}$, $C_{\text{bound,NL}}$, and $C_{\text{bound,NP}}$, we have the following equations for them (substituting X and Y appropriately):[18]

$$C_{\text{free,EW}} = C_{\text{free},p} \qquad C_{\text{free,IW}} = C_{\text{free},p} \cdot \frac{X}{Y}$$

$$C_{\text{bound,NL}} = \frac{P_{o:w} \cdot C_{\text{free},p}}{Y} \qquad C_{\text{bound,NP}} = C_{\text{free},p} \cdot \frac{(0.3P_{o:w} + 0.7)}{Y}$$

Thus our only remaining variable to complete equation (18) is $C_{\text{PR,EW}}$, which can be estimated using the concentration of binding proteins in extracellular water ($[\text{PR}]_{\text{EW}}$), the association constant for binding proteins (Ka_{PR}), and the concentration of free drug in the plasma $C_{\text{free},p}$:

$$C_{\text{PR,EW}} = Ka_{\text{PR}} \cdot [\text{PR}]_{\text{EW}} \cdot C_{\text{free},p}$$

As we saw with $[\text{AP}^-]_{\text{REM}}$ earlier, we know that $[\text{PR}]_{\text{EW}}$ is found by dividing the concentration of binding proteins in the tissue ($[\text{PR}]_{\text{T}}$) by the fractional volume of extracellular water ($f_{\text{EW},i}$).[18] Thus we can replace $[\text{PR}]_{\text{EW}}$ with $[\text{PR}]_i/f_{\text{EW},i}$, so that $f_{\text{EW},i}$ cancels out when eventually plugged into equation (18).

Our final parameter, Ka_{PR}, can be estimated using the following mechanistic equation, which is based on a manipulation of an equation modeling the fraction unbound in the plasma ($f_{u,p}$):[18]

$$Ka_{\mathrm{PR}} = [\frac{1}{f_{u,p}} - 1 - (\frac{P_{o:w} \cdot f_{\mathrm{NL,P}} + (0.3P_{o:w} + 0.7) \cdot f_{\mathrm{NP,P}}}{Y})] \cdot \frac{1}{[\mathrm{PR}]_{\mathrm{P}}}$$

Where $P_{o:w}$ is the n-octanol:buffer partition coefficient seen before, $f_{\mathrm{NL,P}}$ is the fractional volume of neutral lipids in the plasma, $f_{\mathrm{NP,P}}$ is the fractional volume of neutral phospholipids in the plasma, and $[\mathrm{PR}]_{\mathrm{P}}$ is the concentration of binding proteins in the plasma. $f_{\mathrm{NL,P}}$ and $f_{\mathrm{NP,P}}$ are equal to 0.0023 and 0.0013 respectively.[18]

Putting everything together and substituting into equation (18), we now have the following equation for C_i:

$$C_i = C_{\mathrm{free},p} \cdot (\frac{X \cdot f_{\mathrm{IW},i}}{Y} + f_{\mathrm{EW},i} + (\frac{P_{o:w} \cdot f_{\mathrm{NL},i} + (0.3P_{o:w} + 0.7) \cdot f_{\mathrm{NP},i}}{Y} + [(\frac{1}{f_{u,p}} - 1 - (\frac{P_{o:w} \cdot f_{\mathrm{NL,P}} + (0.3P_{o:w} + 0.7) \cdot f_{\mathrm{NP,P}}}{Y})) \cdot \frac{[\mathrm{PR}]_i}{[\mathrm{PR}]_{\mathrm{P}}}]) \quad (19)$$

Now substituting equation (19) into $P_i = C_i / C_{\mathrm{free},p}$, we have the general mechanistic equation for predicting PCs for acids, neutrals, very weak bases, and group 2 zwitterions:

$$P_i = \frac{X \cdot f_{\mathrm{IW},i}}{Y} + f_{\mathrm{EW},i} + (\frac{P_{o:w} \cdot f_{\mathrm{NL},i} + (0.3P_{o:w} + 0.7) \cdot f_{\mathrm{NP},i}}{Y} + [(\frac{1}{f_{u,p}} - 1 - (\frac{P_{o:w} \cdot f_{\mathrm{NL,P}} + (0.3P_{o:w} + 0.7) \cdot f_{\mathrm{NP,P}}}{Y})) \cdot \frac{[\mathrm{PR}]_i}{[\mathrm{PR}]_{\mathrm{P}}}] \quad (20)$$

The physiological parameters for $f_{\mathrm{NL},i}$, $f_{\mathrm{NP},i}$, and $\frac{[\mathrm{PR}]_i}{[\mathrm{PR}]_{\mathrm{P}}}$ can all be found in Tables 1 and 2. The ratio $\frac{[\mathrm{PR}]_i}{[\mathrm{PR}]_{\mathrm{P}}}$ is the tissue-to-plasma albumin or lipoprotein ratio, and it is worth noting that albumin is the main binding protein for acids and very weak bases, while neutrals generally bind with lipoproteins, so these parameters should be chosen accordingly.[18]

Summary of Complex Model

Below is a summary of the mechanistic equations for predicting tissue:blood partition coefficients using the methods from Rodgers et al.[17] and Rodgers and Rowland.[18]

The necessary input parameters are the n-octanol:buffer partition coefficient ($P_{o:w}$), the olive oil:buffer partition coefficient ($P_{vo:w}$), the fraction of drug unbound in the plasma ($f_{u,p}$), the pKa value of the most acidic substituent (if acid), basic substituent (if base), or both (if zwitterionic), and the drug's blood:plasma ratio ($B:P$), which is only relevant for moderate-to-strong bases and group 1 zwitterions.

