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A human stratum corneum–silicone membrane sandwich to simulate drug transport under occlusion

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Summary

A new in vitro method is introduced to study drug penetration through human stratum corneum (SC). Excessive (unphysiological) hydration of the SC is prevented by sandwiching the SC between two silicone membranes. Under these conditions a slow and steady increase in SC water weight fraction was observed (from 45% to 54%) during a 16 h experiment. The silicone membranes also provide mechanical strength to fragile skin preparations, while their diffusional resistance is relatively small and can be dealt with mathematically. The in vitro occlusion simulation model was used to study the influence of a pretreatment of the SC with two different penetration enhancers (Azone and Brij96) on its nitroglycerin (NG) permeability. Because the same donor solution was used in all permeation experiments, the (moderate) effects of the penetration enhancers could be contributed to their interactions with the SC.

Introduction

The percutaneous absorption of drugs and other biologically active agents has been the subject of a number of in vitro and in vivo studies in the pharmaceutical and toxicological fields. An important parameter in skin transport is its state of hydration. It is known that the degree of hydration of the stratum corneum (SC) largely influences the skin permeability for most compounds. In fact, there is a positive relationship between the degree of hydration of the SC and the percutaneous absorption rate of both hydrophilic and lipophilic compounds (see e.g. Barry, 1983). Hence it is very important in in vitro studies to have active control over the SC hydration during permeation experiments.

The rate of drug absorption from a transdermal dosage form is often controlled by the SC. As a consequence, the drug absorption rate is low and there are inter- and intra-individual differences due to biological variability. In order to overcome these obstacles the dosage form should be rate-controlling. Since the resulting transdermal flux may not be too low, a successful approach is to decrease the diffusional resistance of the SC to such an extent that one can control the drug

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release rate with the transdermal dosage form. For that purpose so-called penetration enhancers are developed and studied; these compounds increase the drug permeability of the SC. The development and screening of such compounds involves many in vitro experiments.

In vitro, drug transport through the skin and effects of penetration enhancers are generally determined using the infinite dose technique where the drug permeates from an aqueous donor solution through the (whole or dermatomed) skin into an aqueous acceptor phase (Bennett and Barry, 1986). Such an experimental design causes unphysiological degrees of hydration in the SC, which cannot be controlled during the experiment (Akhter and Barry, 1986; Foreman, 1986). Recent studies of Van der Merwe and coworkers (Van der Merwe and Ackerman, 1987; Van der Merwe et al., 1988) clearly showed the physical deterioration of SC structure during a 24 h permeation experiment in which the skin was in direct contact with aqueous donor and acceptor phases. They observed a time-dependent increase in skin permeability for both water and urea (more than 150 times after 24 h), caused by structural changes in the SC. They concluded that permeability data obtained in this way are not representative of normal skin.

The SC consists of cornified keratinocytes embedded in intercellular lipids; in a physiological state it contains about 15% w/w water. Under occlusion the water content of the SC increases, though the actual degree of in vivo SC hydration under prolonged occlusion has not yet been determined. Under occlusion, endogenous water, normally diffusing through the SC and evaporating from the sweat glands into the environment, will be trapped and hydrate the SC. Potts and coworkers (1985) used attenuated total-reflectance infrared spectroscopy as a non-invasive in vivo technique to measure quantitatively the water content of the uppermost layers (a few microns) of the SC. They showed that during a 5 h occlusion of the skin by petrolatum, the water weight fraction in the surface layers of the SC increased from 7% to 15%, while a further increase up to 20% was observed within 5 min when the skin site was more effectively occluded during successive measurements. Anderson et al. (1973) showed that under moist conditions (a relative humidity of 95%) the water weight fraction of isolated SC will increase up to 45% in one day, and up to 85% in 14 days.

One way of avoiding fast and excessive hydration of the SC in vitro is the use of the finite dose technique to measure the drug transport through the skin (Franz, 1978; Akhter and Barry, 1986). Firstly, a finite dose is applied to the skin; after subsequent evaporation of the volatile vehicle into the ambient atmosphere, the drug transport rate through either occluded or not occluded skin can be measured (Barry, 1983; Foreman et al., 1978).

