Determining Dermal Absorption Parameters *in Vivo* from Tape Strip Data

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Purpose: Tape stripping the outermost skin layer, the stratum corneum (sc), is a popular method for assessing the rate and extent of dermal absorption *in vivo*. Results from tape strip (TS) experiments can be affected significantly by chemical diffusion into the sc during the time required to apply and remove all of the TSs, t_{TS}. Here, we examine the effects of this problem on the interpretation of TS experimental results.

Methods: Dermal absorption of 4-cyanophenol (4CP) in humans was studied using TS experiments to assess conditions in which diffusion alters TS results. Mathematical models were developed to assess the effects of diffusion on parameter estimation.

Results: For an experiment with $t_{TS} > t_{lag}$ (i.e., the lag time for a chemical to cross the sc), the permeability coefficient for 4CP, $P_{sc,v}$, calculated including t_{TS}, was consistent with values from the literature (i.e., 0.0019 cm/h). When diffusion during stripping was not included in the model, $P_{sc,v}$ was 70% smaller.

Conclusions: Calculations show that chemical concentrations in TSs can be affected by diffusion during tape stripping, but if $t_{TS} < 0.2\ t_{lag}$ and the exposure time is $> 0.3\ t_{lag}$, TS concentrations are not significantly affected by t_{TS}.

KEY WORDS: tape strip; stratum corneum; dermal absorption; topical bioavailability; mathematical model.

INTRODUCTION

Many *in vivo* methods for measuring dermal absorption of chemicals are invasive (e.g., blood samples are collected) or slow (e.g., urine samples are collected for extended periods). Tape stripping of the outermost skin layer, the stratum corneum (sc), is a fast and relatively noninvasive technique for measuring the rate and extent of dermal absorption (1,2). Tape strip (TS) data have been used to estimate permeability coefficients and partition coefficients from *in vivo* dermal exposures (3,4). Tape stripping also has been proposed as a method for evaluating bioequivalence of topical dermatological dosage forms (5).

In a TS experiment, an area of skin is exposed to a chemical for a set exposure time and then cleaned. Between 10 and 30 pieces of adhesive tape are applied to and removed from the exposed area in sequence and the mass of chemical determined in each. Although the TS procedure is relatively simple to execute, there are many opportunities for experimental artifacts to develop. For example, TS samples have high surface area-to-volume ratios, and losses by evaporation can be significant even for chemicals with relatively low volatility. Generally, the TS experiment is unsuitable for volatile chemicals, and for all other chemicals analysis should be completed soon after TS removal from the skin (6).

Many studies report the mass of absorbing chemical as a function of TS number. However, the amount of sc removed by each TS generally decreases with increasing depth (6,7). To estimate dermal absorption parameters, the amount of chemical in each TS must be normalized by the amount of sc (4,7). Methods for measuring the amount of sc on each tape include gravimetric analysis (7) and optical spectroscopy (8).

When the mass of sc in the nth TS, $m_{sc,n}$, is determined gravimetrically, the concentration of chemical in the nth TS, C_n, is

$$C_n = m_n\, \rho_{sc}/m_{sc,n} \tag{1}$$

where m_n is the mass of absorbing chemical in the n^{th} TS and ρ_{sc} is the density of the sc (~1 g/cm^3 (4)). The location of C_n as a function of depth in the sc, x_n is:

$$x_n = \frac{1}{A\,\rho_{sc}}\left\{\frac{m_{sc,n}}{2} + \sum_{i=1}^{n-1} m_{sc,i}\right\} \tag{2}$$

where x_n is at the center of $m_{sc,n}$ and A is the area being tape stripped.

Pirot *et al.* (4) determined diffusion and partition coefficients for 4-cyanophenol (4CP) by comparing C_n and x_n data with equations describing dermal absorption as diffusion through a pseudo-homogeneous membrane. A potential problem with this approach is that chemical in the sc when the exposure ends will continue to diffuse during the time that it takes to apply and remove all of the TSs. Unless the TS procedure is fast, the concentration measured in each TS will be different from the concentration at that location when the exposure ended, which would affect estimated values for diffusion coefficients and partition coefficients calculated using the TS data. Experiments and mathematical models have been used to assess the conditions under which diffusion might affect TS results and the magnitude of this effect when it occurs.

MATERIALS AND METHODS

For all experiments, saturated aqueous solutions of 4CP (reagent grade from Aldrich Chemical Co., Milwaukee, Wisconsin; vapor pressure estimated as 0.0065 mm Hg in SRC PhysProp database) prepared with excess 4CP in deionized water (solubility of 0.11 M) were applied to the forearms of human volunteers at the Albany College of Pharmacy with approval of the Albany Medical College Institutional Review Board. A gauze pad (2.5 × 8 cm, CVS, Woonsocket, Rhode Island) soaked with 4CP solution was placed on the application area and held in place by a nonocclusive dressing (5 × 12 cm, Tegaderm, 3M, St. Paul, Minnesota). At the end of the exposure, the corners of the treated area were marked and the skin surface was quickly cleaned with deionized water and

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dried using cotton balls. Preweighed adhesive tapes (2.5 × 8 cm, 3M Book Tape 845) were then applied to and removed from the application site sequentially. Twenty-five TSs were sufficient to remove essentially all of the sc and reveal a glistening surface below. Each TS was weighed after application to the skin surface to determine the mass of sc removed. The mass of 4CP on each TS was determined using attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy as described by others (1,4) shortly after removal from the skin surface.

