On the Volume of Distribution at Steady State and Its Relationship With Two-Compartmental Models

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ABSTRACT: The volume of distribution at steady state is considered to be one of the primary pharmacokinetic measurements obtained from *in vivo* experiments. This quantity is quite commonly calculated using moments of the observed concentration curve, the process being referred to as noncompartmental analysis. In this paper the underlying assumptions of noncompartmental analysis are analysed with regard to the observed behaviour of models with two compartments: one of which has elimination from the central compartment, the other from the peripheral tissue compartment. This is in order to clarify the relationship between volume of distribution and clearance. It is shown that these two models are indistinguishable from measurements in blood. Furthermore relationships between the parameter values for the two models are given so that they produce the same observed profile. Expressions are derived in a novel way that relates the volume of distribution to these model rate constants. The definitions of clearance and volume of distribution at steady state are investigated using several different mathematical techniques, demonstrating the consistency of the derived expressions. It is shown, in a manner that the authors believe is a new approach, that when the assumption of central elimination does not apply, noncompartmental analysis will under estimate the volume of distribution, whereas clearance remains unchanged. This is compared quantitatively with respect to the volume predicted where central elimination holds, and is a result of an extended mean residence time. J Pharm Sci 97:111–122, 2008

Keywords: compartmental modelling; noncompartmental modelling; volume of distribution; clearance; indistinguishability of models; PBPK models

INTRODUCTION

The volume of distribution is used as a measure of the extent to which a pharmaceutical compound distributes itself around the body. It is defined to be the volume of blood necessary to hold the compound present in the body at the concentration observed in the vascular compartment. It is a frequently used measure in the field of pharmaceutical discovery. Too high or low a volume of distribution might cause a reconsideration of a particular compound's potential.

A full understanding of this underlying pharmacokinetic property and its relation to the observed kinetics of blood or plasma concentration as a function of time is obviously needed to allow it to guide good decisions. Therefore, knowing what exactly can be deduced about the disposition of the compound from the blood concentration profile after an i.v. bolus injection is essential.

Compartmental models[1] are commonly used to analyse the observed concentration profile of a

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compound. It has been demonstrated[2] how the multi-exponential output of such models may be used to measure the clearance and volume of distribution of a compound when elimination is assumed to be solely from the compartment observed. Thus the microrate constants associated with the model may be used directly to give an expression for these. However when this assumption is false the 'true' volume of distribution can be different to that observed. There still remains some confusion over this and this paper attempts to clarify the situation.

Whole body physiologically based pharmacokinetic models (PBPK) have been used in a large number of applications in recent years.[3] When comparing the model structure of these with the 'standard' two-compartmental model with central elimination it becomes clear that there are a number of disparities concerning the assumptions upon which they are based. The first is the 'elimination at the point of observation' assumption. This is implicit in the standard two-compartmental model whereas the PBPK models place the major eliminating tissues (liver and the kidneys) in compartments separate to those representing the blood. It is also generally thought that the liver should be 'lumped' in the central compartment (of the two-compartment model) because it is a well-perfused tissue and so should be expected to reach equilibrium with the blood in a relatively short space of time, in comparison to muscle tissues. However, it is possible for the liver to have a time constant comparable with these slower tissues depending upon the pharmacokinetics of the particular compound. This makes the placement of the liver in the central compartment questionable on physiological grounds.

The assumption of central elimination may also be false in the case of antibody pharmacokinetics. Antibodies can undergo elimination by catabolism in peripheral tissues, thus the volume of distribution may be under estimated.[4] With a growing interest in antibodies as therapies, it is thought timely to review and investigate the assumptions of noncompartmental analysis.

In this investigation the case of two-compartmental behaviour is considered because the results can be generalized to any number of compartments using a mamillary model (PBPK models[3] being a particular case). The relationships between the volume of distribution, clearance, tissue–blood partition coefficients and physiological volumes are also investigated. The expression (30) has been stated elsewhere,[5] however, it has been derived here in a mathematically concise manner.

Special consideration is given to the fact that it is impossible from a single i.v. bolus dose experiment to decide which of the competing two-compartmental models represents the *in vivo* distribution and elimination of a compound.[2] This again appears to be a poorly understood concept (as discussed below) within the pharmacokinetics community; though it is well characterised mathematically. A novel expression (38) is derived here that allows the volumes of distribution predicted by different models to be compared in a convenient manner. This enables the pharmacokineticist to evaluate the disparity of measurement between models. It is also demonstrated through this analysis that the measurement of clearance is indeed independent of the placement of drug elimination within the compartmental model. Furthermore, it is demonstrated for the first time that, for a given observed profile, a compartmental model with peripheral elimination only, gives the maximum volume of distribution.

HISTORICAL PERSPECTIVE

Deduction of volume and flow rate in isolated tissues appears to date back to the physiological studies of Stewart[6] and Hamilton et al.[7] These methods involved the measurement of the concentration of some tracer dye and were formulated in a more mathematically formal way in the 1950s by Meier and Zierler.[8] However, the application considered was still of isolated tissue studies. It is unclear to the authors just exactly when mean residence times and clearance measures were first used to measure whole body volumes, however, Oppenheimer et al.[9] and Perl and Samuel[10] offer are two examples of early applications.

The standard 'noncompartmental'[11] expressions for clearance and volume of distribution at steady state are often thought to be model independent. However, over the past 3 decades[12–14] and more recently[15] it has been argued that this is not so. The 'apparent' volume of distribution is based upon the assumption that elimination of a compound is from the same compartment as that being observed. When there is elimination elsewhere than from the compartment under observation then the standard concentration curve moments no longer apply, as will be shown in this article.

