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Faster determination of membrane permeabilities without using the lag time method

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Abstract

A new method of data analysis is presented that allows the determination of membrane permeabilities. The method is applicable to data obtained from a common experimental setup, in which drug dissolved in an inert donor gel diffuses through a membrane, initially void of drug, into a receiver for which sink conditions are maintained. The equations developed can also be used to predict the release of drug from these systems. Fick's Laws are solved, and the early time behavior of the mathematical solution is used to develop the analysis methods. Limitations of the model and their relations to experimental design are determined, and the method of application to experimental data is presented. The method is tested numerically using simulated data generated by a 1-d finite difference program that was used to numerically solve Fick's Laws, and also applied to in vitro human cadaver skin transdermal data for the drugs doxepin, imipramine and amitriptyline. It is concluded that this method can be applied to determine membrane permeabilities and diffusion coefficients with accuracy comparable to other experimental setups, such as lag time experiments and steady state experiments, but requiring experiments that can be significantly shorter.

Keywords: Fick's Laws; Lag time; Membrane; Permeability coefficient; Gels; Transdermal

1. Introduction

A research priority in the field of pharmaceutical technology is to explore routes of administration alternative to oral delivery that overcome potential disadvantages, such as first pass-effect, adverse gastro-intestinal effects and frequency of dosing. Transdermal drug delivery has been shown to be a suitable route of administration for many drugs, giving the ability to control the rate and site of drug absorption over a prolonged period of time, which results in less side effects and improved patient compliance (Walters, 1986; Schaefer and Redelmeier, 1996).

To utilize transdermal delivery systems, it is important to determine the permeability of drugs through synthetic membranes and skin. In order to determine the properties of these membranes, a number of experimental designs have been described in the literature and are commercially

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available (Valia and Chien, 1984; Poulsen and Flynn, 1985; Friend, 1992; Pena, 1995). Most commonly, these experiments involve donor and receiver chambers that are separated by the membrane to be studied. In practice, the donor may be well stirred or may be a gel that is left unstirred, while the receiver is usually well stirred and sink conditions are maintained.

One well-known method of analysis involves the use of the lag time t_{lag}. In this experiment, a drug leaves a donor that is held at a constant concentration, diffuses through a membrane initially void of the drug, and enters a receiver that is held at sink conditions (Flynn et al., 1974; Crank, 1975). The cumulative amount of drug that has entered the receiver M is plotted as a function of time, and the steady state (i.e. linear) part of the M versus t curve is extrapolated back, intersecting the time axis at t_{lag}. The lag time is given as

$$t_{lag} = \frac{h^2}{6D_m} \tag{1}$$

and the steady state slope is

$$\frac{dM}{dt} = \frac{AKD_m C_d}{h} = AP_m C_d \tag{2}$$

Here, h is the membrane thickness, D_m is the membrane diffusion coefficient, K is the membrane-donor partition coefficient, A is the membrane surface area, and C_d is the donor concentration. Knowing the lag time and steady state slope, it is possible to obtain D_m and the membrane permeability P_m. However, the experimental data must be collected over a period that is at least three lag times, to allow the steady state portion of the M versus t to develop (Crank, 1975). In many membranes, including human cadaver skin, lag times can range from less than a minute to several days. When the lag times are long, the experiments require a week or longer, since data must be collected for at least three lag times. This is inconvenient and can lead to possible degradation of biological membranes (Shah, 1993).

In another commonly used experimental method, a drug leaves a donor containing an unstirred polymer gel or matrix, diffuses through a membrane initially void of drug, and enters a receiver held at sink conditions (Nicolettos, 1998). Since the concentration in the donor is not constant, no steady state occurs and the lag time analysis does not apply. Frequently, the M versus t curve will display a nearly linear region, and the permeability of the membrane is estimated by dividing this slope by the area and the donor concentration C_d, as in Eq. (2). However, since the donor concentration is not known at the membrane interface, it is usually approximated as either the original donor concentration, or the original donor concentration corrected for drug that has entered the receiver. When the membrane is highly permeable, these methods overestimate the donor concentration near the membrane interface, which can result in calculated membrane permeabilities that are significantly lower than the true values. On the other hand, for membranes of low permeability, C_d can be estimated accurately enough, but the time required for the linear portion of the curve to develop can be long, leading to problems similar to those of the lag time method when the lag times are long.

In this paper, the latter experimental setup, in which drug leaves an unstirred donor and diffuses through a membrane initially void of drug into a receiver maintaining sink conditions, is studied. The setup is analyzed mathematically by solving Fick's Laws for a transient analysis of the donor and the membrane, and the early time behavior of the solutions is used to develop a data analysis method that allows the determination of membrane permeabilities. The main advantages of this method are as follows: (i) the required experiments can be much shorter than the corresponding lag time experiment; (ii) the method is numerically simple and accurate; and (iii) the method is particularly useful for determinations of the permeability for composite membranes, in which one pathway is clearly rate dominating, such as skin. Equations are developed to allow the prediction of release from similar systems. The method is numerically tested using simulated data, and several tests are developed to check that the experimental data is used correctly. Some application examples are also provided, using data obtained from in vitro experiments measuring

permeation through human cadaver skin for the drugs doxepin, amitriptyline and imipramine.

2. Methods

2.1. Mathematical model

In this model, a membrane of fixed thickness h and initially void of drug is in contact with an unstirred donor gel of thickness L, in which a drug is dissolved with an initially uniform drug concentration C_0. In what follows, the membrane occupies the region $-h < x < 0$, the donor occupies the region $0 < x < L$, and the donor–membrane interface is located at $x = 0$. The subscripts d and m will denote the donor and membrane, respectively.