Two types of experiments were conducted. In both experiments, two sites on the subject's forearm were exposed to 4CP solution for 1 h and then one site was tape-stripped rapidly, taking less than 6 min. Prior experiments have shown that 1 h is long enough for thorough hydration of the sc and for the concentration to reach steady state. In the Type 1 experiment the second site was tape stripped slowly at regular time intervals over 1 h. In the Type 2 experiment, chemical concentration in the sc at the second site was measured after a period of clearance. Specifically, after the exposed sites were cleaned, the second site was occluded for 1 h using a 50 μm-thick polyethylene layer (2.5 × 8 cm, Fisher Scientific, Pittsburgh, Pennsylvania) secured with Tegaderm to maintain hydration and prevent 4CP evaporation and then tape stripped rapidly. Because the polyethylene adhered to the Tegaderm, resulting in poor contact between the skin and polyethylene layer, 4CP transfer from the sc to the polyethylene was unlikely. Also, in separate experiments we found that 4CP absorption into polyethylene was limited.

In both types of experiments, C_n and x_n were calculated using Eqs. (1) and (2), respectively. To locate x_n relative to the total thickness of the sc, x_n/L_{sc}, it was assumed that the sc was completely removed by tape stripping and

$$L_{sc} = \frac{1}{A\,\rho_{sc}} \sum_{n=1}^{N_{TS}} m_{sc,n} = \frac{M_{sc}}{A\,\rho_{sc}} \tag{3}$$

where M_{sc} is the mass of sc removed by the total number of TSs (N_{TS}).

RESULTS

Type 1 experimental results for one subject are presented in Fig. 1. TS concentrations are shown as a function of relative position in the sc. Table 1 lists experimental details and estimated L_{sc}. Concentrations in TSs collected over 1 h are smaller than concentrations in TSs collected in 6 min at a similar position, indicating that 4CP continued to diffuse while TSs were collected. Consistent with the Type 1 experiments, TS concentrations collected after a 1 h delay (i.e., Type 2 experiments) were smaller than those collected immediately after the exposure ended (Fig. 2). Values of L_{sc} were lower than the average value of 12 μm reported by Pirot *et al.* (9).

THEORY

Two types of mathematical models were developed to describe the chemical concentration as a function of position in the sc during the TS procedure. In the first type, equations were developed assuming that the TS procedure could be completed instantaneously. In the second type of model, the concentration within the sc was calculated allowing for diffusion during the TS procedure.

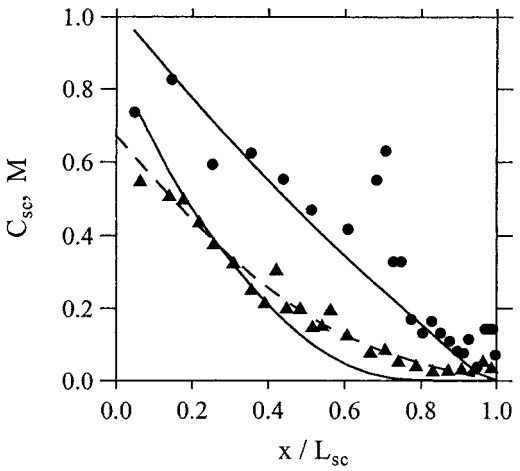

Fig. 1. Experimental and calculated 4CP concentrations in the sc (C_{sc}) as a function of x/L_{sc} for fast (●, < 6 min) and slow (▲, 1 h) tape stripping for the Type 1 experiment. The solids curves were calculated using Eqs. (8), (10), and (13) (i.e., the model that accounted for t_{TS}) and the dashed curve was calculated using Eq. (4).

Although skin is a multilayered membrane, the two outside layers, the sc and the viable epidermis (ve), are the principal barriers for dermal absorption. Although the ve can contribute a significant barrier to extremely lipophilic chemicals, the sc is the rate-limiting barrier for many chemicals (10), including those with properties similar to 4CP. Equations presented here were derived assuming that the sc controls the rate of dermal absorption.

Assuming dermal absorption is passive diffusion through a pseudo-homogenous membrane, the concentration of chemical in the sc (C_{sc}) at the end of an exposure ($t = t_{exp}$) to a constant-concentration vehicle can be estimated by (11):

$$\frac{C_{sc}}{K_{sc/v}C_v^o} = 1 - \frac{x}{L_{sc}} - \frac{2}{\pi}\sum_{n=1}^{\infty}\frac{1}{n}\exp\left(-\frac{n^2\pi^2 t_{exp}}{6t_{lag}}\right)\sin\left(\frac{n\pi x}{L_{sc}}\right) \tag{4}$$

in which C_v^o is the initial concentration of the vehicle, x is the position in the sc, $K_{sc/v}$ is the equilibrium partition coefficient of the absorbing chemical between the sc and the vehicle, and t_{lag} is the lag time for chemical to penetrate the sc, defined as $L_{sc}^2/(6D_{sc})$ where D_{sc} is the effective diffusion coefficient in the sc. Eq. (4) was developed assuming (i) the sc was initially free of chemical, (ii) the outer surface of the sc was in local equilibrium with the vehicle, and (iii) the concentration of absorbing chemical at the sc-ve interface was zero. In the development of Eq. (4) it was also assumed that there is no nonlinear or irreversible binding in the sc. The mass of chemical in the sc at $t = t_{exp}$, M^o, can be calculated as