Though this subject appears to have been well surveyed (Benet and Ronfeld[5] voiced a suspicion of ascribing volumes at all), there appears to have been no real resolution. In fact any discussion of the disparities between models creates confusion in the pharmacokinetics community. Bois et al.[16] stated that the clearance predicted by both central and peripheral clearance models was found to be equal for butadiene. Whether or not this should be expected in general was not discussed.

Further confusion has occurred recently[17] in response to a partition coefficient correction method that allows for the effects of organ elimination.[18] Bjorkman[17] reiterates the claim that the volume of distribution is in no way dependent upon the rate of clearance. It appears that the meaning of the equilibrium partition coefficient causes the confusion. For eliminating tissue this is not purely partitioning due to physiochemical properties (as it would be in noneliminating tissues), but also due to the exit of compound from the tissue via the routes of elimination.[19] This will be reiterated below in the mathematical analysis with the result (Eq. 39), which quantifies the effect peripheral elimination has upon the volume of distribution. Therefore, the partition coefficient measured experimentally for an eliminating tissue is not the physiochemical partition coefficient that may be used in interspecies scaling. This 'apparent' partition coefficient[20] must be corrected for clearance[18] in order to understand the partitioning properties of eliminating tissues.

TWO-COMPARTMENTAL MODELS

In this paper, two-compartment models are considered in order to demonstrate results that may be used for larger systems. Table 1 defines all of the variables and coefficients used throughout this paper. The compartmental systems used to represent PBPK models are of a mamillary structure (a central observed compartment with peripheral tissue compartments connected exclusively to the central compartment). The two-compartmental model of Figure 1 is a particular example.

The compartment numbered 1 is the central compartment whereas compartment 2 is the peripheral compartment. The important distinction is that both input (dosing of drug) and output (observed concentration) of the system are confined to the central compartment. A second distinction for larger systems that have a mamillary structure is that there must be no direct connection between peripheral compartments. Elimination of mass from the system is manifested as the flow to the environment at rates dictated by k_{10}, k_{20}.

Table 1. Notation Used Throughout

Symbol	Meaning
C_i	Concentration in compartment i
X_i	Amount in compartment i
V_i	Physiological volume of compartment i
K_{pi}	Partition coefficient between central compartment and compartment i
k_{ij}	Microrate constant for the flow from compartment i to compartment j.
Q_i	Blood flow rate in compartment/tissue i

The system may be specified by the following equations:

$$\begin{aligned} \frac{dx_1}{dt} &= -(k_{12}+k_{10})x_1 + k_{21}x_2 \\ \frac{dx_2}{dt} &= -(k_{21}+k_{20})x_2 + k_{12}x_1 \\ y &= \frac{x_1}{V_1} \\ x_1(0) &= \text{Dose},\ x_2(0) = 0 \end{aligned} \tag{1}$$

where y is the observed concentration for an *iv* bolus dose. For the cases where there is elimination from one compartment only it may be shown[21] that the resulting system is globally structurally identifiable[22]; the values of the rate constants may be deduced uniquely by observing the concentration of the compound in the first compartment only. In the case of elimination from both compartments it is found that there are an infinite number of solutions for the rate constants, the system becomes structurally unidentifiable.

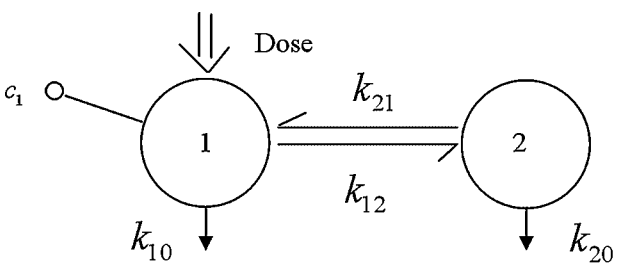

Figure 1. A general two-compartment system. Concentration (c_1) is measured in compartment 1.

THE PHYSIOLOGICAL RELEVANCE OF TWO-COMPARTMENT MODELS

PBPK models[20] provide varying levels of realism dependent on the number of compartments assigned. To make them more accessible for predictive modelling of human data, considerable efforts have been made to reduce the number of compartments and yet retain realistic behaviour with regard to both plasma and other tissues. Typical of the models used in scaling from animals to human is the multi-compartment model of Bernareggi and Rowland.[20] This includes two compartments assigned to the venous and arterial plasma, respectively, on either side of the lung compartment. So fast are the kinetics of these three compartments that they are often 'lumped'[23] as one, making a six-compartment model (Fig. 2).

If one compares this to a 'standard' two-compartment PK model with central elimination, Figure 3 (top), the sequence of model reductions required can be traced as follows. The muscle, bone, skin compartment has been combined with the adipose to form a 'slow' compartment while the remaining four have been lumped into the central 'faster' compartment. This is shown diagrammatically in Figure 4 (left). However, examining the upper grouping in Figure 4 (left), the lung blood flow is in the opposite direction to that of the other compartments, which is illogical.[23] This fact alone makes the two-compartment PK model impossible to scale across species when hepatic metabolism is the main route of clearance. A logical grouping would be as shown in Figure 4 (right), leading to the 'lumped model' shown in Figure 5.