The model is mathematically described by considering the donor and the membrane as separate regions that are linked through boundary conditions at the donor–membrane interface, as shown in Fig. 1. The governing differential equation in each region is Fick's Second Law, which is a partial differential equation that is first order in time and second order in space. The solution of these equations requires one initial and two boundary conditions for each region. The initial conditions are that the membrane is void of drug, and the donor has a uniform concentration C_0. The boundary conditions are as follows: (i) there is no flux of drug into or out of the back face of the donor; (ii) sink conditions exist in the receiver; (iii) at the donor–membrane interface, the flux leaving the donor is equal to the flux entering the membrane; and (iv) across the donor–membrane interface, the concentrations in the two regions are related by partitioning. Mathematically, these are expressed as follows (Crank, 1975):

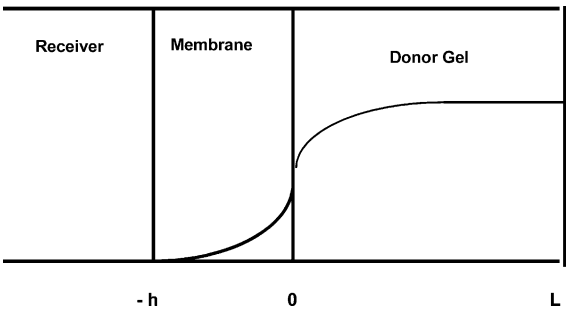

Fig. 1. Schematic diagram of the diffusion model. Fick's second law describes the time dependent concentrations in the donor and the membrane. The region $-h < x < 0$ is the membrane and $0 < x < L$ is the donor.

Governing differential equations

$$\frac{\partial C_d}{\partial t} = D_d \frac{\partial^2 C_d}{\partial x^2} \qquad 0 < x < L \tag{3}$$

$$\frac{\partial C_m}{\partial t} = D_m \frac{\partial^2 C_m}{\partial x^2} \qquad -h < x < 0 \tag{4}$$

Initial conditions

$$C_d(x,\ 0) = C_0 \qquad t = 0 \quad 0 < x < L \tag{5}$$

$$C_m(x,\ 0) = 0 \qquad t = 0 \quad -h < x < 0 \tag{6}$$

Boundary conditions

$$\frac{\partial C_d}{\partial x} = 0 \qquad x = L \tag{7}$$

$$C_m = 0 \qquad x = -h \tag{8}$$

$$D_d \frac{\partial C_d}{\partial x} = D_m \frac{\partial C_m}{\partial x} \qquad x = 0 \tag{9}$$

$$C_m = K C_d \qquad x = 0 \tag{10}$$

Here, C_d and C_m are the concentrations, D_d and D_m are the diffusion coefficients, K is the membrane/donor partition coefficient, x represents the position, and t is the time. The rate of accumulation of drug in the receiver is given by Fick's First Law (Crank, 1975) as

$$\frac{dM}{dt} = A D_m \left.\frac{\partial C_m}{\partial x}\right|_{x=-h} \tag{11}$$

The above system of equations can be solved using either Fourier series or Laplace transform methods, and each method will yield infinite series solutions (Churchill, 1972). However, the properties of the solutions are very different. For the Laplace transform solutions, the series terms are smallest initially and become larger with increasing time, while for the Fourier series solutions, they are largest initially and become smaller at later times (Carslaw and Jaeger, 1959). Thus, the Laplace solution takes on a simpler form at early times and is the method used here to solve for C_m.

In general, the solution to the above system of equations will be a function of the thickness L of the donor region. However, if the experiment is short enough or L is large enough, the dependence on L may be neglected. This condition can be expressed as (Appendix A)

$$\frac{L^2}{D_d} + \frac{2\beta L}{\sqrt{D_d}} > 3t_{max} \tag{12}$$

where t_{max} is the time at which the last data point is taken, and

$$\beta = \frac{h}{2\sqrt{D_m}} \tag{13}$$

If Eq. (12) is satisfied, the above system of equations can be solved, and the cumulative amount in the receiver is given as

$$M = \alpha \sum_{n=0}^{\infty} \delta^n \left[\sqrt{t} \exp\left(-\frac{(2n+1)^2\beta^2}{t} \right) - (2n+1)\beta\sqrt{\pi}\ \mathrm{erfc}\left(\frac{(2n+1)\beta}{\sqrt{t}} \right) \right] \tag{14}$$

where

$$\alpha = \frac{4AKC_0\sqrt{D_d D_m}}{\sqrt{\pi}(\sqrt{D_d} + K\sqrt{D_m})} \tag{15}$$

$$\delta = \frac{\sqrt{D_d} - K\sqrt{D_m}}{\sqrt{D_d} + K\sqrt{D_m}} = 1 - \frac{\alpha\sqrt{\pi}}{2AC_0\sqrt{D_d}} \tag{16}$$

and

$$\mathrm{erfc}\ u = \frac{2}{\sqrt{\pi}} \int_u^{\infty} \exp(-w^2)\mathrm{d}w$$

is the complementary error function. The parameter α has units of mass per square root of time, β^2 has units of time, and δ has no units.

Numerical calculations show that neglecting the $n > 0$ series terms in Eq. (14) results in less than 10% error in the calculated value of M when $t < 6\beta^2$, less than 5% error $t < 4.25\beta^2$, and less than 1% error $t < 2.5\beta^2$. Thus, for early times, the release rate and the cumulative amount of drug in the receiver are accurately approximated by

$$\frac{\mathrm{d}M}{\mathrm{d}t} = \frac{\alpha}{2\sqrt{t}} \exp\left(-\frac{\beta^2}{t} \right) \tag{17}$$

$$M = \alpha \left[\sqrt{t} \exp\left(-\frac{\beta^2}{t} \right) - \beta\sqrt{\pi}\ \mathrm{erfc}\left(\frac{\beta}{\sqrt{t}} \right) \right] \tag{18}$$

Using only these early data points, the values for α and β can be determined from Eq. (18) by nonlinear regression, as discussed in the following section. This avoids the need to calculate δ, which is a significant advantage. If δ is known (which, in general, requires knowing D_d), then Eq. (14) can be used to predict the cumulative amount of drug in the receiver at later times (Fig. 2).

