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Analysis of permeation data: evaluation of the lag time method

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Summary

Equations derived previously for penetration ($C_{(x,t)}$ and $J_{(x,t)}$ vs t), flux and permeation (A vs t) profiles were evaluated numerically to study the process of development of steady-state diffusion across the membrane. The concentration and flux profiles of the diffusant within the membrane indicated that at least three lag times were required to achieve a linear concentration gradient and a steady-state flux across the membrane. The permeation profile becomes truly linear at time greater than three lag times implying that the permeation experiment should be conducted for at least three lag times to achieve steady state which may not be possible with biological membranes. Also, if the experiments were conducted for a duration less than three lag times, the estimated J_{ss} and T_{lag} may be in error. To determine the error introduced, the simulated permeation profile was broken down into data subsets as if the experiments had been conducted for time equal to one to six lag times. The data subsets were then analyzed by the lag time method to estimate the parameters. The error in estimates of J_{ss} and lag time increased significantly as the total experimental time was reduced from six to one lag time. The J_{ss} and T_{lag} were highly underestimated, and for the experiment conducted for one lag time, the percentage errors in J_{ss} and T_{lag} were -60 and -54% from the theoretical values, respectively. The errors in derived values of D, the diffusion coefficient and K_m, the membrane/water partition coefficient were significantly higher (116 and -82%) than the errors in J_{ss} and T_{lag}. These results demonstrated the limitations of the lag time method for analysis of permeation data with particular reference to the duration of the experiment, which should be at least three lag times to achieve steady-state diffusion.

Introduction

Drug permeation through biological barriers such as skin, gastrointestinal mucosa, nasal mu-

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Abbreviations / symbols: D, diffusion coefficient (cm^2/h); K_m, membrane/donor phase partition coefficient; C_s, saturation solubility of diffusant in the donor phase (mmol/ml); h, thickness of the barrier (μm); $C_{(x,t)}$, concentration of the diffusant in the membrane at a distance of x cm from the donor side at time t (mmol/ml); t, time (h); $J_{(x,t)}$, flux of the diffusant in the membrane at a distance of x cm from the donor side at time t (mmol cm^{-2} h^{-1}); J_{ss}, flux of diffusant at steady state (mmol cm^{-2} h^{-1}); T_{lag}, lag time of diffusion (h); A, cumulative amount of diffusant permeated through the membrane until time t (mmol/cm^2).

cosa and buccal mucosa is considered to be a passive diffusion process in general. The mathematical analysis based on diffusion theory serves to correlate drug movement and transport with its physicochemical properties. The simplest model being a homogeneous membrane separated by an infinite reservoir of drug on the donor side and a perfect sink as receptor. Diffusion equations can be derived for this model based on Fick's first and second laws of diffusion (Crank, 1975; Barry, 1983; Durgard, 1983). The most commonly used method for analysis of permeation data from an in vitro experiment with an infinite dose technique is the lag time method (Barry, 1983). The lag time method utilizes the data post-steady state. The data points in the steady-state region are linear and one can determine steady-state flux from the slope of the linear permeation profile, and the x-intercept is the lag time of diffusion. Then one can determine the diffusion coefficient and partition coefficient of a diffusant from lag time and steady-state flux, knowing the donor phase concentration and thickness of the barrier.

This approach has a few problems. First, it is difficult to objectively determine when the permeation profile actually becomes linear, and that steady state is attained (Foreman, 1976; Okamoto, 1986). Secondly, for a new entity with a given biological barrier, it is difficult to predict, a priori, when the steady state will be attained, and hence the duration for which the experiments should be conducted. For a drug with low diffusivity, and for a membrane which may not be viable for a prolonged period of time, the commonly employed duration of experiment may be insufficient to achieve steady state. Besides, the estimation of D and K_m from lag time and steady-state flux are further affected by the inherent difficulty in measuring barrier thickness, which approximates the average diffusional path length of the diffusants. In addition, lag time depends on diffusion coefficient, which is assumed to be constant throughout the entire membrane and independent of diffusant concentration. In some cases, the above assumption is invalid making the lag time approach inappropriate (Frisch, 1957).