$$\frac{M^o}{AL_{sc}K_{sc/v}C_v^o} = \frac{1}{2} - \frac{4}{\pi^2}\sum_{n=0}^{\infty}\frac{\exp[-(2n+1)^2\pi^2 t_{exp}/(6t_{lag,sc})]}{(2n+1)^2} \tag{5}$$

which was derived by integrating C_{sc} as a function of x for $0 \le x \le L_{sc}$. The average concentration of absorbing chemical in the sc at $t = t_{exp}$, $\langle C_{sc}^0 \rangle$, is calculated as $\langle C_{sc}^o \rangle = M^o/(A\,L_{sc})$.

If t_{exp} is long, steady state will be established and Eqs. (4) and 5 become

$$C_{sc} = K_{sc/v}\,C_v^o\,(1 - x/L_{sc}) \tag{6}$$

$$M^o = A\,L_{sc}K_{sc/v}C_v^o/2 \tag{7}$$

TABLE I. Summary of TS Experiments and Parameter Estimation Results

	Experimental details				Estimated parameters			
Experiment	Subject	t_{exp} (min)	t_{delay} (min)	t_{TS} (min)	L_{sc} (μm)	$K_{sc/v}$	t_{lag} (min)	$P_{sc,v} \times 10^4$ (cm/h)
Type 1	A	60	0	<6	7.4[c]	9.1[e]	—	19[h]
〃	〃	〃	0	60	9.2[c]	—	40[f]	
Type 1	A	60	0	60	9.2[c]	6.1[d]	95[d]	5.9[h]
Type 2	A	60	0	<6	11.0[c]	9.5[e]	—	32[h]
〃	〃	〃	60	<6	11.8[c]	—	34[g]	
Type 2	B	60	0	<6	8.5[c]	9.1[e]	—	30[h]
〃	〃	〃	60	<6	8.6[c]	—	26[g]	
Type 2	C	60	0	<6	10.2[c]	8.2[e]	—	26[h]
〃	〃	〃	60	<6	10.8[c]	—	34[g]	
Type 2	A, B, & C	60	0 & 60	<6	10.1 ± 1.5[i]	9.0 ± 0.7[i]	31 ± 5[i]	29 ± 3[i]
Pirot *et al.* (4)	3 subjects	15	0	NA[a]	NA[a]	8.4 ± 3.6	32.5 ± 6.2	37 ± 9[b]

[a] NA = not available.
[b] Calculated using L_{sc} = 15 μm.
[c] Calculated using Eq. 3.
[d] Calculated using Eq. 4.
[e] Calculated using Eq. 16.
[f] Calculated using Eqs. 8, 10 and 13.
[g] Calculated using Eq. 11.
[h] Calculated as $P_{sc,v} = K_{sc,v} L_{sc}/(6\ t_{lag})$. When two values of L_{sc} were available a mean value was used.
[i] Mean value for subjects A, B, and C ± one standard deviation.

C_{sc} depends on both $K_{sc/v}$ and t_{lag} prior to steady state (Eq. [4]), but on only $K_{sc/v}$ after steady state is established (Eq. [6]). When $t_{exp} > 1.7\ t_{lag}$, C_{sc} is essentially linear with position and M^o will increase by < 5% if t_{exp} is increased further.

If sc removal by serial tape stripping occurred immediately at the end of a chemical exposure, then the chemical concentration measured in each TS (i.e., C_n) should be described by Eq. (4). However, if after the chemical is removed there is a delay, t_{delay}, before the sc is tape stripped, then the concentration of chemical in the sc decreases as diffusion proceeds. Again, we describe dermal absorption as Fickian diffusion through a pseudo-homogeneous membrane as follows:

$$\frac{\partial C_{sc}}{\partial t} = D_{sc} \frac{\partial^2 C_{sc}}{\partial x^2} \qquad \text{for } 0 \le x \le L_{sc} \tag{8}$$

The conditions during t_{delay} are described by the following boundary conditions:

$$\text{at } x = 0 \qquad \frac{\partial C_{sc}}{\partial x} = 0 \qquad \text{for } t > t_{exp} \tag{9}$$

$$\text{at } x = L_{sc} \qquad C_{sc} = 0 \qquad \text{for } t > t_{exp} \tag{10}$$

where C_{sc} is given by Eq. (4) for $t = t_{exp}$. Eq. (9) states that flux from the outermost surface of the sc is zero, which is expected for nonvolatile chemicals. The concentration at the sc-ve interface is assumed to be zero (Eq. [10]). When an exposure ends after steady state is established, C_{sc} is described by Eq. (6). The solution of Eqs. (8) through (10) using Eq. (6) as the initial condition is given by (11):

$$\frac{C_{sc}}{K_{sc/v} C_v^o} = \sum_{n=0}^{\infty} \frac{8}{(2n+1)\pi} \cos\left(\frac{(2n+1)\pi}{2} \frac{x}{L_{sc}}\right) \exp\left(\frac{-(2n+1)^2 \pi^2}{24} \frac{t_{delay}}{t_{lag}}\right) \tag{11}$$

where $t_{delay} = t - t_{exp}$. Eq. (11) represents C_{sc} some time after the exposure has ended when the sc is the rate-controlling barrier to dermal absorption for $t_{exp} > 1.7\ t_{lag}$.