Another decisive factor in skin permeation studies is the way in which the skin is prepared. Thin skin preparations (e.g. split epidermal slices or trypsinized SC) are difficult to handle; furthermore it is almost impossible to limit SC hydration if isolated sheets are in direct contact with an aqueous sink (Foreman and Clanachan, 1984). Hence, in many in vitro studies full thickness skin has been used. However, in vitro the dermis (contains $> 85\%$ water, thickness up to 800 μm) may present an unrealistic diffusional resistance for lipophilic drugs, in that the clearance from the lipophilic SC is far less effective than in vivo, where the drug is quickly removed by the capillaries (Barry, 1983). In this way an artefact may be introduced. As a consequence, effects of penetration enhancers observed in such a model may be misleading, because they might not reflect the in vivo situation.

Occlusion simulation in vitro: the SC–silicone membrane sandwich

In this study an alternative method is introduced to study the transport of biologically active compounds through isolated human SC while simulating in vivo occlusion. This technique may be particularly useful in the development of penetration enhancers and transdermal drug delivery systems because unphysiological skin hydration can be avoided. The SC was brought into a well-defined subsaturated state of hydration by equilibration in a constant relative humidity chamber and then sandwiched between two sticky silicone membranes (Fig. 1). In this way the SC is

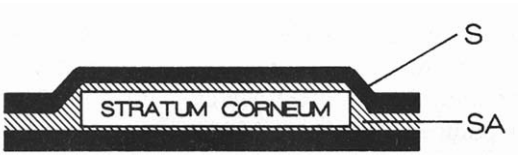

Fig. 1. Stratum corneum–silicone membrane sandwich (see text). S, silicone membrane; SA, silicone adhesive.

protected against excessive maceration by the aqueous donor and acceptor phases throughout the permeation experiments. An important additional advantage of the sandwich model is that the silicone membranes provide mechanical strength to the thin and fragile dermatomed epidermis or SC, while its diffusional resistance for drugs is relatively low (Baker, 1974).

The sandwich model was firstly validated using nitroglycerin (NG) as a relevant model drug. The direct effects of penetration enhancers were studied by *pretreating* the SC with the penetration enhancer in its vehicle without the drug, before it was clamped between the two silicone membranes.

Materials and Methods

Materials

Azone (1-dodecylazacycloheptan-2-one) was synthesized as described elsewhere (Bouwstra et al., 1988). Brij96 (polyoxyethylene(10)oleylether) was a gift from Atlas Chemie, Essen, F.R.G. Both penetration enhancers were used in 10% solutions in propylene glycol (PG) (J.T. Baker, The Netherlands); Brij96/water mixtures were also used; they form transparent liquid crystalline gels (Tiemessen et al., 1988a). The 80:20 surfactant/water mixture has a lamellar gelstructure while the 50:50 surfactant/water mixture exhibits a hexagonal gelstructure.

Skin preparation

Full thickness human abdominal skin samples obtained from cosmetic surgical corrections, were stored less than 18 h at 4°C before they were dermatomed (Padgett electrodermatome, model b) to a thickness of about 120 μm, in order to isolate the epidermis mechanically. The epidermal slices were incubated (SC side up) on filter paper soaked in a 0.2% solution of trypsin (bovine pancreas type III; Sigma) in isotonic phosphate-buffered saline (PBS) pH 7.4 at 37°C. After a 24 h incubation, the SC was peeled off (avoiding shear) and carefully rinsed with distilled water several times. From the obtained hydrated SC a 14 mm diameter disk was punched; the disk was placed on top of a silicone membrane disk (Silastic 500-1, non-reinforced sheeting, 0.127 mm thick, Dow Corning, MI, U.S.A.) with a diameter of 18 mm, which was covered with a thin layer of a silicone adhesive (Medical Adhesive X7-2920, a gift from Dow Corning, MI, U.S.A.) to achieve perfect contact with the SC. The SC–silicone membrane was dried and stored in a desiccator (< 3 months) (Fig. 2). Prior to any experiment the SC was prehydrated in a constant relative humidity (RH) chamber (over a saturated Na_2HPO_4 solution, RH = 95%) at 20°C for 48 h allowing the SC to adopt a well-defined degree of hydration. In some experiments the prehydration step was followed by a pretreatment with a penetration enhancer for 24 h.