Various approaches have been tried to alleviate the above-mentioned problems with the lag time method (Foreman et al., 1976; Okamoto et al., 1986, 1988, 1989; Parry et al., 1990). One of the approaches was to fit the entire permeation data to the analytical solution with an iterative nonlinear least square regression program to obtain parameters from the complete permeation profile (pre- and post-steady states) (Foreman et al., 1976; Okamoto et al., 1986, 1988, 1989). Another approach was to independently determine partition coefficients and then optimize the values of the diffusion coefficient and the effective thickness which gave the best fit of the experimental data to the mathematical description of steady-state and non steady-state equations (Parry et al., 1990).

In the present study, simulations using established equations for diffusion were conducted to study the process of development of steady-state diffusion of a species from an infinite reservoir through a homogeneous membrane into a perfect sink based on Fick's first and second laws of diffusion. These simulations were also used to understand the functional significance of lag time as it relates to development of steady state of diffusion. To determine the effect of the duration of the experiment on estimated J_{ss} and T_{lag}, simulated permeation data were broken down into data subsets and evaluated by the lag time method, and various parameters compared to the theoretical values used to generate the permeation profile.

Theory

Diffusion of a species through a simple homogeneous membrane can be treated with Fick's first and second laws of diffusion (Crank, 1975). In-depth discussion of the process of diffusion and derivation of equations can be found in the literature (Crank, 1975; Barry, 1983; Durgard, 1983). However, for the purpose of clarity, the process is briefly described in this section. The concentration of diffusant is assumed to be constant on the donor side of the membrane (infinite reservoir assumption) and zero on the receptor

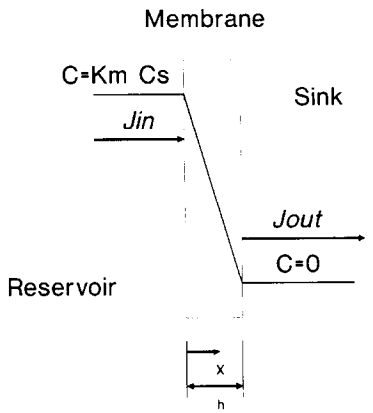

Fig. 1. Schematic model used for derivation of diffusion equations.

side of the membrane (perfect sink), while there is no diffusant present in the membrane initially (Fig. 1). The membrane is the principal barrier with no binding or degradation of diffusant occurring in the membrane. The model also assumes instantaneous equilibrium at the membrane-solution interface. Mathematically, the problem of diffusion in one dimension for the above model can be described by Fick's second law of diffusion as:

$$\frac{\delta C}{\delta t} = D\frac{\delta^2 C}{\delta x^2} \tag{1}$$

with boundary conditions, $C_{(0,t)} = C_s K_m$ at $t \geqslant 0$, and $C_{(h,t)} = 0$ and an initial condition of $C_{(x,0)} = 0$ for $0 < x \leqslant h$. Applying the above-mentioned boundary conditions to the differential, Eqn 1, and using the Laplace transform technique, the analytical solution to Eqn 1 is:

$$C_{(x,t)} = K_m C_s\left(1 - \frac{x}{h}\right) - \frac{2K_m C_s}{\pi}\sum_{n=1}^{\infty}\frac{1}{n}\sin\left(\frac{n\pi x}{h}\right)e^{(-Dn^2\pi^2 t/h^2)} \tag{2}$$

where $C_{(x,t)}$ is the concentration of the diffusant at a distance of x cm from the donor/membrane interface at time t. The flux of the diffusant at any distance within the membrane can be determined from the following equation, which is obtained by differentiating Eqn 2 with respect to x:

$$J_{(x,t)} = -D\frac{\delta C}{\delta x} = \frac{DK_m C_s}{x}\left[1 + 2\sum_{n=1}^{\infty}(-1)^n e^{(-Dn^2\pi^2 t/x^2)}\right] \tag{3}$$

Simulations were conducted using Eqns 2 and 3, with the following values of parameters to develop the penetration profile of the diffusing species in the membrane of unit cross-sectional area, as a function of time: $C_s = 1$ mmol/ml; $K_m = 1$; $h = 10$ μm; and $D = 1 \times 10^{-8}$ cm^2/h.