While it is possible to obtain δ from a three-parameter nonlinear regression of Eq. (14), this would require data from much later times, since the early data points are negligibly dependent on δ, and can lead to significant errors in δ. Therefore, this method will not be considered further here. Instead, the preferred method is to obtain D_d separately from other experimental data and calculate δ from Eq. (16).

From the above considerations, two conditions may be deduced in terms of α and β to check whether the above approximations are valid for the data points used in the analyses.

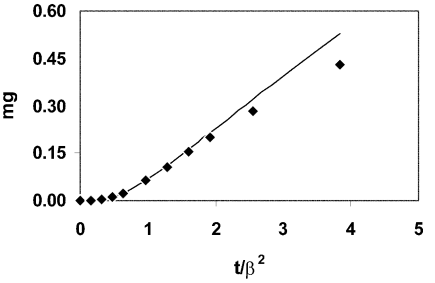

Fig. 2. Cumulative amount released vs. time for simulated data: Data Set A. The diamonds represent the simulated M vs. t data that was generated using a finite difference program, using the parameters listed in Table 1 for Data Set A. The solid line represents the M vs. t curve calculated from Eq. (14), using the values listed in Table 3. The solid line deviates from the data because of the effects of donor depletion at longer times.

1) The maximum time constraint (for ignoring $n > 0$ terms), can be expressed as a maximum cumulative amount of drug in the receiver M_{max} by substituting the time into Eq. (18). This accounts for the effects of partitioning in the membrane, and allows a direct evaluation of which experimental data is appropriate for use in the regression analyses. Using $t_{max} = 2.5\beta^2$ gives $M_{max} < 0.4\alpha\beta$, and using $t_{max} = 6\beta^2$ gives $M_{max} < 1.2\alpha\beta$.
2) Knowing β, Eq. (12) gives the minimum donor depth that is necessary to avoid numerical errors from neglecting donor depletion. Conversely, the time of the latest data point that should be used can be calculated from L and β.

In practice, these conditions are more restrictive than needed, since most of the data points are taken at times that are significantly earlier than the last data point used. In addition, for systems in which $\delta < 1$, the $n > 0$ terms decrease even more rapidly due to the δ^n component in Eq. (14). Typically, acceptable numerical results are obtained when the data points used in the nonlinear regressions satisfy the conditions

$$M_{max} < 2 - 3\alpha\beta \tag{19}$$

$$t_{max} < \frac{L^2}{2D_d} + \frac{\beta L}{\sqrt{D_d}} \tag{20}$$

Once α and β are determined, it is possible to determine the diffusion coefficient D_m and permeability P_m of the membrane from Eqs. (15) and (13) as

$$D_m = \frac{\alpha^2 \pi D_d}{K^2(4AC_0\sqrt{D_d} - \alpha\sqrt{\pi})^2} \tag{21}$$

$$P_m = \frac{KD_m}{h} = \frac{\alpha\sqrt{\pi D_d}}{2\beta(4AC_0\sqrt{D_d} - \alpha\sqrt{\pi})}. \tag{22}$$

2.2. Determination of α, β and P_m

Several methods can be used to determine α and β from experimental data. For instance, a plot of $\ln(2t^{1/2}(dM/dt))$ versus $1/t$ should give a straight line with a slope $= -\beta^2$ and intercept $= \ln \alpha$. In practice, however, obtaining derivatives from a relatively small number of experimental data points typically gives unacceptably large relative numerical errors.

A more accurate method to determine α and β is to perform a two-parameter nonlinear regression to fit Eq. (18) to the M versus t experimental data, by minimizing the sum of squares of the residuals between the predicted and experimental values of M. This method, which is widely used in pharmacokinetics, is accurate and easily done on a personal computer using readily available software. Because of the nonlinear nature of the regression, however, it is important that good initial estimates are used for α and β. (A method to obtain theses estimates is described in Appendix B.) Once the regressions are done, the obtained values of α and β are used to determine if any points used in the regression should be excluded, based on the criteria of Eqs. (19) and (20). The procedure for determining α, β and P_m can be summarized as follows:

1) Obtain appropriate initial estimates for α and β.
2) Fit Eq. (18) to the experimental data to obtain values of α and β, using data from any time range.
3) Use the results to determine whether Eqs. (19) and (20) are satisfied. If both conditions are not satisfied, determine the usable data points be selecting the latest point that satisfies both conditions, and go back to Step 1.
4) If Eqs. (19) and (20) are satisfied, compare the experimental data with predicted data at longer times using the full solution given by Eq. (14). If the agreement is poor, then try including one or two more points, or excluding one or two more points (in case of an outlier data point), and go back to Step 1.
5) Calculate P_m from Eq. (22).

If it is not possible to obtain reasonable agreement between the experimental and predicted data, then the possibility of bad data (such as might occur with a ruptured membrane, etc.) or the wrong choice of model should be considered.