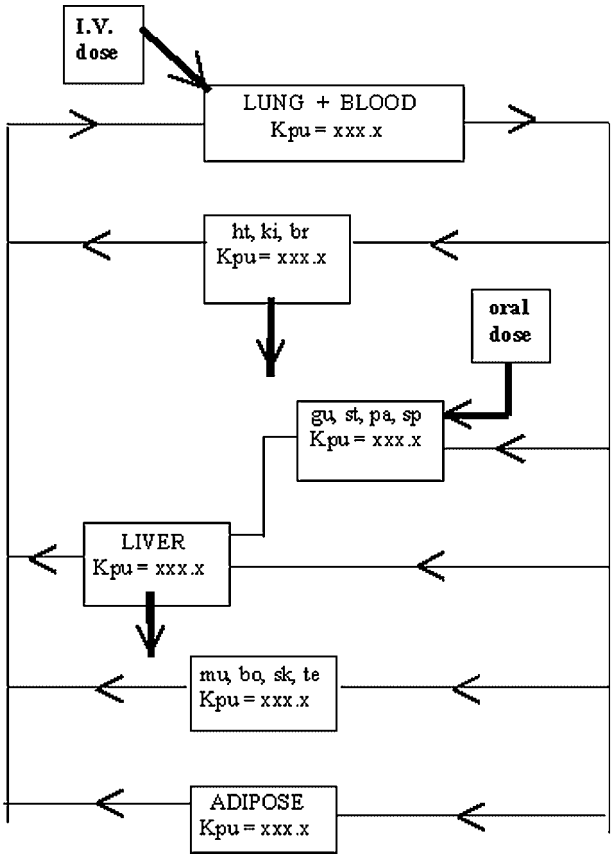

Figure 2. A six-compartment physiologically-based pharmacokinetic model.

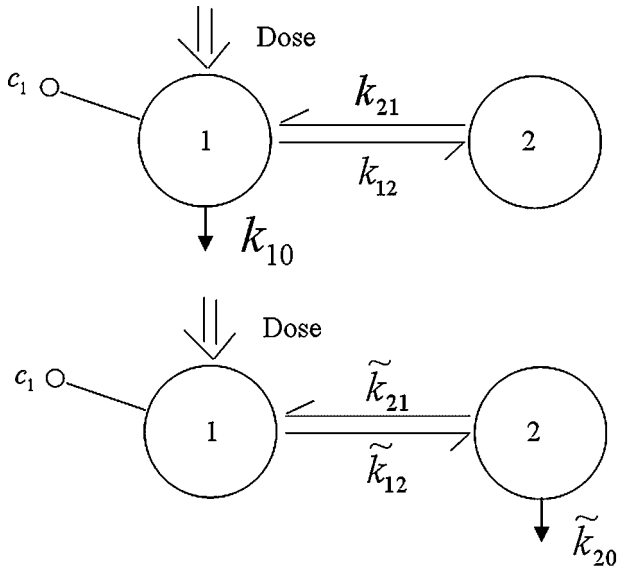

Figure 3. The competing two-compartmental models. Concentration is measured in compartment 1 in both cases.

Clearance (CL) is equal to V_1k_{10} in the standard PK model (Fig. 3, top) so immediately we see that it is not independent of V_1 if clearance is calculated from a plasma profile. However in the reduced PBPK model (Fig. 5), $CL = V_2k_{20}$ so it is independent of V_1, the volume of the observed compartment. This is the feature which enables successful cross species scaling to be produced. The final step in reducing the PBPK diagram (Fig. 4, right) to correspond diagrammatically to the peripheral elimination model (Fig. 3, bottom) is to merge the two-peripheral compartments. The severity of this reduction now becomes clear.

INDISTINGUISHABLE TWO-COMPARTMENT SYSTEMS

The observed behaviours of the two competing models with single point elimination (Fig. 3) are

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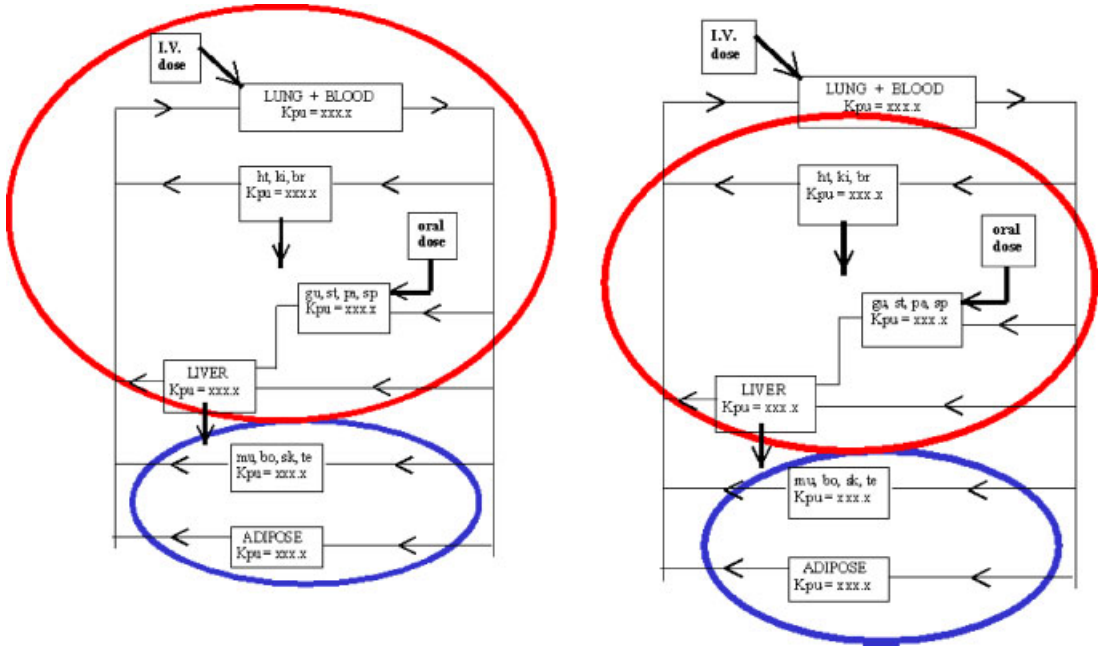

Figure 4. Two possible groupings of tissue compartments. The system on the left corresponds to a two-compartment model with central elimination, the one to the right retains some physiological realism[22] (see text). [Color figure can be seen in the online version of this article, available on the website, www.interscience.wiley.com.]

now considered. It will be shown that the two single point elimination models are indistinguishable in the sense that their input–output behaviours are identical. The relationship between the parameter values in each model for this to occur is shown below.