The pretreatment with penetration enhancers was carried out as follows: a SC disk was immerged in a few millilitres of the enhancer solution or covered with a thick gel layer (about 5 mm), then sealed hermetically and stored at 20°C for 24 h. Subsequently, the SC disk was removed

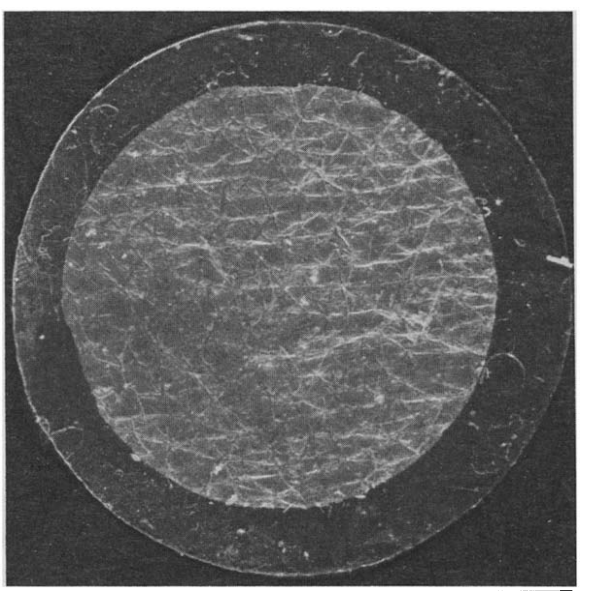

Fig. 2. Stratum corneum (diameter 14 mm) on top of a silicone membrane (diameter 18 mm).

TABLE 1

Stratum corneum treatments with and without penetration enhancers

I. SC (95% RH) *
II. SC (95% RH), 24 hour PG **
III. SC (95% RH), 24 hour (PG/10% Azone) **
IV. SC (95% RH), 24 hour (PG/10% Brij96) **
V. SC (95% RH), 24 hour (Brij96/water 80:20) **
VI. SC (95% RH), 24 hour (Brij96/water 50:50) **
VII. SC 4 h in PBS

* SC is pre-hydrated for 2 days at ambient temperature in a chamber with a RH of 95%.

** After the pre-hydration * the SC is treated with the enhancer (see text).

from the enhancer solution or gel, wiping away residual material with a dry tissue.

Alternatively, in some experiments the SC was fully hydrated by submersion for 4 h into PBS to simulate excessive maceration. Following prehydration or pretreatment the SC was covered with another sticky silicone membrane to restrict further water exchange. All SC pretreatments are listed in Table 1. Instead of SC in some experiments fresh, fully hydrated (4 h submerged in PBS) dermatomed epidermal slices were sandwiched between the two sticky silicone membranes within 24 h after the skin was obtained.

Hydration measurements

To study the water uptake by the SC, located inside the silicone sandwich during the permeation experiment, reference samples of SC were sandwiched between silicone membranes, but these sandwiches were sealed with the adhesive along the edges only. These sandwiches were submerged in water at 32°C. After various periods of time sandwiches were removed from water, dried, cut open and the water weight fraction was determined gravimetrically using a microbalance (Mettler TG50 thermobalance).

Diffusion experiments

The steady-state flux of nitroglycerin (NG) through the sandwich (containing either SC or dermatomed skin) was measured at 32°C using a two-chambered flow-through diffusion cell (Fig. 3; Tiemessen et al., 1988b) which had a diffusion area of 0.63 cm^2 while the volume of each compartment was 16 μl. The aqueous donor solution containing 0.05% NG (Merck) and 5% ethanol, was pumped through the donor compartment at a rate of 35 ml/h. The acceptor perfusate (distilled water) was pumped through the acceptor chamber at a flow rate of 5 ml/h and collected by a fraction collector at one hour intervals. Control experiments were carried out using the silicone double membrane only, without SC or skin.

The NG concentrations in the acceptor perfusate were determined using HPLC. Samples were injected by completely filling a 50 μl loop injection valve (automatic injector Promis, Spark, The Netherlands). The reverse-phase HPLC column (Chromsphere C18, 100 × 30 mm, Chrompack) was eluated at ambient temperature with a mobile phase consisting of methanol/water (50:50 v/v) at a flow rate of 1.1 ml/min (Spectroflow 400, Kratos). The column effluent was monitored at 201 nm (Waters Model 450 variable wavelength detector). The retention time of NG was 1.9 min.

