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Application of kinetic model to in vitro percutaneous permeation of drugs

Jaymin C. Shah[1]

Department of Pharmaceutical Sciences, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425, USA

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Abstract

Kinetic model using inter-compartmental rate constants, for permeation of drug across stratum corneum (k_1), for permeation across viable tissue layer of epidermis (k_2), and for back transfer of drug from viable tissue layer into the stratum corneum (k_3), was applied to in vitro percutaneous permeation process. Permeation data of four drugs, propranolol (PR), triamcinolone acetonide (TA), physostigmine (PHY), and tetrahydroaminoacridine (THA), previously studied across two skin membranes, hairless mouse skin (HMS) and human cadaver skin (HCS) were fitted to the permeation equation based on the kinetic model to obtain optimized values of the rate constants. The permeation profiles were also analyzed by the lag time method to estimate steady state flux (J_{ss}), lag time (T_{lag}), diffusion coefficient (D) and Skin/donor-phase partition coefficient (Km). The D and Km were used to regenerate the entire permeation profile (pre-steady and steady states) using equation based on Fick's laws of diffusion. The kinetic and diffusion models were compared by fits of the observed data to the model predicted values by linear regression, and obtaining R^2 and sum of squared deviations (SSD). Permeation data for all drugs across HMS or HCS, with or without the presence of permeation enhancer, were described very well by the kinetic model ($R^2 > 0.99$), and the SSD's were smaller than that for the diffusion model except for THA. The rate of drug permeation across stratum corneum, k_0 (k_1 X Dose, amount of drug applied/cm^2 of skin in the donor phase) was similar to J_{ss} as expected from the kinetic model. Diffusion coefficient of drug in the viable tissue (D_{vt}) ranged from 0.003–0.23 X 10^{-7} cm^2/s, similar to diffusion coefficient of similar sized molecules in protein gel as would be expected. Also, D_{vt} was four orders of magnitude greater than D, the diffusion coefficient across stratum corneum, confirming stratum corneum to be the rate limiting barrier in the percutaneous permeation of the drugs studied. k_3 for all drugs were negligible indicating that no back diffusion of drug from viable tissue into the stratum corneum was occurring under the sink condition following infinite dose application. Also, except TA, none of the drug studied was lipophilic enough to be retained by the stratum corneum. Therefore, k_3 should not be used in the kinetic model for in vitro percutaneous permeation. For the PR 27-h, TA control and TA with 2% propylene glycol (PG) pretreatment experiments, the experiment duration was less than three T_{lag} and steady state was not achieved, resulting in inaccurate estimates of D and Km and, hence, the regenerated profiles based on diffusion model did not describe the data well. However, kinetic model described these data also very well and SSD's were significantly smaller ($P = 0.05$) than that for the diffusion model based regenerated profiles. The kinetic model is an alternative to diffusion model to describe the percutaneous permeation process, and the results presented here demonstrate the usefulness of the kinetic approach.

[1] Tel.: +803 792 5366; fax: +803 792 0759.

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1. Introduction

Percutaneous permeation has been treated as a diffusion process across a homogeneous membrane and equations have been derived based on Fick's laws of diffusion, to study the permeation of chemicals and drugs across skin (Crank, 1975; Barry, 1983; Durgard, 1983). The diffusion model describes the in vitro percutaneous permeation of drugs fairly well, despite the complex nature and structure of skin. Previously we illustrated with simulations that at least 3–4 lag times were required to achieve steady state diffusion across the membrane, and that the lag time method of data analysis results in significant errors in J_{ss} and T_{lag}, if the experiment had not been conducted for a long enough time period to achieve steady state (Shah, 1993). To confirm the theoretical findings, permeation data of four drugs, propranolol (PR), triamcinolone acetonide (TA), physostigmine (PHY) and tetrahydroaminoacridine (THA), across two model skin membranes, Hairless mouse skin (HMS) and Human cadaver skin (HCS), was obtained (Shah et al., 1994). The in vitro permeation profiles of the above mentioned drugs were evaluated by the lag time method, and the diffusion coefficient (D, cm^2/second) and the skin/donor phase partition coefficient (Km) so obtained were used to regenerate the entire permeation profile (both steady-state and non-steady state regions). The fit of the observed data to the regenerated curve indicated if D and Km were accurately estimated by the lag time method. It was observed again that, overall, the lag time method was successful in the estimation of D and Km, if steady state was achieved at the end of the permeation experiment. Kubota et al. also found that equation for the simple membrane model to estimate permeability coefficient and lag time was warranted, provided that permeation data collected for a sufficiently long time was used (Kubota and Twizell, 1992).

Treating skin as a simple homogeneous membrane enables easy treatment of permeation data using the lag time method to obtain steady-state flux (J_{ss}, $\mu g/cm^2/h$ or $DPM/cm^2/h$), the lag time (T_{lag}, h), D and Km with the above described limitations. However, the assumption of skin being a simple homogeneous membrane can be questioned due to its complex structure and nature. To overcome this limitation, a two-layer diffusion model with polar and non-polar routes in the stratum corneum was used recently for analysis of in vivo and in vitro permeation data (Yamashita et al., 1994). However, it is mathematically very complex, and its biophysical significance has not been thoroughly established. A more correct and rigorous approach would be to solve the differential equations based on Fick's second law using the appropriate boundary and initial conditions, and fitting the data to obtain relevant parameters (Crank, 1975). This is possible, however, it could be complex mathematically and may lose its biological relevance. An alternative approach is the treatment of the drug permeation or absorption across skin as a kinetic process. Guy et al. developed a pharmacokinetic model to describe absorption of various drugs across skin (Guy et al., 1985a; Guy and Hadgraft, 1985; Guy et al., 1985b). This model described the plasma concentration-time profiles following transdermal application of a number of drugs fairly well (Guy et al., 1987). However, this model has not been applied to describe the in vitro percutaneous permeation data of any drug. Nor has it been compared to the rigorous diffusion model for analysis of the permeation data.