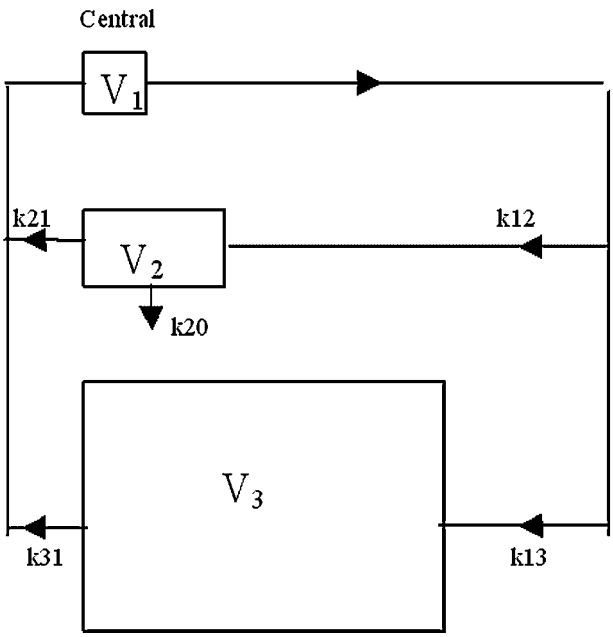

Figure 5. A simplified three-compartment model resulting from 'lumping' of a physiologically-based model.

It may be shown that the transfer functions[1] of the two systems are:

$$G(s) = \frac{\frac{1}{V_1}(s + k_{21})}{s^2 + (k_{12} + k_{21} + k_{10})s + k_{10}k_{21}} \tag{2a}$$

$$\tilde{G}(s) = \frac{\frac{1}{\tilde{V}_1}(s + \tilde{k}_{21} + \tilde{k}_{20})}{s^2 + (\tilde{k}_{12} + \tilde{k}_{21} + \tilde{k}_{20})s + \tilde{k}_{20}\tilde{k}_{12}} \tag{2b}$$

For the two systems to have the same behaviour with respect to the observer of the central compartment it is necessary that

$$G(s) \equiv \tilde{G}(s) \tag{3}$$

This implies

$$\begin{aligned} k_{21} &= \tilde{k}_{21} + \tilde{k}_{20} \\ k_{12} &= \frac{\tilde{k}_{21}\tilde{k}_{12}}{\tilde{k}_{21} + \tilde{k}_{20}} \\ k_{10} &= \frac{\tilde{k}_{12}\tilde{k}_{20}}{\tilde{k}_{21} + \tilde{k}_{20}} \\ V_1 &= \tilde{V}_1 \end{aligned} \tag{4}$$

or equivalently

$$\begin{aligned}\tilde{k}_{21} &= \frac{k_{21}k_{12}}{k_{12}+k_{10}}\\ \tilde{k}_{12} &= k_{12}+k_{10}\\ \tilde{k}_{20} &= \frac{k_{10}k_{21}}{k_{12}+k_{10}}\\ \tilde{V}_1 &= V_1\end{aligned} \tag{5}$$

This means that the two models can be made to fit a given i.v. profile equally well. In fact a change of input type, for example an infusion, will not distinguish between these two models. However, as is shown below, the volume of distribution is different.

NONCOMPARTMENTAL ANALYSIS

It will become important during the course of the discussion to have an unambiguous definition of volume of distribution, mean residence time and clearance. These will be first stated in a model independent manner in order for the model specific expressions to be interpretable.

The volume of distribution at steady state, V_{dss}, is defined to be 'the volume of blood (plasma) apparently necessary, given the amount of compound in the body, to satisfy the observed blood (plasma) concentration'. Mathematically it can be put simply as[15]:

$$V_{\mathrm{dss}} = \frac{A_{\mathrm{ss}}}{C_{\mathrm{ss}}} \tag{6}$$

where A_{ss} is the total amount in the body at steady state and C_{ss} is the observed steady state concentration. A brief summary of basic pharmacokinetic parameters is given below. For further information see Stewart,[6] Hamilton et al.,[7] Wagner,[11] Matis et al.,[24] and Poulin and Theil.[25] The volume of distribution is defined (in terms of partition coefficients) as[18]:

$$V_{\mathrm{dss}} = \sum_i K_{\mathrm{p}i}(1-E_i)V_i \tag{7}$$

where E_i is the extraction ratio. The terms $K_{\mathrm{p}i}V_i$ are referred to as the tissue pharmacokinetic volumes. Alternative definitions are given using moments of the observed concentration curve:

$$\mathrm{AUC} = \int_{\infty} C_1(t)\,\mathrm{d}t \tag{8}$$

$$\mathrm{AUMC} = \int_{\infty} tC_1(t)\,\mathrm{d}t \tag{9}$$

$$\mathrm{CL} = \frac{\mathrm{Dose}}{\mathrm{AUC}} \tag{10}$$

$$\mathrm{MRT} = \frac{\mathrm{AUMC}}{\mathrm{AUC}} \tag{11}$$

With respect to the model output let the volume of distribution be defined as

$$V_{\mathrm{dss}} = \frac{\mathrm{DOSE}\cdot\mathrm{AUMC}}{\mathrm{AUC}^2} = \mathrm{MRT}\times\mathrm{CL} \tag{12}$$

NONCOMPARTMENTAL ANALYSIS AND ITS RELATIONSHIP WITH COMPARTMENTAL MODELS

Curve moments may be derived analytically for two-compartment models. Microrate models are considered as before so as to reduce the visual complexity of the algebra. These parameters may then be substituted for the physiological macrorate parameters to obtain the physiological interpretation of the V_{dss}. The results in this section are not novel,[15] however, the analysis is presented in the appendices for comparison with the results of following sections.