Set:

T	Tissues (abbreviation): {adipose (AD), bone (BO), brain (BR), gut (G), heart (H), kidney (K), liver (L), lung (LU), muscle (M), skin (SK), spleen (S)}

Parameters:

pKa_{acid}	**[Input]** pKa of most acidic substituent (Henderson-Hasselbalch equations for polyprotic acids).
pKa_{base}	**[Input]** pKa of most basic substituent (adjusted using Henderson-Hasselbalch equations for polyprotic bases).
X/Y	Parameter estimating effects of ionization (see Table 3).
$f_{\text{IW},i}$, $i \in T$	Fractional tissue volume intracellular water (Table 2).
$f_{\text{EW},i}$, $i \in T$	Fractional tissue volume extracellular water (Table 2).
$f_{\text{NL},i}$, $i \in T$	Fractional tissue volume neutral lipids (Tables 1 and 2).
$f_{\text{NP},i}$, $i \in T$	Fractional tissue volume neutral phospholipids (Tables 1 and 2).
$[\text{AP}^-]_i$, $i \in T$	Tissue concentration of acidic phospholipids (Table 2, only relevant for moderate-to-strong bases and type 1 zwit.).
Ka_{AP}	Association constant of drug for acidic phospholipids (estimated via $K_{a,\text{BC}}$, see Appendix A; only relevant for moderate-to-strong bases and type 1 zwit.).
$\frac{[\text{PR}]_i}{[\text{PR}]_\text{P}}$	Tissue to plasma albumin (if acid, weak base, or type 2 zwit.) or lipoprotein (if neutral) ratio (Table 2, not relevant for moderate-to-strong bases and type 1 zwit.).
$P_{o:w}$	**[Input]** n-octanol:buffer partition coefficient of non-ionized species at pH 7.4.
$P^*_{vo:w}$	**[Input]** olive oil:buffer partition coefficient of both non-ionized *and* ionized species at pH 7.4.
$f_{u,p}$	**[Input]** Drug fraction unbound in plasma.
$f_{\text{NP,P}}$	Fractional tissue volume neutral phospholipids in Plasma (0.0013).
$f_{\text{NL,P}}$	Fractional tissue volume neutral lipids in Plasma (0.0023).

pH_{IW}	Intracellular pH (7).
pH_{p}	Plasma pH (7.4).
Hematocrit	Erythrocyte volume fraction (0.45)
$B:P$	**[Input]** Drug blood:plasma ratio, used to approximate Ka_{AP} (see Appendix A for usage; only relevant for moderate-to-strong bases and type 1 zwit.).

Parameters marked with **[Input]** must be supplied by the researcher in order to solve the mechanistic equations for PCs.

Mechanistic Equations:

Moderate-to-strong base (pKa $\geq$ 7):

$$P_i = f_{\mathrm{EW}} + \frac{X \cdot f_{\mathrm{IW}}}{Y} + \frac{Ka_{\mathrm{AP}} \cdot [\mathrm{AP}^-]_i \cdot 10^{pKa_{\mathrm{base}} - pH_{\mathrm{IW}}}}{Y},$$

Group 1 Zwitterions (at least one pKa $\geq$ 7. Same equation as moderate-to-strong bases, but incorporates acidic ionization as well):

$$P_i = f_{\mathrm{EW}} + \frac{X \cdot f_{\mathrm{IW}}}{Y} + \frac{Ka_{\mathrm{AP}} \cdot [\mathrm{AP}^-]_i \cdot 10^{pKa_{\mathrm{base}} - pH_{\mathrm{IW}}} + 10^{pH_{\mathrm{IW}} - pKa_{\mathrm{acid}}}}{Y},$$

Acids, very weak bases, neutral drugs, and group 2 zwitterions:

$$P_i = \frac{X \cdot f_{\mathrm{IW},i}}{Y} + f_{\mathrm{EW},i} + \left(\frac{P_{o:w} \cdot f_{\mathrm{NL},i} + (0.3P_{o:w} + 0.7) \cdot f_{\mathrm{NP},i}}{Y}\right) + \left[\left(\frac{1}{f_{u,p}} - 1 - \left(\frac{P_{o:w} \cdot f_{\mathrm{NL,P}} + (0.3P_{o:w} + 0.7) \cdot f_{\mathrm{NP,P}}}{Y}\right)\right) \cdot \frac{[\mathrm{PR}]_i}{[\mathrm{PR}]_{\mathrm{P}}}\right]$$

Defining the Drug (calculating pKa values)

Since this model uses the more complicated model for predicting tissue:blood PCs, one important input parameter is drug identity (as an acid or base) and pKa values.