The objective of this study was to evaluate the applicability of the kinetic model to the in vitro percutaneous permeation data and compare it to the diffusion model used earlier. The kinetic model used was essentially derived from the pharmacokinetic model developed by Guy et al. for transdermal drug delivery in their earlier work (Guy et al., 1985a). The in vitro permeation data of PR, TA, PHY and THA across two skin models, obtained using vertical franz diffusion cells with an infinite dose technique and analyzed earlier by the diffusion model (Shah et al., 1994), were used as described below in the theoretical section. The permeation data were analyzed by the kinetic model and the diffusion model and compared as described below.

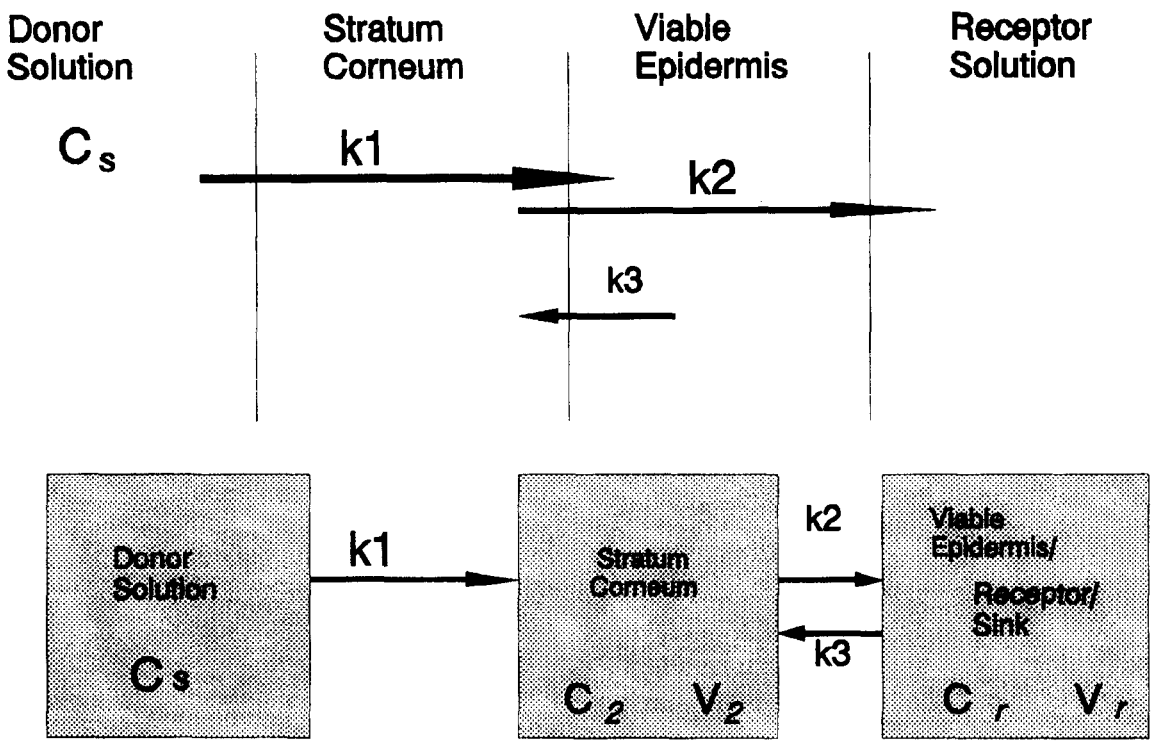

Fig. 1. Schematic figure depicting the kinetic model for in vitro percutaneous permeation. In figures 2–9, the regenerated curve (Eq. 5) is referred to as the diffusion model in the legend and compared to the kinetic model predicted curve (Eq. 1).

2. Theoretical treatment and data analysis

2.1. The kinetic model for percutaneous permeation

Based on the pharmacokinetic model proposed by Guy et al. (Guy et al., 1985a; Guy and Hadgraft, 1985; Guy et al., 1985b), a kinetic model for in vitro percutaneous permeation is proposed in direct analogy as shown in the schematic Fig. 1.

In the kinetic model, k_1 (/second) is the rate constant for diffusion of drug across the stratum corneum. Here the rate of diffusion across stratum corneum is assumed to be first order, however, in infinite dose situation, the rate of absorption or input would be effectively zero order given by $k_0 = k_1 \times$ Dose, the amount of drug applied per cm^2 of skin in the donor solution, which is constant. Therefore, k_0 ($\mu g/cm^2/h$ or $DPM/cm^2/h$) should approximate steady-state flux, J_{ss} ($J_{ss} = C_s(D/h)$, where h is the thickness of the membrane and C_s is the drug concentration in the donor solution).

k_2 (/second) is the first order rate constant for permeation of drug across the viable epidermis. k_3 (/second) is the first order rate constant for back diffusion of drug from viable epidermis into the stratum corneum and thus reflects the affinity of the drug for stratum corneum, and expected to be a function of the oil/water partition coefficient of the drug. k_3/k_2 has been shown to be a function of n-octanol/water partition coefficient (Guy et al., 1985a; Guy and Hadgraft, 1985; Guy et al., 1985b). V's and C's are the volumes and the concentrations of the drug in the respective compartments.

