In Situ Determination of Partition and Diffusion Coefficients in the Lipid Bilayers of Stratum Corneum

Samir Mitragotri[1,2]

Received January 14, 2000; accepted April 27, 2000

KEY WORDS: transdermal; model; diffusion coefficient; partition coefficient; non-intrusive.

INTRODUCTION

Transdermal drug delivery (TDD) offers several advantages over traditional drug delivery systems such as oral delivery and injection including elimination of first pass metabolism, minimization of pain and possible sustained release of drugs (1). However, applications of TDD are limited by the low permeability of stratum corneum (SC), the uppermost layer of the skin. SC consists of about 15 layers of keratin-filled cells (keratinocytes) with the space between the keratinocytes filled with lipid bilayers. Low permeability of the SC originates from the low permeability of its lipid bilayers (2).

Permeability of SC to drugs is determined by two important transport coefficients, that is, partition and diffusion coefficient (3). Numerous reports of permeability measurements of the SC can be found in the literature. However, relatively few measurements of drug partition and diffusion coefficients in the lipid bilayers of the SC have been performed. This follows the fact that direct measurements of drug partition and diffusion coefficients are significantly more challenging than permeability measurements. Direct measurements of solute partitioning into the SC are difficult due to its heterogeneous nature (3). Accordingly, attempts have been made to extract SC lipids and form bilayers of these lipids on a solid support (3). Partition coefficients of solutes into supported lipid bilayers have then been measured. Although this method has been greatly helpful in understanding partition coefficients of drugs in SC lipid bilayers, its applicability is limited due to the requirement of lipid extraction which removes the SC lipids from its natural environment. Furthermore, it is difficult to measure the drug partition coefficient into supported lipid bilayers in the presence of enhancers (chemicals, ultrasound, or electric fields). This follows the fact that the stability of these bilayers in the presence of these enhancers is questionable.

Attempts have also been made to measure diffusion coefficients in bilayers prepared from extracted SC lipids (4). These measurements have been performed using Fluorescent Recovery After Photobleach (FRAP). These measurements have been successfully used for measuring diffusion coefficients of some molecules. However, the applicability of FRAP is limited by the requirement of isolating the SC lipid bilayers, and the requirement of fluorescently labeling drugs, which cannot be performed in many cases. Thus, there is a need for a simple method that can be: i) used to determine drug *partition* and *diffusion* coefficients in the SC without requiring the removal of the lipid bilayers from the SC, and ii) used to understand the dependence of enhancers on the SC transport properties.

In this paper, we describe such a method based on a combined theoretical and experimental approach. The method is based on two independent measurements of the transport properties of the SC: i) steady-state permeability in the presence of a concentration gradient, and ii) release of drug from the SC when the concentration gradient is in the opposite direction. This information is then analyzed using a theory to determine partition and diffusion coefficients. This approach provides a truly non-intrusive determination of solute partition and diffusion coefficients.

THEORETICAL ANALYSIS

Stratum Corneum Permeability

Solute diffusion across stratum corneum (SC) can be described by Fick's law as follows (5):

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

where, D is the solute diffusion coefficient, C is the solute concentration in the SC, and x is the distance. As discussed earlier, the SC consists of several layers of keratin-filled dead cells referred to as keratinocytes (6). The space between the keratinocytes is filled with lipid bilayers. Transdermal transport of drugs (especially hydrophobic drugs) occurs through these lipid bilayers. Hence, only a small fraction of the area is available for drug transport. Furthermore, the drug has to follow a tortuous path to cross the SC. Hence the effective SC thickness for solute transport is τ^*L, where, L is the SC thickness and τ^* is the effective tortuosity factor. Equation 1 can then be solved to obtain the steady-state drug permeability, P, with the boundary conditions, $C(x = 0) = C_0$ and $C(x = L) = 0$ ($x = 0$ corresponds to the SC surface and $x = L$ corresponds to the end of the SC), as follows (7):

$$P = \frac{D_b K_b}{\tau^* L} \tag{2}$$

where D_b is the solute diffusion coefficient in the SC lipid bilayers and K_b is the solute partition coefficient in the SC lipid bilayers. Johnson et al have shown that the value of τ^*L is 3.6 cm (7). Substituting these values into Eq. 2 one obtains:

$$P = \frac{D_b K_b}{3.6} \tag{3}$$

where, P has the units of cm/s and D_b has the units of cm^2/s. K_b is dimensionless. P can be easily measured by performing conventional permeability experiments using diffusion cells. Once that is done, Eq. 3 contains two unknown variables, D_b and K_b. To determine the values of these variables, we need another independent equation relating D_b and K_b. This is discussed next.

