

TUTORIAL

Using partition analysis as a facile method to derive net clearances

Ken Korzekwa  | Jaydeep Yadav | Swati Nagar 

Department of Pharmaceutical Sciences, Temple University, Philadelphia, Pennsylvania, USA

Correspondence

Ken Korzekwa, Department of Pharmaceutical Sciences, Temple University School of Pharmacy, 3307 N Broad Street, Philadelphia, PA 19140, USA.
Email: korzekwa@temple.edu

Present address

Jaydeep Yadav, Department of Pharmacokinetics, Pharmacodynamics, and Drug Metabolism, Merck & Co., Inc., Boston, Massachusetts, USA

Funding information

This work was supported by the National Institutes of Health National Institute of General Medical Sciences (to K.K. and S.N.) Grants 2R01GM104178 and 2R01GM114369

Abstract

Partition analysis has been described previously by W.W. Cleland to derive net rate constants and simplify the derivation of enzyme kinetic equations. Here, we show that partition analysis can be used to derive elimination and transfer (distribution) net clearances for use in pharmacokinetic models. For elimination clearances, the net clearance approach is exemplified with a mammillary two-compartment model with peripheral elimination, and the established well-stirred and full hepatic clearance models. The intrinsic hepatic clearance associated with an observed average hepatic clearance can be easily calculated with net clearances. Expressions for net transfer clearances are easily derived, including models with explicit membranes (e.g., monolayer permeability and blood–brain barrier models). Together, these approaches can be used to derive equations for physiologically based and hybrid compartmental/ physiologically based models. This tutorial describes how net clearances can be used to derive relationships for simple models as well as increasingly complex models, such as inclusion of active transport and target mediated processes.

INTRODUCTION

Pharmacokinetic (PK) parameters are often derived from models in which drug disposition is represented as transfer across different compartments. These compartments can be mathematical or physiological and drug transfer can represent distribution as well as elimination. Drug

transfer rates are routinely represented by first order rate constants (units of time^{-1}) times the drug amount or by clearance (units of volume/time) times drug concentration. In this tutorial, we use a simple method described by Cleland for enzyme kinetics,¹ and apply it to the derivation of net clearance (CL_{net}) terms for use in PK models. Possibly the most well-known example of a CL_{net} in PKs

Abbreviations: CL_{bile} , Active efflux into the bile; CL_{dif} , Passive diffusional clearance across a membrane.; CL_{eff} , Active efflux clearance; CL_{H} , Hepatic clearance; $CL_{\text{H,av}}$, Average hepatic clearance; CL_{i} and CL_{o} , Clearances in and out of a membrane; $CL_{\text{int,H}}$, Intrinsic hepatic clearance; CL_{met} , Intrinsic hepatic metabolic clearance; $CL_{\text{netx,y}}$, Net clearances from compartment x to compartment y; CL_{s} , Systemic clearance (dose/AUC); CL_{up} , Active uptake clearance; f_{ub} , fraction of drug unbound in blood; f_{ui} , Fraction unbound in interstitial fluid; P_{app} S, Permeability Surface area product across a cell monolayer; Q_{H} , Hepatic blood flow.

Ken Korzekwa and Swati Nagar contributed equally to this work.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. *Clinical and Translational Science* published by Wiley Periodicals LLC on behalf of American Society for Clinical Pharmacology and Therapeutics.

is the hepatic clearance equation based on the well-stirred model. This equation can be derived with simple mass balance considerations and algebra. The more complex full clearance equation can be derived with determinants. This tutorial uses an alternative approach—partition analysis—to derive in a more straightforward manner, CL_{net} as described below.

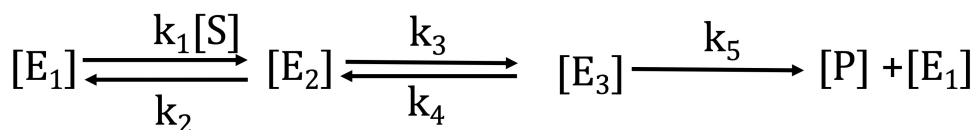
Net clearances can be used to combine clearance terms and eliminate compartments to simplify a model. If a compartment or compartments do not significantly contribute to drug disposition, compartments can be removed (i.e., mathematically ignored) and CL_{net} can be used to represent the clearance (or flow) through these compartments. These clearances, whether they are transfer clearances, elimination clearances, or systemic clearances, can be used in PK equations. The clearances alone may not be particularly useful, but can be used to build compartmental, physiologically-based pharmacokinetic (PBPK), or hybrid compartmental-PBPK models. These PK models are usually solved directly from their ordinary differential equations (ODEs) using numerical methods.

With regard to notations and assumptions in PKs, we note that throughout this paper, the term “clearance” is used to denote volume flow per unit time, and a “clearance” term can be a transfer rate for drug elimination or drug distribution. In the discussion below, several clearances are defined for specific physiological processes (e.g.,

clearances for diffusion, transport, metabolism, etc.) as well as the systemic clearance (CL_s) which is a primary PK parameter. CL_s is defined as the volume of blood or plasma cleared of drug per unit time. It is calculated as the rate of drug elimination (dX/dt) divided by the concentration in the reference fluid. The reference fluid is assumed to be in the central compartment (sampling site). The importance of clearly defining the reference fluid is discussed later in the context of average clearances as well as distribution volumes when elimination occurs from the peripheral compartment. Although the discussion below uses clearance to derive relationships, as with all PK models, these relationships can also be derived with rate constants and relevant volumes.

PARTITION ANALYSIS

In 1975, Cleland published a method to derive steady-state rate equations for enzymes using partition analysis.¹ This method uses net rate constants to greatly simplify complex kinetic schemes. Basically, the Cleland method calculates a net rate constant based on partition analysis. The net rate constant through a species is the rate constant to the species times the fraction of that species that moves forward. An example of the use of partition analysis to simplify an enzyme kinetic scheme is shown in [Figure 1](#). In [Figure 1](#),

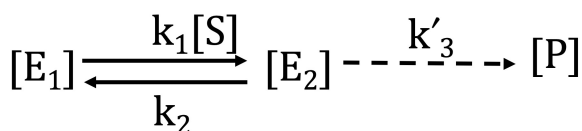


The net rate constant k_3' from $[E_2]$ to $[P]$ is:

$$k_3' = \frac{k_3 k_5}{k_4 + k_5}$$

Product of all rate constants going from $[E_2]$ to $[P]$

Sum of all rate constants going away from $[E_3]$



The net rate constant k_1' from $[E_1]$ to $[P]$ is:

$$k_1' = \frac{k_1[S]k_3'}{k_2 + k_3'}$$

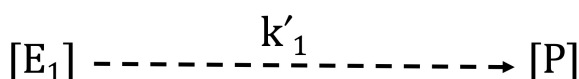


FIGURE 1 Simple enzyme kinetic scheme. Net rate constants k_3' and k_1' are used to simplify the kinetic scheme resulting in a net rate constant from (E_1) to P . E_1 , E_2 , and E_3 , enzyme species; S , substrate; P , product.

the net rate constant for E_2 to P, k_3' , is the rate constant from E_2 to E_3 , k_3 , times the fraction of E_3 that moves on to P, $k_5/(k_4 + k_5)$. The net rate constant from E_1 to P, k_1' , can then be calculated in the same way using k_3' . Cleland provides the methods and rationale to calculate net rate constants for complex systems, including branched and alternate pathways, and uses these net rate constants to easily derive equations for maximum value (V_{\max}) and kinetic metabolite (K_m ; denoted as V and K by Cleland). This method is substantially simpler than using determinants or graphical methods, such as the King-Altman method.

We have been using net rate constants to simplify enzyme kinetic analyses for drug metabolism. For example, we have used net rate constants to calculate inactivation rate constants for mechanism-based inactivation of the cytochrome P450s.²⁻⁵ The kinetic schemes for these processes can be very complicated with sequential steps and branched pathways. Calculating a net rate constant for inactivation allows the standard equations to be used to predict drug-drug interactions. As can be seen below, partition analysis can also be used to easily derive equations for organ (e.g., hepatic) clearances, transport, and tissue partitioning, in order to build compartmental and physiological PK models. Examples of the use of this technique with specific models are presented below.