There are several standard methods for determining the pKa values of a drug. First, one may choose to perform a potentiometric titration, in which a sample is simply titrated

with an acid or base while monitored by a pH electrode. The pKa can then be calculated from the from the titration curve.[20] Alternatively, one may choose to find pKa values via spectroscopy, NMR titration, liquid chromatography, or capillary electrophoresis.[20] With all of these available techniques, potentiometric titration is the most common method for determining pKa values.[20] If these laboratory techniques are unavailable, still another method of generating these values involves predicting pKa values; this can be done with products such as ChemAxon.[21]

Finding $P_{o:w}$ and $P_{vo:w}$

The next input parameters useful for estimating PCs (using either the simple or more complex model) are the n-octanol:water and olive oil:water partition coefficients. Although simple numbers, the implications of a the $P_{o:w}$ and $P_{vo:w}$ are quite powerful since they provide insight into the lipophilicity of the drug, which will ultimately determine how it interacts with various tissues.[14,15,17–19]

The process for determining the $P_{o:w}$ and $P_{vo:w}$ is actually quite straight forward. First, we must create an octanol-water system, then dissolve a known amount of drug into that system and allow it to equilibrate. Since the octanol is essentially non-polar and water is very polar, the drug will separate into each fraction according to its chemical properties (e.g. more hydrophilic molecules will be more dissolved in the aqueous phase). Next, we use high-performance liquid chromatography to analyze the concentration of drug in each phase.[22] Finally, the $P_{o:w}$ is determined by taking the ratio of the concentration of drug in the octanol phase to the concentration of drug in the aqueous phase (at pH 7.4, physiological pH).[22] That is:

$$P_{o:w} = \frac{[\text{drug in octanol}]}{[\text{drug in aqueous phase (at pH 7.4)}]}$$

In order to determine the $P_{vo:w}$ - which will be used to find a more accurate PC for adipose tissues[14,15,17–19] - we can simply repeat the experiment replacing the octanol buffer with olive oil in order to simulate the drug partitioning into neutral lipids.[14] If lab

materials are unavailable, platforms like ChemAxon[21] are similarly useful for predicting $P_{o:w}$, which could then be used to predict a value for $P_{vo:w}$.[14]

Blood:Plasma Ratio ($B:P$)

The blood:plasma ratio and fraction unbound (described in the following subsection) are another set of important parameters used to predict the system of tissue:blood PCs. Specifically, this ratio is important for estimating Ka_{BC}, which is used as an estimation of $Ka_{[\mathrm{AP}]}$ in the complex model for calculating PCs. Note that this parameter is only relevant for estimating PCs for drugs defined as a moderate-to-strong base or type 1 zwitterion (meaning they have at least one pKa ≥ 7).

There are a few ways to generate the $B:P$ using *in vitro* and *ex vivo* techniques. Similar to $P_{o:w}$, this parameter is simply the ratio of the drug's concentration between two mixtures, however the mixtures involve a combination red blood cells (RBC) or whole blood, and plasma.[23–25] This ratio can be found using a high performance liquid chromatography assay,[24] or liquid chromatography-tandem mass spectrometry (LC/MS/MS).[25]

An even newer version of this method improves on the LC/MS/MS method by using an LC/MS/MS depletion assay to measure drug concentration in plasma that is equilibrating with RBCs.[23] This method can be more efficient than HPLC and standard LC/MS/MS since it measures concentrations in one matrix (rather than separating the RBC and plasma) and eliminates the need for calibration standards while maintaining an R^2 value of 0.9396.[23] If none of these methods are available to a researcher, the $B:P$ parameter can also be determined through companies such as Cyprotex.[26]

Fraction Unbound in Plasma ($f_{u,p}$)

The fraction of drug unbound in the plasma ($f_{u,p}$) presents a very valuable parameter for PBPK modeling by allowing us to begin to understand the drug's efficiency.

In the context of this model, $f_{u,p}$ plays a crucial role in estimating tissue:blood partition coefficients using either the simple or more complex.

There are several effective *in vitro* methods for determining $f_{u,p}$. One such method involves equilibrium dialysis, as well as a gas chromatographic analysis in order to speed up equilibrium time to improve results.[27] Other methods using equilibrium dialysis are also possible.[28] In the absence of the technology to carry out such experiments (which may cost a significant time and money[27]), one could again use methods produced by companies like Cyprotex[29] for their plasma protein binding assay, which is based on the methods using a novel equilibrium dialysis plate.[30]

Absorption (k_a) and Bioavailability (f)

Two of the most important parameters for modeling pharmacokinetic behavior of orally administered drugs are absorption and bioavailability. As used in equation (8), this model assumes a first-order plasma absorption rate constant described by k_a and a fractional bioavailability described by f.