[1] Department of Chemical Engineering, University of California, Santa Barbara, California 93106.

[2] To whom correspondence should be addressed. (e-mail: samir@engineering.ucsb.edu)

0724-8741/00/0800-1026$18.00/0 © 2000 Plenum Publishing Corporation

Solute Release from the Stratum Corneum

In these experiments, a piece of SC is soaked in a solution of radiolabeled solute in PBS. SC is allowed to equilibrate with the solution. SC is then removed from this solution and transferred into a known volume of PBS. Release of the drug from the SC is measured by measuring the concentration of the solute in PBS as a function of time. The concentration of solute in PBS, $C_s(t)$, increases with time before reaching an equilibrium value, C_∞. The rate of drug release is also determined by Fick's law as described in Eq. 1. The boundary conditions are given by:

$$C(t = 0) = C(0)$$

$$V_{PBS}\left(\frac{\partial C}{\partial t}\right)_{x=0,L} = \pm D_b K_b A \left(\frac{\partial C}{\partial x}\right)_{x=0,L}$$

where A is skin area. This equation can be solved to arrive at the following equation (5):

$$\frac{C_s(t)}{C_\infty} = 1 - \sum_{n=0}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n} e^{-D_b q_n^2 t/(L\tau^*)^2} \quad (4)$$

where,

$$\alpha = \frac{K_b V_{SC} f}{V_{PBS}} \quad (5)$$

where, V_{PBS} is the volume of PBS into which the drug is released (5 ml) and V_{SC} is the volume of the SC used for experiments ($V_{SC} = L \times A$) (typically 0.0026 cm^3, that is, $A = 2\ cm^2$ and $L = 13\ \mu m$ [7]), and f is the fractional volume of lipids in the SC (0.1 (7)). Equation 4 can be simplified for *short times* as follows (5):

$$\frac{C_s(t)}{C_\infty} = (1+\alpha)\left(1 - e^{D_b t/(L\tau^*)^2\alpha^2}\, erfc\left[\frac{D_b t}{(L\tau^*)^2\alpha^2}\right]^{0.5}\right) \quad (6)$$

Equations 3 and 6 respectively describe the permeability of the SC and the release from the SC. Both these quantities can be experimentally measured and substituted in Eqs. 3 and 6 respectively. In that case, Eqs. 3 and 6 have only two unknown variables, that is, D_b and K_b. Hence, these equations can be solved to obtain the values of these variables.

EXPERIMENTAL METHODS

Measurements of SC Permeability

Permeability experiments were performed with heat-stripped human epidermis using previously published methods (8). Skin was obtained from National Disease Research Institute. Subcutaneous fat was removed and the epidermis was separated using heat stripping (9,10). Transport experiments were performed using side-by-side diffusion cells (Crown Glass Company). The epidermis was placed between the donor and the receiver compartments. The compartments were filled with Phosphate Buffered Saline at a pH of 7.4 (PBS). Skin electrical resistance was measured using previously published methods (11). Skin samples with resistivity less than 50 kohm-cm^2 were considered damaged and were discarded. The donor compartment was then emptied and was filled with a solution of radiolabeled solute (estradiol, testosterone, corticosterone, progesterone, aldosterone, napthol, lidocaine, octanol, and decanol, all obtained from New England Nuclear, MA) in PBS. The concentration of the solute in the donor compartment was 1 μCi/ml in each case. Samples were taken from the receiver compartment periodically over 48 hours. Radioactivity in the collected samples was measured using a scintillation counter (Packard 2000 CA). Skin permeability was calculated based on the equation, $P = J/C_d$, where J is the steady-state transdermal solute flux and C_d is the solute concentration in the donor compartment (assuming that C_d is less than the saturation concentration). All experiments were repeated at least 4 times.

