Diffusion modelling of percutaneous absorption kinetics: 4. Effects of a slow equilibration process within stratum corneum on absorption and desorption kinetics

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Abstract

One of the main functions of the skin is to control the ingress and egress of water into and out of the body. The transport kinetics of water in the stratum corneum, the dominant site of resistance in the skin, is normally described assuming a homogeneous membrane model. In the present work, the desorption of water from stratum corneum was studied and profiles obtained for amount desorbed versus time profiles that were more consistent with water transport occurring in a heterogeneous membrane. Analysis of the resulting profiles yields a model that is consistent with a slow equilibration/slow binding of water within stratum corneum as well as its permeation through the stratum corneum. Diffusion model solutions were used to derive the steady-state flux, lag time and mean desorption time for water in stratum corneum. The slow binding kinetics of water in the stratum corneum are limited and most pronounced in the early transient stages of transport and are not easily discerned using steady state penetration studies. The practical importance of this work is in its use of desorption experiments to recognise and define the skin reservoir for water and other solutes as well as penetration parameters in defining their transdermal kinetics.

Keywords: percutaneous penetration, stratum corneum desorption, slow binding, skin reservoir effect

Introduction

Water is the key component in the body and the skin has a major role in maintaining homeostasis. Most studies on water transport in the skin have been undertaken using skin penetration studies, evaporimetry or trans-epidermal water loss technique. The transport kinetics of water in the stratum corneum (SC), the dominant site of resistance in the skin, is normally described assuming a homogeneous membrane model.[1] However, the SC morphology is that of a heterogeneous multi-phase tissue consisting of intracellular lipids and corneocytes hold together by desmosomes.[2] Accordingly, as illustrated in Fig1A, diffusion through SC may be affected by binding to various components in SC or slow permeation into and out of corneocytes. Indeed, significant amounts of water are taken up by the stratum corneum and can be up to 5 times the weight of the stratum corneum. It has been suggested that water exists in SC in both the free and bound forms,[3] although this two state description is probably an oversimplification and continuous spectrum from bulk to strongly bound water is the correct representation. It is possible that large pools of water[4] formed during extended hydration of SC may be an additional source of slow equilibration within SC.

The effect of binding on transdermal transport is well recognised and in the context of the epidermal penetration has been recently discussed by Roberts et al.[5] In addition, we have also examined the kinetics associated with the reservoir effect of the stratum corneum in simple terms, recognising the more complex, convection diffusion modelling undertaken by Reddy et al.[6] In each of these models, instantaneous binding, that is equilibration between bound and unbound states is fast compared to diffusion, has been assumed. The advantage of such an approach is that the mathematical modelling associated with instantaneous binding is relatively simple in that it enables the diffusion coefficient (D) in the diffusion equation to be replaced by an effective diffusion coefficient (D_{eff}), where $D_{eff}=f_u D$ and f_u is the fraction unbound. As the fraction unbound is less than unity, binding leads to slower diffusion, and therefore longer lag times. For water desorption experiments presented in this work it is shown that the assumption of an instantaneous equilibration is invalid. This could be due to water molecules slowly partitioning into corneocytes. In this work, we develop solutions for the amount penetrated and desorbed in the Laplace domain for the case of slow equilibration. We then demonstrate that experimental data is consistent with the effect of slow equilibration. Using the solutions we also derive equations for the lag time and mean desorption time and analyse potential effects of slow binding/equilibration on these parameters.

Theory

Fig. 1A shows potential origins of slow binding/equilibration within SC and Fig. 1B shows a schematic diagram that simplifies SC transport as the slow binding/equilibration diffusion model. Generally, the processes illustrated in Fig. 1A is quite complicated to model mathematically as molecules that enter a corneocyte will diffuse, possibly bind and unbind from keratin fibres, then eventually exit from an arbitrary location on the corneocyte boundary. This aspect of potential transport through corneocyte is not explicitly captured in the simple model presented here, and therefore the model is just an approximation of the overall complexity of SC transport. The diffusion transport of bound and unbound solutes in the membrane taking in consideration slow binding is mathematically described by partial differential equations:

$$\frac{\partial C_u}{\partial t} = D\frac{\partial^2 C_u}{\partial x^2} - k_{on}C_u + k_{off}C_b \qquad 1$$