Equation (4) could represent C_n poorly if the tape stripping was slow compared with the rate that chemical penetrates the sc, because chemical will diffuse across the remaining sc during the TS procedure. In this case, Eq. (8) describes the diffusion of chemical for $x_{TS} \le x \le L_{sc}$, the part of the sc remaining after a portion has been removed by the TS pro-

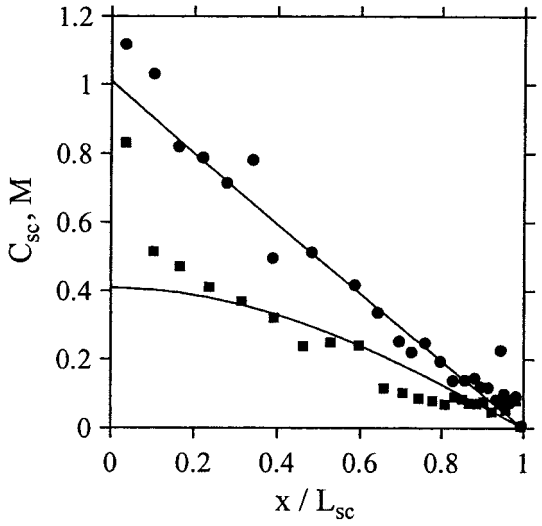

Fig. 2. Experimental and calculated 4CP concentrations in the sc (C_{sc}) without (●) and with (■) a 1 h delay before tape stripping as a function of x/L_{sc} for the Type 2 experiment for subject A. Curves through the data with no delay and with a 1 h delay were calculated using Eqs. (6) and (11), respectively.

cedure, with the outside of the sc located at x_{TS} (Fig. 3). As each TS is removed, the position of the outside edge of the sc moves inward (i.e., x_{TS} increases) until all of the sc is removed. To simplify the analysis, we assumed that the time required to clean the skin is insignificant, the time between TS applications is constant, and that the same amount of sc is removed by each TS. Consequently, the location of x_{TS} relative to L_{sc} is described by

$$\frac{x_{TS}}{L_{sc}} = \frac{1}{N_{TS}} \sum_{j=1}^{N_{TS}} u\left[t - t_{exp} - j \frac{t_{TS}}{N_{TS}} \right] \qquad (12)$$

in which t_{TS} is the total time to complete the TS process. The unit step function, u[t-a] , is defined as 0 for $t \leq a$ and 1 for $t > a$.

For the model including diffusion during stripping, Eq. (8) was solved numerically with C_{sc} given by Eq. (4) at $t = t_{exp}$, restricted by Eqs. (10) and (13),

$$\text{at } x = x_{TS} \qquad \frac{\partial C_{sc}}{\partial x} = 0 \qquad \text{for } t > t_{exp} \qquad (13)$$

which states that flux is zero from the outer sc surface after each TS is removed. These equations were solved numerically as described in the Appendix. Once C_n is calculated, the total mass of chemical in all TSs, M_{TS}, is given by

$$M_{TS} = \frac{AL_{sc}}{N_{TS}} \sum_{n=1}^{N_{TS}} C_n \qquad (14)$$

which is less than or equal to M^o.

DISCUSSION

Figure 4 presents model calculations comparing the concentration in TSs (represented by data points) as a function of x/L_{sc} for three values of the total time to tape strip, t_{TS}, relative to how rapidly the chemical can penetrate the sc, represented by t_{lag}. The concentration profile in the sc at the end of the exposure is represented by the solid curve. In these calculations and all others shown here, $N_{TS} = 30$. Whether an exposure ends before (i.e., $t_{exp} = 0.3\ t_{lag}$) or after (i.e., $t_{exp} > 1.7\ t_{lag}$) steady state was established, diffusion can alter TS experimental results if tape stripping takes a long time relative to t_{lag}.

Significantly, when $t_{TS} > t_{lag}$ the concentration profile measured by tape stripping can be nonlinear even if t_{exp} was long enough to achieve steady state (Fig. 4b). These calculations are consistent with the experimental results presented in Fig. 1, and may explain the 4CP data reported by Higo *et al.*

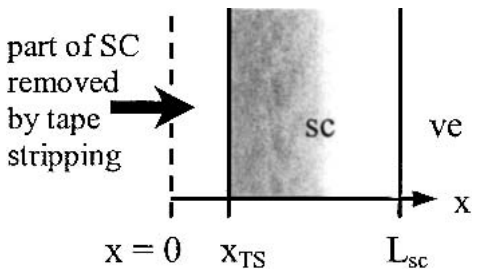

Fig. 3. Schematic diagram of the sc during a TS experiment.