The above parameters result in an effective steady-state flux of $J_{ss} = 1 \times 10^{-5}$ mmol cm^{-2} h^{-1} and lag time (T_{lag}) for diffusion of 16.66 h. The results of simulations have been plotted in Figs 2 and 3. The overall diffusant flux into the

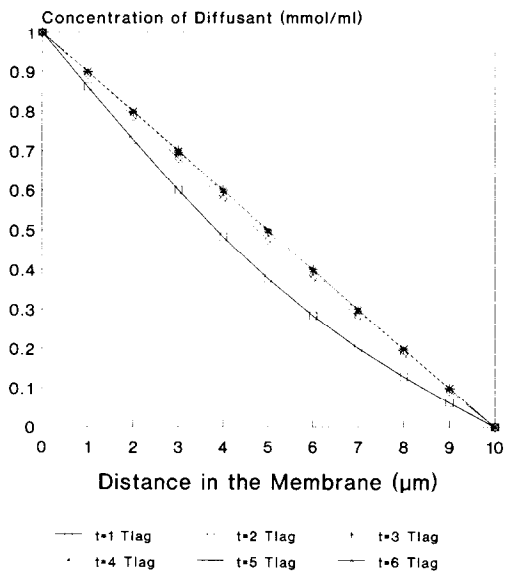

Fig. 2. Simulated concentration gradient in the membrane as a function of time, using Eqn 2 ($D = 1 \times 10^{-8}$ cm^2/h, $h = 10$ μm, $K_m = 1$, $C_s = 1$ mmol/ml, $J_{ss} = 1 \times 10^{-5}$ mmol cm^{-2} h^{-1} and $T_{lag} = 16.66$ h).

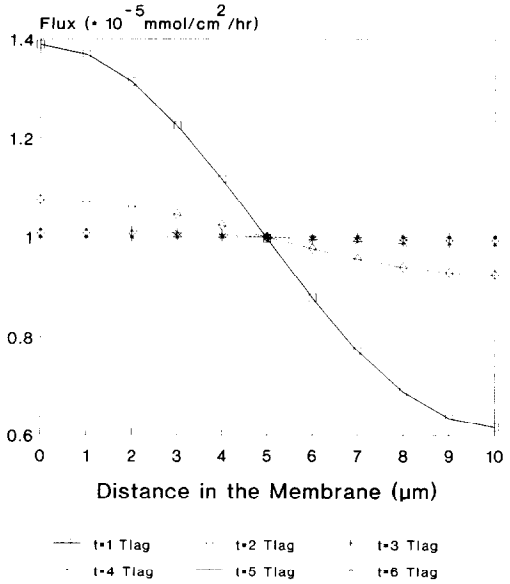

Fig. 3. Simulated flux of the diffusant within the membrane as steady state was attained, using Eqn 3 ($D = 1 \times 10^{-8}$ cm^2/h, $h = 10$ μm, $K_m = 1$, $C_s = 1$ mmol/ml, $J_{ss} = 1 \times 10^{-5}$ mmol cm^{-2} h^{-1} and $T_{lag} = 16.66$ h).

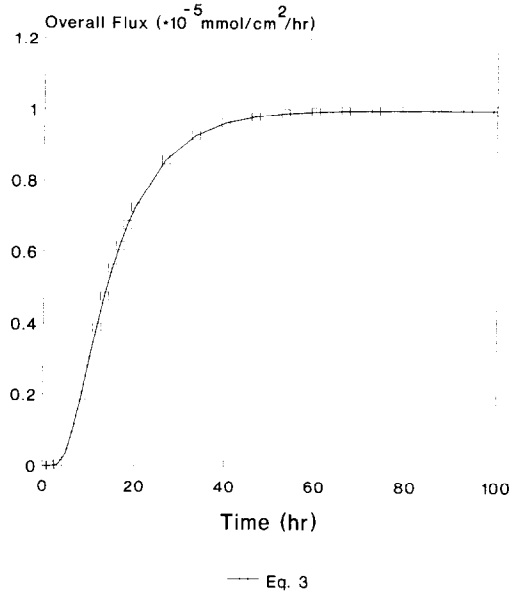

Fig. 4. Diffusant flux across the membrane as steady state was attained, evaluated using Eqn 3 ($J_{ss} = 1 \times 10^{-5}$ mmol cm^{-2} h^{-1} and $T_{lag} = 16.66$ h).

receptor compartment was calculated using Eqn 3 at $h = 1$ at the receptor side of the membrane (Fig, 4).