3. Testing and application of the method

The above analysis was numerically tested by applying it to simulated data that was generated by numerical solution of Fick's Laws, using known values for the diffusion parameters. Values of α and β were determined by nonlinear regressions, and various comparisons were made between the simulated data and the regression results. The method was also applied to experimental data to assess the applicability to real data. For rough comparisons, values for the permeability were calculated using Eq. (22) for the fit P_m and Eq. (2) for the steady state P_m (denoted in the tables as SS P_m). In calculating the SS P_m, the dM/dt was taken as the slope of the nearly linear part of the M versus t curve. Whenever possible, this was done starting with the first data point for which $t > 2\beta^2$ and ending with the first data point for which $t > 5\beta^2$. The average donor concentration was estimated as $C_d \approx C_0 - 0.5M_{max}/V_d$, where M_{max} is the amount of drug in the receiver at the last time used, and V_d is the donor volume.

3.1. Application to simulated data

The simulated data was generated using a finite difference program (Bellantone, 1999) to solve Eqs. (3)–(10), using a well-known method based on a forward time, centered space (FTCS) algorithm (Fletcher, 1988; Press et al., 1992). Three of these M versus t data sets (denoted as Data Sets A, B and C) were generated for a finite donor using the parameters listed in Table 1, and are shown in Table 2. Each set corresponded to different limitations and conditions for testing the method.

Table 1
Constants used to generate simulated data

Data Set	D_m (cm^2 h^{-1})	K	α (mg h$^{-1/2}$)	β^2 (h)
A	1.0×10^{-6}	100	0.312	6.25
B	5.0×10^{-5}	1	0.030	0.125
C	1.0×10^{-7}	100	0.125	62.5

The following were used in all generated data sets: Area $A = 2.0$ cm^2, $D_d = 0.05$ cm^2 h^{-1}, $C_0 = 1.0$ mg ml^{-1}, $h = 0.005$ cm, $L = 0.5$ cm. For convenience, the theoretical values of α and β^2 are also given. D_m is given in cm^2 h^{-1}, α in mg h$^{-1/2}$, and β^2 in hours.

The parameters used to generate Data Set A were chosen so that the condition of Eq. (19) was satisfied for all times listed in Table 2, while the requirement of Eq. (20) was satisfied at early times and broke down later. For this set of data, a theoretical value of $\beta^2 = 6.25$ h was calculated. From Eqs. (19) and (20), estimates were calculated of $t_{max} = 8.1$ h and $M_{max} = 0.59$ mg. A plot of the data is given in Fig. 2. Two nonlinear regressions were done using Eq. (18), to test the effects of using different numbers of data points in the regression analysis. The first used data from 0–4 h, and resulted in values of $\alpha = 0.311$ mg h$^{-1/2}$, $\beta^2 = 6.26$ h. A fit $P_m = 0.0199$ cm h^{-1} was obtained from Eq. (22). The second regression used data from 0–12 h, and gave $\alpha = 0.296$ mg h$^{-1/2}$, $\beta^2 = 6.20$ h, and a fit $P_m = 0.0186$ cm h^{-1}. Both of the fit P_m values were in agreement with the theoretical value of 0.02 cm h^{-1}, but the agreement was weaker with the 0–12 h data. This is because, even though Eq. (19) is satisfied for times used above ($M_{max}/\alpha\beta = 0.27$ for the 0–12 h data), Eq. (20) indicates that data taken later than 8 h will begin to introduce errors due to donor depletion. This is illustrated in Fig. 3, in which the fitted values of α and β from the 0–4 data were used in Eq. (14) to generate predicted values of M versus t. There, it can be seen that there is excellent agreement between the data generated using the finite difference program and the predicted line drawn using the fitted values at early times ($t/\beta^2 \sim 1$). By the 12 h ($t/\beta^2 \sim 2$) point, however, the predicted line deviates above the "data" points, due to neglecting the effects of donor depletion. For comparison, the permeability calculated using the steady state gave a value of SS $P_m = 0.0103$ cm h^{-1}, which is significantly lower than the theoretical values. This is because the estimated value of C_d is an average value over the donor region, and is higher than the actual value at the donor–membrane interface.

The points generated from Data Set B represent a case in which β^2 is small ($\beta^2 = 7.5$ min). Using data from 0–1 h ($t_{max} = 8\beta^2$) in the nonlinear regressions resulted in the fit $P_m = 0.0116$ cm h^{-1}, while 0–2 h data resulted in the fit $P_m = 0.0123$ cm

Table 2
Simulated data using parameters listed in Table 1: cumulative amount released in milligrams vs. time in hours

Data Set A		Data Set B		Data Set C	
h	mg	h	mg	h	mg
0.0	0.00	0.00	0.000	0.0	0.00
1.0	3.88×10^{-5}	0.25	3.23×10^{-3}	8.0	7.58×10^{-6}
2.0	2.19×10^{-3}	0.50	8.03×10^{-3}	12.0	1.77×10^{-4}
3.0	1.02×10^{-2}	0.75	1.28×10^{-2}	16.0	9.42×10^{-4}
4.0	2.39×10^{-2}	1.00	1.76×10^{-2}	24.0	5.66×10^{-3}
6.0	6.23×10^{-2}	1.50	2.70×10^{-2}	32.0	1.50×10^{-2}
8.0	1.07×10^{-1}	2.00	3.63×10^{-2}	40.0	2.82×10^{-2}
10.0	1.54×10^{-1}	3.00	5.47×10^{-2}	48.0	4.39×10^{-2}
12.0	1.99×10^{-1}	4.00	7.27×10^{-2}	60.0	7.06×10^{-2}
16.0	2.85×10^{-1}	5.00	9.04×10^{-2}	72.0	9.92×10^{-2}
24.0	4.31×10^{-1}	6.00	1.08×10^{-1}	84.0	1.28×10^{-1}
36.0	5.96×10^{-1}	8.00	1.41×10^{-1}	96.0	1.57×10^{-1}

These data points were included in the regressions of Eq. (18) that led to the calculated values of P_m shown in Table 1.