While *in vivo* animal models are usually used to predict human absorption and fractional bioavailability rates,[5] there have also been efforts to estimate these values using *in vitro* methods. Conceptually, *in vitro* models for predicting bioavailability aim to simulate the effects of first-pass metabolism by exposing the drug to a synthetic intestinal wall and measuring its permeability. Most often this is done using Caco-2 cells, which are derived from the human colon and can be cultured to imitate the function of the human intestine.[31]

By modeling the drug's interactions within the intestine, we can estimate the effective permeability (P_{eff}) of the drug, which is strongly correlated to absorption parameters.[32,33] Some effective techniques for estimating bioavailability include estimations based on Caco-2 cell permeability;[31] rat jejunum, the Caco-2 model, and excised intestinal segments in the Ussing chamber;[32,33] drug structure and computer modeling;[5,34,35] and CYP3A concentrations[36] (in order to account for the major drug-metabolizing enzyme in

first-pass).

There is much less consensus on how to predict absorption, and due to the more crucial assumptions made when predicting k_a, these estimations tend to be less accurate than estimations of f.[5] That being said, most of these estimations are also usually based on P_{eff} because of its relevance to physical uptake from the intestine. One of the simplest methods for estimating k_a is the following model:[5,37]

$$k_a = 2P_{\text{eff}}/\text{intestinal radius}$$

More complicated methods estimate segment-specific absorption rates along the GI tract[38] (which for the sake of this model could reasonably be weighted and averaged into a single $\bar{k_a}$). Another method estimates the plasma absorption rate (k_a) by assuming it is equal to the rate of drug dissolution (k_d).[39] That is to say that this model assumes that the rate of drug absorption to the plasma is the same as the rate of drug disappearance from the intestinal lumen. While in many cases this may work with great accuracy, it may also create wildly inaccurate results for drugs that are significantly retained in tissue during first-pass before joining systemic circulation.[39]

Clearance (CL)

Unlike most tissues, the liver possesses many enzymes responsible for destructive metabolism, and serves as the site of metabolism and elimination in our model. Due to the many interactions that account for the true process of metabolism, the rate of clearance is difficult to model without *in vivo* testing. Still, due to the extensive metabolism of most drugs in the liver (specifically by enzyme CYP3A), most predictive models for CL involve *in vitro* testing of hepatocytes - the building blocks of the liver - in order to simulate how quickly a drug becomes metabolized *in vivo*.

The term for the first-order clearance rate that is estimated during either *in vivo* or *in vitro* procedures - and what we refer to as CL - is called the "intrinsic clearance"

(CL_{int}). This constant captures the "pure measure of enzyme activity toward a drug", uninfluenced by blood flow to the liver.[40] The rate of metabolism can be described by equation (21) below.[40] As used in this model, we combine metabolism and elimination such that the drug no longer exists in the system once it is metabolized; therefore, equation (21) captures the function of R_{out} from our system of differential equations.

$$\text{Rate of metabolism} = CL_{int} \cdot C_{\text{liver}} \tag{21}$$

As seen earlier, C_{liver} describes the concentration of free drug in the liver. Thus the goal for estimating metabolism and elimination is simply to estimate the value of CL_{int}, which has been explored through a few different techniques.

One method for predicting CL_{int} uses estimated[41] enzymatic parameters for maximum rate of reaction (V_{max}) and affinity (K_M) to predict clearance based on the relationship: $CL_{int} = V_{max}/K_M$.[41–43] Another method, dubbed the "in vitro $T_{1/2}$ method," involves predicting the human half life ($T_{1/2}$) of the drug and using a verified mechanistic equation to predict CL_{int} from the half life.[43] A third method involves exposing the drug to human hepatocytes *in vitro* and measuring the rate of metabolism,[44] yielding an R^2 value of 0.87 as compared to *in vivo* data.

Additional Parameters

There are only a few remaining parameters necessary in order to create a functioning PBPK model. Firstly, the model requires knowledge of the drug's molecular weight in order to produce and maintain the correct, desired dimensions for tissue concentrations over time. Next, the patient's body weight is also necessary. Additionally, overall cardiac output is assumed to be 6.5 L/min (an average healthy male),[45] and individual tissue blood flow rates (Q_i) were found by multiplying this overall cardiac output with percentage of blood flow to tissues from Poulin and Theil[19] (see Table 1). Also, tissue volumes in Liters were found by multiplying the body weight (in kilograms) with the fractional body weight per tissue in L/kg found in Poulin and Theil[15] (see Table

1). Finally, the number of doses, dosing interval (in hours), and dose amount (in milligrams) are necessary to create a single or multiple-dose PBPK profile.

Results

Implementation

Due to its user-friendly interface and script-writing components, I elected to use MATLAB to implement both the PBPK model and the partition coefficient predicting algorithm. This code can be provided upon request, and is a modified version of "pbpk4DAE.m" from Gieschke and Serafin's book *Development of Innovative Drugs via Modeling with MATLAB: A Practical Guide*.[6] The function outputs a time series with corresponding blood concentrations individually, as well as the arterial and venous concentrations for the 11 tissues used in the model. In order for the function to work, the user must input the relevant parameters as indicated in earlier sections. Further detail on how to properly use the function is provided in Appendix B.