The total amount of diffusant which passes through the membrane in time t, i.e., the cumulative amount of drug (A) permeated into the receptor compartment is obtained by integrating flux (Eqn 3) with respect to time, as shown in Eqn 4:

$$A = \frac{DK_mC_s}{h}\left(t - \frac{h^2}{6D}\right) - \frac{2hK_mC_s}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}e^{(-Dn^2\pi^2t/h^2)} \qquad (4)$$

Eqn 4 was used to generate a permeation profile for 100 h ($6 \times T_{lag}$), as shown in Fig. 5.

Equations similar to Eqns 2–4 have been derived in the past (Crank, 1975; Barry, 1983; Durgard, 1983), but they have been re-derived here in order to demonstrate, using simulations, the rela-

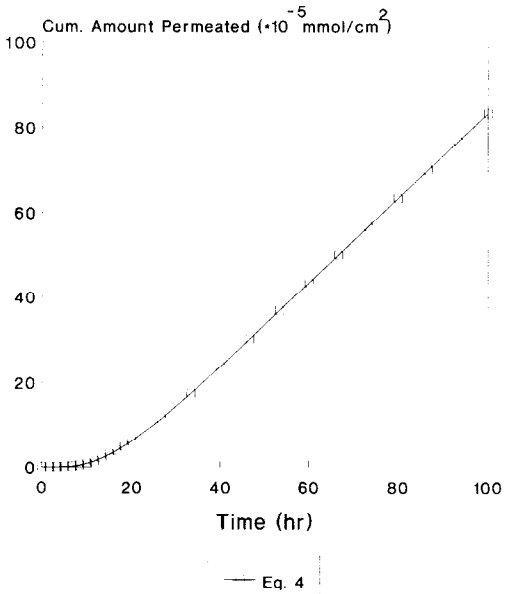

Fig. 5. Simulated permeation profile generated using Eqn 4 ($J_{ss} = 1 \times 10^{-5}$ mmol cm^{-2} h^{-1} and $T_{lag} = 16.66$ h).

tionship between time to achieve steady state and lag time. Eqn 4 can be rewritten as follows:

$$A = J_{ss}(t - T_{lag}) - (12/\pi^2)J_{ss}T_{lag}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}e^{[-(n^2\pi^2/6)(t/T_{lag})]} \quad (5)$$

where

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

and

$$J_{ss} = \frac{DK_mC_s}{h} \quad (7)$$

At longer times ($t > 3\text{–}4 \times T_{lag}$), the second term of Eqn 5 becomes negligible and the equation simplifies to:

$$A = J_{ss}(t - T_{lag}) \quad (8)$$

Traditionally, the data from the membrane permeation experiments are analyzed using Eqn 8 above, and D and K_m are calculated from the values of T_{lag} and J_{ss}, knowing C_s and h using Eqns 6 and 7. However, the accuracy of this analysis will depend upon how accurately Eqn 8 approximates Eqn 5. This will depend upon how fast the exponential terms become insignificant and the rate of convergence of the series in Eqn 5. The convergence and elimination of the second term in Eqn 5 will depend upon the ratio, t/T_{lag}. At relatively short times, Eqn 5 cannot be approximated to Eqn 8, but at larger values of t/T_{lag}, Eqn 5 will degenerate into Eqn 8.