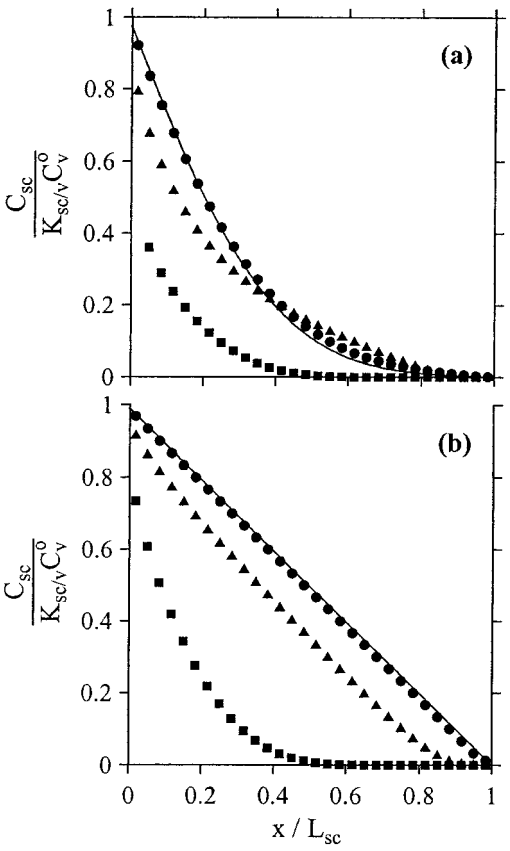

Fig. 4. The concentration in the sc at $t = t_{exp}$ (curves) compared with calculated concentrations in 30 TSs as a function of x/L_{sc} when $t_{TS} = 0.1\ t_{lag}$ (●), $1\ t_{lag}$(▲) and $10\ t_{lag}$ (■) for t_{exp} (a) $= 0.3\ t_{lag}$ and (b) $> 1.7\ t_{lag}$.

(7), which were nonlinear even for exposure times of 3 h. In these experiments subjects placed their tape-stripped forearms directly onto the ATR crystal of the FTIR after each TS was applied and removed. Based on experimental results in Fig. 1 and calculations shown in Fig. 4, we suspect that t_{TS} was greater than t_{lag} for this procedure.

The effect of diffusion on TS concentrations can be studied using the calculated differences between the concentration in each TS at the end of the exposure (C_n^o, calculated using Eq. [4]) and at the time of tape stripping (i.e., C_n). Figure 5 shows the average absolute value of $(C_n^o - C_n)$ for all TSs calculated as

$$\langle \left| C_n^o - C_n \right| \rangle = \frac{1}{N_{TS}} \sum_{i=1}^{N_{TS}} \left| C_n^o - C_n \right| \qquad (15)$$

and normalized by $\langle C_{sc}^o \rangle$. The quantity $\langle \left| C_n^o - C_n \right| \rangle$ is a measure of the effect of t_{TS} on the concentration profile measured by tape stripping. Based on the calculations shown in Fig. 5, the average change in the concentration profile during t_{TS} is less than about 10% when $t_{exp} > 0.3\ t_{lag}$ if $t_{TS} < 0.2\ t_{lag}$. If $0.03 < t_{exp}/t_{lag} < 0.3$, then the average change in the concentration profile is less than 10% if $t_{TS} < 0.05\ t_{lag}$. The curves for small values of t_{exp}/t_{lag} have inflection points because for exposures that end prior to steady state, $(C_n^o - C_n)$ can be <0 for interior TSs, but for steady-state exposures, $(C_n^o - C_n)$ is always > 0.

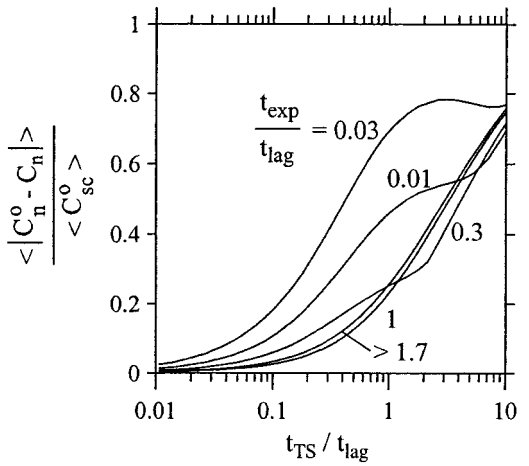

Fig. 5. Normalized $\langle|C_n^o - C_n|\rangle$ as a function of t_{TS}/t_{lag} for several values of t_{exp}/t_{lag}.

As shown in Fig. 6, diffusion during the TS procedure will reduce the total mass of chemical in the sc measured by tape stripping (i.e., $M_{TS}/M^o < 1$). For any value of t_{TS}/t_{lag}, the effect of t_{exp} on M_{TS}/M^o is < 15%. As long as $t_{TS} < 0.3\ t_{lag}$, $M_{TS} > 0.9\ M^o$ for any t_{exp}. If t_{TS} and t_{lag} were equal, as they might have been for chloroform in the TS measurements by Islam *et al.* (6), then the amount of chloroform measured by tape stripping could have been 10–25% smaller than the amount in the sc at the end of the exposure, even if no chloroform were lost from the TSs by evaporation.