Single-Dose Simulation

In order to assess the success of the PBPK model, five well-studied compounds (Sectral, Carvedilol, Verapamil-R, Etodolac - Extended Release, and Cyclosporine) were modeled using parameters from literature. These drugs cover a spectrum of compounds including acids, bases, and neutrals, but not zwitterions. Table 4 includes all of the physiologically-relevant drug parameters for this set of drugs, as found in literature; whenever possible, the mean and standard deviation for a parameter was used. In the one case in which no $P_{vo:w}$ was available, $P_{o:w}$ was used to predict the adipose tissue:blood partition coefficient instead. Information regarding k_a and CL was provided from population pharmacokinetic (pop-PK) studies, which aim to assess the pharmacokinetics of drugs in specific populations. Any pop-PK data used here was taken from the "healthy"

population. Table 5 includes each drugs' molecular weight, as well as the average body weight (with standard deviations when possible), dose amount and dosing interval used to recreate the clinical data.

Success was determined based on the model's ability to duplicate empirically determined $C_{\max}$, or maximum plasma concentration following a single dose, as well as Area Under Curve (AUC) when possible. AUC is used to describe a patient's total exposure to the drug after a single dose by measuring the total area under the blood vs. time concentration curve, which has serious pharmacological implications.

To test the model for accuracy and consistency, each drug was subject to a 24 hour simulation with 150 subjects, whose resulting blood concentration profiles can be seen in Figure 8. In order to introduce variation among subjects and perform a sensitivity analysis on the model with respect to absorption, body weight, and clearance rates, these three parameters were drawn from a normal distribution based on a mean and standard deviation whenever possible (see Tables 4 and 5). The results of these simulations are listed in Table 5 under "PBPK single-dose $C_{\max}$" and "PBPK AUC".

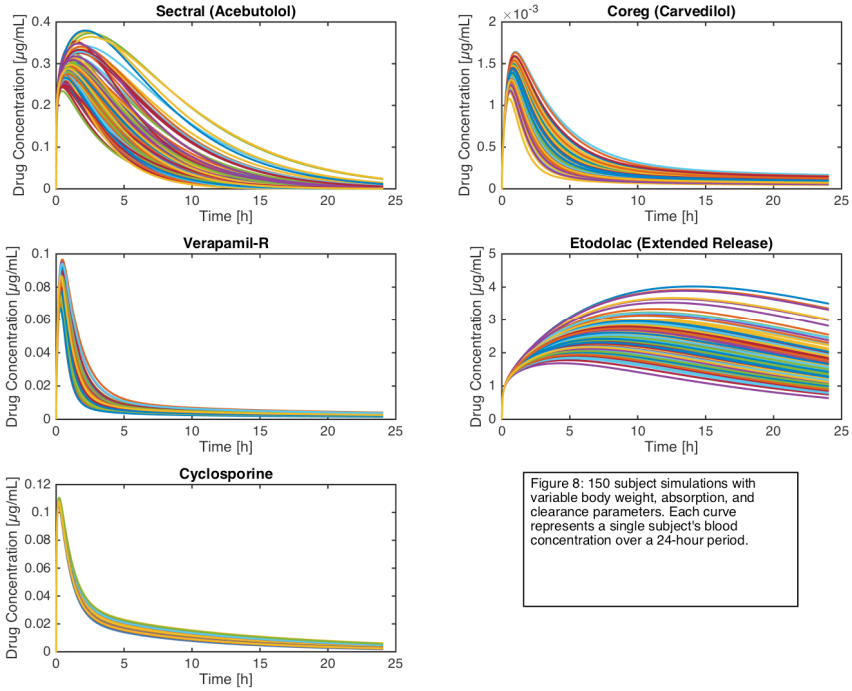

Figure 8: 150 subject simulations with variable body weight, absorption, and clearance parameters. Each curve represents a single subject's blood concentration over a 24-hour period.

Simply based on the plots generated, we can see that this model is able to capture varying profiles. For instance, Verapamil-R is nearly entirely eliminated after 5 hours, whereas Etodolac Extended Release retains high blood concentrations even 24 hours later; both of these profiles are consistent with the behaviors we would expect from a night-time drug and extended release tablet respectively. In a pre-clinical setting, these profiles would be important for understanding a drug's behavior and determining possible dosing strategies. The single-dose $C_{\max}$ results showed varying degrees of success. For Sectral (Acebutolol), the predicted $C_{\max}$ value fell within a standard deviation of the reported value, whereas the other simulations were less successful. Coreg (Carvediolol) and Etodolac-R cam very short of their reported $C_{\max}$ values, achieving only 2.9% and 27.4% respectively. Verelan PM (Verapamil-R) was slightly more successful, by predicting a $C_{\max}$ value of about 141% what we expect. Lastly, the PBPK model predicted Cyclosporine $C_{\max}$ within 68.9% of of the reported value.

Consistent with the $C_{\max}$ results, the results for AUC simulations were within the same realm of accuracy. The PBPK model predicted Sectral's AUC within two standard deviations of the reported mean, but for Coreg and Verelan PM the predicted results were 3.5% and 21% of the reported AUCs, respectively.