Measurements of Solute Release from the SC

Release kinetics was measured from the human cadaver stratum corneum (SC). SC was prepared from heat-stripped skin that was in turn prepared using methods described above. To separate SC from the heat-stripped skin, heat-stripped epidermis was placed in trypsin solution for 24 hours (12). Epidermis was then washed off using Phosphate Buffered Saline (PBS). This procedure yielded clean SC samples. These samples were placed onto a Teflon board and cut into 2 cm^2 disks carefully using a metal punch. Solutions of radiolabeled solutes (estradiol, testosterone, corticosterone, progesterone, aldosterone, napthol, lidocaine, octanol, and decanol) were prepared in PBS at a concentration of 1 μCi/ml. SC disks were placed in 5 ml of

Table 1. List of Model Solutes and Their Molecular Properties

Permeant	MW	Permeability (cm/hr)	K_b (this study)	D_b cm^2/s (this study)	K_b Ref. (3)	D_b cm^2/s Ref. (3)	$K_{octanol/water}$ Ref. (3)
Aldosterone	360	3.0×10^{-05}	16	1.8×10^{-09}	8	4.5×10^{-09}	12
Corticosterone	346	1.0×10^{-04}	39	2.5×10^{-09}	33	3.3×10^{-09}	87
Decanol	158	9.0×10^{-02}	189	4.7×10^{-07}	—	5.3×10^{-08}	17378
Estradiol	272	3.0×10^{-03}	85	3.5×10^{-08}	177	3.5×10^{-09}	7244
Lidocaine	234	3.0×10^{-03}	59	5.0×10^{-08}	24	3.9×10^{-08}	302
Napthol	144	2.6×10^{-02}	513	5.0×10^{-08}	954	1.8×10^{-07}	692
Octanol	130	7.0×10^{-02}	173	4.0×10^{-07}	—	2.8×10^{-07}	1413
Progesterone	314	2.0×10^{-02}	329	6.0×10^{-08}	1060	2.7×10^{-08}	5888
Testosterone	288	2.2×10^{-03}	108	2.0×10^{-08}	81	6.7×10^{-09}	2042

solution for a period of 24 hours. This was done to load the SC samples with the solute. The solution temperature was maintained at 4°C. At the end of 24 hours, SC samples were tapped dry on a paper towel to remove surface radioactivity. These samples were quickly placed in a glass container containing 5 ml of PBS at room temperature. A stir bar was placed in PBS to maintain good mixing (same speed for all experiments). Concentration of solutes in PBS was measured every 5–10 minutes for the first hour and once every 30 minutes thereafter for another 3 hours. A final sample was taken after 48 hours. No physical degradation of the SC was observed in this period. This data is consistent with our previous experience regarding the stability of the SC and the epidermis in PBS over this period (8). Each experiment was repeated at least 4 times. The concentration of the solute in PBS was plotted as a function of time. The data for the first three hours, along with the permeability data was fitted to Eqs. 3 and 6. The mean error associated with the fit was about 5%. Note that the model is based on permeability measurements of the epidermis while release measurements of the SC. Hence, the model is applicable for solutes for which SC is the rate-limiting barrier.

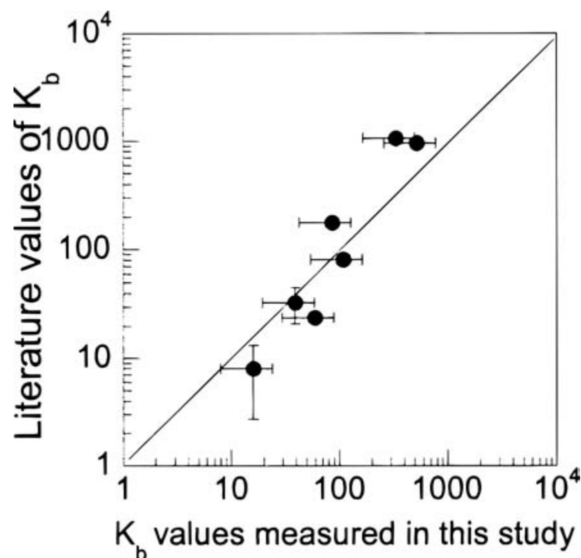

Fig. 2. Comparison of K_b values measured by our method with those reported by Johnson *et al.* (3). Error bars on values measured by our methods correspond to a 40% error in measurement of skin permeability.