$$\frac{\partial C_b}{\partial t} = k_{on}C_u - k_{off}C_b \qquad 2$$

where C_b and C_u are concentrations of bound and unbound solutes, D is diffusion coefficient of the unbound solute (bound solute is assumed immobile, or its diffusion is so slow that it can be neglected) and k_{on} and k_{off} are binding and unbinding rate constants. Further, t is time and x is the distance in the membrane. Equations similar to 1 and 2 were considered previously for drug release from two-phase systems,[7,8] and more recently for the lag time and higher moments analysis of transport in membranes with linear reversible binding and irreversible removal.[9] We note that equations 1 and 2 are only one of many possible ways to mathematically model binding/slow equilibration/trapping in the SC. Some other more general mathematical approaches for such diffusional transport in generalised membranes,[10] SC[11] and two-phase polymer hydrogels[12] were investigated previously.

For permeation studies there is no solute initially present in the membrane (for water experiments in this work this means no tritiated water present initially) and the initial condition is:

$$C_u(x,0) = 0; \quad C_b(x,0) = 0 \qquad 3$$

For the desorption case, the membrane is initially saturated with the solute by equilibrating it with the large volume of the donor solution with concentration C_0. Therefore, the initial condition for the desorption experiment is:

$$C_u(x,0) = C_0 K_{eff} f_u; C_b(x,0) = C_0 K_{eff}(1 - f_u) \qquad 4$$

where K_{eff} is the effective partition coefficient of bound and unbound fractions with the donor solution and f_u is the fraction unbound at the equilibrium between bound and unbound fractions. The fraction unbound is related to binding and unbinding rate constants:

$$f_u = \left(1 + \frac{k_{on}}{k_{off}}\right)^{-1} \quad 5$$

In this work we will consider simplest boundary conditions for the permeation and desorption. In the permeation case, the boundary condition at the donor cite (x=0) and receptor (x=h) are:

$$C_u(0,t) = K_{eff}\, f_u C_0\,;\; C_u(h,t) = 0 \quad 6$$

where h is the membrane thickness.

For desorption experiments the boundary conditions are:

$$C_u(0,t) = 0; \quad C_u(h,t) = 0 \quad 7$$

Taking into account initial condition (3), for the absorption experiment, the Laplace transform of eqs 1, 2 and boundary conditions 6 yields:

$$D\frac{d^2\hat{C}_u}{dx^2} = s\hat{C}_u + k_{on}\hat{C}_u - k_{off}\hat{C}_b \quad 8$$

$$\hat{C}_b = \frac{k_{on}\hat{C}_u}{s + k_{off}} \quad 9$$

$$\hat{C}_u(0,s) = \frac{1}{s}K_{eff}\, f_u C_0 \quad 10$$

$$\hat{C}_u(h,s) = 0 \quad 11$$

where s is the Laplace variable and ^ over functions denotes its Laplace transform.

Solving eqs 8 and 9 with boundary conditions 10 and 11 yields:

$$\hat{C}_u(x,s) = \frac{K_{eff}\, f_u C_0}{s} \times \left(\cosh\left(\sqrt{g(s)t_d}\,\frac{x}{h}\right) - \frac{\sinh\left(\sqrt{g(s)t_d}\,\frac{x}{h}\right)}{\tanh\left(\sqrt{g(s)t_d}\right)} \right) \quad 12$$

where t_d=h^2/D is the characteristic time of diffusion and g(s) is defined as

$$g(s) = s + k_{on} - \frac{k_{on}k_{off}}{s + k_{off}} \quad (13)$$

We note that $\hat{C}_b(x,s)$ can be found using eq 9. The amount of solute permeating into the receptor phase can be found as:[13]

$$\hat{Q}(s) = -\frac{DA}{s}\frac{d\hat{C}_u(x,s)}{dx}\bigg|_{x=h} \quad (14)$$

where A is the area of the membrane. Substitution of $\hat{C}_u(x,s)$ from eq 12 to 14 yields after some algebra:

$$\hat{Q}(s) = \frac{1}{s^2}\frac{C_0 K_{eff} f_u DA}{h}\frac{\sqrt{g(s)t_d}}{\sinh\sqrt{g(s)t_d}} \quad (15)$$