Using partition analysis to derive PK clearances is simpler than deriving rate equations for enzymes. For enzymes, there is a limited total concentration of enzyme (Et), and the concentration of the various forms of the enzyme must be considered when deriving rate equations. In PK models, drug distributes into different compartments instead of from different enzyme species. When calculating CL_{net} , we do not “use up” compartmental space. This is similar to V_{\max}/K_m kinetics for an enzyme, when substrate concentrations are very low. For the scheme in Figure 1, only the net rate constants that contain S will contribute to V_{\max}/K_m , at low substrate concentrations. Therefore, the first order rate constant (V_{\max}/K_m) is k_1'/S (see Cleland¹ for a detailed discussion). When calculating CL_{net} for compartmental models, only the CL_{net} of the reference compartment (i.e., the one by which we will be multiplying the drug concentration) is used.

Another obvious difference between in vitro enzyme kinetics and cell or whole-body PKs involves volumes of distribution. Specifically, whereas all enzyme and substrate species in an in vitro assay are present in a single volume, we must consider rate and extent of drug distribution across multiple compartments in cell/body drug disposition. Distribution is discussed in detail in a subsequent section below.

Consider the model in Figure 2, where a drug must traverse three compartments (2, 3, and 4) to move from compartment one to compartment five. This could represent

a model where the plasma is compartment one and the drug is irreversibly eliminated from compartment four. The simplest method to calculate the CL_{net} for a drug moving from compartment one to compartment five, is to begin at the irreversible step, $CL_{4,5}$, and work from right to left (from $5 \rightarrow 1$, Figure 2a and b). We first calculate the CL_{net} from three to five: $CL_{\text{net}3,5}$ is $CL_{3,4}$ times the fraction moving from four to five (i.e., $CL_{\text{net}3,5} = CL_{3,4} CL_{4,5}/(CL_{4,3} + CL_{4,5})$). We then calculate the net clearance from two to five: $CL_{\text{net}2,5} = CL_{2,3} CL_{\text{net}3,5}/(CL_{3,2} + CL_{\text{net}3,5})$. Finally, we calculate the net clearance from one to five: $CL_{1,5} = CL_{1,2} CL_{\text{net}2,5}/(CL_{2,1} + CL_{\text{net}2,5})$.

A certain direction or order of the derivation is not required. Calculating in the direction from $1 \rightarrow 5$ (Figure 2c), we first calculate the net clearance from one to three, $CL_{\text{net}1,3}$. However, we also need to calculate the net clearance from three to one, $CL_{\text{net}3,1}$, to calculate the net clearance from one to four, $CL_{\text{net}1,4}$. Likewise, we need to use $CL_{\text{net}4,1}$ when calculating $CL_{\text{net}1,5}$. The final equations for $CL_{\text{net}1,5}$ requires five net clearances in the $1 \rightarrow 5$ direction (see Figure 2b) compared to three in the $5 \rightarrow 1$ direction (see Figure 2c).

One could also calculate three net clearances, $CL_{\text{net}1,3}$, $CL_{\text{net}3,1}$, and $CL_{\text{net}3,5}$, and then calculate $CL_{\text{net}1,5}$ as $CL_{\text{net}1,3} CL_{\text{net}3,5}/(CL_{\text{net}3,1} + CL_{\text{net}3,5})$. The same equation for $CL_{\text{net}1,5}$ will be obtained using all three methods. However, for more complicated models, some approaches are much simpler than others (see examples below). It should be noted that all the above methods calculate a net clearance ($CL_{\text{net}1,5}$) with the concentration in compartment one as the driving concentration.

Calculating a net clearance removes a compartment from the model. For example, calculating $CL_{\text{net}1,3}$ in Figure 2 removes compartment two. This is equivalent to the system in Figure 2 at the limit of $V_2 \rightarrow 0$, or when compartment two is at steady-state (i.e., rate into compartment 2 = rate out of compartment 2 and drug concentration in compartment 2 is constant). Cleland discusses a net rate constant as a “conductance” through an enzyme species, and it is tempting to think of the compartments as a series of resistances. Resistances are additive, and the usual definition of conductance as the inverse of resistance would suggest that the net conductance for a drug moving from one to three in Figure 2 would be $1/(1/CL_{1,2} + 1/CL_{2,3})$. The result, $CL_{1,2} CL_{2,3}/(CL_{1,2} + CL_{2,3})$ is only true when $CL_{1,2} = CL_{2,1}$. For passive diffusion across a barrier, this may be the case, but it is not always true (see discussion below).

ELIMINATION

The simplest PK model with kinetically distinct compartments is the mammillary two-compartment model. If

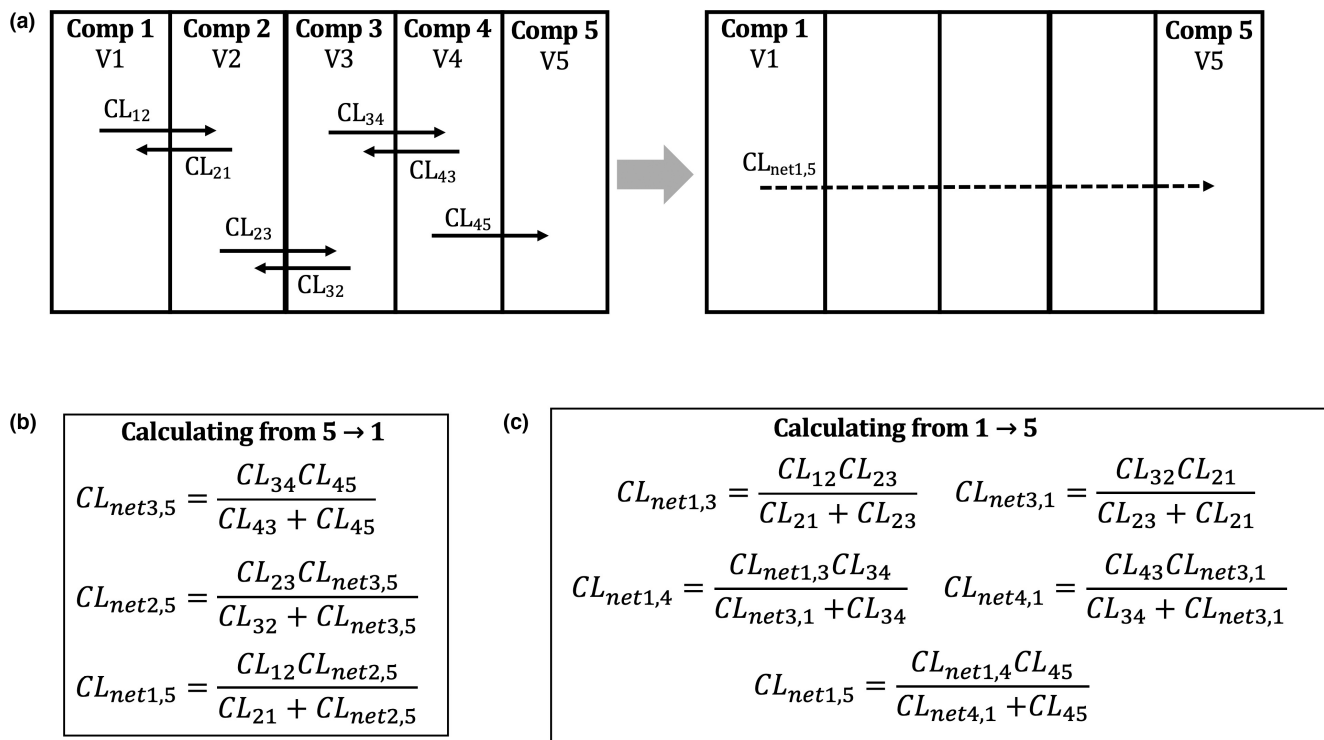


FIGURE 2 Calculating net clearances for a drug that moves from compartment 1–5. (a) Model for collapsing a 5-compartment model. (b) Net CL equations calculated from compartment 5 to 1. (c) Net CL equations calculated from compartment 1 to 5. All CL terms are as defined in the Glossary. V1–V5: compartment volumes. CL, clearance.