Calculations

The permeabilities of the sandwich and the SC were calculated using Eqns. 1 and 2 (Flynn et al., 1974):

$$P_s = J/C_d \quad (1)$$

$$1/P_{sc} = 1/P_s - 1/P_m \quad (2)$$

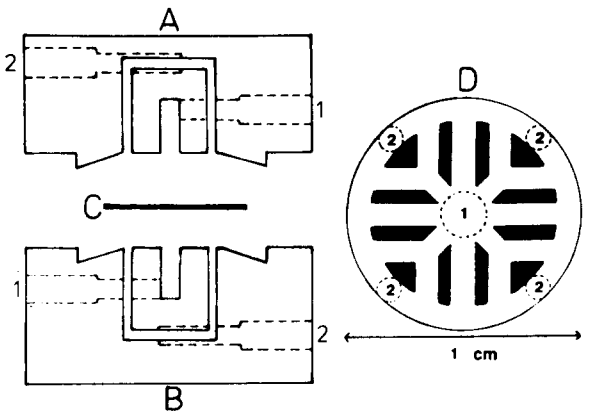

Fig. 3. A schematic cross-section of the two-chambered flow-through diffusion cell (A) acceptor chamber, (B) donor chamber, (C) membrane, (D) surface profile, (1) inlet, (2) outlet.

where J = the flux of the drug through the sandwich; P = the permeability; C_d = the drug concentration in the donor solution; s, denotes sandwich; m, silicone doublemembrane; sc, stratum corneum.

Light microscopy

Stratum corneum and dermatomed skin samples were fixed in 4% formaldehyde, embedded in paraffin and stained with haematoxylin and eosin, for light microscopic observation.

Results

Validation of the skin–silicone membrane sandwich model

Table 2 shows the permeabilities of the silicone membrane and the fully hydrated skin from 2 sources, determined from the steady-state NG fluxes using Eqns. 1 and 2. The permeability of the two supporting membranes is much higher than the permeabilities of the epidermal slices. Therefore, the skin preparation is the rate-controlling barrier in the sandwich while the small contribution of the supporting membranes to the permeability of the sandwich can be separated from the SC permeability mathematically using Eqn. 2. By separating the contributions of the skin and the silicone membranes to the total permeability of the sandwich, any unnegligible effects of stagnant water layers in the diffusion cell are implicitly dealt with, i.e. hidden in the apparent permeability of the silicone membranes.

TABLE 2

Permeabilities of silicone membranes and dermatomed skin from two sources

Membrane(s) + treatment	Permeability ± S.D. (10^{-6} cm/s)	
	Source A	Source B
2 adhesive silicone membranes	36 ±2 ($n = 3$)	
Fully hydrated dermatomed skin (120 ± 10 μm)	7.6 ± 2.0 ($n = 4$)	7.2 ± 1.2 ($n = 3$)

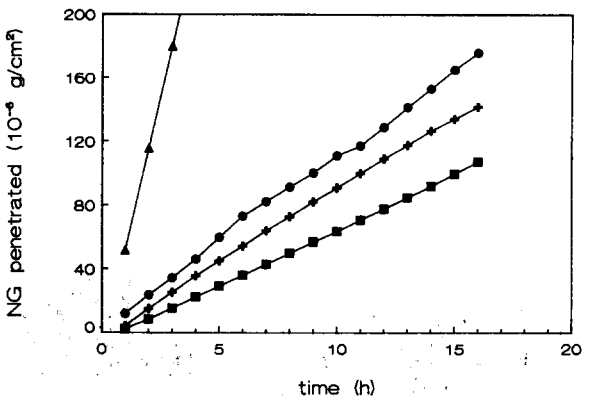

Fig. 4. Typical curves showing the NG permeation through (▲) silastic sheeting; (●) hydrated dermatomed skin from source A; (+) hydrated SC from source A; (■) SC from source A (occlusion simulation).