For instantaneous binding we have k_{on}, $k_{off} \to \infty$, and therefore $g(s) \to s/f_u$ as can be seen from eqs 13 and 5. Accordingly, the amount of solute permeating into the receptor phase becomes:

$$\hat{Q}(s) = \frac{1}{s^2}\frac{C_0 K_{eff} D_{eff} A}{h}\frac{\sqrt{st_d^{eff}}}{\sinh\sqrt{st_d^{eff}}} = \frac{C_0 A k_p}{s^2}\frac{\sqrt{st_d^{eff}}}{\sinh\sqrt{st_d^{eff}}} \quad (16)$$

where $D_{eff} = f_u D$ is effective diffusion coefficient, $t_d^{eff} = h^2/D_{eff} = t_d/f_u$, and k_p ($=K_{eff}D_{eff}/h$) is permeability coefficient. As expected, for instantaneous binding, equation 16 corresponds to a case of permeation through a homogeneous membrane. [14]

For the desorption experiment, taking into account initial conditions 4, the Laplace transform of eqs 1, 2 and boundary conditions 7 yields:

$$D\frac{d^2\hat{C}_u}{dx^2} = s\hat{C}_u - K_{eff} f_u C_0 + k_{on}\hat{C}_u - k_{off}\hat{C}_b \quad (17)$$

$$\hat{C}_b = \frac{k_{on}\hat{C}_u + K_{eff}(1-f_u)C_0}{s + k_{off}} \quad (18)$$

$$\hat{C}_u(0,s) = 0; \quad \hat{C}_b(0,s) = 0 \quad (19)$$

Solving ordinary differential eqs 17 and 18 with boundary conditions 19 for $\hat{C}_u(x,s)$ yields:

$$\hat{C}_u(x,s) = \frac{K_{eff}C_0}{g(s)}\left(f_u + \frac{k_{off}(1-f_u)}{s+k_{off}}\right)\left[1 - \cosh\left(\sqrt{g(s)t_d}\,\frac{x}{h}\right) + \frac{\cosh\sqrt{g(s)t_d} - 1}{\sinh\sqrt{g(s)t_d}}\sinh\left(\sqrt{g(s)t_d}\,\frac{x}{h}\right)\right] \quad (20)$$

We note that $\hat{C}_b(x,s)$ for the case of desorption can be found using eq 18. The amount of solute desorbed into the receptor phase in this case can be found as:

$$\hat{Q}(s) = \frac{DA}{s}\left(\left.\frac{d\hat{C}_u(x,s)}{dx}\right|_{x=0} - \left.\frac{d\hat{C}_u(x,s)}{dx}\right|_{x=h} \right) \qquad 21$$

and yields after some algebra:

$$\hat{Q}(s) = \frac{AhC_0K_{eff}}{s}\left[f_u + \frac{k_{off}(1-f_u)}{s+k_{off}} \right] \frac{2\tanh\left(\frac{1}{2}\sqrt{g(s)t_d}\right)}{\sqrt{g(s)t_d}} \qquad 22$$

For instantaneous binding (k_{on}, $k_{off} \rightarrow \infty$) again $g(s) \rightarrow s/f_u$ and the term in square brackets becomes unity and the amount of solute permeating into the receptor phase becomes:

$$\hat{Q}(s) = \frac{AhC_0K_{eff}}{s} \frac{2\tanh\left(\frac{1}{2}\sqrt{st_d^{eff}}\right)}{\sqrt{st_d^{eff}}} = \frac{AC_0k_pt_d^{eff}}{s} \frac{2\tanh\left(\frac{1}{2}\sqrt{st_d^{eff}}\right)}{\sqrt{st_d^{eff}}} \qquad 23$$

This equation describes desorption from a homogeneous membrane.

Methods and numerical analysis

Desorption. Pieces of human SC from one subject, obtained using trypsin treatment of heat-separated epidermis as previously described,[15] were suspended on wire rings in separate airtight vials (volume 14ml) over 400µl of 0.9% NaCl deionised water solution. After 40 hours SC membranes were transferred into similar vials with 400µl of 0.9% NaCl deionised water solution spiked with ^3H_2O and saturated with ^3H_2O vapours for 5 hours at room temperature. Total ^3H activity of the solution used was about 20µCi and the equilibrium relative humidity above it was ≈99.4% (assuming Raoult's Law). SC pieces were then quickly removed from vials and installed separately (within 5 seconds) into fresh receptor solutions (volume 1.7ml) in glass vials. During desorption experiments receptor solutions were continually stirred with magnetic bars. Aliquots of 200µl were taken from the receptor phase at designated times and replaced with an equal amount of fresh solution and assayed by liquid scintillation counting. Desorption experiments were performed at room temperature.

