A Simplified Approach for Estimating Skin Permeation Parameters from *In Vitro* Finite Dose Absorption Studies

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ABSTRACT: Historically, percutaneous absorption permeation parameters have been derived from *in vitro* infinite dose studies, yet there is uncertainty in their accuracy if the applied vehicle saturates or damages the *stratum corneum*, or when the permeation parameters are inappropriately derived from cumulative absorption data. An approach is provided for determining penetration parameters from *in vitro* finite dose data. Key variables, and equations for their derivation, are identified from the literature and provide permeation parameters that use only T_{max}, AUC, and AUMC from finite dose data. The equations are tested with computer-generated model data and to actual study data. Derived permeation parameters obtained from the computer model data match those used in generating the simulated finite dose data. Parameters obtained from actual study data reasonably and acceptably model the penetration profile kinetics of the study data. From *in vitro* finite dose absorption data, three parameters can be obtained: the diffusion transit time (t_d), which characterizes the diffusion coefficient, the partition volume ($V_m P$), which characterizes the partition coefficient, and the permeation coefficient (K_p). These parameters can be obtained from finite dose data without having to know the length of the diffusion pathway through the membrane. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:4048–4057, 2014

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INTRODUCTION

The kinetic profiling of *in vitro* infinite dose steady-state percutaneous absorption has been most often characterized by Fick's Laws of diffusion[1–3] as shown in Eqs. (1–4).

$$J_{ss} = \frac{PDC}{l} \tag{1}$$

$$K_p = \frac{PD}{l} \tag{2}$$

$$J_{ss} = K_p C \tag{3}$$

$$T_{lag} = \frac{l^2}{6D} \tag{4}$$

where J is flux, P is the partition coefficient, D is the diffusion coefficient, C is the concentration of drug in the donor phase (assuming infinite sink in the receptor phase), l is the diffusional pathway length, and K_p is the permeability constant.

When cumulative absorption (often used in the vernacular as "cumulative penetration") from an infinite dose study is plotted, the slope of the asymptotic linear portion of the curve represents the steady-state flux (dQ/dt), and its x-axis intercept, the lag time (T_{lag}). From T_{lag} and J_{ss} (see Table 1 for variable definitions), and a measured or estimated length of the diffusion pathway (e.g., *stratum corneum* thickness), a diffusion coefficient and partition coefficient can be derived.

Franz,[4,5] publishing on the relevance of the *in vitro* finite dose model, also demonstrated the influence of each permeation coefficient on the shape of the kinetic absorption profile. At that time, the finite dose model was defined as being applicable when the applied dose is considered clinically relevant (e.g., 1–10 mg/cm^2) and where the kinetic absorption profile demonstrates a depletion of the applied dose over time. This model has become a widely recognized method[6,7] as it better represents the actual exposure one encounters in the use of cosmetics and topical pharmaceuticals. One solution of the finite dose model is shown in Eq. (5) from Carslaw and Jaeger.[8]

$$J = 2hpDC_0 \sum_{n=1}^{\infty} \frac{\alpha_n e^{-D\alpha_n^2 t}}{\sin \alpha_n l \,[l(\alpha_n^2 + h^2) + h]} \tag{5}$$

where v is vehicle dose layer thickness, $h = \frac{p}{v}$, and $\alpha_n =$ *roots of* $[\alpha l \tan \alpha l] = hl$.

What is so often misunderstood in consideration of infinite and finite dose data analysis is that a small donor volume (applied dose) does not *a priori* define it to be a finite dose, and conversely, a large donor volume does not *a priori* define it to be an infinite dose. To be a finite dose, it must demonstrate dose depletion kinetics resulting from absorption, volatilization, or precipitation of the solute of interest. To be an infinite dose, it must maintain a constant concentration of diffusible solute in the applied vehicle to sustain a steady-state flux. Further, a clinically relevant dose does not *a priori* define it to be a finite dose. Though many clinically relevant dose applications do result in a finite dose delivery profile, Figure 1 demonstrates two examples from the author's files that show an infinite

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Table 1. Variables Used in the Equations and Text with Brief Definitions

Variable	Units	Description
A_m	cm^2	Area of the membrane
AUC_{0-t}	Mass/cm^2	Area under the flux curve; $t = 0$ to $t =$ last
$AUMC_{0-t}$	Mass–time/cm^2	Area under the flux first moment curve; $t = 0$ to $t =$ last
$AUC_{0-\infty}$	Mass/cm^2	Area under the flux curve; $t = 0$ to $t =$ infinity
$AUMC_{0-\infty}$	Mass–time/cm^2	Area under the flux first moment curve; $t = 0$ to $t =$ infinity
C	Mass/cm^3	Concentration
D	cm^2/h	Diffusion coefficient
Dose	Mass	Amount applied. $AUC_{0-\infty}$ for finite dose model
J_{max}	Mass/cm^2/time	Peak observed flux
J_{ss}	Mass/cm^2/time	Steady-state flux
K_p	cm/h	Permeability coefficient
l	cm	Diffusional pathway or *stratum corneum* thickness
MTT	h	Mean transit time
P	–	Partition coefficient (membrane to vehicle)
t_d	h	Diffusion transit time
T_{max}	h	Time of peak flux
v	cm	Donor vehicle thickness
V_d	cm^3	Volume of donor vehicle
V_m	cm^3	Volume of membrane
V_mP	cm^3	Partitioning volume
V_{dN}	–	Donor volume number