Estimating Dermal Absorption Parameters

The parameters $K_{sc/v}$ and t_{lag} can be estimated by comparing theoretical and experimental TS concentrations. Values for L_{sc} are needed to locate x within the sc (i.e., to specify x/L_{sc}) and to calculate the permeability coefficient, $P_{sc,v}$, from estimates of $K_{sc/v}$ and t_{lag}. Often in estimating x / L_{sc}, it is assumed (as in Figs. 1 and 2) that the sc is completely removed by tape stripping and one uses Eq. (3) to calculate L_{sc} (4). Also, estimates of $P_{sc,v}$ have been calculated assuming that L_{sc} was 15 μm even when Eqs. (2) and (3) were used to calculate x/L_{sc} (4). A new method for estimating L_{sc} involves combining measurements of $m_{sc,n}$ and transepidermal water loss (9,12), but the method cannot be used at the exposed site if the skin is hydrated during the exposure as in the experiments described here.

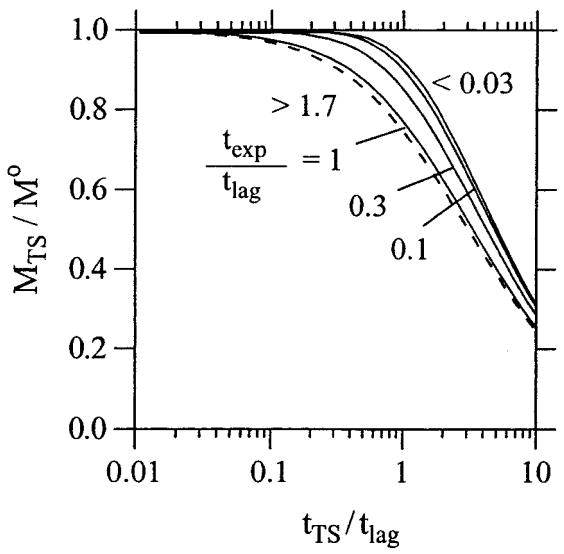

Fig. 6. M_{TS} / M^o as a function of t_{TS}/t_{lag} for several values of t_{exp}/t_{lag}.

If TS data were collected after steady state was established (i.e., $t_{exp} > 1.7\ t_{lag}$), then $K_{sc/v}$ but not t_{lag} could be estimated. As shown in Eq. (7), $K_{sc/v}$ can be calculated from steady-state TS data as follows:

$$K_{sc/v} = \frac{2}{L_{sc}\ A\ C_v^o} \sum_{i=1}^{N_{TS}} m_i \qquad (16)$$

Eq. (16) is only valid when the sc is the primary barrier for dermal absorption.

If TSs are collected rapidly, then diffusion during TS collection will be insignificant and the concentration profile represented by the TSs should fairly represent the concentration profile in the sc at t_{exp}. If the concentration profile was not at steady state (e.g., t_{exp} was less than t_{lag}, or t_{delay} after the exposure ended was ≠ 0), then both $K_{sc/v}$ and t_{lag} can be estimated by comparing C_n and x_n/L_{sc} with the appropriate theoretical equation (e.g., Eq. [4] or [11]). Often more than one pair of values for $K_{sc/v}$ and t_{lag} will provide an acceptable fit of unsteady TS data. This difficulty is avoided if $K_{sc/v}$ is known from a steady-state experiment, and then t_{lag} can be estimated from the unsteady-state data. However, steady-state measurements are not always possible, particularly for chemicals with large t_{lag}.

Equations developed assuming that the sc is the controlling mass transfer resistance (e.g., Eq. [4] or [11]), can be used for short exposures to highly lipophilic chemicals (for which the ve contributes a significant resistance), because when $t_{exp} < t_{lag}$, the ve has not affected C_{sc}. Although Eq. (16) is not valid for extremely lipophilic chemicals, it is possible to calculate $K_{sc/v}$ from steady-state data when the ve is also a significant barrier to transport (13).

Data Analysis

The data shown in Fig. 1 were analyzed in two ways, and the results are summarized in Table 1. In the first analysis, data from both the rapid and slow TS experiments were used. After a 1 h exposure, the 4CP concentration in TSs that were removed rapidly vary linearly with position in the sc, suggesting that 1 h was long enough to establish steady state. After estimating the value of $K_{sc/v}$ to be 9.1 using Eq. (16), the t_{lag} (= 40 min) was determined by regressing the mathematical model calculations that included t_{TS} (i.e., the numerical solution of Eqs. [8] , [10] , and [13] combined with Eq. [A3]) to the slow TS data. The solid curves in Fig. 1 represent model calculations based on these values of $K_{sc/v}$ and t_{lag}, which are consistent with TS experimental results reported elsewhere (4).

Notably, for the fast TS experiment, $t_{TS}/t_{lag} \simeq$ (6 min/40 min) = 0.15, which meets the criteria developed from Fig. 5 for neglecting diffusion in the TS results. By comparison, $t_{TS}/t_{lag} \simeq 1.5$ for the slow TS data, indicating that diffusion during the TS procedure would be significant. Furthermore, although t_{exp}/t_{lag} = 60 min/40 min = 1.5 is almost enough

time to establish steady state, the concentration profile from the slow TS experiment does not look linear.