RESULTS AND DISCUSSION

The method proposed in this paper was tested for nine solutes, estradiol, testosterone, aldosterone, corticosterone, progesterone, napthol, lidocaine, decanol, and octanol. These solutes were chosen sine the SC lipid partition coefficients as well as permeabilities for most of these solutes have been previously measured (3). A list of measured permeabilities for the solutes used in this study is shown in Table 1. Figure 1 shows the relative rate of release for two drugs, that is, testosterone (circles) and progesterone (squares), from the SC (measured according to methods described in the previous section). The rate of release of progesterone is lower than that of testosterone. This data is consistent with the higher lipophilicity of progesterone. Specifically, transfer of lipophilic drugs from SC into PBS is unfavorable, thus retarding their release from the SC.

The permeability and release data was analyzed respectively using Eqs. 3 and 6 to arrive the values of K_b and D_b. These values are listed in Table 1. The Table also shows K_b values directly measured by Johnson *et al.* (3) in bilayers of extracted SC lipids and D_b values predicted using the model

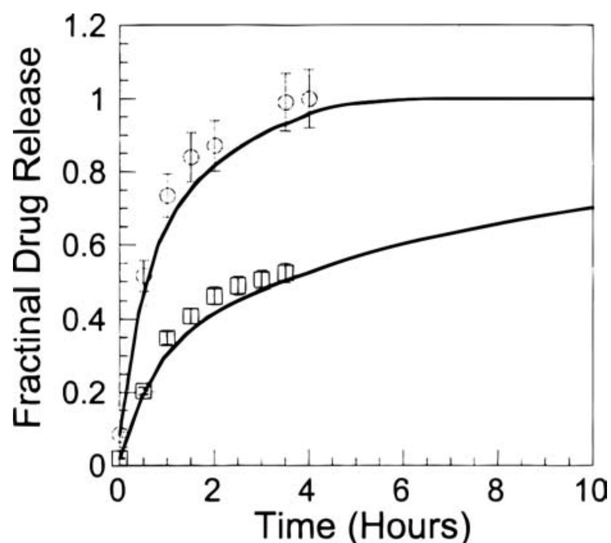

Fig. 1. Fractional release of testosterone (circles) and progesterone (squares) from the SC. Fractional release was calculated by dividing the total amount released at time, *t*, by that released at infinite time (48 hours) (n = 4–5).

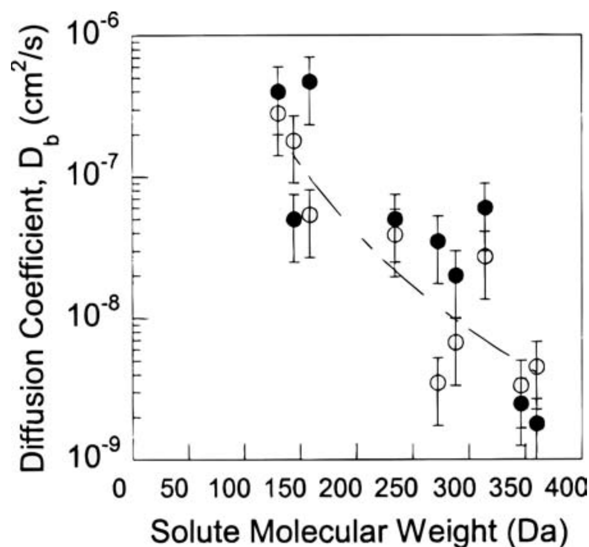

Fig. 3. Dependence of diffusion coefficients on solute molecular weight. Values indicated by closed circles are those measured by methods described in this paper. Values indicated by open circles correspond to those calculated by Johnson *et al.* (4). Typical error associated with our measurements as well as with the calculations of Johnson *et al.* is about 40%.