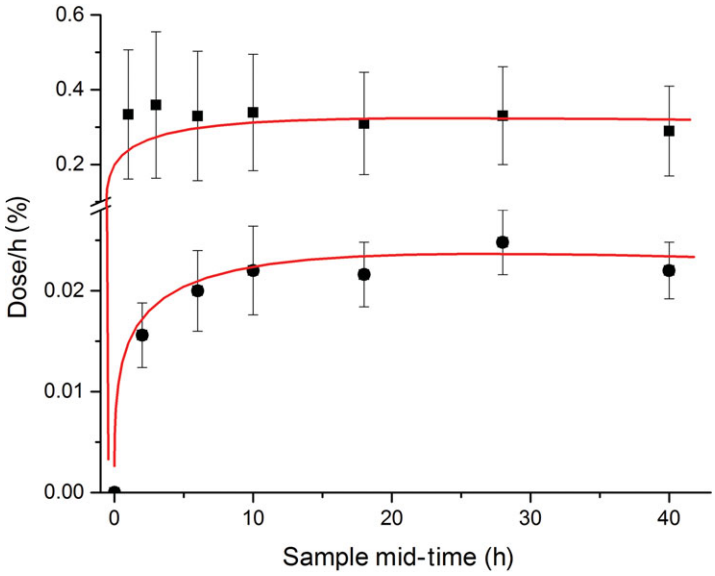

Figure 1. Apparent steady-state absorption from a clinically relevant applied dose (5 μL/cm^2) on dermatomed human skin *in vitro* (mean ± SE, $n = 6$ donors each in triplicate). (■) 5% minoxidil from a commercial aerosol formulation, and (●) 5% imiquimod from a commercial cream formulation. Solid lines represent estimated fit of the data.

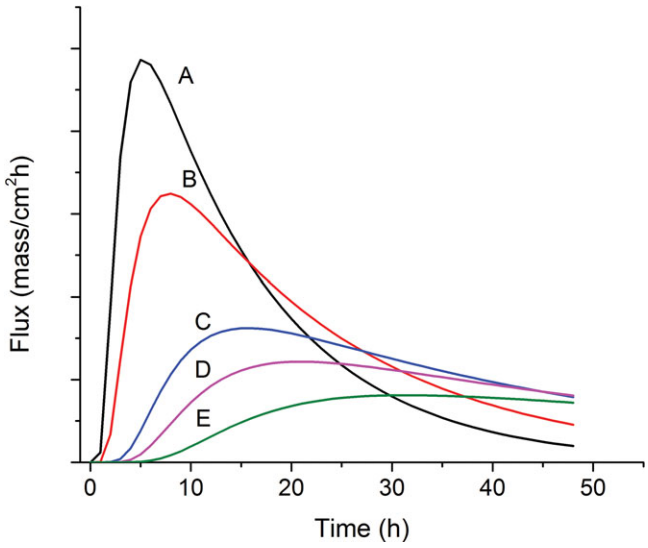

Figure 2. Computer-generated finite dose flux profiles, 0–48 h, generated using Eq. (5).

dose steady-state absorption profile from a small applied dose volume.

The use of the infinite dose study design for determining permeation coefficients of solutes has proven problematic as the *stratum corneum* is often damaged, saturated, or modified by the continuous exposure to the dosing vehicle. Derived diffusion parameters are less likely a characteristic of the permeating compound but more likely a representation of its diffusion through a vehicle-modified membrane.[9] This issue is of lesser concern for finite dose studies as the applied volume of vehicle is typically very small and often contain volatile excipients that evaporate rapidly (such as water and alcohol). As a result, the potential for damage or alteration to the *stratum corneum* barrier is appreciably reduced, negligible, or inconsequential. More importantly, any change that is induced to the membrane by the vehicle or its excipients would be clinically relevant, such as would be intended from a penetration enhancer.

Data from *in vitro* absorption studies are frequently presented as cumulative absorption, which is then used to derive K_p and T_{lag} values. However, without also analyzing the data as flux versus time, the true nature of the actual kinetic profile may not be realized. To demonstrate this, a series of finite dose modeled flux curves were generated using Eq. (5). As seen in Figure 2, each curve demonstrates a finite dose absorption profile with a rise to a peak flux (J_{max}), as penetration increases, followed by a decline in flux as the applied vehicle is depleted of the permeating compound.

If this were a study that was terminated at 12, 24 (Fig. 3), 36, or 48 h and profiled only as cumulative absorption versus time, one would falsely interpret the results as demonstrating steady-state flux because of a visualized asymptotic linearity to the data. Even through 48 h, curves D and E would still suggest a steady-state rate of absorption when in fact the finite dose flux profile is a broad curve with a protracted T_{max}.

The consequence of relying only on cumulative absorption to calculate K_p, when in fact the data represent a finite dose absorption profile is demonstrated in Table 2. Permeation