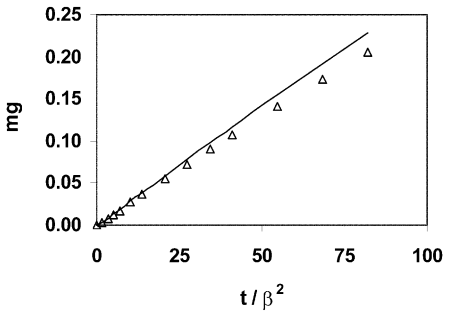

Fig. 3. Cumulative amount released vs. time for simulated data: Data Set B. The diamonds represent the simulated M vs. t data that was generated using a finite difference program, using the parameters listed in Table 1 for Data Set B. The solid line represents the M vs. t curve calculated from Eq. (14), using the values listed in Table 3. The solid line deviates from the data because of the effects of donor depletion at longer times.

h^{-1}. As shown in Table 3, Eqs. (19) and (20) are satisfied in both cases, and both values are in reasonable agreement with the theoretical value of 0.0100 cm h^{-1}. In this case, however, α and β are both small, so that small absolute errors in the values obtained from the nonlinear regression can result in significant relative errors. For comparison, the SS $P_m = 0.0096$ cm h^{-1}. In cases such as this, where α and β are very small, the SS P_m can be obtained relatively quickly and may be more accurate than the newer method. This is shown in Fig. 3.

The points generated from Data Set C represent a case in which β^2 is large ($\beta^2 = 62.5$ h). Using data from 0–16 h ($t_{max} = 0.25\beta^2$) resulted in the fit $P_m = 0.00194$ cm h^{-1}, which is in good agreement with the theoretical value of 0.002 cm h^{-1}. Using data from 0–60 h resulted in a calculated value of the permeability was $P_m = 0.00176$ cm h^{-1}. This value deviates from the theoretical value because some of the points used in the nonlinear regression corresponded to times that exceeded the estimated t_{max} of ~ 20 h, thus violating the condition given by Eq. (20). In this case, the SS P_m was calculated using times from 40 to 96 h (0.64–$1.54\beta^2$), and gave a value of SS $P_m = 0.00126$ cm h^{-1}.

3.2. Application to in vitro data: permeation of human cadaver skin

To verify that the method is applicable to permeation experiments, analyses were done on data that was obtained from in vitro transdermal experiments using human cadaver skin and modified Franz diffusion cells (Nicolettos, 1998). The drugs used were doxepin HCl (DOX), imipramine HCl (IMI) and amitriptyline HCl (AMI). In all cases, the donor gel consisted of an aqueous solution of 0.4% Methocel® (hydroxypropylmethylcellulose), the initial drug concentration was $C_0 = 100$ mg ml^{-1}, and the diffusion coeffi-

Table 3
Results of fitting the simulated data

Data Set	Data times	α	β^2	$M_{max}/\alpha\beta$	Calc. t_{max}	Fit $P_m \times 100$	SS $P_m \times 100$
A	0–4	0.311	6.26	0.03	8.1	1.99	1.03
	0–12	0.296	6.20	0.27	8.1	1.86	
B	0–1	0.038	0.153	1.20	3.4	1.16	0.96
	0–2	0.042	0.175	2.05	3.4	1.23	
C	0–16	0.122	62.5	<0.01	20.2	1.94	1.26
	0–60	0.111	61.7	0.08	20.1	1.76	

The units for the range of data times, β^2 and t_{max} are in hours, α is in mg $h^{-1/2}$, and the permeabilities are in cm h^{-1}. The calculated t_{max} was the smaller of $2\beta^2$ and the value for t_{max} that was obtained from Eq. (20). The fit P_m was calculated using Eq. (22) and the SS P_m was calculated using Eq. (2), as described in Section 3.

cient of drug in the donor $D_d > 0.01$ cm^2 h^{-1} at the experimental temperature of 37 °C. (A full report will be made in a follow-up paper.) Values of α and β were calculated by the procedure described above. The SS P_m was calculated using Eq. (2) as described in the previous section, using data starting from the first point for which $t > 2\beta^2$ through the first point for which $t > 5\beta^2$. The data is given in Table 4 and Figs. 5 and 6.

For the DOX release, using data from 0–12 h, the fitted values were $\alpha = 0.10$ mg $h^{-1/2}$ and $\beta^2 = 1.5$ h, which gave a value of fit $P_m = 1.1 \times 10^{-4}$ cm h^{-1}. Using data from 0–8 h gave nearly identical results for the fit P_m, although the values of α and β changed somewhat. Using data from 0–20 h gave a fit $P_m = 1.2 \times 10^{-4}$ cm h^{-1}. For all three ranges, the predicted data was in good agreement with the experimental data, as shown in Figs. 3 and 4. For comparison, the SS $P_m = 1.2 \times 10^{-4}$ cm h^{-1}.

For the IMI release, using data from 0–20 h, the fitted values were $\alpha = 0.14$ mg $h^{-1/2}$ and $\beta^2 = 6.6$ h, and the fit $P_m = 0.66 \times 10^{-4}$ cm h^{-1}. Using data from 0–28 h gave nearly identical results for the fit P_m. Using data from 0–12 h gave a consistent but different value for the permeability (fit $P_m = 0.44 \times 10^{-4}$ cm h^{-1}). The 0–12 h results were rejected, however, because the agreement

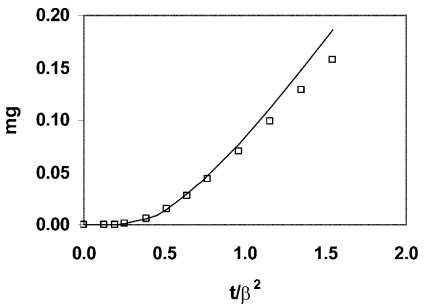

Fig. 4. Cumulative amount released vs. time for simulated data: Data Set C. The squares represent the simulated M vs. t data that was generated using a finite difference program, using the parameters listed in Table 1 for Data Set A. The solid line represents the M vs. t curve calculated from Eq. (14), using the values listed in Table 3. The solid line deviates from the data because of the effects of donor depletion at longer times.