If only the slow TS data in Fig. 1 had been collected, then there would be no evidence that chemical had diffused during the tape stripping procedure. If Eq. (4) was regressed to only the slow TS data, then $K_{sc/v}$ and t_{lag} are calculated to be 6.1 and 95 min, respectively (Table 1). Compared with the data analysis using both fast and slow TS data, the resulting estimate for the permeability coefficient is 70% smaller (i.e., 5.9×10^{-4} compared with 19×10^{-4} cm/h). Although these calculations, shown as the dashed curve in Fig. 1, appear reasonable, the calculation $t_{TS}/t_{lag} = 60$ min/95 min $= 0.6$ indicates that diffusion probably affected the TS results. Notably, if t_{TS} is greater than the actual value of t_{lag}, the apparent t_{lag} will be overestimated, and $K_{sc/v}$ and $P_{sc,v}$ will be underestimated, consistently.

The Type 2 experiments (Fig. 2) were analyzed assuming that $t_{TS} < 6$ min was fast enough that diffusion was insignificant during tape stripping. After Eq. 16 was used to calculate $K_{sc/v}$ with the TS data collected immediately at t_{exp}, t_{lag} was determined by regressing Eq. (11) to the TS data collected 1 h after t_{exp}. Model calculations are consistent with the Type 1 experiment analysis that included diffusion during the TS procedure (Table 1). For the Type 2 experiments, $t_{TS} = 6$ min was fast enough that diffusion should not have affected the data and $t_{exp} = 1$ h was long enough for steady state (i.e., $t_{TS}/t_{lag} < 0.2$ and $t_{exp}/t_{lag} > 1.7$).

During the clearance time after an exposure ends, C_{sc} decreases, thereby reducing the driving force for diffusion. Consequently, TS data after a delay are less affected by diffusion than data collected immediately at the end of the exposure. Thus, the criteria that $t_{TS} < 0.2\ t_{lag}$ if $t_{exp} > 0.3\ t_{lag}$ and $t_{TS} < 0.05\ t_{lag}$ if $0.03\ t_{lag} < t_{exp} < 0.3\ t_{lag}$ are adequate for analyzing data after a period of clearance.

RECOMMENDATIONS

If TS data are collected immediately at t_{exp}, experiments should be conducted at two different t_{exp}. At least one of these must be an unsteady-state experiment in which $0.06 \leq t_{exp}/t_{lag} \leq 0.6$. The concentration profile is insensitive to t_{lag} when $t_{exp} > t_{lag}$, but when $t_{exp} < 0.06\ t_{lag}$, the concentration profile is too sensitive to t_{lag} and small changes in TS data would produce large variations in estimates of t_{lag}. If possible, the second experiment should be long enough to establish steady state (i.e., $t_{exp} > 1.7\ t_{lag}$). If $t_{TS} < 0.2\ t_{lag}$ for $t_{exp} > 0.3\ t_{lag}$, then diffusion during the TS procedure should not significantly affect TS concentrations. During short exposures relative to t_{lag}, the driving force for mass transfer is greater and chemical movement into the sc is faster. As a result, for $0.003 < t_{exp}/t_{lag} < 0.3$, trs need to be < approximately $0.05\ t_{lag}$.

An alternative strategy is to conduct two TS experiments, one with and one without a delay after t_{exp}. For the simplest data analysis, t_{exp} should be selected to establish steady state. The delay time after the exposure has ended should be long enough for the concentration profile to change appreciably (t_{delay} greater than about $0.3\ t_{lag}$), but not so long that the analytical errors become significant relative to C_n. If $t_{TS} < 0.2\ t_{lag}$ when $t_{exp} > 0.3\ t_{lag}$, TS results should not be affected by t_{TS}.

The data analysis requires that $P_{sc,v}$ be the same for the long and short exposures or at the end of the exposure and after the delay. For vehicles that significantly alter the skin (e.g., hydration of the sc by an aqueous solution), the experimental procedure should be designed to ensure that the skin condition is the same for all experiments used in the data analysis (e.g., by prehydrating).

NOTATION

A	surface area of chemical exposure (area being tape stripped)
C_n	average concentration of the absorbing chemical in the nth TS
C_n^o	average concentration of the absorbing chemical in the nth TS at $t = t_{exp}$
C_{sc}	concentration of absorbing chemical in the sc as a function of position and time
$\langle C_{sc}^o \rangle$	position-averaged concentration of absorbing chemical in the sc at $t = t_{exp}$
C_v^o	initial concentration of the absorbing chemical in the vehicle
D_{sc}	effective diffusion coefficient of the absorbing chemical in the sc
$K_{sc/v}$	partition coefficient of the absorbing chemical between the sc and the vehicle
L_{sc}	effective thickness of the sc
M^o	mass of absorbing chemical in the sc at $t = t_{exp}$
m_n	mass of absorbing chemical in the nth T_S
M_{sc}	total amount of sc removed by all TSs
$m_{sc,n}$	mass of sc removed in the nth tape strip
M_{TS}	mass of absorbing chemical in the sc as measured by tape stripping
N	number of nodes in the sc for the finite-difference solution
N_{TS}	number of TSs used to completely remove the sc by tape stripping
$P_{sc,v}$	permeability coefficient of chemical through the sc from the vehicle
sc	stratum corneum
t	time
t_{delay}	period of delay before the sc is tape stripped
t_{exp}	duration of the exposure
t_{lag}	lag time for chemical penetrating through the sc, $L_{sc}^2/(6D_{sc})$
t_{TS}	period of time required to completely remove the sc by tape stripping
u[t-a]	unit step function defined as 0 for $t \leq a$ and 1 for $t > a$
ve	viable epidermis
x	position in the sc
x_n	location of the center of the nth TS
x_{TS}	location of the outside edge of the sc which moves inward as TSs are removed
ρ_{sc}	density of the sc