To quantify the extent of keratin-water tritium exchange separate experiments were conducted. The protocol is similar to that of the desorption experiment, except that after saturation of SC pieces

with ^3H_2O vapours for 5 hours they were left in an oven and kept at 45^oC overnight before conducting desorption. Desorption in this case was conducted for 27 hours.

Permeation. Water permeation experiments were performed on the SC obtained from the same subject as for desorption experiments. Membranes were installed in side-by-side diffusion cells with donor and receptor filled with 0.9% NaCl deionised water solution (1.5 ml) and equilibrated overnight at room temperature. After equilibration, donor compartment was emptied and filled with 0.9% NaCl deionised water solution spiked with ^3H_2O (total activity about 1 μCi). Aliquots of 200μl were taken from the receptor phase at designated times and replaced with an equal amount of fresh solution and assayed by liquid scintillation counting. Permeation experiments were performed at room temperature.

Numerical inversions of Laplace domain solutions and nonlinear regressions were performed using the program SCIENTIST (MicroMath Scientific software, Salt Lake City). Weighting of $1/y_{\text{observed}}$ was used for nonlinear regressions.

Results

Experimental data analysis

Fig 2 shows experimental amount desorbed–time profile (squares, n=3) and amount penetrated–time profile (circles, n=5) for water. Although regression of desorption and penetration data individually with homogeneous membrane model (eq 16 and 23 respectively) results in an apparently good fit (curves not shown), the fitting parameters obtained for desorption (k_p=3.05±0.07 μm/h, t_d^{eff} =300±8.3 min) and penetration (k_p =6.23±0.12 μm/h, t_d^{eff} = 146±8.3 min) are significantly different. The differences in the fitting parameters for the two analyses could not be justified given that identical parameters are anticipated for SC samples being used from the same subject in both experiments. A simultaneous fitting of desorption and penetration data was therefore undertaken to ensure parameter consistency for a given SC sample, irrespective of analysis method, to reflect SC samples from the same subject's were used in both desorption and penetration experiments. However, a simultaneous fitting with homogeneous membrane model yields a poor regression (dashed line, Fig. 2) with a non-random distribution of residuals and is evidence that a more complex model than had previously been assumed is required.

We have previously suggested that a heterogeneous two-slab model in which the stratum corneum was represented as two layers (which imitate stratum disjunctum and stratum compactum) with different diffusion and partition coefficients is required to describe the heterogeneity in steroid distribution within the stratum corneum.[16] This model has two extra parameters that describe heterogeneity or non-uniformity of the SC: $\xi_D = D_0/D_1$ and $\xi_K = K_0/K_1$ where D_0, D_1 and K_0, K_1 are diffusion and partition coefficients in the first and second layers of the SC respectively. In Fig. 2 the dot-dashed line represents the result of fitting the experimental data with the two-slab model with the ratio of thickness of two layers fixed to 2 ($=h_0/h_1$). It can be seen that it represents an improvement on fitting with homogeneous membrane model, but still a non-random distribution of residuals is evident. Importantly, the regression with two-slab model resulted in extremely high heterogeneity parameters ($\xi_D = 0.0059$ and $\xi_K = 184$), whereas it is non-physiological to expect such extreme heterogeneity for water transport in SC, especially for the partition coefficient. If the partition coefficient heterogeneity parameter is fixed to unity ($\xi_K = 1$), the quality of regression deteriorates even further (graph not shown).