elimination occurs from the central compartment, CL_s will be constant. Figure 3a shows a mammillary two-compartment model with elimination from the peripheral compartment. Because the model eliminates drug from the peripheral compartment (see Figure 3a), the systemic clearance changes with time until distribution equilibrium is achieved, and then plateaus to a constant value. The CL_{net} derived below is therefore an exposure-averaged clearance, which is also the clearance at steady-state.^{6–8} Partition analysis can be used to calculate the CL_{net} from compartment one (central) to compartment three (elimination). The CL_{net} is the systemic clearance CL_s , and can be derived as the clearance from compartment one to compartment two (CL_d) times the fraction in compartment two that is eliminated ($CL_{int}/(CL_d + CL_{int})$, or $CL_s = CL_d CL_{int}/(CL_d + CL_{int})$. This concept is further detailed in Figure 3b and c. An instantaneous i.v. bolus dose or dosing to steady-state (Figure 3b) can be considered. Upon an i.v. bolus injection at time $t = 0$, there is no drug concentration in the eliminating peripheral compartment, a maximum concentration in the central compartment, and $CL_H = 0$. With time, CL_H will achieve a constant value above $CL_{H,av}$ (Figure 3b, middle). In addition, upon an i.v. infusion to steady-state, the steady-state clearance is the same as the average clearance. It is important to note that dosing and sampling are assumed to be from the central compartment (reference fluid). Figure 3c shows the same model (Figure 3a) now with decreasing

peripheral volume V_2 (i.e., to the limit of $V_2 \rightarrow 0$). As $V_2 \rightarrow 0$, the concentration–time profile approaches monophasic kinetics, and the clearance becomes constant with time. For all i.v. bolus models with peripheral elimination, CL_{av} will equal dose/area under the curve (AUC).

Figure 4a shows the liver as a well-stirred model for consideration of hepatic clearance^{9,10} where Q_H is liver blood flow and $f_{ub} CL_{int,H}$ is the unbound intrinsic clearance of the liver. This model assumes rapid equilibration in the liver. We can easily see that the net hepatic clearance from blood, CL_H , is simply the liver blood flow, Q_H , times the fraction moving forward, $f_{ub} CL_{int,H}/(Q_H + f_{ub} CL_{int,H})$.

The full model for hepatic clearance^{11–15} that includes membrane diffusion (CL_{dif}) and transporter-mediated uptake (CL_{up}) and efflux (CL_{eff}) in and out of the liver (Figure 4b), can be similarly derived easily. First, the blood to elimination CL_{net} is calculated as the clearance into the liver ($f_{ub} CL_{dif} + f_{ub} CL_{up}$) times the fraction that is eliminated ($CL_{eff} + CL_{met}$)/($CL_{dif} + CL_{eff} + CL_{met}$). This simplifies the model to one analogous to the model in Figure 4a, where the net elimination clearance $CL_{net1,3}$ is used instead of $CL_{int,H}$. These relationships are not new. These CL_{nets} have been described previously by Gillette and Pang as apparent clearances¹¹ and by Yamazaki et al. as $CL_{int,all}$.¹² Using partition analysis, these and more complex equations can be rapidly derived.

It should be noted that transfer clearances (involved only in distribution) that are not in the path

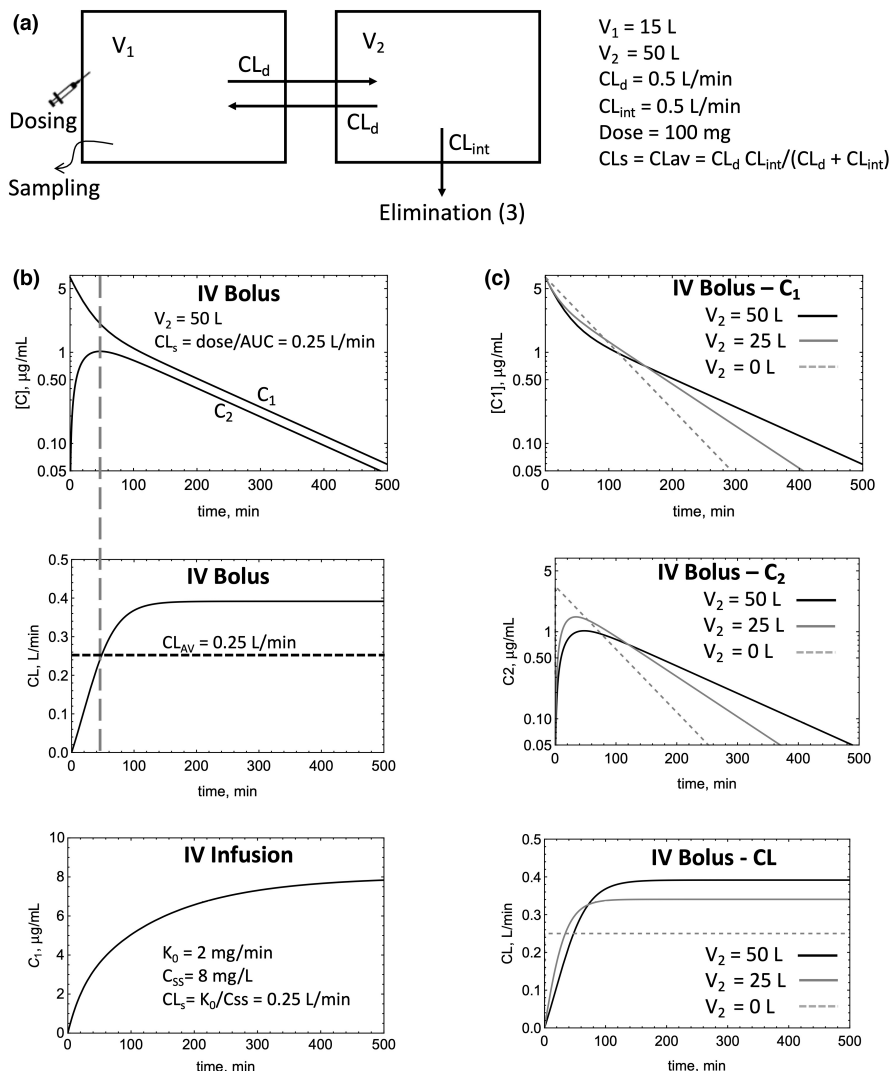


FIGURE 3 A two-compartment model with peripheral elimination. (a) Two-compartment mammillary model with elimination from the peripheral compartment. Dosing and sampling are from the central compartment. All parameters for the simulation are listed along with the expression for net clearance. (b) (top) Simulated concentration–time profiles in compartments one and two upon an i.v. bolus dose. (middle) Clearance as a function of time for an i.v. bolus. The exposure-averaged clearance is shown with the horizontal dashed line. The vertical dashed line indicates the time at which the peripheral compartment is at steady-state and the clearance is the average clearance. (bottom) Simulated concentration–time profile in compartment 1 upon an i.v. infusion dosed to steady-state. (c) (top) C_1 and (middle) C_2 concentration–time profiles upon an i.v. bolus as V_2 approaches zero. (bottom) Clearance as a function of time an i.v. bolus as V_2 approaches zero. CL_{av} , average clearance; CL_{dif} , passive diffusional clearance across a membrane; CL_{int} , intrinsic clearance; CL_s , systemic clearance; CL_{ss} , clearance at steady-state.