Rate equations were developed for concentrations in each compartment. The rate equations were solved using the laplace transform technique to obtain the equation for A (μg or DPM), the cumulative amount of drug permeated into the receptor. The details of the derivation is not provided however the final equation is as follows:

Table 1
Rate constants and permeation parameters of various drugs obtained by analyses of the profiles by the kinetic and diffusion models

Drug	Kinetic model					Diffusion model		
	k_0 DPM/cm^2/h	k_2 h^{-1}	k_3 h^{-1}	D_{vt} X 10^7 cm^2/s	[c]R^2	J_{ss}^b DPM/cm^2/hr	D^b X 10^{11} cm^2/s	Km
PR, 27 h	5.17 X 10^2 ± 6.22 X 10^2	0.028 ± 0.041	2.60 X 10^{-27} ± 3.59 X 10^{-16}	0.020	0.990	4.48 X 10^2	4.53	2.04
PR, 72 h	4.72 X 10^2 ± 3.86 X 10^2	0.008 ± 0.008	7.26 X 10^{-28} ± 2.37 X 10^{-17}	0.005	0.990	1.91 X 10^2	2.58	1.65
TA, Control	5.97 X 10^2 ± 0.68 X 10^2	0.371 ± 0.117	1.28 X 10^{-29} ± 1.35 X 10^{-18}	0.23	0.995	5.63 X 10^2	30.83	0.08
TA, 2% PG Pretreatment	8.24 X 10^3 ± 1.05 X 10^3	0.143 ± 0.03	7.36 X 10^{-28} ± 3.20 X 10^{-17}	0.089	0.999	6.39 X 10^3	18.88	1.53
TA, 2% AZ Pretreatment	1.47 X 10^5 ± 0.05 X 10^5	2.158 ± 1.10	3.39 X 10^{-27} ± 1.65 X 10^{-15}	1.35	0.997	1.44 X 10^5	255.55	2.56
TA, 2% AZ Copenetrant	2.20 X 10^5 ± 1.05 X 10^5	0.052 ± 0.03	1.52 X 10^{-28} ± 4.91 X 10^{-18}	0.033	0.997	0.82 X 10^5	18.88	19.71
PHY[a]	37.48 ± 27.80 μg/cm^2/h	0.004 ± 0.003	1.77 X 10^{-30} ± 4.44 X 10^{-20}	0.003	0.999	7.48 μg/cm^2/h	3.33	1.66
THA[a]	45.55 ± 3.52 μg/cm^2/h	0.063 ± 0.012	2.60 X 10^{-15} ± 4.06 X 10^{-4}	0.039	0.999	42.31 μg/cm^2/h	6.14	2.63

[a] These studies used human cadaver skin, other studies used hairless mouse skin.
[b]J_{ss} was obtained using the lag time method (Eq. 2). D was obtained from Tlag using Eq. 4 and taking the thickness of stratum corneum to be 40 μm (Scheuplein, 1967, Higuchi and Yu, 1987; Shah et al., 1994).
[c]All rate constants reported as mean ± S.D. with $n = 3$, and R^2 is for the fit of the observed data to the kinetic model.
D_{vt} was calculated from k_2 using the eq.: $D_{vt} = k_2$ X $(h_{vt})^2$; Where h_{vt} is the thickness of the viable tissue and taken to be approximately 150 μm (Guy et al., 1985a; Guy et al., 1985b).

$$A = \frac{k_0 k_2 t}{(k_2 + k_3)} + \frac{k_0 k_2 [e^{(-(k_2 + k_3)t)} - 1]}{(k_2 + k_3)^2} \quad (1)$$

Using Eq. 1, the permeation profile can be constructed or simulated with various values of k_0, k_2 and k_3. Similarly, the observed permeation profile can be fitted to Eq. 1 using a nonlinear regression program to obtain the best fit and the optimized values of the rate constants, k_0, k_2 and k_3.

The permeation profiles of all drugs studied were fitted to Eq. 1 using a nonlinear regression algorithm and the optimized values of the rate constants so obtained were listed in Table 1. The fit of the curve predicted by the kinetic model to the data was compared to the regenerated curve based on the diffusion model in Figs. 2–9. The R^2 and the sum of squared differences (SSD) for the kinetic models were also calculated, and compared to that obtained for analysis of data by the diffusion model as seen in Fig. 10. Analysis of residuals was conducted to determine if the residuals were scattered randomly about zero as estimated by serial correlations using MINSQ (Micromath Inc., Utah). Also, the residual plots were constructed with time, and the observed values as independent variables, and evaluated visually for any systematic trend.

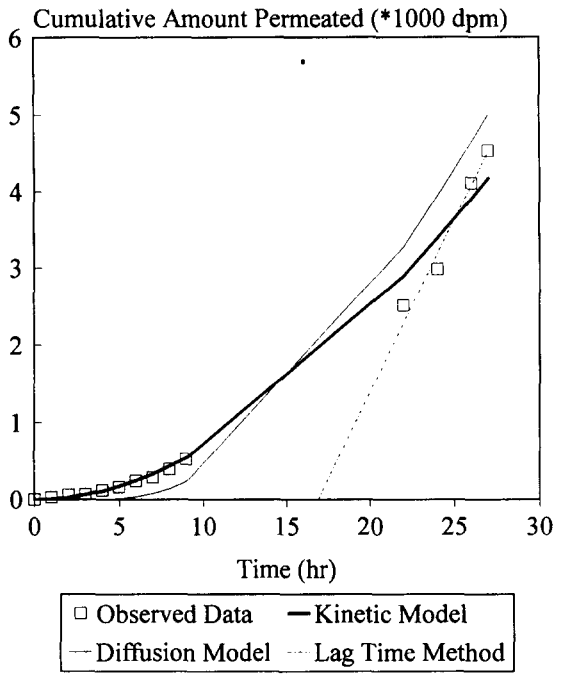

Fig. 2. Propranolol permeation profile (27-h experiment) across hairless mouse skin (n = 4).