In the following, k_{ij} is used to denote the microrate constants for the case of central elimination only, similarly $\tilde{k}_{ij}$ is used for the case of peripheral elimination only. Thus for the two competing models considered in this article, $k_{20} = \tilde{k}_{10} = 0$; there is elimination from one compartment only. For a model with central elimination we have:

$$\mathrm{CL}_{\mathrm{nca,cen}} = \frac{\mathrm{Dose}}{\mathrm{AUC}} = V_1k_{10} \tag{13}$$

$$\mathrm{MRT}_{\mathrm{nca,cen}} = \frac{\mathrm{AUMC}}{\mathrm{AUC}} = \frac{1}{k_{10}}\left[1+\frac{k_{12}}{k_{21}}\right] \tag{14}$$

$$V_{\mathrm{dss,nca,cen}} = V_1\left(1+\frac{k_{12}}{k_{21}}\right) \tag{15}$$

If the rate constants are written with respect to physiological constants:

$$k_{12} = \frac{Q_2}{V_1} \tag{16}$$

$$k_{21} = \frac{Q_2}{V_2K_{\mathrm{p2}}} \tag{17}$$

where Q_2 is the blood flow rate, V_2 is the volume of the tissue, and K_{p2} is the blood/tissue partition coefficient. The coefficient K_{p2} would have to be corrected for elimination were there any from the peripheral compartment, however there is elimination from the central compartment only. Substituting these into Eq. (19) gives

$$V_{dss,nca,cen} = V_1 + K_{p2}V_2 \tag{18}$$

Which, for the two-compartment central elimination case, is Eq. (7). It is also apparent that the above analysis can be extended for any number of peripheral compartments in parallel. Hence for elimination in the central compartment only, the volume of distribution is expressed by Eq. (7).

Physiologically, it is apparent that the major organs of elimination are the liver and the kidneys and these tissues might find their representations as peripheral compartments with elimination. For a compartment with peripheral elimination we have:

$$CL_{nca,per} = \tilde{V}_1\tilde{k}_{12}\left(\frac{\tilde{k}_{20}}{\tilde{k}_{21}+\tilde{k}_{20}}\right), \tag{19}$$

$$MRT_{nca,per} = \frac{1}{\tilde{k}_{20}}\left[\frac{\tilde{k}_{21}+\tilde{k}_{20}}{\tilde{k}_{12}} + \frac{\tilde{k}_{21}}{\tilde{k}_{21}+\tilde{k}_{20}}\right], \tag{20}$$

$$V_{dss,nca,per} = \tilde{V}_1\left[1 + \frac{\tilde{k}_{12}\tilde{k}_{21}}{(\tilde{k}_{21}+\tilde{k}_{20})^2}\right]. \tag{21}$$

The relationship between the two-compartmental volume (15) and (21) is now analysed. Considering these two models from the point of view of indistinguishability means that the same value for the measured volume of distribution will be obtained when using noncompartmental analysis. Assume that the system with peripheral elimination is identified using available pharmacokinetic data to give values for $\tilde{k}_{ij}$. This situation may be represented by applying Eq. (4) to Eq. (15) to give

$$\begin{aligned} V_{dss,nca,cen} &= V_1\left(1+\frac{k_{12}}{k_{21}}\right) \\ &= \tilde{V}_1\left[1 + \frac{\tilde{k}_{12}\tilde{k}_{21}}{(\tilde{k}_{21}+\tilde{k}_{20})^2}\right] = V_{dss,nca,per} \end{aligned} \tag{22}$$

which is Eq. (28). Hence by calculating the volume of distribution at steady state using a noncompartmental method, the volume calculated is the same, as has been noted previously.[14] It is also impossible to tell noncompartmentally whether peripheral or central elimination is appropriate. It will be shown in the next two sections that Eq. (21) is a 'false' value in the sense that, for the case of peripheral elimination, it does not give the full extent of the distribution of the compound as defined by Eq. (6). We do not consider simultaneous elimination because not only is it an unidentifiable system, but also it has been shown by Vaughn[14] that it may be rewritten as either one of the two cases shown in Figure 2.

STOCHASTIC MEAN RESIDENCE TIME

An altered expression for the MRT is derived for the case of peripheral elimination which, when coupled with the indistinguishability results, shows quantitatively how the volume of distribution is increased when elimination is in the peripheral compartment.

Let Eq. (A1) or Eq. (B1) be rewritten in the form

$$\dot{X} = KX \tag{23}$$

as in Ref. 18 Then if

$$\{\theta_{ij}\} = -(K^T)^{-1} \tag{24}$$

then[1] θ_{ij} is the mean time that a drug molecule dosed into compartment i resides in compartment j prior to elimination. Hence for both cases of two-compartmental models considered in this paper, the total mean residence time is the sum of time spent in compartments 1 and 2,

$$MRT = \theta_{11} + \theta_{12} \tag{25}$$

In the case of central elimination

$$\begin{aligned} K &= \begin{bmatrix} -(k_{12}+k_{10}) & k_{21} \\ k_{12} & -k_{21} \end{bmatrix} \Rightarrow -(K^T)^{-1} \\ &= \begin{bmatrix} k_{10}^{-1} & \dfrac{k_{12}}{k_{10}k_{21}} \\ k_{10}^{-1} & \dfrac{k_{12}+k_{10}}{k_{10}k_{21}} \end{bmatrix} \end{aligned} \tag{26}$$