of elimination will not impact CL_s (Dose/AUC). The model in Figure 4c includes an explicit membrane compartment into which hydrophobic drugs can partition. Membrane partitioning is modeled with using clearance in and out terms, CL_i and CL_o , respectively. This model is similar to any physiologically based liver model that is both perfusion and permeability limited. Although only a phospholipid compartment is shown in Figure 4c, any number or type of intracellular distribution compartments could be added (e.g., neutral lipids, lysosomes, etc.).^{16–20} Although partitioning into an intracellular compartment can affect the rate and extent

of distribution (see distribution below), it will not affect the average elimination clearances. This can be easily understood at steady-state when there is no net movement of drug into distribution-only compartments. Because the liver compartment in Figure 4c is modeled with a distribution volume, the liver becomes kinetically distinct from the blood compartment, and the CL_{net} obtained is an exposure-averaged hepatic clearance, $CL_{H,av}$ (Figure 3d). In the absence of any other organ clearance, $CL_{H,av} = CL_s = \text{Dose/AUC}$. This is the average organ clearance because elimination occurs from a peripheral compartment made kinetically distinct from the central

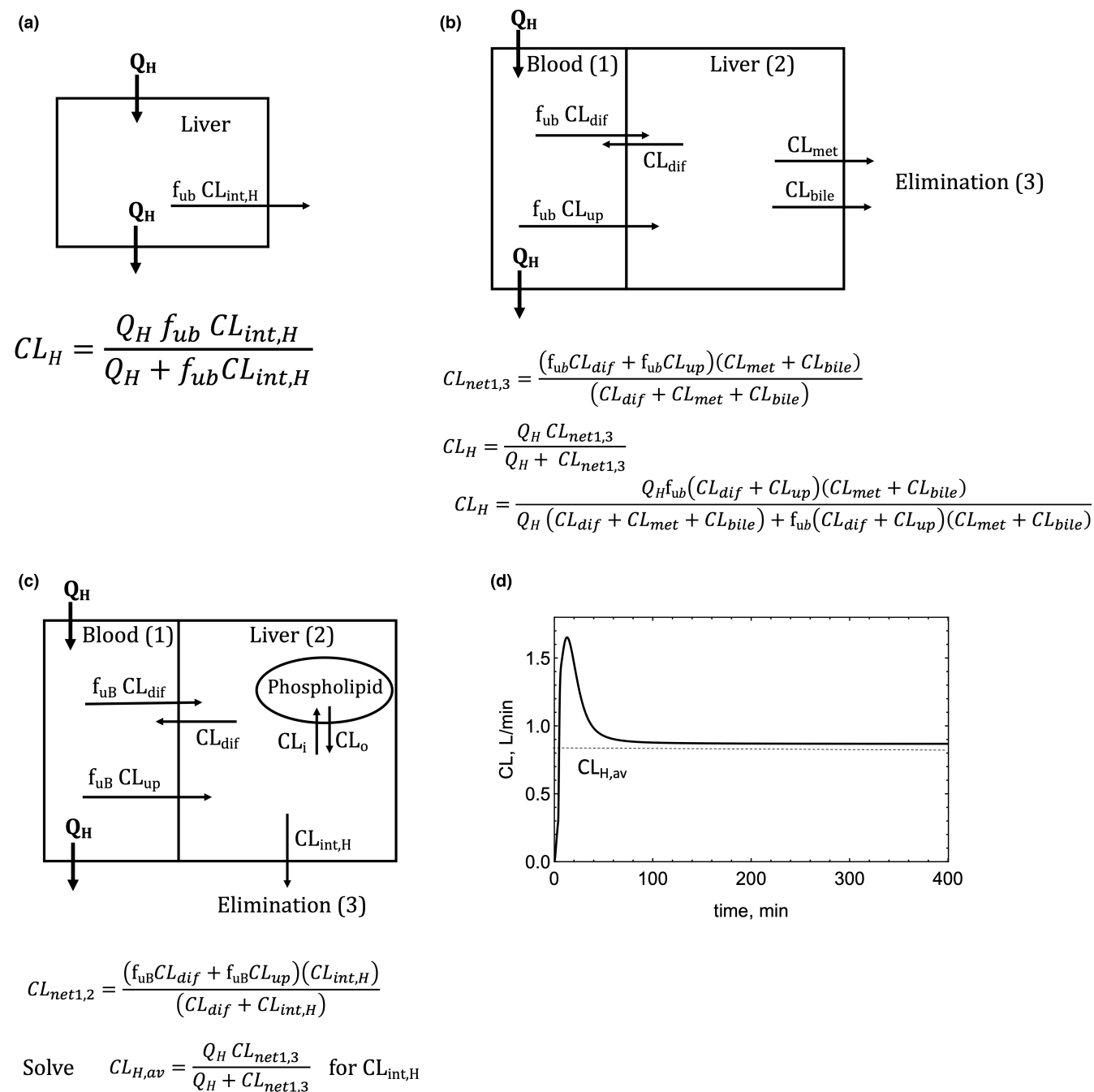


FIGURE 4 Well-stirred models for hepatic clearance. Equations show how the intrinsic clearance can be calculated from a known systemic clearance. (a) Simple liver model. Q_H , liver blood flow; $CL_{int,H}$, hepatic intrinsic clearance; f_{ub} , fraction unbound in blood; CL_H , hepatic clearance. (b) Full clearance model. CL_{dif} , diffusional clearance through a membrane; CL_{up} , uptake transporter clearance into the liver; CL_{bile} , efflux transporter clearance into the bile; CL_{met} , hepatic metabolic clearance. (c) Liver model with a phospholipid distribution compartment. (d) Simulation of clearance (CL) as a function of time for an i.v. bolus dose of verapamil (a high P_{app} , high K_p drug) with the standard Rodgers and Rowland PBPK model, assuming $fe = 0$. The horizontal dashed line is the $CL_{H,av} = \text{dose}/\text{AUC}$.

compartment by, for example, limited permeability or high partitioning.

Net clearances are applicable for the schemes in Figures 4a and b, because Q_H is either part of a blood compartment or connected to a central compartment. However, in the kinetic scheme for Figure 4a, the liver has no explicit volume and CL_H is the steady-state clearance.

Similarly for the scheme in Figure 4b, equilibrium is essentially instantaneous and clearance varies minimally with time. For Figure 4c, partitioning into the phospholipid compartment delays distribution equilibrium, and the hepatic clearance is an average hepatic clearance. For this system, the smaller the value of CL_{dif} and the greater the partitioning into the phospholipid compartment, the

greater the variability of clearance with respect to time (as seen in the simulation in Figure 4d). If the average systemic clearance is known, CL_{nets} can be used to easily solve for the required $CL_{\text{int,H}}$. For the model in Figure 4c, first the CL_{net} from blood ($CL_{\text{net1,2}}$) is calculated, then the expression $CL_{\text{H}} = Q_{\text{H}} CL_{\text{net1,2}} / (Q_{\text{H}} + CL_{\text{net1,2}})$ is solved for $CL_{\text{int,H}}$. If hepatic clearance is the only clearance mechanism, the calculated $CL_{\text{int,H}}$ value will reproduce systemic clearance in a complete PK model (dose/AUC).

As expected, tissue volumes and partition coefficients (CL_i/CL_o) will not affect the average systemic clearance because clearance and volume (V) are independent.²¹ In Figure 4c, CL_{dif} , CL_{up} , CL_i , CL_o , and Q_{H} all affect distribution, although CL_{dif} , CL_{up} , and Q_{H} can also affect $CL_{\text{h,av}}$. Factors that impact both CL_s and V in no way undermine the independence of these parameters. This is revisited in the discussion of Equation 1 below.