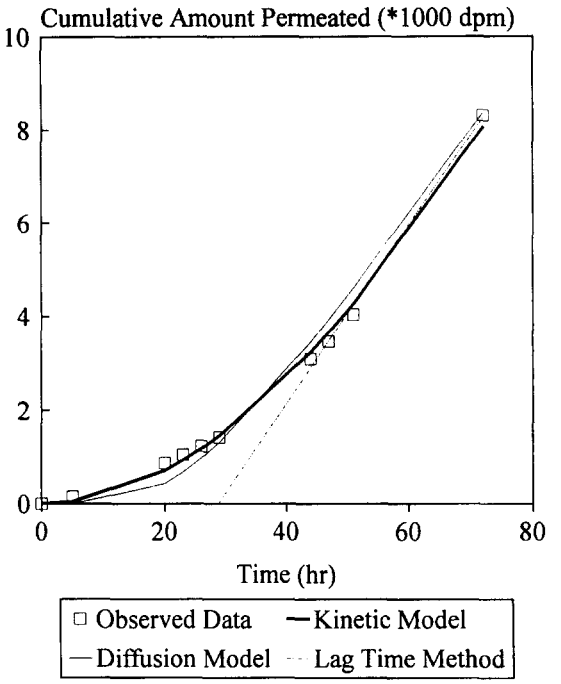

Fig. 3. Propranolol permeation profile (72-h experiment) across hairless mouse skin (n = 4).

2.2. The diffusion model; the lag time method

This data treatment is described in detail in the earlier publications (Shah, 1993; Shah et al., 1994). The apparent terminal linear (steady state) portion of the observed permeation profile was fitted to the following equation:

$$A = J_{ss}(t - T_{lag}) \quad (2)$$

Where,

$$J_{ss} = \frac{DKmCs}{h} \quad (3)$$

and

$$T_{lag} = \frac{h^2}{6D} \quad (4)$$

D was calculated from T_{lag}, taking the value of thickness of fully hydrated stratum corneum to be 40 μm (Scheuplein, 1967; Bronaugh and Maibach, 1983; Higuchi and Yu, 1987). From the values of J_{ss}, D, h, and the drug concentration in donor solution (Cs), Km was calculated. The estimated parameters were listed in Table 1.

To determine if J_{ss} and T_{lag} were accurate and reflected the true permeation parameters (D and Km) of the respective drugs, D and Km obtained from J_{ss} and T_{lag} were used in Eq. 5 to regenerate the complete permeation profile (pre-steady and steady states). The cumulative amount of drug permeated (A) at various sampling time period was calculated numerically using D, Km, Cs and h in Eq. 5. An equation has been obtained previously for permeation of diffusant across a membrane of finte thickness (Crank, 1975). Similarly, the following equation was obtained by Laplace transform technique on the appropriate differential equation based on Fick's laws of diffusion, using the computer program, LAPLACE (Micromath Inc., Utah):

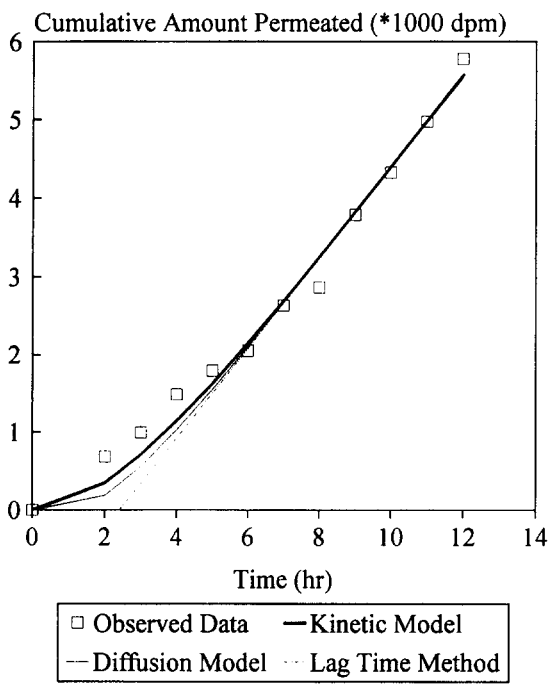

Fig. 4. Triamcinolone Acetonide permeation profile (Control) across hairless mouse skin (n = 6).

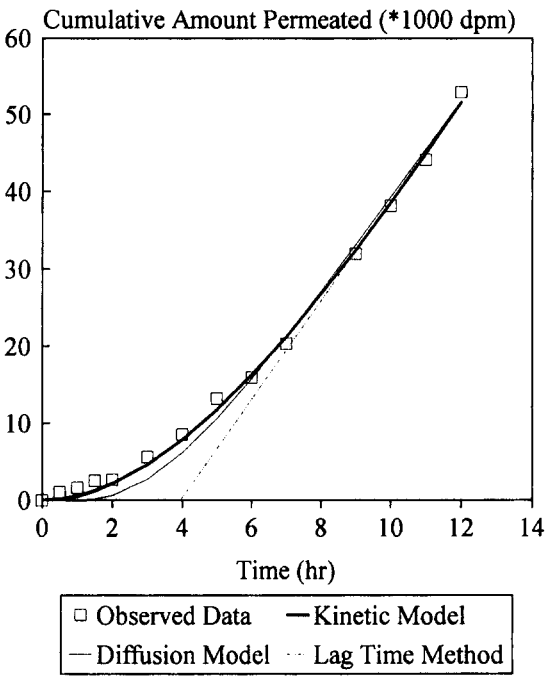

Fig. 5. Effect of 2% PG pretreatment on triamcinolone acetonide permeation across hairless mouse skin (n = 2).