Contrary to the homogeneous and the two-slab models the slow equilibration model (eqs 15, 22), yielded an excellent simultaneous regression of the experimental profiles for desorption and penetration (solid line, Fig. 2) with a random distribution of residuals. Parameter estimates of k_{on}=0.045±0.026 min^{-1}, k_{off}=0.045±0.016 min^{-1} and t_d=81±11 min were obtained. These estimates correspond with an unbound water fraction in SC of f_u=0.50 and the lag time of 27 min. If a hydrated SC thickness of h=30μm is assumed,[3] a diffusion coefficient of unbound water in human SC of $D=h^2/t_d$=1.8x10^{-9}cm^2/s is estimated. This value is similar to water diffusion coefficients obtained using the transepidermal water loss technique in vivo (D=2.2±1.1x10^{-9}cm^2/s).[17]

Tritium exchange between water and keratin can be potentially misinterpreted as slowly exchanging/bound water. Water-keratin tritium exchange experiment was conducted to measure the extent of this exchange. After drying SC samples overnight at 45^{o}C it can be assumed that only tritium associated with keratin will remain in SC. During desorption this tritium will reassociate with water and result in tritium in the receptor phase. It was established that after drying SC the amount of tritium in the receptor phase corresponded to only 0.0055±0.0007 mg/cm^2 of tritiated water. This is only about 0.3% of total water recovered from SC in the desorption experiment (1.5± 0.1 mg/cm^2) and does not contribute significantly to slow equilibration/ binding of water.

Desorption

A useful summary model independent parameter to describe desorption kinetics is the mean desorption time (*MDT*) as it can be defined as the first moment of the flux versus time profile. This moment can also be defined in terms of an underlying model using equation 24:

$$MDT = \frac{\int_0^\infty J(t)tdt}{\int_0^\infty J(t)dt} = \lim_{s\to 0}\frac{d}{ds}\ln \hat{J}(s) = \lim_{s\to 0}\frac{d}{ds}\ln s\hat{Q}(s) \qquad 24$$

where J is the desorption flux. Using $\hat{Q}(s)$ from the slow binding model derived in this work (eq 22), MDT can be expressed as:

$$MDT = \frac{t_d^{eff}}{12} + \frac{1-f_u}{k_{off}} \qquad 25$$

The first term in this equation represents *MDT* for instantaneous equilibration and the second term an increase in the mean desorption time due to the slow binding. It is to be emphasised that, if only the slow phase of water transport is measured, as is normally the case with water penetration kinetics, only the first, diffusion, term will be seen. However, using eq 25, it can be concluded that the contribution due to slow equilibration to *MDT* is about 26% even for moderately bound solutes (f_u=0.7) and relatively fast equilibration ($k_{off}t_d^{eff}=10$). For stronger bound solutes and lower k_{off} values the effect of slow equilibration on *MDT* becomes more pronounced. Thus, the slow equilibration term is most likely to be evident when the reservoir effect for a solute in the stratum corneum is large.

Fig. 3 shows theoretical profiles for amount desorbed versus time emphasising the importance of the reservoir effect in skin transport based on the role of slow equilibration and the extent of binding. It is evident that the effect of slow binding on amount–time profiles is more pronounced for the strongly (f_u=0.01, A) and moderately (f_u=0.7, B) bound solutes and when k_{off} and k_{on} are small. Fig 3 also shows that the amount–time profiles are significantly affected by slow equilibration even for moderately (f_u=0.7) bound solutes. The skin reservoir effect is most likely to be evident with strong binding where (f_u>0.01, A) and a slow equilibration/binding process in SC.

A specific parameter defining this equilibrium process is the characteristic time of equilibration for water in SC ($t_{eq)}$ is approximately given by $1/k_{off}$ ($\approx 1/k_{on}$) and for water is 1/0.045≈22min. When

slow equilibration is due to diffusion in the keratin matrix of the corneocytes, an order of magnitude $h_c^2/2D_k$, where h_c is the thickness of corneocytes and D_k is the diffusion coefficient in the keratin matrix of the corneocytes, may be anticipated. If we assume h_c=3μm and D_k=10^{-5}cm^{2}s^{-1}, [18] we obtain a $h_c^2/2D_k$ =0.0045 sec, which is five orders of magnitude less than t_{eq}. It is therefore unlikely that slow equilibration of water in the SC is due to the diffusion process in the keratin matrix of the corneocytes. We believe that more likely explanation for slow equilibration of water in SC is rate-limiting penetration of the corneocyte envelope. Some contribution to the slow equilibration of water could also be due to slow diffusion out of water pools that form within hydrated SC.[4]