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APPENDIX

Equations (8), (10), and (13) were solved by representing the sc with N nodes equally spaced in x and approximating spatial derivatives using finite difference formulas:

$$\frac{\partial^2 C_{sc,i}}{\partial x^2} = \frac{C_{sc,i-1} - 2C_{sc,i} + C_{sc,i+1}}{(\Delta x)^2} \quad \text{(A1)}$$

$$\left.\frac{\partial C_{sc,i}}{\partial x}\right|_{x=x_{TS}} = \frac{-3C_{sc,i} + 4C_{sc,i+1} - C_{sc,i+2}}{2\Delta x} \quad \text{(A2)}$$

where i is the node number from 1 to N, and $\Delta x = L_{sc}/(N - 1)$. The resulting equations were solved with a Fortran program and the IVPAG routine from the IMSL library. N was either 121 or 301 nodes corresponding to either 5 or 11 nodes for each TS if $N_{TS} = 30$. Stability of the numerical solution was confirmed by determining that increasing N did not change results and by requiring that the sum of M_{TS} and the mass that diffused through the sc was within 0.4% of M^o.

C_n was calculated by averaging C_{sc} using Simpson's rule as follows:

$$C_n = \frac{N_{TS}}{L_{sc}} \int_{(n-1)L_{sc}/N_{TS}}^{n\,L_{sc}/N_{TS}} C_{sc}\,dx \quad \text{(A3)}$$

Equation (A3) was derived assuming the same amount of sc is removed with every TS, which does not occur experimentally. To use C_n predicted by Eq. (A3) to analyze data, values of concentrations between TSs that corresponded to the x_n/L_{sc} position of the data were interpolated.

REFERENCES

1. A. L. Stinchcomb, F. Pirot, G. D. Touraille, A. L. Bunge, and R. H. Guy. Chemical uptake into human stratum corneum *in vivo* from volatile and nonvolatile solvents. *Pharm. Res.* **16**:1288–1293 (1999).
2. F. P. Schwarb, B. Gabard, T. Rufli, and C. Surber. Percutaneous absorption of salicylic acid in man after topical administration of three different formulations. *Dermatology* **198**:44–51 (1999).
3. K. Tojo and A.-R. C. Lee. A method for predicting steady-state rate of skin penetration *in vivo*. *J. Invest. Dermatol.* **92**:105–108 (1989).
4. F. Pirot, Y. N. Kalia, A. L. Stinchcomb, G. Keating, A. L. Bunge, and R. H. Guy. Characterization of the permeability barrier of human skin *in vivo*. *Proc. Nat. Acad. Sci. USA* **94**:1562–1567 (1997).
5. V. P. Shah, G. L. Flynn, A. Yacobi, H. I. Maibach, C. Bon, N. M. Fleischer, T. J. Frantz, S. A. Kaplan, J. Kawamoto, L. J. Lesko, J. P. Marty, L. K. Pershing, H. Schaefer, J. A. Sequeira, S. P. Shrivastava, J. Wilkin and R. L. Williams. Bioequivalence of topical dermatological dosage forms—methods of evaluation of bioequivalence. *Pharm. Res.* **15**:167–171 (1998).
6. M. S. Islam, L. Zhao, J. N. McDougal, and G. L. Flynn. Uptake of chloroform by skin on brief exposures to the neat liquid. *Am. Ind. Hyg. Assoc. J.* **60**:5–15 (1999).
7. N. Higo, A. Naik, D. B. Bommannan, R. O. Potts, and R. H. Guy. Validation of reflectance infrared spectroscopy as a quantitative method to measure percutaneous absorption *in vivo*. *Pharm. Res.* **10**:1500–1506 (1993).
8. H. Weigmann, J. Lademann, H. Meffert, H. Schaefer, and W. Sterry. Determination of the horny layer profile by tape stripping in combination with optical spectroscopy in the visible range as a prerequisite to quantify percutaneous absorption. *Skin Pharmacol. Appl. Skin Physiol.* **12**:34–45 (1999).
9. F. Pirot, E. Berardesca, Y. N. Kalia, M. Singh, H. I. Maibach, and R. H. Guy. Stratum corneum thickness and apparent water diffusivity: facile and noninvasive quantitation *in vivo*. *Pharm. Res.* **15**:492–494 (1998).
10. R. J. Scheuplein and R. L. Bronaugh. Percutaneous absorption. In L. A. Goldsmith (ed), *Biochemistry and Physiology of the Skin*, vol. II, Oxford University Press, New York, 1983, pp. 1255–1295.
11. J. Crank. *The Mathematics of Diffusio*n, 2nd ed., Clarendon Press, Oxford, United Kingdom, 1975.
12. Y. N. Kalia, F. Pirot, and R. H. Guy. Homogenous transport in a heterogeneous membrane: water diffusion across human stratum corneum *in vivo*. *Biophys. J.* **71**:2692–2700 (1996).
13. M. B. Reddy. Examining Issues in Percutaneous Transport Using Mathematical Models, PhD thesis, Colorado School of Mines, 2000.