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Note

The determination of a diffusional pathlength through the stratum corneum

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Abstract

The stratum corneum possesses a very heterogenous structure. As such a diffusing molecule can access a number of different pathways. It is probable that the excellent barrier properties of the stratum corneum result from a tortuous diffusional pathway around the dead cells. However, there are considerable problems in designing diffusion experiments and analysing the data to prove, without doubt, which is the predominant pathway. The mathematical problems posed are discussed in this article.

Keywords: Diffusional pathlength; Human stratum corneum

The mechanism by which xenobiotics passively permeate the skin's least permeable layer, the stratum corneum (SC), remains a subject of considerable debate. The heterogeneous nature of the SC suggests that mass transfer can occur (at least) via transcellular and intercellular pathways

Abbreviations: A_L, area of lipid fraction of SC in contact with vehicle; A_{sc}, area of SC in contact with vehicle; C_v, concentration of permeant in the vehicle (assumed time invariant); D, diffusion coefficient of permeant in an unspecified path through the SC; D_L, diffusion coefficient of permeant in the intercellular pathway across the SC; D_{sc}, apparent diffusion coefficient in the SC considered as a pseudo-homogeneous membrane; h, length of an unspecified pathway through the SC; h_L, length of the intercellular pathway through the SC; h_{sc}, apparent length (i.e. thickness) of the SC; k_P, molecular permeability coefficient across the SC; $K_{L/v}$, equilibrium partition coefficient representing the distribution of permeant between the lipid fraction of the SC and vehicle; $K_{sc/v}$, equilibrium partition coefficient representing the apparent distribution of permeant between the SC and vehicle; $\dot{M}_{SS}$, steady-state rate of permeant penetration through the SC; V_L, volume of the intercellular pathway across the SC $= A_L \times h_L$; V_{sc}, volume of the SC $= A_{sc} \times h_{sc}$.

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(Michaels et al., 1975). Furthermore, the intercellular regions are full of lipids structured in multilamellar arrays, through which more than one route of molecular diffusion may be envisaged. Arguments over the relative importance of the various possible routes have continued for more than 25 years—to say nothing of the potential impact of shunt diffusion via appendageal structures such as hair follicles.

Lately, though, there has been a preponderance of opinion leaning towards the dominance of intercellular diffusion, based on supporting (if sometimes only circumstantial) evidence from diverse sources. For example, Albery and Hadgraft (1979a,b) applied a detailed mathematical analysis to the results of in vivo skin permeation experiments to deduce that the intercellular path was likely to dominate for small nonelectrolytes; Nemanic and Elias (1980) and Bodde et al. (1991) used sensitive electron microscopic techniques to localize penetrants within the intercellular domains; and the most compelling argument originated from combined permeability and SC biophysical measurements which led, ultimately, to the deduction that the molecular diffusion pathlength (h) of water across the SC is manifold greater than the membrane's simple thickness (h_{sc}) (Potts and Francoeur, 1990, 1991). However, recently, Pellett et al. (1997) re-examined the issue of diffusion pathlength, and determined a value of h much closer to that of h_{sc}, opening the question once more of the importance of transcellular diffusion.

In their study, Pellett et al. (1997) combined the results of a classic in vitro skin permeation measurement with those from a novel attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) experiment to derive the value for h. Specifically, the diffusion cell data yielded the permeant's steady-state permeability coefficient ($\{k_P\}_{\text{diff cell}}$), while the ATR-FTIR experiment generated, at steady state, the equilibrium partition coefficient ($\{K_{sc/v}\}_{\text{ATR-FTIR}}$) of the chemical between the total volume of the SC and the vehicle (in this case water), and, from the transient period, the chemical's characteristic transport parameter ($\{D/h^2\}$), where D is its diffusivity along the diffusion pathlength within the SC. Pellett et al. (1997) specified that h could then be calculated from Eq. (1):

$$h = \frac{\{k_P\}_{\text{diff cell}}}{\{K_{sc/v}\}_{\text{ATR-FTIR}}\ \{D/h^2\}} \tag{1}$$

which explicitly assumes

$$\{k_P\}_{\text{diff cell}} = \frac{DK_{sc/v}}{h} \tag{2}$$

However, ($\{k_P\}_{\text{diff cell}}$ characterizes SC uptake and penetration by all possible routes (i.e. intracellular and transcellular) into and through the heterogeneous SC barrier of thickness h_{sc} and this means, as we will now prove, that h must equal h_{sc}, irrespective of the actual route of transport. In other words, the method outlined by Pellett et al. (1997) cannot unequivocally identify the molecular diffusion pathlength across the SC.