DISTRIBUTION

Net transfer clearances can be useful to simplify PK models in order to focus on compartments that contribute to drug distribution. For example, a drug's physicochemical properties and transporter phenotype may either necessitate or obviate the need to model drug partitioning into and permeability across an explicit membrane compartment. We will begin the discussion of clearances in drug distribution (i.e., transfer across compartments) using a model for membrane partitioning and permeability.²² Figure 5 shows a model for membrane permeability either (a) with or (b) without an explicit membrane compartment. In Figure 5a, a molecule that enters the membrane compartment can either move forward or backward, both with clearances CL_o . Therefore, intuitively and by calculating the CL_{net} , CL_{dif} across a membrane is $CL_i/2$. This is also the relationship that is obtained for a mathematical model for Figure 5a as the limit of $V_{\text{mem}} \rightarrow 0$. For the

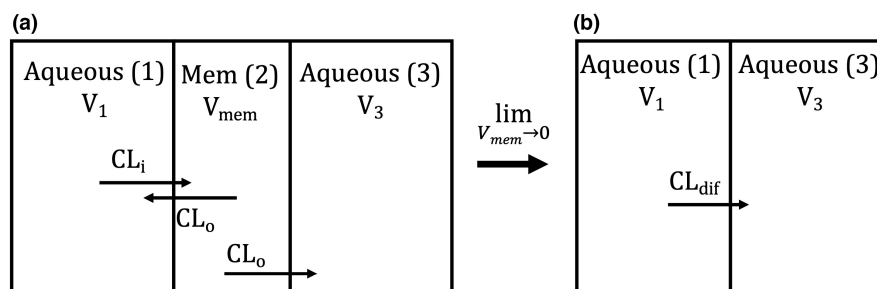
explicit membrane model (see Figure 5a), the distribution volume that the membrane compartment adds to the system is $V_{\text{mem}} K_{\text{p,mem}}$ or $V_{\text{mem}} (CL_i/CL_o)$. For the model in Figure 5b, there is no membrane volume.

When considering drug distribution, removal of a compartment with CL_{nets} is appropriate only when the volume being removed does not contribute significantly to the drug's volume of distribution. For most physiological organ models, the plasma membrane only constitutes 1% of total cell membranes (based on a 25 μm cell and 7% phospholipid content²³) and can be removed without impacting the volume of distribution. As discussed below, explicit membrane compartments will also be required when the drug concentration in a membrane drives specific processes (e.g., efflux transport from the membrane).

Apparent permeability (P_{app}) is usually measured for a cell monolayer at steady-state and sink conditions. Under these conditions, for every molecule that enters the cell from the exposed plasma membrane, a molecule exits into the receiver compartment. Therefore, we can easily use CL_{net} to derive the relationships among P_{app} , membrane permeability, and membrane partitioning (Figure 6). Calculating the CL_{net} across the cell, crossing two membranes (in the absence of transporter activity; see Figure 6), gives $P_{\text{app}} S = CL_i/4$, where S is the surface area of the monolayer, and $CL_i = 4 P_{\text{app}} S$. If $K_{\text{p,mem}}$ is the partition coefficient for phospholipid partitioning, $CL_o = 4 P_{\text{app}} S / K_{\text{p,mem}}$. It is interesting to consider that there may be more than two mandatory membranes for transcellular permeability. If three membranes must be crossed, $CL_i = 6 P_{\text{app}} S$, and for four membranes, $CL_i = 8 P_{\text{app}} S$. We can use these relationships to include any number of explicit membrane compartments in a model.

For most models, explicit plasma membrane compartments are not necessary if intracellular partitioning is modeled with other compartments.^{14,17–20,24–26} The actual volume of the plasma membrane is very small compared to the other phospholipid volumes in the cell.²³ However,

FIGURE 5 Collapse of a 3-compartment membrane model to a diffusional clearance, CL_{dif} (a) Three-compartment model. (b) Two-compartment model. CL_i - clearance into a membrane; CL_o - clearance out of a membrane. "Aqueous" compartments can denote any compartment on either side of the membrane (e.g., cytosol, ISF, lumen). "Mem" compartment denotes membrane. CL, clearance; ISF, interstitial fluid.



$$CL_{\text{net1,3}} = \frac{CL_i CL_o}{CL_o + CL_o} = \frac{CL_i}{2} = CL_{\text{dif}}$$

$$CL_i = 2 CL_{\text{dif}} \quad K_{\text{p,mem}} = \frac{CL_i}{CL_o} \quad CL_o = 2 CL_{\text{dif}} / K_{\text{p,mem}}$$

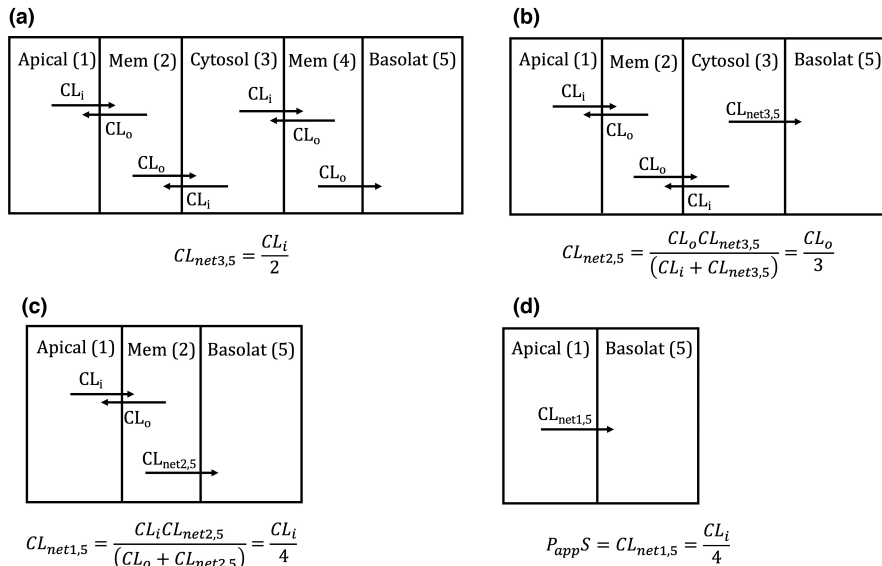


FIGURE 6 Sequentially removing compartments (4 to 2) for a monolayer permeability model. (a) Five compartment model. (b) Removal of compartment 4. (c) Removal of compartments 3 and 4. (d) Removal of compartments 2, 3, and 4. CL, clearance.