Table 4: Physiologically relevant drug-specific parameters:

Compound & Refs	pKa	$\log[P_{o:w}]$	$\log[P_{vo:w}]$*	Blood:plasma ratio ($B:P$)	Fraction unbound ($f_{u,p}$)	Absorption constant (k_a, hr^{-1})	Bioavail. (f)	Clearance constant (CL, L/hr)
Moderate-to-strong bases:								
Sectral (Acebutolol)[17,46,47]	9.7	1.87	0.74	1.09	0.79	0.451 (0.125)	0.4	81.14 (9.936)
Coreg (Carvedilol)[17,48–50]	8.1	4.19	3.32	0.81	0.019	0.81 (0.1471)	0.3	0.89 (9.94)
Verelan PM (Verapamil-R)[17,51,52]	8.5	3.79	2.88	0.85	0.05	1.72 (0.31)	0.49	91.7 (5)
Acid:								
Etodolac-R (ER)[18,53,54]	4.7	3.6	-	-	0.0108	0.172 (0.0413)	0.8	3.68 (0.405)
Neutral:								
Cyclosporine[18,55,56]	-	2.9	-	-	0.083	1.28	0.3	43.6 (16.4)

Data presented as: mean (standard deviation), when possible. If multiple values were reported, the average value reported was used. * If $\log[P_{vo:w}]$ data was unavailable, $\log[P_{o:w}]$ was used to calculate P_{AD}.

Table 5: Results from various drug simulations.

Compound & Refs	Molecular Weight	Dose (mg)	Dosing Interval (h)	Body Weight (kg)	Experimental single-dose C_{max}	PBPK single-dose C_{max}	Experimental AUC	PBPK AUC
Sectral (Acebutolol)[46,47]	372.89	200	12	68 (9)	342 (113) μg/L	285.03 (27.24) μg/L	2465 (344) μg·h/L	1837.58 (936.92) μg·h/L
Coreg (Carvedilol)[48–50]	406.5	3.125	12	70*	46.7 ng/ml	1.34 (0.105) ng/ml	165 ng·h/mL	5.84 (1.41) ng·h/mL
Verelan PM (Verapamil-R)[51,52]	454.6	120	24 (taken before bed)	74 (4)	56.43 ng/ml	79.36 (5.93) ng/ml	875.96 ng·h/ml	184.20 (36.82) ng·h/ml
Etodolac-R (ER)[53,54]	287.37	400	24	71	9.1 mg/L	2.48 (0.439) mg/L	-	-
Cyclosporine[55,56]	1202.63	156	12	70*	156 ng/ml	107.48 (2.88) ng/ml	-	-

Data presented as: mean (standard deviation), when possible. * If mean or median body weight was not specified, 70 (10) kg was used.

Discussion

While the results suggest that this model may not predict a drug's pharmacokinetic profile with consistent accuracy, they do suggest that the model provides useful insight into how the drug will distribute throughout the body, and general trends in the drug's behavior. Figures 9 and 10 show the whole-body PK profiles compared to the blood compartment for Carvedilol and Etodolac following 5 consecutive doses. Profiles like these can be useful for understanding which tissues are particularly exposed to the drug, as well as the amount of doses necessary to reach steady state concentrations ($C_{\text{max,ss}}$ and $C_{\text{trough,ss}}$) in both tissues and blood. Especially when a threshold concentration should be met and maintained, this model can provide insight into the appropriate dosing regimen.

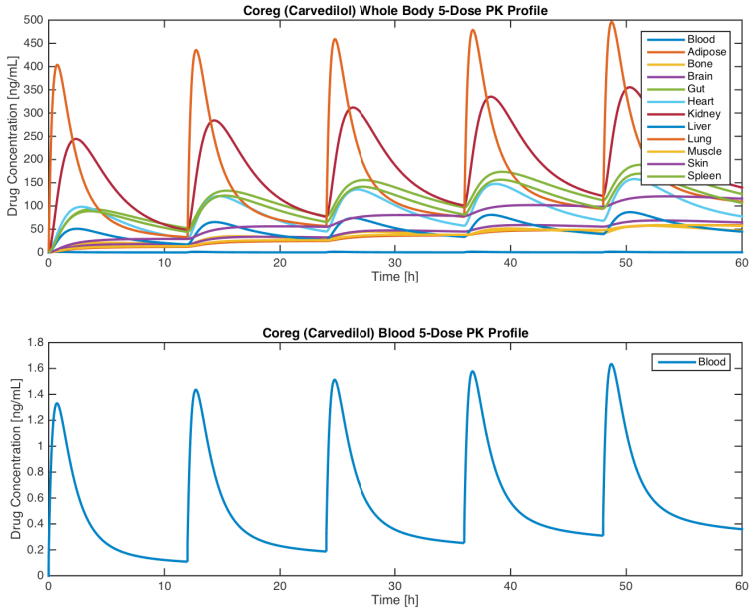

Figure 9: Carvedilol PK profiles following 5 doses (12 hour dose intervals) using mean values from Tables 4 and 5. Top: whole body profile for 12 tissues, bottom: blood concentrations over same time span.