Consider first the steady-state rate ($\dot{M}_{ss}$, mass/time) of permeant transport across the SC into an infinite sink. A general definition, which makes no judgement on the route of permeation, based upon Fick's first law of diffusion, would be:

$$\dot{M}_{ss} = A_{sc}\, C_v\, \frac{K_{sc/v}\, D_{sc}}{h_{sc}} \tag{3}$$

where C_v is the permeant's concentration in the vehicle (assumed to be a constant), A_{sc} is the area of SC in contact with the vehicle, $K_{sc/v}$ is the equilibrium partition coefficient of the permeant between the total volume of the SC and the vehicle, and D_{sc} is the apparent diffusion coefficient of the chemical in the SC considered as a pseudo-homogeneous membrane of thickness h_{sc}. The permeability coefficient of the SC is defined as:

$$\{k_P\}_{\text{diff cell}} = \frac{\dot{M}_{ss}}{A_{sc}\, C_v} \tag{4}$$

It follows from Eq. (3) that

$$\{k_P\}_{\text{diff cell}} = \frac{D_{sc}\ K_{sc/v}}{h_{sc}} \tag{5}$$

Now consider a permeant that diffuses across the SC exclusively via the intercellular pathway. In this case, it is appropriate to rewrite Eq. (3) solely in terms of the intercellular lipid (L) pathway:

$$\dot{M}_{ss} = A_L\, C_v\, \frac{K_{L/v}\, D_L}{h_L} \tag{6}$$

in which A_L represents the area of the lipid fraction of the SC in contact with the vehicle, i.e.

$$A_L = V_L/h_L \tag{7}$$

where V_L is the volume of the lipid domain of the SC and h_L is the length of the intercellular lipid pathway through the SC. D_L is the diffusivity of the permeant in the lipid path across the barrier and $K_{L/v}$ is the equilibrium partition coefficient representing the distribution of the permeant between the lipid fraction of the SC and the vehicle. As determined from mass balance, $K_{L/v}$ is related to $K_{sc/v}$ by Eq. (7):

$$K_{L/v} = K_{sc/v}\frac{V_{sc}}{V_L} \tag{8}$$

where V_{sc} is the total volume of the exposed SC ($V_{sc} = A_{sc} \times h_{sc}$).

By substituting Eq. (6) into Eq. (4), it is evident that $\{k_P\}_{\text{diff cell}}$ for permeation through the uniquely intercellular route is

$$\{k_P\}_{\text{diff cell}} = \frac{A_L}{A_{sc}}\ \frac{K_{L/v}\, D_L}{h_L} \tag{9}$$

indicating that a correct representation of the permeability coefficient in terms of the properties of the actual diffusion route must include the area fraction of the pathway and the partition coefficient between the region through which diffusion occurs and the vehicle.

In a similar fashion, one can address the other experimental value determined by Pellett et al. (1997), i.e. the characteristic transport parameter $\{D/h^2\}_{\text{ATR-FTIR}}$. This quantity must be equivalent to both the corresponding parameter characterizing diffusion through the actual path (i.e. D_L/h_L^2 for the exclusively intercellular lipid pathway) and the ratio of the apparent diffusivity (D_{sc}) to the square of the membrane's thickness (h_{sc}^2):

$$\{D/h^2\}_{\text{ATR-FTIR}} = D_L/h_L^2 = D_{sc}/h_{sc}^2 \tag{10}$$

In fact, for chemicals permeating through only the intercellular pathway, this is simply the definition of D_{sc}, the apparent diffusion coefficient when the SC is treated as a pseudo-homogeneous membrane of thickness h_{sc}.

The inevitability that Pellett et al. (1997) would conclude that $h = h_{sc}$, regardless of the nature of the true pathway (i.e. even if h is h_L), can now be illustrated by substituting into Eq. (1), the correct definitions of the experimentally measured parameters (i.e. Eqs. (9) and (10)) when the diffusion pathlength is uniquely via the intercellular lipids:

$$h = \frac{A_L}{A_{sc}}\frac{K_{L/v}\, D_L}{h_L}\ \frac{h_L^2}{K_{sc/v}\, D_L} = \frac{K_{L/v}}{K_{sc/v}}\ \frac{A_L}{A_{sc}}\ h_L \tag{11}$$

which can be simplified using Eqs. (7) and (8) and $V_{sc} = A_{sc} \times h_{sc}$ to give:

$$h = \frac{V_{sc}}{V_L}\ \frac{A_L}{A_{sc}}\ h_L = h_{sc} \tag{12}$$

Consequently, it is incorrect to conclude from the calculation described by Pellett et al. (1997), that molecular transport occurs via a specific route. Stated differently, it is not possible to determine unequivocally the length of the actual SC diffusion pathway from the values of $\{k_P\}_{\text{diff cell}}$, $\{K_{sc/v}\}_{\text{ATR-FTIR}}$, and $\{D/h^2\}_{\text{ATR-FTIR}}$ as measured using the methods described by Pellett et al. (1997).

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