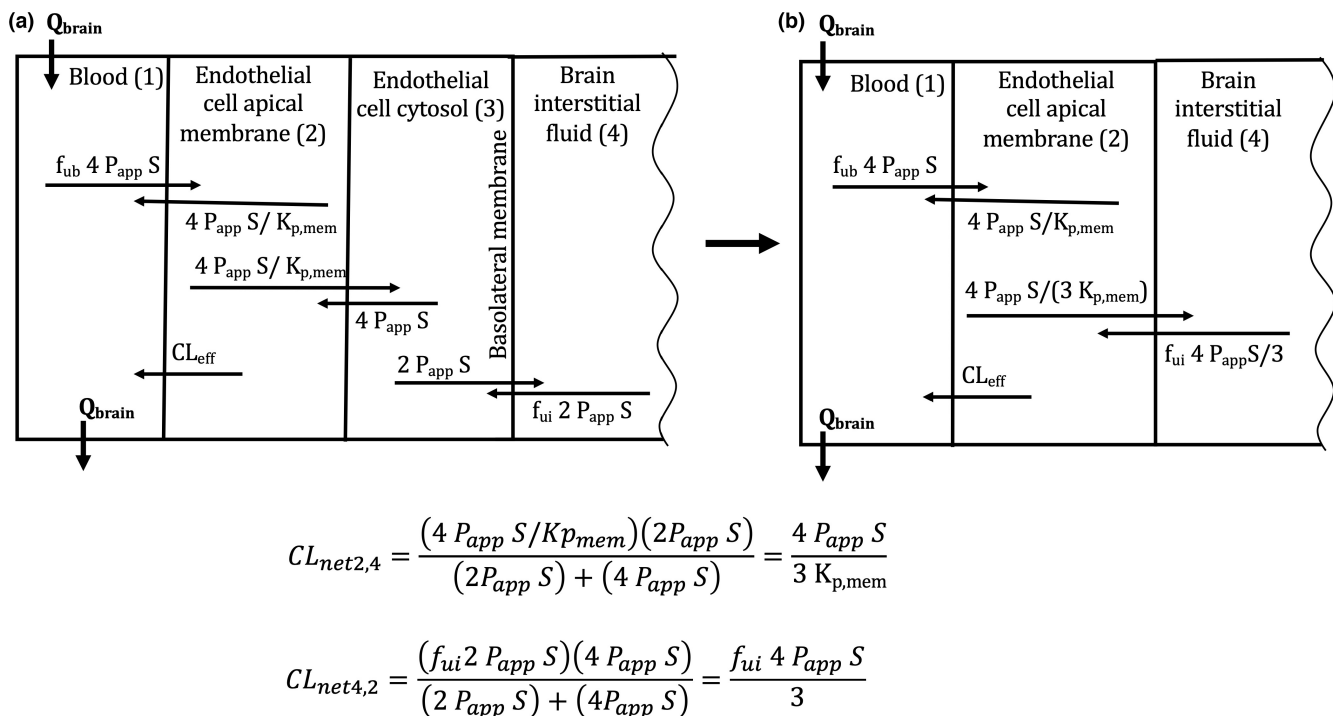


FIGURE 7 Blood to brain interstitial fluid (ISF) models with explicit apical membrane compartments. (a) Four compartments of a brain model. (b) Removal of the endothelial cell cytosol compartment. S, brain capillary surface area; f_{ub} , fraction unbound in blood; f_{ui} , fraction unbound in the ISF; $K_{p,mem}$, membrane partition coefficient; CL_{eff} , Apical efflux clearance.

the apical efflux transporter P-gp effluxes drugs directly from the apical membrane.²⁷ This necessitates the inclusion of an explicit apical membrane compartment when modeling P-gp (and probably BCRP) mediated efflux when the drug enters the cell from the apical membrane.^{23,28} For the gut or the blood–brain barrier, efflux transport by P-gp should be modeled from the apical membrane and not the cytosol, because the transporter prevents substrates from reaching the cytosol. For the liver, exposure is from the

basolateral membrane and apical efflux modeled from the cytosol or apical membrane will provide similar results.²⁸

We can use models for apical efflux at the blood–brain barrier to show how CL_{nets} can be used to simplify distribution models. Figure 7a is a model for distribution of drug from the blood to the brain interstitial fluid (ISF). An explicit apical membrane is included and active apical efflux (CL_{eff} , e.g., by P-gp) is modeled from that compartment. We want to include a basolateral membrane as well,

because a drug must cross both membranes to reach the brain ISF. The endothelial cell cytosol is included, and basolateral diffusion is modeled as CL_{dif} without an explicit membrane compartment. We can simplify the model by removing the endothelial cell cytosol compartment and using a net clearance from the apical membrane to the ISF (see [Figure 7b](#)). This will provide identical clearances to the model in [Figure 7a](#), but the endothelial cell cytosol volume is now zero. A model can be simplified by removing the compartments only when the distribution characteristics of the compartment being removed can be ignored.

USE OF NET CLEARANCES IN THE CONSTRUCTION OF COMPLEX MODELS

PK models can be constructed with varying levels of complexity. Compartmental models are simple and have the advantage of more accurately describing concentration-time profiles than PBPK models.^{25,29} However, compartmental models have the disadvantage that compartments and distribution rates have no physiological meaning.⁶ PBPK models are more complex and are useful for modeling complexities, such as special population predictions. Although a compartmental model is empirical, it can usually recapitulate observed rate of distribution very well, whereas rate of distribution is often poorly predicted especially with perfusion-limited PBPK models. One may want to construct complex models that include compartmental models along with physiological models for specific organs. For example, we may want to predict intracellular liver concentrations when drug diffusion is slow and/or if transporters are involved.³⁰ Hybrid models can be constructed in which a physiological organ model is combined with a compartmental model ([Figure 8a](#)). In this model, a liver is constructed with compartments for liver blood, cytosol, and lipids. If the drug partitions significantly into membranes, the distribution volume of the liver will be determined by the lipid compartment (V_6) and the blood and cytosol compartments can be removed using net clearances ([Figure 8b, c](#)). In [Figure 8b](#), we have removed the blood compartment with CL_{nets} allowing us to model cytosolic concentrations and liver distribution. If we want to focus on characterizing the distribution into the liver, we can further simplify the model with elimination from the central compartment, as shown in [Figure 8c](#).

For these models, the systemic clearance is simply the CL_{net} from the central compartment to elimination ($CL_{net1,0}$). Volume of distribution is a little more complicated when elimination occurs from a peripheral compartment.³¹ When this occurs, the elimination

pathway must be considered when calculating the apparent volume of the peripheral compartment, because the elimination changes the drug concentration in that compartment. Again, consideration of the reference fluid compartment relative to the compartment of interest is important. Exact volumes of distribution cannot be calculated from plasma concentration data when elimination occurs from a peripheral compartment,³¹ but if the liver is considered a well-stirred compartment, and is the major eliminating organ, the errors will be small.³² However, if active uptake increases intracellular liver concentrations (e.g., see [Figure 8](#)), greater differences will be observed.

For a mammillary model with elimination from a peripheral compartment (p), the distribution volume of the peripheral compartment can be calculated as⁷:

$$V_{p,app} = \frac{V_p CL_{cp}}{CL_{pc} + CL_e} \quad (1)$$

where $V_{p,app}$ is the distribution volume of the peripheral compartment with reference fluid in the central compartment, V_p is the distribution volume of the peripheral compartment if the reference fluid were in the peripheral compartment, CL_{cp} , CL_{pc} , and CL_e are clearances to and from the central compartment and elimination from the peripheral compartment, respectively. [Equation 1](#) shows the relationship between $V_{p,app}$ and V_p (i.e., the intrinsic clearance out of a peripheral compartment will result in a decrease in the drug's mean residence time in that compartment), and a central compartment reference fluid will calculate a lower peripheral distribution volume ($V_{p,app}$) compared to the true V_p . It is useful to realize that while this peripheral intrinsic clearance will impact both the systemic clearance and the V_{ss} of a drug, the two primary PK parameters remain independent of one another.²¹

In [Figure 8c](#), we have removed the volumes of the liver blood and cytosol leaving partitioning into the liver lipid as the only contribution to distribution. Using net clearances in [Figure 8](#), the distribution volume of V_6 ($V_{6,app}$) will be:

$$V_{6,app} = \frac{V_6 CL_{net1,6}}{CL_{net6,1} + CL_{net6,0}} \quad (2)$$

There are two ways to simplify [Figure 8a–c](#). In [Figure 8](#) and the equations therein, we first remove V_4 , using the net clearances $CL_{net1,5}$ and $CL_{net5,1}$. Next, we remove V_5 using the net clearances $CL_{net1,6}$ and $CL_{net6,1}$. This is the simpler approach to calculate $V_{6,app}$ using [Equation 2](#). A second method is to first eliminate V_5 using $CL_{net4,6}$ and $CL_{net6,4}$ and then eliminating V_4 . However, this requires