Permeation

We now consider the applicability of this analysis to skin permeation results. Relevant permeation parameters such as the coefficient of permeability (k_p) and the lag time (t_{lag}), deduced from equation 15 are:[13]

$$k_p = \frac{\lim_{s\to 0} s^2\hat{Q}(s)}{C_0 A} = \frac{Df_u K_{eff}}{h} \qquad 26$$

$$t_{lag} = -\lim_{s\to 0}\frac{d}{ds}\ln\left(s^2\hat{Q}(s)\right) = \frac{h^2}{6D}\left(1+\frac{k_{on}}{k_{off}}\right) \qquad 27$$

This lag time is identical to that derived by Siegel. [9]

Importantly, the effective diffusion coefficient is a function of the fraction unbound, as shown previously:[5]

$$D_{eff} = Df_u = D\left(1+\frac{k_{on}}{k_{off}}\right)^{-1} \qquad 28$$

Substitution of D_{eff} into eqs 26 and 27 also result in the usual definitions[5] for the permeability coefficient and the lag time:

$$k_p = \frac{D_{eff} K_{eff}}{h} \qquad 29$$

$$t_{lag} = \frac{h^2}{6D_{eff}} = \frac{t_d^{eff}}{6} \qquad 30$$

where $t_d^{eff} = h^2/D_{eff} = t_d/f_u$. This analysis shows that the same steady-state flux is found in penetration studies for both slow and instantaneous binding in the membrane. This result makes

sense intuitively, as at the steady-state (long times) enough time has lapsed for an equilibrium between the bound and unbound phases to have been reached even if slow binding were evident.

Slow binding is most evident in the transient stage of penetration studies, i.e. before the steady state is developed. In Fig 4 the amount penetrated vs. time is presented for strongly (f_u=0.01, A) and moderately (f_u=0.7, B) bound solutes and different values of k_{off}. The corresponding values for k_{on} are defined by eq 5 and the fixed value of f_u. It can be seen in Fig 4 (A) that for strongly bound solutes (f_u=0.01), and relatively fast equilibration ($k_{off} t_d^{eff} \geq 10$) profiles are very close to that for the instantaneous equilibration at long times ($t / t_d^{eff} \geq 0.6$). At early times a greater amount of a slowly equilibrating and highly bound solute will penetrate relative to a rapidly equilibrating drug so that for $t \leq 0.2 t_d$ and relatively fast equilibration ($k_{off} t_d^{eff} = 20$), the amount penetrated is significantly higher than predicted by the instantaneous equilibration. In contrast, the initial phase is very similar for moderately bound solutes (eg f_u=0.7, Fig 4 (B)) for a range of $k_{off} t_d^{eff}$ values defining very slow to instantaneous equilibration. Hence, we conclude that slow equilibration effects will only be observed in penetration experiments for highly bound solutes.

Discussion

In this work, we have examined the transport of water within the SC by a model which allows for the slow binding/equilibration of water in the SC as well as diffusion within the SC itself. All previous studies appear to have used a non- steady solution of Fick's law of diffusion which assumed instantaneous distribution of water between the various phases during the diffusion process.[19] A single instantaneous diffusion process was also used to describe the water evaporation rate from SC lipids.[20] Pirot et al[21] showed that the transepidermal water loss could be directly related to the diffusivity of water in the SC and its thickness after tape stripping assuming a homogenous diffusion model. This work assumed a homogeneous transport for water in its transport across human SC in vivo.[22] They suggest that transepidermal water loss and impedance spectra provide complementary methods for looking at transport of water in the stratum corneum.

In the present work all studies were conducted with fully hydrated SC from normal human skin. It is recognised that the kinetics of water uptake in the SC is dependent on the SC water content. Accordingly, different values for the water diffusion coefficient and its binding may be found depending on the SC level of hydration and on its prior treatment. Water is a well known plasticizer of the SC,[23] with the amount of water bound in the SC being dependent on the amount of

hygroscopic water substances present.[24-26] Kasting and Barai have pointed out that SC water sorption is similar to that into keratinized tissues (i.e., wool and horn) at low water activities but, at high water activities, it is similar to that in polymeric hydrogels.[27] They showed that a number of theoretical water sorption models provide a satisfactory description of the equilibrium water content of human SC over the water activity range 0.03-1.0. Formulations can also greatly affect SC water content.[28-31]