Table 4
Results of fitting in vitro human cadaver skin data

Drug	Data times	α	β^2	$M_{max}/\alpha\beta$	Calc. t_{max}	Fit $P_m \times 10^4$	SS $P_m \times 10^4$
DOX	0–12	0.10	1.5	1.4	14.7	1.1	1.1
IMI	0–20	0.14	6.6	0.54	21.4	0.66	0.70
AMI	0–12	0.028	4.1	0.93	35.7	0.20	0.18

Data obtained for the release of doxepin (DOX), imipramine HCl (IMI) and amitriptyline HCl (AMI) from a donor gel of 0.4% Methocel® through human cadaver skin. In all cases, the concentration of drug in the donor was 100 mg ml^{-1}, the donor volume was 2 ml and the thickness L of the donor was ~ 1.1 cm. All values for SS P_m were calculated using data from $> 2\beta^2$ through $> 5\beta^2$, as described in Section 3.

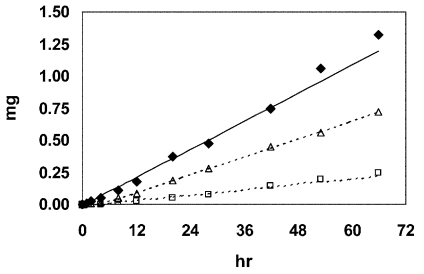

Fig. 5. Permeation through human cadaver skin: cumulative amount of drug in the receiver vs. time for doxepin HCl, Imipramine HCl and amitriptyline HCl. Data obtained for the release of doxepin (DOX), imipramine HCl (IMI) and amitriptyline HCl (AMI) from a donor gel of 0.4% Methocel® through human cadaver skin. In all cases, the concentration of drug in the donor was 100 mg ml^{-1}, the donor volume was 2 ml and the thickness L of the donor was ~ 1.1 cm. The filled diamonds represent experimental data for DOX, the triangles represent experimental data for IMI, and the squares represent data for AMI. The lines represent predicted values using the fitted values of α and β obtained by nonlinear regressions.

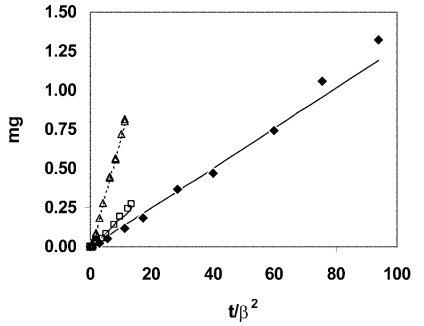

Fig. 6. Permeation through human cadaver skin: cumulative amount of drug in the receiver vs. t/β^2 for doxepin HCl, Imipramine HCl and amitriptyline HCl. Data obtained for the release of doxepin (DOX), imipramine HCl (IMI) and amitriptyline HCl (AMI) from a donor gel of 0.4% Methocel® through human cadaver skin. In all cases, the concentration of drug in the donor was 100 mg ml^{-1}, the donor volume was 2 ml and the thickness L of the donor was ~ 1.1 cm. The filled diamonds represent experimental data for DOX, the triangles represent experimental data for IMI, and the squares represent data for AMI. The lines represent predicted values using the fitted values of α and β obtained by nonlinear regressions.

between the predicted and experimental data at later times was significantly better using the results of the 0–20 h fits. For comparison, the SS $P_m = 0.70 \times 10^{-4}$ cm h^{-1}.

For the AMI release, using data from 0–12 h, the fitted values were $\alpha = 0.028$ mg $h^{-1/2}$ and $\beta^2 = 4.1$ h, and the fit $P_m = 0.20 \times 10^{-4}$ cm h^{-1}. Using data from 0–20 h gave nearly identical results for the fit P_m. For comparison, the SS $P_m = 0.18 \times 10^{-4}$ cm h^{-1}.

4. Discussion

The method described here can be used to obtain membrane permeabilities using release data from unstirred donors, such as gels. Mathematically, the assumptions about the membrane are the same those of the lag time model (although the boundary conditions are different), in that a single layer membrane with constant diffusion parameters is assumed. However, the method can be used successfully for composite membranes (such as skin), when one layer or pathway is clearly rate determining. For these cases, it is more meaningful to determine the permeability of the membrane instead of the total membrane thickness and the component diffusion and partition coefficients. The applicability of the method to composite membranes was demonstrated using human cadaver skin transdermal data.

Analyses have shown that accurate determinations of P_m can be made using data taken exclusively from early time points. For systems in which the concentration profile develops slowly in the membrane, this can be an important advantage over other methods. For instance, obtaining the permeability from steady state data can require long experiments before the steady state portion of the curve is reached. Similar remarks apply to lag time experiments. For systems such as these, the method presented here might require experiments that can be $\sim 1/5$ as long as classic lag time experiments or steady state experiments. Even for systems where β is small, the results are more accurate than those obtained using steady state approximations unless α is also small. For those cases, in which α and β are both small, the method is applicable but less advantageous.