Effect of the duration of the experiment on parameter estimation by the lag time method

The meaningful data obtained from a permeation experiment are usually the cumulative amount of diffusing species permeated into the receptor side, i.e., the permeation profile. The permeation profile is used to obtain J_{ss} and T_{lag}; subsequently, D and K_m are obtained using Eqns 6 and 7 knowing the membrane thickness, h. Implicit in this method of data analysis are the assumptions that steady state has been achieved and only post-steady-state data are used. A simulated permeation profile was generated using the parameters listed above. The data were broken down into data subsets as if the experiments were conducted for 16 h ($= T_{lag}$), 32 h ($2 \times T_{lag}$), and up to 100 h ($6 \times T_{lag}$). The terminal apparent linear region of the permeation profile was used

TABLE 1

Parameters obtained by analysis of the simulated permeation data by the lag time method after breaking the data into subsets, each representing an experiment with a decreasing duration of time

Duration of experiment (h)	T/T_{lag}	Apparent J_{ss} (mmol cm^{-2} h^{-1}) ($\times 10^{-5}$)		Apparent T_{lag} (h)		R^2	Apparent D (cm^2/h) ($\times 10^{-8}$)		Apparent K_m	
			%Error		%Error			%Error		%Error
100	6.00	1	0	16.61	0	1	1.00	0	0.99	−1
62	3.72	0.97	−3	15.55	−7	1	1.07	7	0.91	−9
50	3.00	0.90	−10	13.38	−20	1	1.25	25	0.72	−28
38	2.28	0.85	−15	12.78	−23	0.999	1.30	30	0.65	−35
32	1.92	0.77	−23	11.54	−31	0.998	1.44	44	0.54	−47
26	1.56	0.68	−32	10.43	−37	0.997	1.60	60	0.43	−57
20	1.20	0.54	−46	9.09	−45	0.996	1.83	83	0.29	−71
16	0.96	0.40	−60	7.73	−54	0.993	2.16	116	0.19	−82

The simulated data were generated with $J_{ss} = 1 \times 10^{-5}$ mmol cm^{-2} h^{-1} and $T_{lag} = 16.667$ h ($D = 1 \times 10^{-8}$ cm^2/h, $K_m = 1$, $C_s = 1$ mmol/ml, $h = 10$ μm).

to calculate J_{ss} and T_{lag} by fitting it to Eqn 8. The results of these analyses are depicted in Fig. 6a–e. D and K_m were calculated from J_{ss} and T_{lag} using Eqns 6 and 7. The percentage errors in the estimated values of the parameters were calculated and have been listed in Table 1.