A number of papers have shown that diffusivity of the water in SC increases with water concentration.[19,32,33] A careful examination of the work of Liron et al[32,33] suggest that there was not a random distribution of residuals for the predicted model of water uptake/desorption in porcine SC at 25° C compared to the actual data and that a more complex model than the one described may be required. Most recently Kasting et al[18] showed that diffusion of water within the SC is water concentration dependent. The results of this analysis combined with previous spectroscopic analyses is consistent with lipids providing most of the SC water barrier and the diffusion pathway for water is mainly transcellular. The diffusivity of water in the SC is also dependent on the source of the SC and its diseases. Schwindt et al[17], for instance, showed that the transepidermal water loss could be correlated to both the gender and site of the body. Tagami et al[34] noted that the water holding capacity of thick scaly skin and psoriatic skin was much less than for normal skin, most likely reflecting the lower amount of lipid soluble and water soluble substances in the stratum corneum.

Our analysis indicates that about half of water in SC is unbound (f_u=0.50), this corresponds to about 50% of slow binding/equilibrating water in the SC and agrees favourably with 30% of water in SC reported as non freezable and therefore representing “bound” water.[35] This agreement must be interpreted with caution though, as slow binding/equilibration in this work is likely to be due to a combination of processes as outlined schematically in Fig 1A. At this stage it is difficult to attribute apparent slow binding/equilibration of water in SC to a single process (like binding) and more work is warranted to establish this.

Water sorption and desorption processes have been studied previously by Scheuplein and Morgan using a microbalance technique.[3] They had previously suggested that both bound and free water existed in the stratum corneum and had affected the water diffusivity profiles. More recent data by Pieper et al[36] suggests that the three phases of water exist and include bound, weakly-bound and free water. It is well known intercellular lipids can facilitate water binding in the SC.[31,37] Analysis

of the Scheuplein and Morgan[3] data using our slow binding diffusion model is presented in Fig. 5. Equation 22 was modified to describe water remaining in the SC and desorption from one side of the SC only. Further, as the beginning of desorption from SC is not determined precisely, an extra parameter (time shift) was introduced to compensate for this uncertainty. It could be seen in Fig. 5 that the quality of regressions is good. Some deviation for early data points may be due to a rate-limiting water vapour diffusion in unstirred air surrounding SC sample for the fast initial phase of the desorption process.[32] The fitting yields following parameters averaged for 3 experimental profiles: k_{on}=0.0056±0.0036 min^{-1}, k_{off}=0.018±0.008 min^{-1} and t_d=7.4±2 min which are very different to those obtained in our experiments. These very different diffusion parameters are most likely the result of changing hydration of SC in Scheuplein and Morgan[3] experiments, as SC samples dry during the desorption process, and in this case more complex model with diffusion coefficient changing in time is required to model the data.

Conclusions

In this work the effect of slow equilibration/binding within SC on solute penetration through and desorption from stratum corneum assuming diffusion transport have been considered. Solutions in the Laplace domain for the amount of solute penetrated and desorbed have been derived for slow equilibration/binding. Analysis of these solutions shows that the cumulative amount of solute penetrated is less affected by slow equilibration as compared to the amount of solute desorbed. Based on the analysis of our own and previously published data of water desorption form SC, we conclude that the slow equilibration model adequately describes desorption of water.

Acknowledgements

We are grateful to the financial support of the National Health & Medical Research Council of Australia and the Queensland and New South Wales Lions Medical Research Foundation.

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Figure legends

Fig 1. (A) Schematic diagram of SC illustrating potential origins of slow binding/equilibration within SC and (B) schematic diagram of the slow equilibration model.

Fig 2. Experimental SC water desorption (squares, n=3) and penetration (circles, n=5) profiles and their simultaneous regression for homogeneous membrane model (dashed line), heterogeneous two-slab model (dot-dashed line) and slow binding model (solid line).

Fig 3. Amount desorbed vs. time profiles for fu=0.01 (A) and fu=0.7 (B) for $k_{off} t_d^{eff} = 3, 10, 20, \infty$ starting from lower curve.

Fig 4. Amount penetrated vs. time profiles for fu=0.01 (A) and fu=0.7 (B) for $k_{off} t_d^{eff} = 3, 10, 20, \infty$ starting from upper curve.

Fig 5. Scheuplein and Morgan[3] SC water desorption data (SC hydration time 25h - circles, 5h - squares and 0.5h -triangles) fitted using slow binding model (solid lines).