Since the above analyses involve truncating infinite series solutions to differential equations, it is important to evaluate when the necessary

mathematical conditions are met in terms of the experimental data. Eqs. (19) and (20) provide two tests that can be done directly on the analysis results to verify that these mathematical restrictions are met. In addition, Eq. (14) allows the comparison of the experimental data and the predicted M versus t using the results of the analysis, which provides a third guideline for choosing the range of data to be used in the regressions. This can be important when analyzing data with experimental variability, or an occasional bad data point. An example of this was seen for the imipramine release data. Once an appropriate range of data points is selected, however, the values of P_m obtained from the nonlinear regressions typically do not change much due to changing the range of data points used (as long as the other restrictions are met). Typically, these variations in P_m are less than 10%, which is within acceptable error.

An important result is provision of a means for predicting the performance of other systems, given a knowledge of α and β. This is not possible knowing only P_m and using a steady state model. For instance, if the area or initial donor concentration are changed, α may be recalculated from Eq. (15) to predict the M versus t curve from Eq. (14). It should be noted that, in some cases, the predicted data may be a better indicator of in vivo performance than the experimental data. For instance, α and β may characterize skin in contact with a transdermal patch better than skin that is in contact with a donor solution for a week.

It is also possible to use data obtained from lag time data to predict release from different unstirred donors of known D_d. Since $\beta^2 = 1.5$, t_{lag} and P_m can be obtained from Eq. (2), $K\sqrt{D_m} = 2\beta P_m$, and α may then be calculated from Eq. (15). (It should be noted that Eqs. (1) and (2) can be applied to different experimental data than Eqs. (13) and (15), but the two pairs are not mathematically independent. Thus, no new physical information would be obtained if lag time data were combined with data applicable to the model presented in this paper.)

Eq. (20) expresses the condition for which neglecting donor depletion can be neglected. This is analogous to the model for the release of drugs from ointments (Higuchi, 1962), which holds only while no more than approximately 1/3 of the drug has been released. In this model, however, it should be noted that the amount of drug that has left the donor might not equal the amount that has been released into the receiver. If the membrane is thick enough or the drug partitions enough into the membrane (so that Kh is large), a significant membrane depot effect may occur. As a result, the donor depletion restriction is best expressed in terms of L and β instead of M.

Unlike the lag time experiments, this method requires that the diffusion coefficient of the drug in the donor gel D_d be known. However, for most skin experiments, the values of P_m calculated from Eq. (22) are relatively insensitive to errors in D_d, since δ is typically close to 1. Physically, this is equivalent to saying that the membrane is rate controlling.

The parameters α and β are functions of K, D_m and h. Eqs. (1) and (2) are also functions of these parameters, but they are not independent of Eqs. (13) and (15). Thus, no new physical information would be obtained if lag time experiments were also done on similar membranes. Thus, it is possible to determine only two parameters from the release experiments, and the third must be determined by some other type of measurement. However, if the membrane permeability is measured from a constant donor experiment using Eq. (2), then a third parameter can then be deduced. This is of limited usefulness, however, for composite membranes, since the meanings of h and K are not well defined in that case.

5. Summary

A new method for the determination of membrane permeabilities has been developed, based on the common experimental technique of release of drug from an unstirred donor gel. The method was numerically tested on simulated data obtained from numerical solutions to Fick's Laws for a number of conditions. The method was also found to be applicable to experimental transdermal data obtained using human cadaver skin. The method

has the advantage that accurate determinations of membrane permeabilities can be done using much shorter experiments than might be required using other methods (such as the classic lag time experiment or steady state experiments). In addition, the equations developed can be used to predict the release from other systems at early times. For homogeneous membranes or composites in which one layer or pathway is definitely rate limiting, the analysis gives excellent determinations of the membrane permeability. For composite membranes in which there is no clear rate-determining component, the method must be modified. This analysis will be presented in a future paper.

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Appendix A: Notes on simplifying the series solution leading to Eq. (18)

Eqs. (3)–(10) can be solved by the method of LaPlace transforms (Eqs. (9) and (10)) and combined with Fick's First Law to give the LaPlace transform of the rate of accumulation in the receiver as

$$L\left\{\frac{dM}{dt}\right\} = \frac{2AKC_0\sqrt{D_m D_d}}{\sqrt{s}(K\sqrt{D_m}+\sqrt{D_d})}\,(y^{1/2}-zy^{1/2}) \times\left[\sum_{m=0}^{\infty}(-1)^m y^m z^m\right] \times\sum_{n=0}^{\infty}\left[\delta^n(y+z)^n\left(\sum_{l=0}^{\infty}(-1)^l y^l z^l\right)^n\right]$$

where s is the LaPlace variable that is conjugate to the time, and y and z are defined as

$$y=\exp\left(-2h\sqrt{\frac{s}{D_m}}\right)\ \text{and}\ z=\exp\left(-2L\sqrt{\frac{s}{D_d}}\right).$$

This solution can be inverted by straightforward expansion and use of the inverse LaPlace Transformation property (Churchill, 1972)

$$f(s)=\frac{1}{\sqrt{s}}\exp(-k\sqrt{s})\rightarrow F(t)=\frac{1}{\sqrt{\pi t}}\exp\left(-\frac{k^2}{4t}\right)$$

This solution, which includes the products of several infinite series, can be simplified in two ways. The first is to determine the conditions under which the dependence on L can be neglected, which corresponds to neglecting all of the terms containing z. The second is to determine the range of times for which the higher order terms ($n>1$) in the resulting infinite series can be neglected, which corresponds to neglecting all terms in y and z. These are discussed below.

It is sufficient to examine when the z terms in $(y^{1/2}-zy^{1/2})$ can be neglected, since terms including any powers in $(y+z)$ can be neglected by similar arguments to those that follow.