$$A = \frac{DKmCs}{h}\left(t - \frac{h^2}{6D}\right) - \frac{2hKmCs}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}e^{\left(-\frac{Dn^2\pi^2t}{h^2}\right)} \quad (5)$$

The regenerated permeation profiles were plotted and compared to the observed data for all the four drugs (Figs. 2–9). In Figs. 2–9, the regenerated curve (Eq. 5) is referred to as the diffusion model in the legend and compared to the kinetic model predicted curve (Eq. 1). To determine how closely the regenerated permeation profile correlate to the observed permeation profile, linear regression was conducted with observed values as the independent variable as for the kinetic model. The R^2 and SSD between the observed and the regenerated amounts of drug were calculated for each profile (Fig. 10), and compared to that obtained for the kinetic model. The residuals were evaluated in a similar fashion as for the kinetic model. If true steady state was achieved at the end of the experiment, the lag time method would result in fairly accurate estimates of D and Km.

Hence, the regenerated profile from these estimated values of D and Km should match with the observed permeation profile.

3. Results and discussion

We have used a kinetic model based on a compartmental model developed earlier for percutaneous absorption of drugs (Guy et al., 1985a). The model uses inter-compartmental transfer rate constants with some physico-chemical basis to describe movement of drug across skin. The permeation data of four drugs across two skin models was fitted to Eq. 1, the analytical solution for the cumulative amount permeated to obtain rate constants listed in Table 1. The fit of the data for all the drugs across HMS or HCS, studied under various conditions, to the curve based on kinetic model was superior to that of the regenerated curve based on diffusion model (Eq. 5) as seen in Figs. 2–9. The kinetic model based curves (Eq. 1)

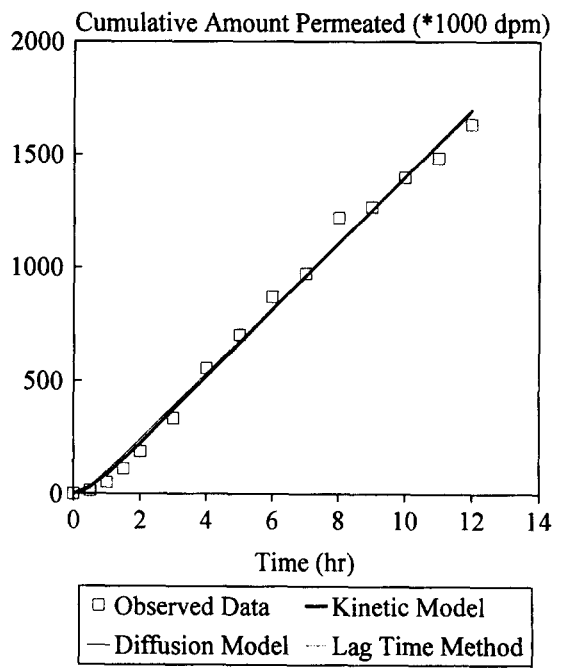

Fig. 6. Effect of 2% Azone (AZ) pretreatment on triamcinolone acetonide permeation across hairless mouse skin (n = 3).

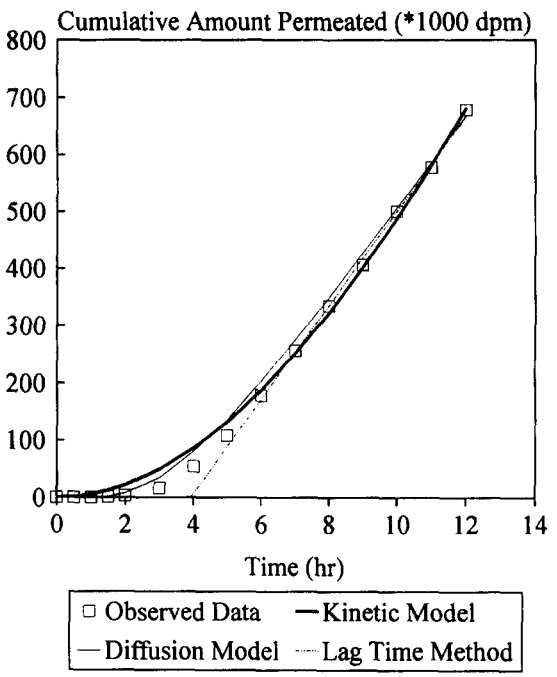

Fig. 7. Effect of 2% Azone (AZ) copenetration on triamcinolone acetonide permeation across hairless mouse skin (n = 6).

in Figs. 2–9 described the observed permeation profiles better than the curves regenerated using Eq. 5, derived using application of Fick's laws of diffusion to percutaneous permeation. The R^2 for fit of the observed data to the kinetic model Eq. 1 was greater than 0.99 for all the profiles (Table 1). The SSD's for the kinetic model based curve fits to the permeation data were smaller (statistically significant at (P = 0.05) than that for the regenerated curves for all the profiles except for THA as seen in Fig. 10. Both R^2 and SSD indicated that kinetic model predicted the curve described, and fitted the observed data better than the diffusion model-based regenerated curve (Fig. 10).