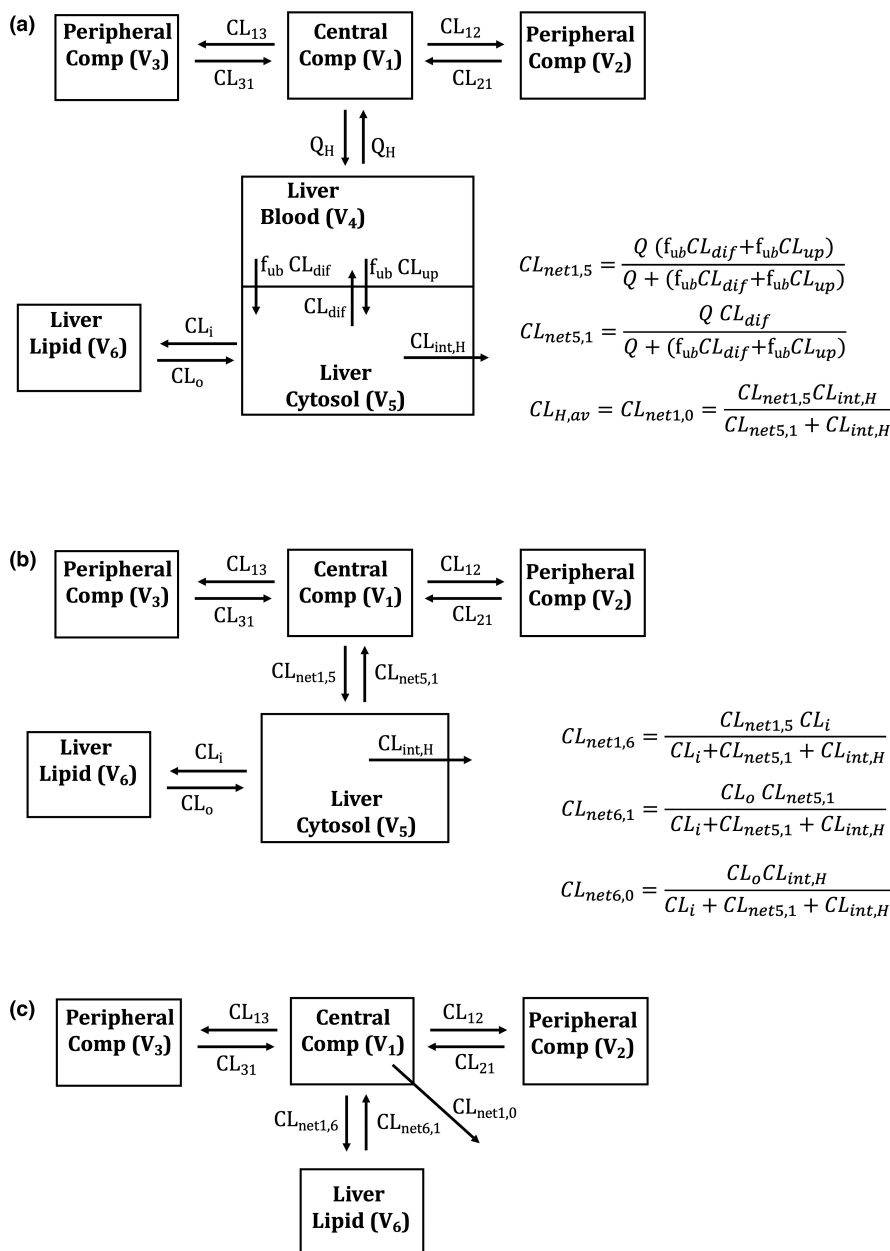


FIGURE 8 Hybrid compartmental model with a well-stirred physiological liver. (a) Complete model. (b) Using net clearances to eliminate V₄. (c) Using net clearances to eliminate V₄ and V₅. CL, clearance.

calculating the CL_{net} from V₄ to elimination (CL_{net4,0}) to account for all the drug that moves from V₄ to V₅:

$$CL_{net4,6} = \frac{(f_{uB} CL_{dif} + f_{uB} CL_{up}) CL_i}{CL_i + CL_{dif} + CL_{int,H}} \quad (3)$$

$$CL_{net4,0} = \frac{(f_{uB} CL_{dif} + f_{uB} CL_{up}) CL_{int,H}}{CL_i + CL_{dif} + CL_{int,H}} \quad (4)$$

$$CL_{net1,6} = \frac{Q CL_{net4,6}}{Q + CL_{net4,6} + CL_{net4,0}} \quad (5)$$

$$CL_{net6,4} = \frac{CL_o CL_{dif}}{CL_i + CL_{dif} + CL_{int,H}} \quad (6)$$

$$CL_{net6,1} = \frac{Q CL_{net6,4}}{Q + CL_{net4,6} + CL_{net4,0}} \quad (7)$$

Again, V_{6,app} can be calculated using Equation 2. Equations 5 and 7 will be identical to the equations in Figure 8, but the derivation removing V₅ first is more difficult than removing V₄ first.

UTILITIES AND LIMITATIONS

CL_{net}s can be used to derive transfer and elimination clearance equations for use in PK models. In the accompanying paper, the use of this technique is exemplified with the development of a new PBPK framework.

Specifically, (1) the equations in Figure 4 are used to calculate the required hepatic intrinsic clearances, (2) the relationships in Figure 6 allow use of P_{app} values as experimental inputs to model membrane partitioning and permeability, and (3) the relationships derived in Figure 7 are used to model brain distribution. The partition analysis technique makes derivation of relationships within the overall model facile. Although computational power is no longer an issue with complex modeling, we find this technique very useful in deriving meaningful relationships that may be intuitive and therefore helpful to scientists in the field.

The overall method is broadly applicable in simplifying the derivation of complex PK models. Most current models (e.g., PBPK, PK/pharmacodynamic [PD], etc.) use differential equations and numerical methods to directly model PK processes. The clearance terms in these models can be simplified with CL_{nets} when either volumes can be ignored, equilibration is fast, or if steady-state parameters are desired. For example, CL_{nets} can be used to convert a hybrid model (Figure 8a) to a three-compartment model with elimination from the central compartment, while maintaining distribution into liver lipids (see Figure 8c). Although the examples in this tutorial use first-order processes, saturable processes, such as metabolism, transport, and PD response, can easily be included.^{33,34} For example, metabolism can be modeled as a first-order process using V_{max}/K_m , or as a saturable process using $V_{max}/(K_m + C)$. The appropriate relationships can be included in the derivation of a CL_{net} relationship, keeping in mind that the compartment driving the saturable process cannot be removed from the overall model. Given the emerging importance of more complex models, such as target-mediated drug disposition of biologics, PK/PD models, systems biology, etc., using net clearances to simplify complex models can be useful.

The examples above use clearances to model drug transfer and elimination. All models can be similarly constructed using first order rate constants (k) instead of clearances. The resulting net rate constants (k_{net}) will be equal to the net clearance divided by the volume of the driving compartment. This net rate constant will be multiplied by the amount of drug in the driving compartment to obtain the rate of drug transfer. When mathematically converting net rate constants to net clearances, all volumes will cancel except for the volume of the driving compartment.

As with any model, it should be noted that limitations of the derived equations will depend on the validity of underlying assumptions. Thus, for example, reversible transfer rates cannot be correctly modeled if a unidirectional irreversible process is assumed. Similarly, a saturable process or a second-order process should be modeled as such

instead of assuming linear first-order reactions. In addition, the quality of the experimental input will dictate the overall model performance, as discussed in detail in the accompanying paper.

CONCLUSIONS

This tutorial by no means provides new approaches to PK modeling. It simply uses the partition analysis method described previously by Cleland to derive PK equations easily.¹ Today's PK models are becoming more complex. However, the complexity of a model should be determined by the questions being asked, the mechanistic knowledge of the modeled processes, and the experimental data available for these processes. We should observe "the law of parsimony or Occam's razor" that states that models should be as simple as possible.³⁵ If the goal is to model elimination clearances, all volumes can be ignored, and CL_{nets} can be used to easily derive the correct relationships. The elimination clearance models developed previously by Wilkinson and Shand,⁹ Pang and Rowland,¹⁰ Gillette and Pang,¹¹ and Yamazaki et al.¹² have been a cornerstone for PK modeling. If we need to understand intracellular concentrations for specific organs, easily calculating elimination clearances and distribution characteristics may be useful. As we transition to more complex models with additional clearances (e.g., additional transport processes, target mediated distribution, etc.), CL_{nets} can simplify the derivation of these relationships. The last two sentences of Cleland's paper¹ state: "In this laboratory this technique has become the method of choice for routine derivations where it is desired to determine quickly the result of expanding a mechanism by adding extra steps with extra rate constants associated with them, and an immense amount of time has been saved thereby. Hopefully this paper will serve to make the method equally available to others with an interest in enzyme kinetics." Hopefully this tutorial will be useful to others with an interest in deriving PK relationships.