Rate control inside the skin

Further experiments were carried out to determine whether the SC is indeed the rate-controlling domain inside the skin. Fig. 4 shows some typical curves of the cumulative amount of NG which penetrated through dermatomed skin and sc sandwiches obtained from source A, compared with the silicone double membrane. The constancy of NG penetration rates in all cases shows that the barrier properties with respect to NG remain constant throughout the experiments. The permeabilities, calculated from these experiments, using skin from source A are shown in Fig. 5. These results indicate that there is no significant difference in permeability between fully hydrated dermatomed skin and fully hydrated SC. A reduction in SC

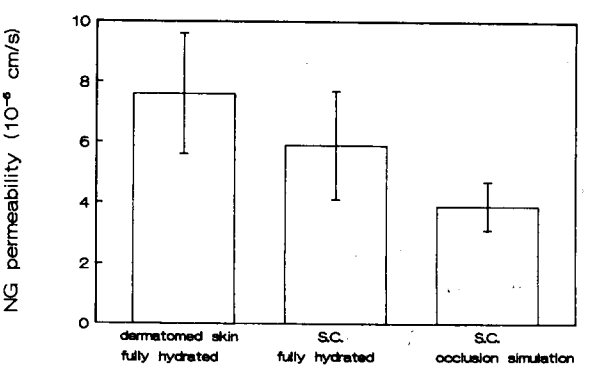

Fig. 5. Permeabilities of skin samples from source A. The data are presented as the mean ± S.D. ($n = 3$ or 4).

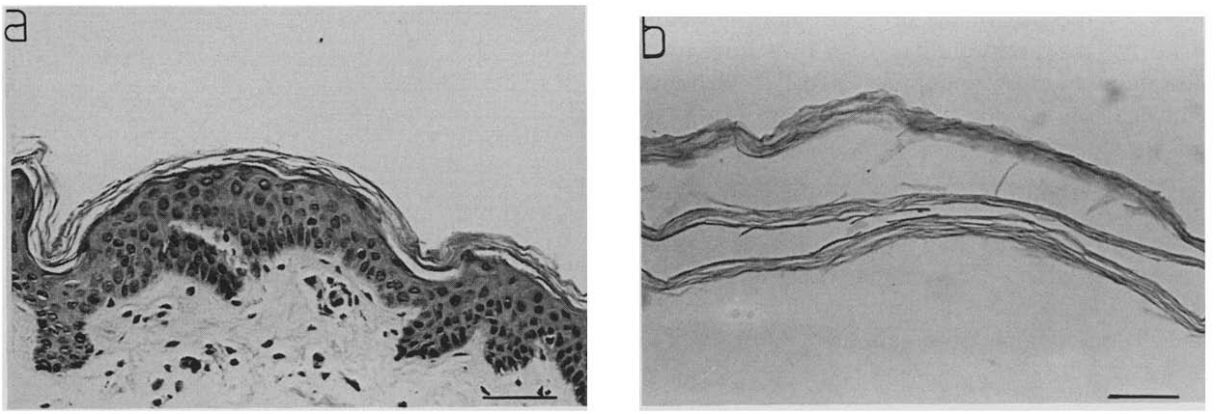

Fig. 6. Light microscopic pictures of (a) dermatomed skin and (b) SC taken after a permeation experiment. Bar = 50 μm.

hydration (occlusion simulation model) inside the sandwich does not significantly decrease its permeability. Using skin preparations obtained from source B, fully hydrated skin (water content > 80%) and "occluded" SC (final water weight fraction of $57 \pm 6\%$ (S.D., $n = 6$)) appeared to have the same permeability.

These results confirm that the SC is the rate-controlling region inside the skin, even when its permeability may be overestimated because of excessive hydration, as in case A. Furthermore the data show that the diffusional barrier remains intact upon drying and rehydrating. Under physiological conditions the influence of the SC on the skin permeability may be even more pronounced.

Skin appearance and water content in the "occlusion simulation model"

Fig. 6a and b show light microscopic pictures of the dermatomed skin and SC samples, respectively, upon termination of a permeation experiment. The appearance of the skin under the light microscope was identical to the appearance of untreated specimens. These pictures confirm that the trypsin treatment of the dermatomed skin does not disrupt the SC structure and that there is no physical deterioration of the two skin preparations as a result of the permeation experiment.