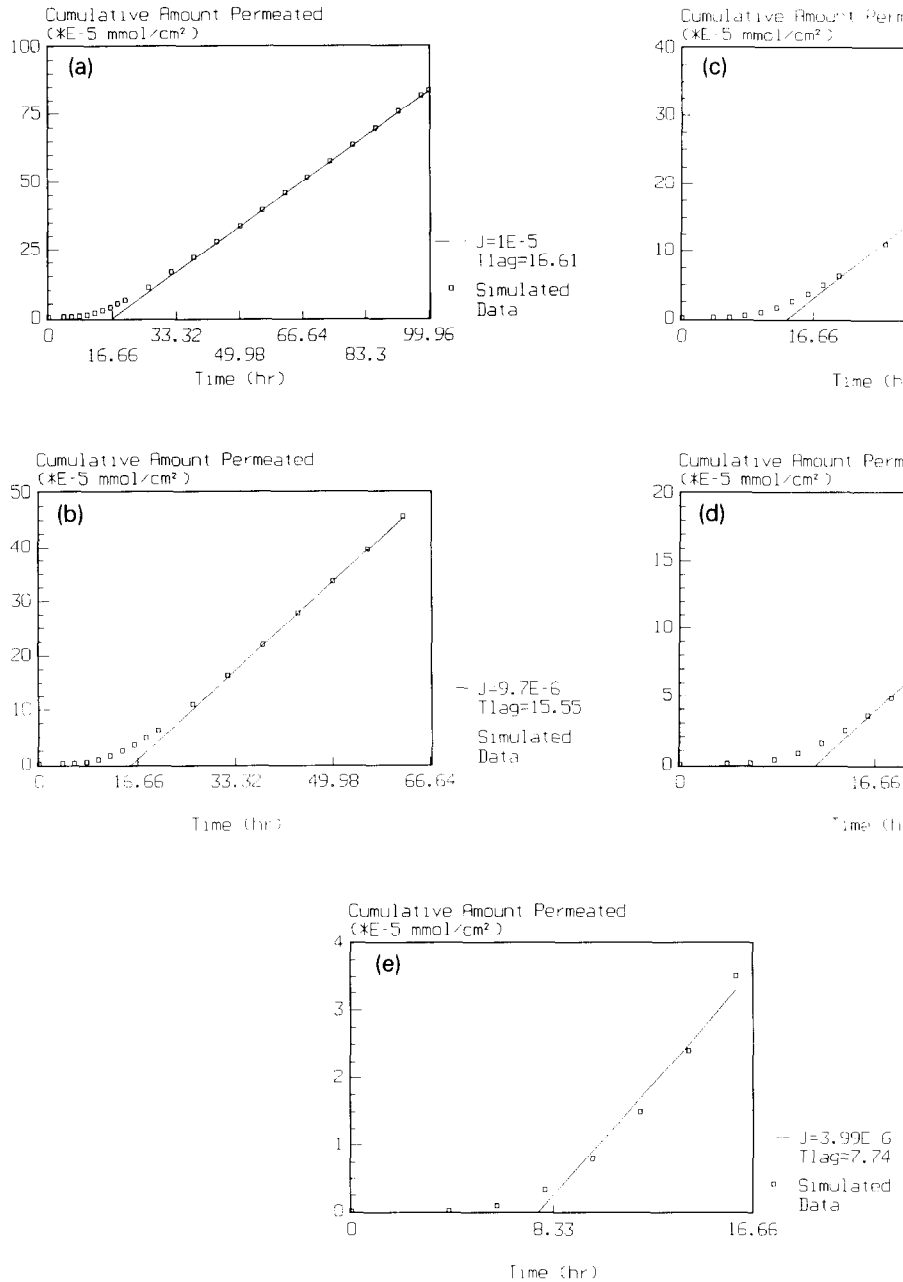

Fig. 6. Analysis of the permeation profile by the lag time method, when the experiments were conducted for (a) $6 \times T_{lag}$, (b) $4 \times T_{lag}$, (c) $3 \times T_{lag}$, (d) $2 \times T_{lag}$, and (e) $1 \times T_{lag}$. (The simulated permeation profiles were generated using values of $J_{ss} = 1 \times 10^{-5}$ mmol cm^{-2} h^{-1} and $T_{lag} = 16.66$ h.)

Results

As seen from the penetration profile ($C_{(x,t)}$ vs x, Fig. 2), at time equal to $1-3 \times T_{lag}$, the concentration gradient across the membrane was nonlinear. At times greater than $3 \times T_{lag}$, the concentration gradient of the diffusant approached linearity and was superimposable subsequently, indicating the achievement of steady-state diffusion.

The flux ($J_{(x,t)}$) of the diffusant at various planes in the membrane perpendicular to the direction of diffusion at various time intervals decreases along the concentration gradient at early times as expected (Fig. 3). However, as more diffusant diffuses into the membrane, the flux tends to plateau throughout the entire membrane. At the end of $4 \times T_{lag}$, the flux is constant throughout the membrane and is equal to the steady-state value, $J_{ss} = 1 \times 10^{-5}$ mmol cm^{-2} h^{-1}, indicating the attainment of steady state. This is also seen in Fig. 4, which shows the overall flux of diffusant into the receptor reaching almost 100% of steady-state flux at the end of $4-5 \times T_{lag}$. However, at one lag time only 61.7% steady state was achieved but if the experiment was carried out for $3 \times T_{lag}$, almost 90% steady state was achieved by then. The overall flux increases in a sigmoidal manner in the earlier time period (Fig. 4).