ACKNOWLEDGEMENTS

The authors acknowledge Drs. K. Sandy Pang and Priyanka Kulkarni for critically reading the manuscript and providing helpful comments to improve the content. We also acknowledge Dr. Jeffrey Jones for introducing us to the method of partition analysis for the derivation of enzyme kinetic equations.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

ORCID

Ken Korzekwa  <https://orcid.org/0000-0002-7119-9200>

Swati Nagar  <https://orcid.org/0000-0003-2667-7063>

REFERENCES

- Cleland WW. Partition analysis and the concept of net rate constants as tools in enzyme kinetics. *Biochemistry*. 1975;14:3220-3224.
- Yadav J, Paragas E, Korzekwa K, Nagar S. Time-dependent enzyme inactivation: Numerical analyses of in vitro data and prediction of drug–drug interactions. *Pharmacol Ther*. 2020;206:107449.
- Yadav J, Korzekwa K, Nagar S. Impact of lipid partitioning on the design, analysis, and interpretation of microsomal time-dependent inactivation. *Drug Metab Dispos*. 2019;47:732-742.
- Yadav J, Korzekwa K, Nagar S. Improved predictions of drug–drug interactions mediated by time-dependent inhibition of cyp3a. *Mol Pharm*. 2018;15:1979-1995.
- Barnaba C, Yadav J, Nagar S, Korzekwa K, Jones JP. Mechanism-based inhibition of cyp3a4 by podophyllotoxin: aging of an intermediate is important for in vitro/in vivo correlations. *Mol Pharm*. 2016;13:2833-2843.
- Rowland M, Benet LZ, Graham GG. Clearance concepts in pharmacokinetics. *J Pharmacokinetic Biopharm*. 1973;1:123-136.
- Källén A. Modelling the distribution process. *Computational pharmacokinetics*. Chapman & Hall/CRC; 2008:97-136.
- Berezhkovskiy LM. Prediction of the possibility of the secondary peaks of iv bolus drug plasma concentration time curve by the model that directly takes into account the transit time through the organ. *J Pharm Sci*. 2009;98:4376-4390.
- Wilkinson GR, Shand DG. Commentary: a physiological approach to hepatic drug clearance. *Clin Pharmacol Ther*. 1975;18:377-390.
- Pang KS, Rowland M. Hepatic clearance of drugs. I. Theoretical considerations of a “well-stirred” model and a “parallel tube” model. Influence of hepatic blood flow, plasma and blood cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance. *J Pharmacokinetic Biopharm*. 1977;5:625-653.
- Gillette JR, Pang KS. Theoretic aspects of pharmacokinetic drug interactions. *Clin Pharmacol Therapeut*. 1977;22:623-639.
- Yamazaki M, Suzuki H, Sugiyama Y. Recent advances in carrier-mediated hepatic uptake and biliary excretion of xenobiotics. *Pharm Res*. 1996;13:497-513.
- Sirianni GL, Pang KS. Organ clearance concepts: New perspectives on old principles. *J Pharmacokinetic Biopharm*. 1997;25:449-470.
- de Lannoy IA, Pang KS. Presence of a diffusional barrier on metabolite kinetics: enalaprilat as a generated versus preformed metabolite. *Drug Metab Dispos*. 1986;14:513-520.
- Sato H, Sugiyama Y, Miyauchi S, Sawada Y, Iga T, Hanano M. A simulation study on the effect of a uniform diffusional barrier across hepatocytes on drug metabolism by evenly or unevenly distributed uni-enzyme in the liver. *J Pharm Sci*. 1986;75:3-8.
- Korzekwa K, Nagar S. Drug distribution part 2. Predicting volume of distribution from plasma protein binding and membrane partitioning. *Pharm Res*. 2017;34:544-551.
- Rodgers T, Rowland M. Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. *J Pharm Sci*. 2006;95:1238-1257.
- Rodgers T, Leahy D, Rowland M. Physiologically based pharmacokinetic modeling 1: predicting the tissue distribution of moderate-to-strong bases. *J Pharm Sci*. 2005;94:1259-1276.
- Geng W, Schwab AJ, Horie T, Goresky CA, Pang KS. Hepatic uptake of bromosulfophthalein-glutathione in perfused eisai hyperbilirubinemic mutant rat liver: a multiple-indicator dilution study. *J Pharmacol Exp Ther*. 1998;284:480-492.
- Schwab, A.J., F. Barker, 3rd. Goresky, C.A. & Pang, K.S. Transfer of enalaprilat across rat liver cell membranes is barrier limited. *Am J Physiol-Gastrointest Liver Phys Ther* 258, G461-G75 (1990).
- Gibaldi M, Koup JR. Pharmacokinetic concepts – drug binding, apparent volume of distribution and clearance. *Eur J Clin Pharmacol*. 1981;20:299-305.
- Nagar S, Korzekwa K. Commentary: nonspecific protein binding versus membrane partitioning: It is not just semantics. *Drug Metab Dispos*. 2012;40:1649-1652.
- Nagar S, Tucker J, Weiskircher EA, Bhoopathy S, Hidalgo IJ, Korzekwa K. Compartmental models for apical efflux by p-glycoprotein-part 1: evaluation of model complexity. *Pharm Res*. 2014;31:347-359.
- Kulkarni P, Korzekwa K, Nagar S. Intracellular unbound atorvastatin concentrations in the presence of metabolism and transport. *J Pharmacol Exp Therapeut*. 2016;359:26-36.
- Holt K, Ye M, Nagar S, Korzekwa K. Prediction of tissue-plasma partition coefficients using microsomal partitioning: incorporation into physiologically based pharmacokinetic models and steady-state volume of distribution predictions. *Drug Metab Dispos*. 2019;47:1050-1060.
- Poulin P, Theil FP. A priori prediction of tissue:plasma partition coefficients of drugs to facilitate the use of physiologically-based pharmacokinetic models in drug discovery. *J Pharm Sci*. 2000;89:16-35.
- Jin MS, Oldham ML, Zhang Q, Chen J. Crystal structure of the multidrug transporter p-glycoprotein from caenorhabditis elegans. *Nature*. 2012;490:566-569.
- Korzekwa K, Nagar S. Compartmental models for apical efflux by p-glycoprotein: Part 2-a theoretical study on transporter kinetic parameters. *Pharm Res*. 2014;31:335-346.
- Ye M, Nagar S, Korzekwa K. A physiologically based pharmacokinetic model to predict the pharmacokinetics of highly protein-bound drugs and the impact of errors in plasma protein binding. *Biopharm Drug Dispos*. 2016;37:123-141.
- Kulkarni P, Korzekwa K, Nagar S. A hybrid model to evaluate the impact of active uptake transport on hepatic distribution of atorvastatin in rats. *Xenobiotica*. 2020;50:536-544.
- Berezhkovskiy LM. Volume of distribution at steady state for a linear pharmacokinetic system with peripheral elimination. *J Pharm Sci*. 2004;93:1628-1640.
- Berezhkovskiy LM. On the accuracy of calculation of the mean residence time of drug in the body and its volumes of distribution based on the assumption of central elimination. *Xenobiotica*. 2016;46:477-482.
- Sato M, Toshimoto K, Tomaru A, et al. Physiologically based pharmacokinetic modeling of bosentan identifies the saturable

- hepatic uptake as a major contributor to its nonlinear pharmacokinetics. *Drug Metab Dispos.* 2018;46:740-748.
34. Kushihara H, Sugiyama Y. Pharmacokinetic modeling of the hepatobiliary transport mediated by cooperation of uptake and efflux transporters. *Drug Metab Rev.* 2010;42:539-550.
 35. Bonate PL. The art of modeling. *Pharmacokinetic-Pharmacodynamic Modeling and Simulation.* Springer; 2011: 1-60.

How to cite this article: Korzekwa K, Yadav J, Nagar S. Using partition analysis as a facile method to derive net clearances. *Clin Transl Sci.* 2022;15: 1867-1879. doi:[10.1111/cts.13310](https://doi.org/10.1111/cts.13310)