developed by Johnson *et al.* (4). Their model utilized Eq. 3 in combination with the correlation $K_b = K_{o/w}^{0.76}$ to predict D_b. The relationship between K_b values measured using our method with those measured by Johnson et al is shown in Fig. 2. The error bars on our values of K_b correspond to the error in the prediction introduced by a 40% error in the experimental measurement of skin permeability. Overall, the agreement between the two values is good. Figure 3 shows the variation of D_b measured by our method with solute molecular weight (closed circles). The Figure also shows D_b values calculated by Johnson *et al.* (7) (open circles). Overall, the variation of the diffusion coefficient with solute molecular weight is consistent in both methods. Solute diffusion coefficient decreases with increasing molecular weight, although the exact relationship between the two needs further investigations. Thus, the partition and diffusion coefficients predicted by our method compare well with the literature reports. Yet, the method is simple to use (especially for radiolabeled drugs) and can be utilized for a broad variety of drugs. Furthermore, it allows in situ determination of solute partition and diffusion coefficients. This is especially important since our method determines the transport properties of lipid bilayers while they are within the SC. Most importantly, this method can be easily utilized to determine the effect of enhancers (chemicals or ultrasound) on solute partition and diffusion coefficients. For example, permeability as well as release of drugs from the SC can be easily measured in the presence of enhancers and utilized to determine K_b and D_b values. Such studies should assist in developing a better understanding of the mechanisms of the effect of enhancers on transport properties of SC lipids.

REFERENCES

1. R. L. Bronaugh and H. I. E. Maibach. *Percutaneous Absorption: Mechanisms-Methodology-Drug Delivery*, Marcel Dekker, New York, 1989.
2. S. Mitragotri, M. E. Johnson, D. Blankschtein, and R. Langer. A theoretical analysis of partitioning, diffusion, and permeation across lipid bilayers. *Biophys. J.* **77**:1268–1283 (1999).
3. M. E. Johnson. Ph.D. Thesis in *Chemical Engineering*, Massachusetts Institute of Technology, Cambridge (1996).
4. M. E. Johnson, D. A. Berk, D. Blankschtein, and R. Langer. Lateral diffusion of small compounds in human stratum corneum and model lipid bilayer systems. *Biophys. J.* **71**:2656–2668 (1996).
5. J. Crank. *Mathematics of Diffusion*, Oxford Publishers, 1975.
6. P. M. Elias, E. R. Cooper, A. Korc, and B. E. Brown. Percutaneous transport in relation to stratum corneum structure and lipid composition. *J. Invest. Dermatol.* **76**:297–301 (1981).
7. M. E. Johnson, D. Blankschtein, and R. Langer. Evaluation of solute permeation through the stratum corneum: Lateral bilayer diffusion as the primary transport mechanism. *J. Pharm. Sci.* **86**:1162–1172 (1997).
8. M. E. Johnson, S. Mitragotri, A. Patel, D. Blankschtein, and R. Langer. Synergistic effect of ultrasound and chemical enhancers on transdermal drug delivery. *J. Pharm. Sci.* **85**:670–679 (1996).
9. S. Mitragotri, D. Blankschtein, and R. Langer. Transdermal drug delivery using low-frequency sonophoresis. *Pharm. Res.* **13**:411–420 (1996).
10. S. Mitragotri, D. Edwards, D. Blankschtein, and R. Langer. A mechanistic study of ultrasonically enhanced transdermal drug delivery. *J. Pharm. Sci.* **84**:697–706 (1995).
11. S. Mitragotri, J. Farrell, H. Tang, T. Terahara, J. Kost, and R. Langer. Determination of the threshold energy dose for ultrasound-induced transdermal drug delivery. *J. Control. Rel.* **63**:41–52 (2000).
12. B. D. Anderson, W. I. Higuchi, and P. V. Raykar. Heterogenity effects of permeability-partition coefficient relationship in human stratum corneum. *Pharm. Res.* **5**:566–573 (1988).