The value of k_0, the zero order stratum corneum transfer rate matched closely with the J_{ss} value obtained by the lag time method as expected (Table 1). Therefore, k_0, the zero order transfer rate constant in the kinetic model is equivalent to steady state flux in the diffusion model. This should be expected since both define the rate at which drug permeates across stratum corneum at

steady state, in the respective models. k_2, the first order rate constant for permeation of drug across the viable epidermis, were in the range of 0.1–59.9 X 10^{-5} /second. These values are in the same range as reported k_2 for testosterone, hydrocortisone and benzoic acid (Guy and Hadgraft, 1985). D_{vt} (cm^2/second), the diffusion coefficient of drug in the viable tissue was calculated from k_2 using the eq.: $D_{vt} = k_2 \text{ X } (h_{vt})^2$, where h_{vt} is the thickness of the viable tissue and taken to be approximately 150 μm (Guy et al., 1985a; Guy et al., 1985b). As seen in Table 1, D_{vt} ranged from 0.003–1.35 X 10^{-7} cm^2/second. The magnitude of these values supports the assumption that k_2 reflects the rate constant for transport across the viable epidermis layer, since, Scheuplein found diffusion coefficient across epidermis in the range of 10^{-6}–10^{-7} cm^2/second (Scheuplein, 1967). Guy et al. considered diffusion across viable tissue equivalent to diffusion across a protein gel, and found D_{vt} to be about 10^{-7} cm^2/second for benzoic acid and testosterone (Guy et al., 1985a). The

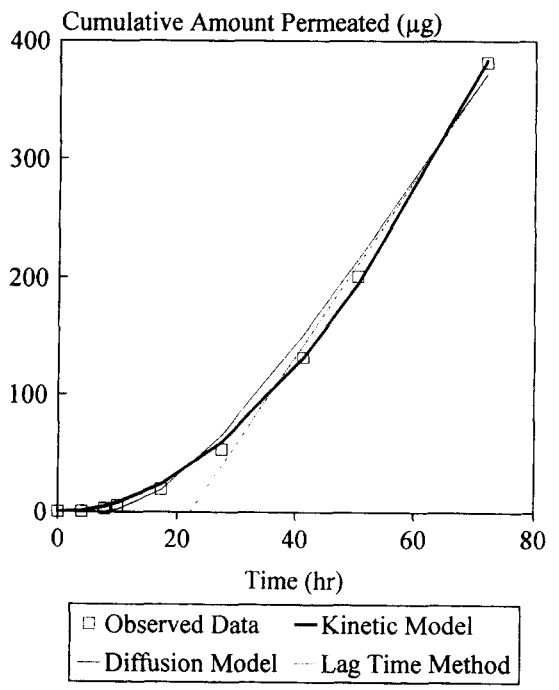

Fig. 8. Physostigmine permeation profile across human cadaver skin (n = 5).

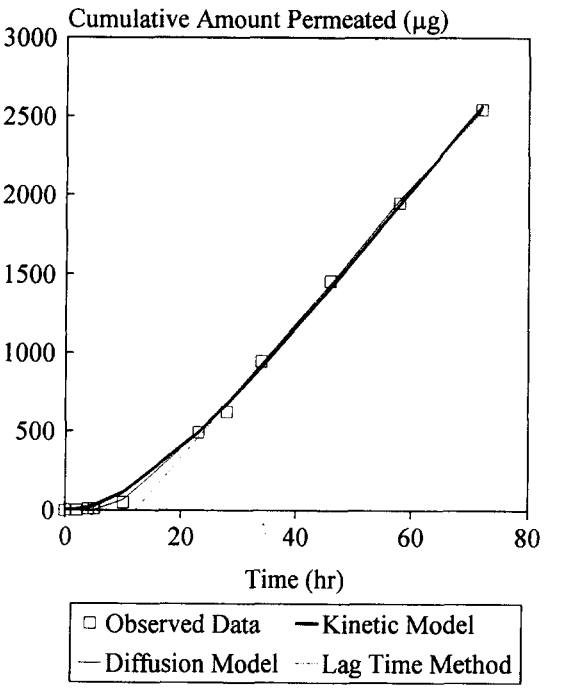

Fig. 9. Tetrahydroaminoacridine permeation profile across human cadaver skin (n = 7).

diffusion coefficient of KCl across a protein gel is 6 X 10^{-7} cm^2/second (Cussler, 1991). Thus, the observed D_{vt} for the drugs studied are of the same magnitude as the diffusion coefficients for similarly sized permeants across protein gel and viable epidermis. However, the wide range of the D_{vt} observed suggest that to consider diffusion of permeant across viable epidermis as diffusion across protein gel solely dependent on penetrant size is a very rough approximation. K_2 involves the partitioning of the drug between stratum corneum and the viable epidermis and, therefore, drug lipophilicity will influence K_2 and hence D_{vt}. It is also well known that many drugs and chemicals interact specifically with skin to result in altered diffusional resistance. Therefore, it is simplistic to assume that D_{vt} reflects diffusion of drug across protein gel and is solely dependent on its molecular size. D_{vt} will depend on the lipophilicity and the molecular size of the drug and on any specific interaction of drug with the viable epidermis. This may explain partially the large range of D_{vt} values observed for drugs with varying physic-