Which means that

$$MRT_{cen} = \frac{1}{k_{10}}\left[1+\frac{k_{12}}{k_{21}}\right] \tag{27}$$

which is entirely consistent with Eq. (14) (the subscript cen denotes the mean residence time assuming central elimination). However, in the case of

[1]Here the standard matrix element convention $\theta_{row,column}$ is used.

peripheral elimination

$$K = \begin{bmatrix} -\tilde{k}_{12} & \tilde{k}_{21} \\ \tilde{k}_{12} & -(\tilde{k}_{21}+\tilde{k}_{20}) \end{bmatrix} \Rightarrow -(K^{\mathrm{T}})^{-1} = \begin{bmatrix} \dfrac{\tilde{k}_{21}+\tilde{k}_{20}}{\tilde{k}_{20}\tilde{k}_{12}} & \tilde{k}_{20}^{-1} \\ \dfrac{\tilde{k}_{21}}{\tilde{k}_{20}\tilde{k}_{12}} & \tilde{k}_{20}^{-1} \end{bmatrix} \quad (28)$$

and thus

$$\mathrm{MRT}_{\mathrm{per}} = \frac{1}{\tilde{k}_{20}}\left[1+\frac{\tilde{k}_{21}+\tilde{k}_{20}}{\tilde{k}_{12}}\right] \quad (29)$$

Thus we have that Eq. (29) is inconsistent with Eq. (20) (per denotes the assumption of peripheral elimination). The expression (29) can be taken to be the true value as it is derived using the definition of mean residence time.[26] Thus, assuming the definition of clearance as shown Eq. (10) is applicable, using Eqs. (12), (19), and (29):

$$\begin{aligned} V_{\mathrm{dss,per}} &= \tilde{V}_1\frac{\tilde{k}_{12}}{\tilde{k}_{20}}\left(1-\frac{\tilde{k}_{21}}{\tilde{k}_{21}+\tilde{k}_{20}}\right) \times \left(1+\frac{\tilde{k}_{21}+\tilde{k}_{20}}{\tilde{k}_{12}}\right) \\ &= \tilde{V}_1\left(1+\frac{\tilde{k}_{12}}{\tilde{k}_{21}+\tilde{k}_{20}}\right) \end{aligned} \quad (30)$$

Similarly

$$V_{\mathrm{dss,cen}} = V_1\left[1+\frac{k_{12}}{k_{21}}\right] \quad (31)$$

Both Eqs. (30) and (31) are consistent with Eq. (7). By applying relations (4) to (29) to obtain the $\mathrm{MRT}_{\mathrm{per}}$ in terms of the central elimination parameter values, the following is obtained:

$$\begin{aligned} \mathrm{MRT}_{\mathrm{per}} &= \frac{1}{\tilde{k}_{20}}\left[1+\frac{\tilde{k}_{21}+\tilde{k}_{20}}{\tilde{k}_{12}}\right] \\ &= \frac{k_{12}+k_{10}}{k_{10}k_{21}}\left[1+\frac{k_{21}}{k_{12}+k_{10}}\right] \\ &= \frac{1}{k_{10}}\left[1+\frac{k_{12}+k_{10}}{k_{21}}\right] \\ &= \mathrm{MRT}_{\mathrm{cen}}+\frac{1}{k_{21}} \\ &= \mathrm{MRT}_{\mathrm{cen}}+\frac{1}{\tilde{k}_{21}+\tilde{k}_{20}} > \mathrm{MRT}_{\mathrm{cen}} \end{aligned} \quad (32)$$

Thus the mean residence time is increased in comparison to Eq. (27). It is evident from the above that by applying Eq. (5) to Eq. (19), Eq. (13) will be obtained. Assuming that expression (10) for the clearance is still valid then for the case of peripheral elimination, applying Eq. (4) to Eq. (30) suggests that

$$\begin{aligned} V_{\mathrm{dss,per}} &= \tilde{V}_1\left(1+\frac{\tilde{k}_{12}}{\tilde{k}_{21}+\tilde{k}_{20}}\right) \\ &= V_1\left[1+\frac{k_{12}+k_{10}}{k_{21}}\right] \\ &= V_{\mathrm{dss,cen}}+V_1\frac{k_{10}}{k_{21}} > V_{\mathrm{dss,cen}} \end{aligned} \quad (33)$$

Thus, it can be seen that the volume of distribution, using the noncompartmental method, is underestimated for the case of peripheral elimination.[15]

VOLUME OF DISTRIBUTION USING THE FINAL VALUE THEOREM FOR LAPLACE TRANSFORMS

The expression (33) was derived under the assumption that the clearance is correctly measured using the first moment of the observed concentration curve. However this assumption has not been formally checked. The derivation for the clearance expression (10) would involve associating a volume with the unobserved compartment.[8] This is paradoxical as there are no concentration data for this compartment. Another method must be used to check the assumptions of the expression for clearance. The volume of distribution is calculated using the model independent definition (6).

The model independent expression for the volume of distribution (6) may be written in the Laplace transform domain:

$$V_{\mathrm{dss}} = \frac{A_{\mathrm{ss}}}{C_{\mathrm{ss}}} = \frac{\lim\limits_{s\to 0} sA(s)}{\lim\limits_{s\to 0} sC(s)} \quad (34)$$

where the system is assumed to be under constant infusion of rate R. This is to obtain a true steady state. Substituting for the transfer function of these quantities in the case of peripheral elimination, it can be seen that under constant infusion,

the following expression is thus derived:

$$V_{\mathrm{dss,per}} = \frac{\lim_{s\to 0} s(X_1(s)+X_2(s))}{\lim_{s\to 0} sC_1(s)} = \frac{\lim_{s\to 0} s\frac{R}{s}\left(\frac{s+(\tilde{k}_{21}+\tilde{k}_{12}+\tilde{k}_{20})}{\Delta(s)}\right)}{\lim_{s\to 0} s\frac{R}{s}\left(\frac{\frac{1}{\tilde{V}_1}(s+(\tilde{k}_{21}+\tilde{k}_{20}))}{\Delta(s)}\right)} = \frac{\tilde{k}_{21}+\tilde{k}_{12}+\tilde{k}_{20}}{\frac{1}{\tilde{V}_1}(\tilde{k}_{21}+\tilde{k}_{20})} = \tilde{V}_1\left(1+\frac{\tilde{k}_{12}}{\tilde{k}_{21}+\tilde{k}_{20}}\right) \quad (35)$$

where $\Delta(s) = s^2 + (\tilde{k}_{21}+\tilde{k}_{12}+\tilde{k}_{20})s + \tilde{k}_{12}\tilde{k}_{20}$ is the characteristic equation of the system. Notice this is the same as Eq. (30). Thus it can be deduced that Eq. (30) is valid and that the expression for the clearance (10) is applicable as well for the case of peripheral elimination. A similar treatment of the central elimination model results in Eq. (31). Thus noncompartmental clearance is model independent whereas volume of distribution is model dependent.

QUASI-IDENTIFIABILITY ANALYSIS OF THE MAXIMUM POSSIBLE VOLUME OF DISTRIBUTION

The Laplace transform of the general two-compartmental model with elimination from both compartments is:

$$G(s) = \frac{\frac{1}{V}(s+k_{21}+k_{20})}{s^2+(k_{12}+k_{21}+k_{20}+k_{10})s+(k_{21}+k_{20})(k_{12}+k_{10})-k_{21}k_{12}} \quad (36)$$

It may be then shown, in a similar manner to above, that the volume of distribution for the general two-compartment model with elimination from both compartments (1) is also given by Eq. (35). Examining Eq. (36) it can be seen that the only uniquely estimable parameter combinations from the *iv* experiment are:

$$\Psi_1 = k_{10}+k_{12} \quad (37)$$

$$\Psi_2 = k_{20}+k_{21} \quad (38)$$

$$\Psi_3 = k_{12}k_{21} \quad (39)$$

It is clear that Eq. (35) is maximised when k_{12} is largest and $k_{21}+k_{20}$ is smallest within the confines of Eqs. (37–39). Thus Eq. (35) is maximised when clearance is attributed to the peripheral compartment only (that is $k_{10}=0$). Thus for a given observed profile the peripheral elimination model results in the largest volume of distribution, whereas the central elimination model has the smallest volume of distribution.

DISCUSSION

Expressions (19) and (22) relate two important 'noncompartmental' measures to the case of elimination in a peripheral compartment.

The first observation is that the clearance is still measurable directly by using the AUC of the central compartment concentration profile.

The second observation is that the relationship between the volume of distribution and the model parameters alters Eq. (30). Noncompartmental analysis under-estimates the volume of distribution at steady state when peripheral elimination best describes the disposition of a compound.

This has implications for the prediction of the volume of distribution using such methods as those suggested by Poulin et al.[25] Notice that K_{p2} in Eq. (18) is the partition coefficient that governs the transfer of mass between the central and peripheral compartment; it still characterises the kinetics of the exchange between compartments. If there is elimination from the peripheral compartment then K_{p2} will have to be amended as per Eq. (7).

The expression (7) is used to estimate the volume of distribution from the estimated partition coefficients. The analysis above suggests that the partition coefficient for eliminating tissue should be modified. It also suggests that otherwise there will be an under-prediction of the value of V_{dss} and this error will increase with the clearance. The error can be quantified using Eq. (33).

The volume of distribution derived noncompartmentally is often used to estimate partition coefficients for PBPK models.[22,27,28] Failure to use the correct estimate of volume may result in a

suboptimal set of partition coefficients being used in the PBPK model. This may result in a reduced precision in the predictions that may be derived from such models.

It should also be noted that Eq. (19) implies that distribution can be the rate limiting step of clearance as if

$$\tilde{k}_{20} \gg \tilde{k}_{12} \tag{40}$$

then

$$\mathrm{CL}_{\mathrm{app}} \approx \tilde{V}_1 \tilde{k}_{12} \tag{41}$$

This result is entirely to be expected because it is analogous to the well-stirred liver model,[29] whereby inter-compartmental flow is a rate-limiting step of elimination for highly cleared compounds.

In conclusion, when elimination is from a peripheral compartment, the standard noncompartmental method underestimates the extent of distribution of the compound. A direct result of this is that the quantity of drug remaining in the body at a given time is underestimated. Most tissues accumulate compound due to the physiochemical partition coefficient between the tissue and the plasma. If elimination occurs here then the tissue is like a sink, 'trapping' the compound and eliminating it to create a larger apparent partitioning.

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APPENDIX A: NONCOMPARTMENTAL ANALYSIS WITH CENTRAL ELIMINATION

Consider the system shown in Figure 3 with elimination from the central compartment only. Using this, model dependent expressions for the clearance and volume of distribution will be obtained via the noncompartmental definitions (10,12). Consider an i.v. bolus injection:

$$\begin{aligned} V_1 \frac{\mathrm{d}C_1}{\mathrm{d}t} &= -V_1(k_{12}+k_{10})C_1 + V_2 k_{21} C_2 \\ V_2 \frac{\mathrm{d}C_2}{\mathrm{d}t} &= V_1 k_{12} C_1 - V_2 k_{21} C_2 \\ V_1 C_1(0) &= \mathrm{Dose} \\ V_2 C_2(0) &= 0 \end{aligned} \tag{A1}$$