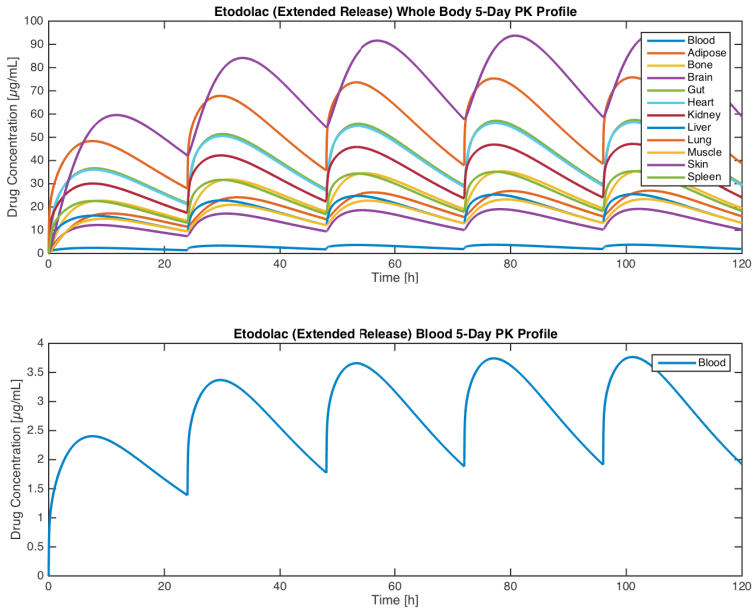

Figure 10: Etodolac-Extended Release PK profiles following 5 doses (24 hour dose intervals) using mean values from Tables 4 and 5. Top: whole body profile for 12 tissues, bottom: blood concentrations over same time span.

Assumptions and Limitations

As with any predictive model, this PBPK model has several assumptions and limitations that contribute to any inaccuracies we see. Firstly, due to the availability of relevant physiological data, this model assumes the system of interactions to only include the blood and 11 tissues. Ideally, a whole body PBPK model would include relevant data for each individual tissue in the human body. Another assumption used in this model is the fact that nearly the entire GI tract is treated as one tissue (the gut), as opposed to being broken down to its various compartments (e.g. duodenum, jejunum, etc.). Again, more specific breakdowns of individual tissue should lead to more accurate results.

Two important assumptions in this model are the absorption and clearance. As mentioned in the Methods, absorption is considered constant and is simulated as a steady stream of drug moving from some external "compartment" straight into systemic circulation. In order to account for first-pass metabolism, we assessed the oral bioavailability and simply adjusted the available dose by multiplying it with the fraction of drug that is orally bioavailable. The model would perhaps be better served if it incorporated a compartment in which we actually modeled first-pass metabolism, and passed the drug through the liver and then into systemic circulation. This compartment would also account for the lag time we see in orally administered drugs, whereas this model immediately introduces the drug to the blood stream following administration. For clearance, this model assumed that the drug was entirely metabolized and cleared from the liver, which is not always the case.

Another assumption of this model is that each tissue receives the same arterial concentration during the distribution process. For some tissues there are barriers that affect how much drug will actually interact with the tissue. For instance, the blood-brain barrier allows for only certain types of molecules to transport to the brain.[13] Understanding how a drug will interact with this barrier is difficult, and could have a great impact on brain tissue concentrations depending on the nature of the drug.

Most remaining assumptions come from the method for predicting partition

coefficients. In this algorithm, some physiological parameters ($\frac{[\mathrm{PR}]_i}{[\mathrm{PR}]_\mathrm{P}}$, $[\mathrm{AP}^-]_i$, and fractional tissue volumes) come from rat tissues as opposed to humans. While these should be relatively consistent, given the functions of tissues and organs are consistent, any inconsistencies between human and rat tissue composition could have serious repercussions when predicting tissue:blood partition coefficients, which then directly impact the distribution of the drug.

Further limitations to this model revolve around its simplicity. As of now, the model does not incorporate any patient-specific details (e.g. height, gender, age) besides body weight. The model also cannot account for the effects of drug-drug interactions (DDI) without explicit information as to how DDIs affect first order absorption and clearance. Likewise, this model assumes that k_a is constant over all doses unless otherwise known, so there is no accounting for dose-dependent absorption rates.

Conclusion

In this paper, I have presented a physiologically-based pharmacokinetic model that functions using parameters that can be estimated without *in vivo* data and incorporates an established algorithm for predicting tissue:blood partition coefficients. While the PBPK model used here is variable in its ability to accurately predict pharmacokinetic outcomes, we have shown here that it can be used in some cases to accurately predict a drug's $C_{\max}$ and AUC data. Additionally, the concentration vs. time profiles generated by this model can provide important insight into distribution of the drug, and highlight sites of pooling that could lead to adverse effects. Likewise, dosage size and dosing intervals necessary to maintain various threshold concentrations can be explored once information regarding k_a and CL is estimated.