Using the inverse transforms above, the z terms can be neglected to no more than 5% error when

$$0.05\exp\left(-\frac{h^2}{4D_m t}\right)>\exp\left(-\frac{1}{4t}\left[\frac{h}{\sqrt{D_m}}+\frac{2L}{\sqrt{D_d}}\right]^2\right)$$

This is equivalent to the condition that

$$\frac{Lh}{\sqrt{D_d D_m}}+\frac{L^2}{D_d}>3t$$

When the z terms can be neglected, the above series simplifies to

$$L\left\{\frac{dM}{dt}\right\}=\frac{2AKC_0\sqrt{D_m D_d}}{\sqrt{s}(K\sqrt{D_m}+\sqrt{D_d})}\exp\left(-h\sqrt{\frac{s}{D_m}}\right)\times\sum_{n=0}^{\infty}\delta^n y^n$$

Performing the inverse Laplace transform of this equation gives Eq. (14). By inspection, it can be calculated that neglecting the $n>0$ terms in Eq. (14) results in 1% error when $t=2.43\beta^2$, 5% error when $t=4.34\beta^2$, 10% error when $t=6.38\beta^2$, 15%

error when $t = 8.61\beta^2$, and 20% error when $t = 11.24\beta^2$.

Appendix B: Methods for obtaining initial estimates for α and β

Initial estimates for α and β can be obtained in a number of ways. For instance, they are often obtained simply from trial and error, by comparing plots of Eq. (14) and the experimental data. However, occasions arise when more than one reasonable range of values exists for the initial estimates. For these cases, an alternative method is described below.

Multiplying Eq. (17) by $(2/3)t^{-(3/2)}$ and integrating with respect to time from t_0 to t gives

$$\frac{2}{3}\int_{t_0}^{t}\frac{\mathrm{d}M}{\mathrm{d}t}\,t^{-3/2}\,\mathrm{d}t = \int_{t_0}^{t}\frac{\alpha}{3t^2}\exp\left(-\frac{\beta^2}{t}\right)\mathrm{d}t$$

Calling the left-hand side $I(t)$ and integrating the right-hand side by parts gives

$$I(t) = \int_{t_0}^{t} Mt^{-5/2}\,\mathrm{d}t + \frac{2}{3}\left(M(t)t^{-3/2} - M(t_0)t_0^{-3/2}\right)$$

$$= \frac{\alpha}{3\beta^2}\left[\exp\left(-\frac{\beta^2}{t}\right) - \exp\left(-\frac{\beta^2}{t_0}\right)\right]$$

where t_0 is chosen to be the earliest time for which reliable experimental data can be measured.

Taking the natural log of this equation, and noting that (from L'Hospital's Rule of calculus) $M(t_0)t_0^{-3/2} \to 0$ and $\exp(-(\beta_2/t_0)) \to 0$ when $t_0 \to 0$ gives

$$\ln I(t) = \ln\left(\frac{\alpha}{3\beta^2}\right) - \frac{\beta^2}{t}$$

$I(t)$ can be determined from the data by numerical integration of the data, and estimates for α and β can be determined from a plot of $\ln I(t)$ versus $1/t$, which gives a straight line with slope $= -\beta^2$ and intercept $= \ln(\alpha/(3\beta^2))$. It should be pointed out that the values of α and β are typically not accurate enough to use as final numbers, but work well as initial estimates.

References

Bellantone, R. 1999. Finite difference program for transient diffusion calculations in 1-d using the explicit FCTS algorithm.

Carslaw, H.S., Jaeger, J.C., 1959. Conduction of Heat in Solids. Clarendon, New York.

Churchill, R.V., 1972. Operational Mathematics. Wiley, New York.

Crank, J., 1975. The Mathematics of Diffusion, second ed.. Oxford, New York.

Fletcher, C.A.J., 1988. Computational Techniques for Fluid Dynamics, vol. I. Springer-Verlag, New York.

Flynn, G.L., Yalkowsky, S.H., Roseman, T.J., 1974. Mass transport phenomena and models: theoretical concepts. J. Pharm. Sci. 63, 479–510.

Friend, D.R., 1992. In vitro skin permeation techniques. J. Controlled Release 18, 235–248.

Higuchi, W.I., 1962. Analysis of data on the medicament release from ointments. J. Pharm. Sci. 51, 802–804.

Nicolettos, N., 1998. Transdermal delivery of certain tricyclic antidepressants: in vitro/in vivo evaluations. Ph.D. Dissertation, Department of Pharmaceutics and Industrial Pharmacy, Arnold and Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, New York.

Pena, E.L., 1995. Gel dosage forms: theory, formulation, and processing. In: Osborne, E.W., Amann, A.H. (Eds.), Topical Drug Delivery Formulations. Marcel Dekker, New York, pp. 381–388.

Poulsen, B.J., Flynn, G.L., 1985. In vitro methods to study dermal delivery and percutaneous absorption. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption: Mechanism-Absorption-Drug Delivery. Marcel Dekker, New York, pp. 431–459.

Press, W.H., Teukolsky, S.A., Vetterling, W.T., Flannery, B.P., 1992. Numerical Recipes in Fortran 77: The Art of Scientific Computing. Cambridge University Press, New York.

Schaefer, H., Redelmeier, T., 1996. Skin Barrier: Principles of Percutaneous Absorption. Karger, Basel.

Shah, J.C., 1993. Analysis of permeation data: evaluation of the lag time method. Int. J. Pharm. 90, 161–169.

Valia, K.H., Chien, Y.W., 1984. Development of a dynamic skin permeation system for long-term permeation studies. Drug Dev. Indust. Pharm. 10, 579–599.

Walters, K.A., 1986. Percutaneous absorption and transdermal therapy. Pharm. Tech. 10, 30–42.