$C_1(t)$ is the plasma concentration and the system (A1) is open and so

$$V_1 C_1(\infty) = V_2 C_2(\infty) = 0 \tag{A2}$$

Integrating the differential equations of Eq. (A1) yields

$$-\mathrm{Dose} = -V_1(k_{12}+k_{10})\mathrm{AUC} + V_2 k_{21} \int_0^\infty C_2(t)\,\mathrm{d}t \tag{A3}$$

$$0 = V_1 k_{12}\mathrm{AUC} - V_2 k_{21} \int_0^\infty C_2(t)\,\mathrm{d}t \tag{A4}$$

Thus by substituting Eq. (A4) into Eq. (A3) and rearranging,

$$\mathrm{CL} = \frac{\mathrm{Dose}}{\mathrm{AUC}} = V_1 k_{10} \tag{A5}$$

The first step in calculating the MRT is to multiply the differential equations in system (A1) by t and integrating by parts with respect to time.

$$-V_1\mathrm{AUC} = -V_1(k_{12}+k_{10})\mathrm{AUMC} + V_2 k_{21} \int_0^\infty t C_2(t)\,\mathrm{d}t \tag{A6}$$

$$-V_2 \int_0^\infty C_2(t)\,\mathrm{d}t = V_1 k_{12}\mathrm{AUMC} - V_2 k_{21} \int_0^\infty t C_2(t)\,\mathrm{d}t \tag{A7}$$

Thus, by adding Eq. (A6) to Eq. (A7), applying Eq. (A4) and rearranging,

$$\mathrm{MRT} = \frac{\mathrm{AUMC}}{\mathrm{AUC}} = \frac{1}{k_{10}}\left[1 + \frac{k_{12}}{k_{21}}\right] \tag{A8}$$

The expression for the apparent volume of distribution is thus

$$V_{\mathrm{dss}} = V_1\left(1 + \frac{k_{12}}{k_{21}}\right) \tag{A9}$$

APPENDIX B: NONCOMPARTMENTAL ANALYSIS WITH PERIPHERAL ELIMINATION

Consider the case of a two-compartment model with elimination from the peripheral compart-

ment only:

$$\begin{aligned}\tilde{V}_1\frac{dC_1}{dt} &= -\tilde{V}_1\tilde{k}_{12}C_1 + \tilde{V}_2\tilde{k}_{21}C_2\\ \tilde{V}_2\frac{dC_2}{dt} &= \tilde{V}_1\tilde{k}_{12}C_1 - \tilde{V}_2(\tilde{k}_{21}+\tilde{k}_{20})C_2\\ \tilde{V}_1C_1(0) &= \text{Dose}\\ \tilde{V}_2C_2(0) &= 0\end{aligned} \quad \text{(B1)}$$

Notice that again the system is open and so Eq. (A2) holds. The analysis is as before. Integrating the differential equations of system (B1)

$$\begin{aligned}-\text{Dose} &= -V_1C_1(0)\\ &= -V_1k_{12}\text{AUC} + V_2k_{21}\int_0^\infty C_2(t)\,dt\end{aligned} \quad \text{(B2)}$$

$$0 = \tilde{V}_1\tilde{k}_{12}\text{AUC} - \tilde{V}_2(\tilde{k}_{21}+\tilde{k}_{20})\int_0^\infty C_2(t)\,dt \quad \text{(B3)}$$

Thus

$$\begin{aligned}\text{CL}_{\text{app}} &= \tilde{V}_1\tilde{k}_{12}\left(1-\frac{\tilde{k}_{21}}{\tilde{k}_{21}+\tilde{k}_{20}}\right)\\ &= \tilde{V}_1\tilde{k}_{12}\left(\frac{\tilde{k}_{20}}{\tilde{k}_{21}+\tilde{k}_{20}}\right)\end{aligned} \quad \text{(B4)}$$

This is the same relationship as that found in Eq. (4). Here the app subscript refers to that observed from the central compartment, it is shown later that this expression does apply to the case of peripheral elimination. The mean residence time is calculated in the same manner as before. Multiplying the differential equations of system (B1) by t and integrating for all time

$$\begin{aligned}&-\tilde{V}_1\text{AUC}\\ &= -\tilde{V}_1\tilde{k}_{12}\text{AUMC} + \tilde{V}_2\tilde{k}_{21}\int_0^\infty tC_2(t)\,dt\end{aligned} \quad \text{(B5)}$$

$$\begin{aligned}&-\tilde{V}_2\int_0^\infty C_2(t)\,dt\\ &= \tilde{V}_1\tilde{k}_{12}\text{AUMC}\\ &\quad -\tilde{V}_2(\tilde{k}_{21}+\tilde{k}_{20})\int_0^\infty tC_2(t)\,dt\end{aligned} \quad \text{(B6)}$$

Using Eqs. (B5), (B6) and (B3), the expression for MRT is

$$\begin{aligned}\text{MRT} = \frac{\text{AUMC}}{\text{AUC}} &= \frac{\left[1+\dfrac{\tilde{k}_{12}\tilde{k}_{21}}{(\tilde{k}_{21}+\tilde{k}_{20})^2}\right]}{\tilde{k}_{12}\left[1-\dfrac{\tilde{k}_{21}}{\tilde{k}_{21}+\tilde{k}_{20}}\right]}\\ &= \frac{1}{\tilde{k}_{20}}\left[\frac{\tilde{k}_{21}+\tilde{k}_{20}}{\tilde{k}_{12}}+\frac{\tilde{k}_{21}}{\tilde{k}_{21}+\tilde{k}_{20}}\right]\end{aligned} \quad \text{(B7)}$$

Thus the noncompartmental volume of distribution is given by

$$V_{\text{dss}} = \tilde{V}_1\left[1+\frac{\tilde{k}_{12}\tilde{k}_{21}}{(\tilde{k}_{21}+\tilde{k}_{20})^2}\right] \quad \text{(B8)}$$

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