Despite its various assumptions and limitations, this model can serve well as a simple, pre-clinical tool for gaining general information about a drug's ADME. Intended to provide insight for researchers on newly developed extended release (XR) antiretroviral (ARV) drugs, this model has demonstrated a capacity to capture behaviors that will prove

important in the drug development process. In the future, this model can be expanded upon to include more tissues, drug-drug interactions, and more accurate measures of pre-clinically determined pharmacokinetic parameters in order to serve as an accurate predictor for clinical outcomes.

Appendix

A.

The equation to approximate $K_{a,\mathrm{BC}}$ is as follows:

$$K_{a,\mathrm{BC}} = [P_{\mathrm{BC}} - (\frac{1+10^{\mathrm{pKa}-pH_{\mathrm{BC}}}}{1+10^{\mathrm{pKa}-pH_p}} \cdot f_{\mathrm{IW,BC}}) - (\frac{P_{o:w} \cdot f_{\mathrm{NL,BC}} + (0.3P_{o:w}+0.7)f_{\mathrm{NP,BC}}}{1+10^{\mathrm{pKa}-pH_p}})] \cdot (\frac{1+10^{\mathrm{pKa}-pH_p}}{[\mathrm{AP}^-]_{\mathrm{BC}} \cdot 10^{\mathrm{pKa}-pH_{\mathrm{BC}}}})$$

Where $pH_{\mathrm{BC}} = 7.22$, $pH_p = 7.4$, $[\mathrm{AP}^-]_{\mathrm{BC}} = 0.5$, $f_{\mathrm{IW,BC}} = 0.603$, $f_{\mathrm{NL,BC}} = 0.0035$, $f_{\mathrm{NP,BC}} = 0.0029$, pKa is the pKa of the most basic substituent (adjusted for polyprotic bases).[17]

In order to estimate P_{BC}, we use the following mechanistic equation:[18]

$$P_{\mathrm{BC}} = \frac{\mathrm{Hematocrit} - 1 + B:P}{f_{u,p} \cdot \ \mathrm{Hematocrit}}$$

Where Hematocrit = 0.45,[19] $B:P$ is the drug's blood to plasma ratio (determined *in vitro*), and $f_{u,p}$ is the fraction of drug unbound in the blood (determined *in vitro*).

B.

The MATLAB function, titled **pbpkmultidose.m** works in the following way:

```
[time,blood,concs,molarblood,molar_conc] = pbpkmultidose(DrugIsAcid,DrugIsBase,DrugIsNeutral,...
    pKa,pKb,LogP,LogVP,DrugBloodPlasmaRatio,DrugFractionUnbound,molweight,dose,ka,...
    bw,bioavail,clearance,doseint,doses)
```

Below is a table containing an explanation for each input, note that zwitterions are both basic and acidic, so DrugIsAcid and DrugIsBase would both be 1, and relevant pKa's must also be provided:

B.

Table B1. PBPK Function Input Parameters

Parameter	Symbol in MATLAB Function	Input Details/ Dimension	Description
Acidic Drug	DrugIsAcid	Binary: 1 if Yes, 0 if No	Identifies the drug as acidic, to be used for predicting PCs
Basic Drug	DrugIsBase	Binary: 1 if Yes, 0 if No	Identifies the drug as basic, to be used for predicting PCs
Neutral Drug	DrugIsNeutral	Binary: 1 if Yes, 0 if No	Identifies the drug as neutral, to be used for predicting PCs
Drug pKa	pKa	Dimensionless Number	pKa associated with an acidic compound.
Drug pKb	pKb	Dimensionless Number	pKa associated with a basic compound
n-Octanol:Water Partition Coefficient	LogP	Dimensionless Number	Logarithm of experimentally determined $P_{o:w}$, used for predicting PCs.
Olive Oil:Water Partition Coefficient	LogVP	Dimensionless Number	Logarithm of experimentally determined $P_{vo:w}$, used for predicting PCs.
Blood to Plasma Concentration Ratio	DrugBloodPlasmaRatio	Dimensionless Number	Only necessary for moderate-to-strong bases and group 1 zwit.
Fraction of Drug Unbound in Plasma	DrugFractionUnbound	Dimensionless (percentage - between 0 and 1)	Experimentally determined $f_{u,p}$, used for predicting PCs.
Molecular Weight	molweight	grams / mole	Standard term for molecular weight.
Dose	dose	milligrams (mg)	Dosage to be given during simulation.
Absorption Constant	ka	1 / hour	Experimentally determined k_a, used to represent plasma absorption rate.
Body Weight	bw	kilograms (kg)	Body weight of simulated patient, assumed to be 70 kg.
Bioavailability	bioavail	Dimensionless (percentage - between 0 and 1)	Fractional bioavailability for oral dose; can be determined experimentally using techniques from methods section.
Clearance Constant	clearance	Liters / hour	Experimentally determined CL, used to represent metabolism and elimination from the system.
Dosing Interval	doseint	hours	Number of hours between doses.
Number of doses	doses	Dimensionless number	Describes the number of dosing intervals to simulate.

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