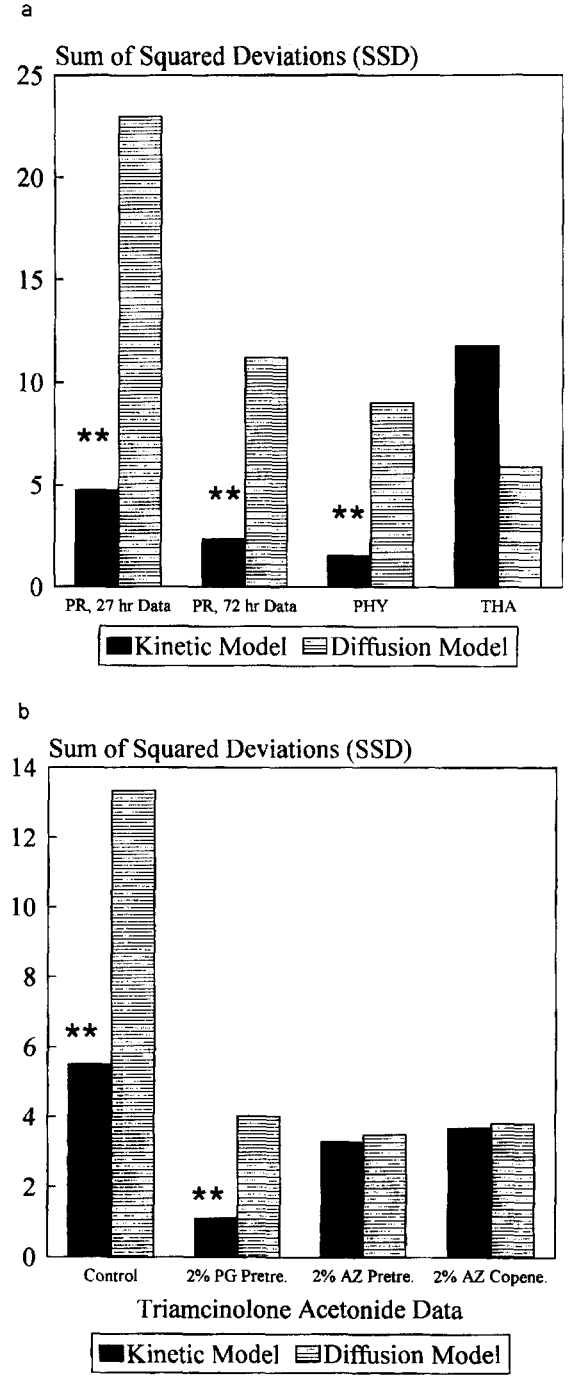

Fig. 10. Comparison of the fits of the kinetic model based curve (Eq. 1) and the regenerated curve (Eq. 5) based on diffusion model, as evaluated by SSD of (a) PR, PHY and THA permeation data and (b) TA permeation data. ** Significantly better fit of the kinetic model predicted curve to the observed data by F test at $P = 0.05$. The SSDs were plotted and compared after normalizing with their exponential terms.

ochemical characteristics with and without permeation enhancers.

D was also calculated from T_{lag} taking the thickness of hydrated stratum corneum to be 40 μm. Scheuplein reported the thickness of hydrated and unhydrated human stratum corneum to be 38 μm and 13 μm, respectively (Scheuplein, 1967). Higuchi and Yu used 40 μm as the thickness of hairless mouse stratum corneum in their work with Ara-A prodrugs (Higuchi and Yu, 1987). The thickness of unhydrated human and hairless mouse stratum corneum sections were reported to be 16.8 μm and 8.9 μm, respectively (Bronaugh and Maibach, 1983). Considering that stratum corneum has enormous capacity to hydrate and swell, the thickness of hydrated stratum corneum could easily be 40 μm. Therefore, the calculated D value thus reflects the diffusion coefficient of drug across hydrated stratum corneum of HMS or HCS. The observed D values were indeed much smaller than D_{vt} by 3–4 orders of magnitude as seen in Table 1. D should be smaller than D_{vt} since, diffusion across stratum corneum is considerably slower than that through viable epidermis for majority of drugs and, hence, stratum corneum is considered to be the permeation rate-limiting barrier (Scheuplein, 1967; Barry, 1983; Durgard, 1983).

k_3 values were very small (10^{-15} to 10^{-30} /second) in absolute terms, and also much smaller in comparison to other rate constants, $k_0(k_1)$ and k_2 as seen from Table 1. k_3 was proposed to be a rate constant for the back-transfer of diffusant from viable epidermis to stratum corneum. Hence, k_3 is a measure of the affinity of the drug for stratum corneum and that ratio k_3/k_2 should be a direct function of the octanol/water partition coefficient of the permeant. In our results, we find that for all the drugs studied under various conditions, the values of k_3 were almost negligible compared to that of k_2, and no correlations appear to exist between k_3 or k_3/k_2 and the lipophilicity of the permeant. These findings are very unlike the results obtained by Guy et al. (Guy et al., 1985a) for various permeant, where a rank-order correlation was observed between the ratio of k_3/k_2 and the octanol/water partition coefficient. However, they had obtained k_3 values from application of the analogous pharmacoki-

netic model to the plasma concentration time profile of the drugs following transdermal application.

In this study, we applied the kinetic model to in vitro permeation data obtained using franz diffusion cell following application of infinite dose and maintaining sink conditions in the receptor compartment. The concentrations in the receptor solution were very low in comparison to donor concentration throughout the duration of the experiment. This suggests that the sink condition, and the persistently high concentration gradient from SC to the dermis precludes possibility of back diffusion of drug into the stratum corneum from the underlying viable epidermis. If we assume that there is no back diffusion of drug from viable epidermis into the stratum corneum, k_3 can be eliminated from the model, i.e. k_3 is zero or negligible in comparison to other rate constants. In the above scenario, the kinetic model is not altered significantly, and Eq. 1 will become:

$$A = k_0 t + \frac{k_0[e^{(-k_2 t)} - 1]}{k_2} \quad (6)$$

The above equation can be further simplified to,

$$A = k_0\left[t - \frac{1}{k_2}\right] + k_0[e^{(-k_2 t)}] \quad (7)$$

At long time intervals, post-steady state, the exponential term will become negligible and Eq. 7 will be similar to Eq. 2, used for the lag time method. Thus, comparing Eq. 2 and Eq. 7 at longer times, $1/k_2$ may approximate lag time, and as seen earlier k_0 does approximate J_{ss}, the steady state flux. From the values of k_3, it appears to be of no significance for the drugs studied or of consequence to their permeation. Secondly, the elimination of k_3 results in equations similar to those for the diffusion model. Therefore, as suggested earlier, there may not be significant back diffusion of the drugs studied, into the stratum corneum in the in vitro permeation experiments. None of the drug studied here except TA, is very lipophilic, however, for absorption of very lipophilic drugs across skin, k_3 could be significant. Even without k_3, the kinetic model for percutaneous permeation can describe the data well and thus low values of k_3 does not invalidate nor reduce the applicability of the kinetic model.