The permeation profile calculated using Eqn 4 is demonstrated in Fig. 5. Although the profile appears to be linear at times shorter than $3 \times T_{lag}$, the profile was truly linear only at times beyond $3 \times T_{lag}$. Since Eqn 4 describes the complete permeation profile, fitting the permeation data to Eqn 4 or 5 would yield the most accurate estimates of the parameters; J_{ss}, T_{lag}, D and K_m. However, Eqn 4 is a series with an exponential function which converges very slowly at very small values of time. Thus, use of a nonlinear regression program to obtain optimized values of the parameter is very slow and difficult. Hence, traditionally, the lag time method which is mathematically very simple is used for data analysis. The validity of the T_{lag} method was tested by breaking the simulated permeation profile into data subsets as if the experiments were conducted for $1-6 \times T_{lag}$. The data subsets were then analyzed by the T_{lag} method as shown in Fig. 6a–e, the parameters obtained being listed in Table 1. Although there were fewer data points in the shorter duration experiments, the R^2 for regression was greater than 0.99 for all the data subsets. The percentage errors in J_{ss} and T_{lag} increased dramatically as the overall experimental time was reduced from 100 h ($6 \times T_{lag}$) to 16 h ($1 \times T_{lag}$). If the experiment was conducted for only one lag time, although the permeation profile may appear to have achieved linearity ($R^2 = 0.993$), the percentage errors in J_{ss} and T_{lag} values were -60 and -54%, respectively. Thus, J_{ss} and T_{lag} were highly underestimated and strongly subject to error depending on the total experimental time. However, more alarming was the percentage error in the values of D and K_m calculated from T_{lag} and J_{ss} using Eqns 6 and 7. The percentage error for D ranged from 0.32 to 115%, depending on the duration of the experiment.

Discussion

In the literature (Flynn et al., 1988), the lag time of diffusion has been defined as a measure of the time it takes for the permeant's concentration gradient to become stabilized across the membrane. In another article (Walters, 1986), the lag time of diffusion was defined as the period required for a permeant to establish a linear concentration gradient across the membrane; the time of onset of maximal flux. However, as seen from Figs 2 and 3, a duration equal to at least three lag times was required to achieve a linear concentration gradient and a uniform steady-state flux across the membrane, i.e., achieving J_{ss}. The permeation profile (Fig. 5) also demonstrated that linearity was achieved only after three lag times. These results suggest that to use the lag time method, the permeation experiment should be carried out for at least $3-4 \times T_{lag}$, to achieve steady state and accurately estimate J_{ss} and T_{lag}. Although this fact is known (Crank, 1975; Barry, 1983; Durgard, 1983), the vast literature on percutaneous permeation has never addressed this problem.

The diffusion coefficients of some drugs across biological membranes are very low yielding very long lag times of diffusion. The diffusion coefficients of commonly used drugs across skin range from 10^{-6} to 10^{-13} cm^2/s (Barry, 1983; Martin et al., 1983). The lag times of diffusion across skin thus range from a few minutes to several days (Durgard, 1983). However, the structural integrity and viability of biological barriers such as human cadaver skin in vitro after such prolonged periods of time is questionable (Foreman et al., 1976). Thus, permeation studies across biological membranes for such prolonged periods of time are often impossible. The lag times of diffusion of sulfanilic acid, indomethacin, and butylparaben across male guinea pig skin were in the range of 12–42 h (Okamoto et al., 1986). Steady state was not achieved because the experiments were conducted for 30 h. Hence, nonlinear regression was used to estimate D and K_m from the fit of the complete profile to the analytical solution of the permeation equation, rather than using the lag time method.

Another disadvantage of the lag time method to estimate D and K_m is the requirement to know the thickness of the barrier, which can be difficult to estimate for a biological barrier. Secondly, if skin were the barrier, the thickness would vary significantly depending on the anatomical site, the degree of hydration, and various physiological and pathological factors (Durgard, 1983). The errors in the values of J_{ss} and T_{lag} when combined with the inaccurate estimates of barrier thickness may result in very significant errors in estimates of D and K_m. In this paper, using simulations, the significant effect of the duration of the experiment was demonstrated on values of D and K_m calculated by the lag time method (Table 1). As the duration of the experiment decreased, the percentage error in D increased significantly and up to 115%, was significantly greater than the errors in the values of J_{ss} and T_{lag}. Thus, the D and K_m estimates were more sensitive to the duration of experiment than J_{ss} and T_{lag}. In addition, the lag time method is subjective due to the required judgement of a researcher on linearity of the permeation profile. Based upon the judgement, some data points are selected to calculate J_{ss} and T_{lag}, the rest that appear to fall in the pre-steady state are discarded. In fact, most membranes of biological origin such as skin are non-homogeneous, multi-phasic and hence complex. Parameters like D and K_m are only effective or observed parameters and not very realistic and should not be used for interpretation of structure of membrane or the mechanism of diffusion. A mass transfer coefficient such as the permeability coefficient is more meaningful and useful.