Previously, we have described in detail the application of the diffusion model via the lag time method to in vitro percutaneous permeation data (Shah, 1993; Shah et al., 1994). Overall the lag time method was successful in the estimation of permeation parameters, D and Km from which the entire profile could be regenerated for all the drugs studied (Figs. 2–9). However, the lag time method did result in inaccurate estimates of D and Km if steady state was not achieved as in the case of PR 27h (Fig. 2), TA control (Fig. 4), and TA with 2% PG pretreatment (Fig. 5). Regenerated profiles in these three cases did not correlate well with the observed data. This was due to the fact that steady state may not have been achieved at the end of the experiment, thus, the lag time method resulted in values of D and Km with higher errors as demonstrated earlier (Shah, 1993). However, the kinetic model described even these profiles very well (Figs. 2 and 4 and 5), with significantly lower SSD's (Fig. 10), and reliable values for k_0, k_2, and D_{vt}. Therefore, the kinetic model is able to describe the permeation data well and obtain reliable rate constants, even when steady state diffusion of drug may not have been achieved during the experiment.

The kinetic model described all permeation data better than the diffusion model as seen from the fit of the curves to the data and the values of R^2 and SSDs in Fig. 10. Even when lag time method resulted in errors in D and Km (PR 27h, TA control and TA with 2% PG pretreatment), the kinetic model described the data very well (Figs. 2–4 and 10). Using kinetic model, k_0 (J_{ss}), and D_{vt}, the diffusion coefficient in the viable tissue could be obtained, in addition to D and Km even when steady state of diffusion may not have been achieved, indicating the strengths of the kinetic model. The inherent limitation of the kinetic model is to treat stratum corneum and the viable epidermis as well strirred compartments (uniform drug concentration), which is incorrect since it is a known fact that concentration gradient develops across strtum corneum at steady state. The results presented here attempted to demonstrate the applicability, and evaluate the

usefulness of the kinetic model for in vitro percutaneous permeation. These results in no way invalidate the diffusion model so widely used for description of percutaneous permeation process. From a mechanistic point, diffusion model using the Fick's two laws of diffusion and using the correct analytical solution thereof may be the most appropriate model to describe permeation of drugs through skin. However, the kinetic model is an alternative approach at describing the same process in an easily understood manner.

References

Barry, B.W., *Dermatological Formulations*, Marcel Dekker, New York, 1983, pp. 49–94.

Bronaugh, R.L. and Maibach, H.I., *Dermatotoxicology*. In Marzulli, F.N. and Maibach, H.I. (Eds.), 2nd edition, Hemisphere Publishing Corp., New York, 1983, pp. 117–146.

Crank, J., *The Mathematics of Diffusion*, Oxford University, London, 1975, pp. 42–61.

Cussler, E.L., *Diffusion, Mass Transfer in Fluid Systems*, Cambridge University Press, New York, 1991, pp. 172–193.

Durgard, P.H., *Dermatotoxicology* Marzulli, F.N., and Maibach, H.I. (Eds.), 2nd edition, Hemisphere Publishing Corp., New York, 1983, pp. 91–116.

Guy, R.H., Hadgraft, J. and Maibach, H.I., A Pharmacokinetic Model for Percutaneous Absorption. *Int. J. Pharm.*, 11 (1985a) 119–129.

Guy, R.H. and Hadgraft, J., Transdermal Drug Delivery: A Simplified Pharmacokinetic Approach. *Int. J. Pharm.*, 24 (1985) 267–274.

Guy, R.H., Hadgraft, J. and Maibach, H.I., Percutaneous Absorption in Man: A Kinetic Approach. *Toxicol. Appl. Pharmacol.*, 78 (1985b) 123–129.

Guy, R.H., Hadgraft, J., Hintz, R.S., Roskos, K.V. and Bucks, A.W., In Vivo Evaluations of Transdermal Drug Delivery in *Transdermal Controlled Systemic Medications*. In Chien, Y.W. (Ed.), Marcel Dekker, Inc., New York, 1987, pp. 179–224.

Higuchi, W.I. and Yu, C.D., *Prodrugs in Transdermal Delivery in Transdermal delivery of Drugs*, Volume 3. In Kydonieus, A.F. Berner, B. (Eds.), CRC Press, Inc. Boca Raton, Fl., 1987, pp. 43–83.

Kubota, K. and Twizell, E.H., A Nonlinear Numerical Model of Percutaneous Drug Absorption. *Math. Biosci.*, 108 (1992) 157–178.

Scheuplein, R.J., Mechanism of Percutaneous Absorption II: Transient Diffusion and The Relative Importance of Various Routes of Skin Penetration, *J. Invest. Derm.*, 48 (1967) 79–88.

Shah, J., Analysis of Permeation Data: Evaluation of The Lag Time Method. *Int. J. Pharm.*, 90 (1993) 161–169.

Shah, J., Kaka, I., Tenjarla, S., Lau, S.W.J. and Chow, D., Analysis of Percutaneous Permeation Data II: Evaluation of The Lag Time Method. *Int. J. Pharm.*, 109 (1994) 283–290.

Yamashita, F., Bando, H., Koyama, Y., Kitagawa, S., Takakura, Y. and Hashida, M., *In Vivo* and *In Vitro* Analysis of Penetration Enhancement Based on a Two-Layer Diffusion Model with Polar and Nonpolar Routes in the Stratum Corneum. *Pharm. Res.*, 11 (1994) 185–191.