The best approach would be to fit the complete permeation profile to Eqn 4 or 5 to yield the most accurate estimates of the parameters. Recently, several articles have demonstrated the use of different nonlinear regression methods to analyze the complete percutaneous permeation profiles (Foreman et al., 1976; Okamoto et al., 1986, 1988, 1989; Parry et al., 1990). An analytical solution for a model involving non-steady-state diffusion for a finite dose across skin has also been used to estimate D and K_m (Bhatt et al., 1989). In the study by Okamoto et al. (1986), reliable estimates of D and K_m were obtained despite the fact that in certain experiments steady state was not attained in the duration of the experiment.

In this paper, only the case where the membrane is at initial zero concentration of diffusant was considered. Detailed mathematical analysis and equations have been developed for the more general case of membrane diffusion where either the membrane was presaturated with diffusant or the receptor was not under sink conditions or both (Crank, 1957; Jenkins, et al., 1970). Even in the above-mentioned situations, in a more general case, a finite amount of time is required to achieve steady state and develop a linear concentration gradient across the membrane (Crank, 1975). However, in the most commonly used permeation experiment protocol to measure drug transport characteristics, the membrane is not presaturated with drug. Thus, the results discussed in this paper are more relevant and applicable to permeation studies designed to estimate drug absorption across biological barriers in which scenario, the membrane is always at zero initial drug concentration.

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References

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

Bhatt, P.H., Hanna, M.S., Szeptycki, P. and Higuchi, T., Finite dose transport of drugs in liquid formulations through stratum corneum: Analytical solution to a diffusion model. *Int. J. Pharm.*, 50 (1989) 197–203.

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

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

Flynn, G.L. and Stewart, B., Percutaneous drug penetration: Choosing candidates for transdermal development. *Drug Dev. Res.*, 13 (1988) 169–185.

Foreman, M.I. and Kelly, I., The diffusion of nadrolone through hydrated human cadaver skin. *Br. J. Dermatol.*, 95 (1976) 265–268.

Frisch, H.L., The time lag in diffusion. *J. Phys. Chem.*, 61 (1957) 91–95.

Jenkins, R.C.L., Nelson, P.M. and Spirer, L., Calculation of transient diffusion of a gas through a solid membrane into a finite outflow volume. *Trans. Faraday Soc.*, 66 (1970) 1391–1401.

Martin, A., Swarbrick, J., Cammarata, A. and Chun, A.H., C., *Physical Pharmacy: Physical Chemical Principles in The Pharmaceutical Sciences*, Lea & Febiger, Philadelphia, 3rd Edn, 1983, pp. 399–444.

Okamoto, H., Komatsu, H., Hashida, M. and Sezaki, H., Effects of β-cyclodextrin and di-*O*-methyl-β-cyclodextrin on the percutaneous absorption of butylparaben, indomethacin and sulfanilic acid. *Int. J. Pharm.*, 30 (1986) 35–45.

Okamoto, H., Hashida, M. and Sezaki, H., Structure-activity relationship of 1-alkyl-or 1-alkenylazacycloalkanone derivatives as percutaneous enhancers. *J. Pharm. Sci.*, 77 (1988) 418–424.

Okamoto, H., Yamashita, F., Saito, K. and Hashida, M., Analysis of drug penetration through the skin by the two-layer skin model. *Pharm. Res.*, 6 (1989) 931–937.

Parry, G.E., Bunge, A.L., Silcox, G.D., Pershing, L.K. and Pershing, D.W., Percutaneous absorption of benzoic acid across human skin. I: In vitro experiments and mathematical modeling. *Pharm. Res.*, 7 (1990) 230–236.

Walters, K.A., Percutaneous absorption and transdermal therapy. *Pharm. Technol.*, 10 (1986) 30–46.