The time-dependent uptake of water by the SC (source C) inside the silicone membrane sandwich in a waterbath at 32° C is shown in Fig. 7. Since the hydrophobic silicone membranes only have a limited permeability (Barrie, 1968) for water, the SC water content inside the sandwich increased only very slowly. During a period of 29 h a slow and linear increase in SC water content is observed. During the first 16 h of a permeation experiment the SC water weight fraction increases from 46% to 55%. This gradual increase in water content is not accompanied by an increase in SC permeability as can be concluded from the constant penetration rate evident from Fig. 4. After 65 h (separate experiment) the water weight fraction of the SC inside the sandwich is $72 \pm 1\%$ (S.D., $n = 3$). Direct submersion of SC in phosphate-buffered saline resulted in a significantly higher water weight fraction: 83 ± 3 ($n = 3$) after 5 h at 32° C.

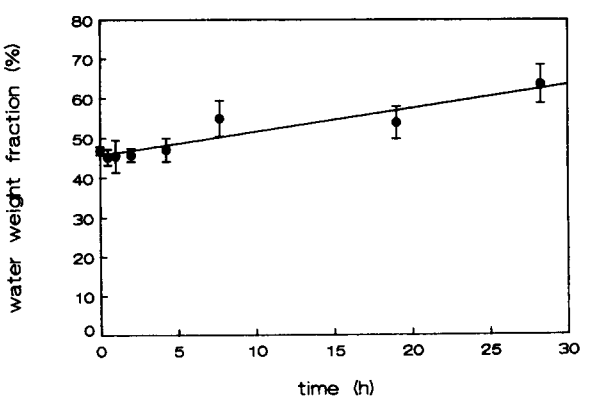

Fig. 7. Time-dependent water uptake by the stratum corneum (source C) inside the silicone sandwich, submerged in water at 32° C. The data are presented as the mean ± S.D. ($n = 3$ or 4).

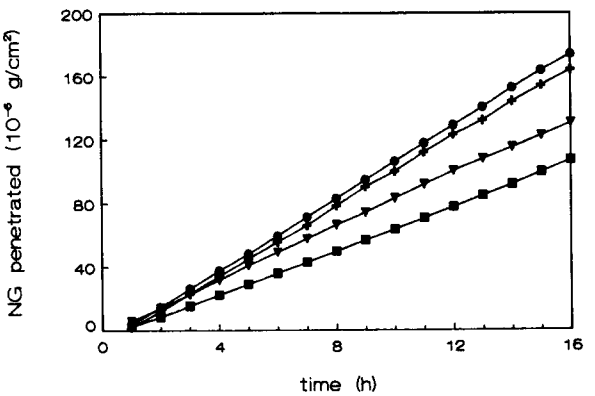

Fig. 8. Typical curves illustrating the influence of the penetration enhancers (see Table 1) on the NG permeation through the stratum corneum: (■) untreated SC; (▼) treatment IV; (+) treatment VI; (●) treatment III.

SC pretreatment with penetration enhancers

Because the SC is the rate-controlling domain inside the skin, the experiments with the penetration enhancers were performed on isolated SC. Fig. 8 illustrates the effects of various SC pretreatments on the cumulative SC penetration of NG; the calculated SC permeabilities are summarized in Fig. 9. These data show, that for SC from both sources the PG treatment did not cause a significant increase of the SC permeability, while the addition of Azone in both cases increased the permeability. An optimal penetration enhancement by the non-ionic surfactant Brij96 was observed after it had been applied under occlusive conditions in a 50 : 50 mixture with water.

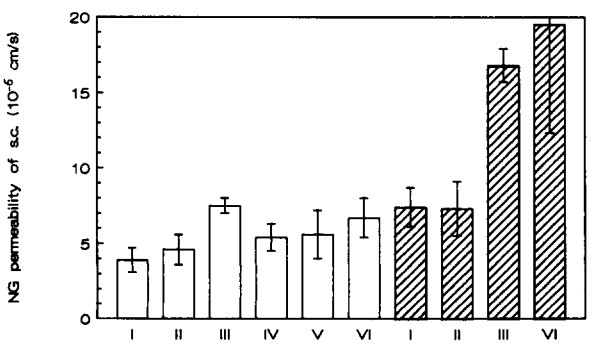

Fig. 9. Permeabilities of SC from sources A and B (shaded) after pretreatment with penetration enhancers (see Table 1). The data are presented as the mean ± S.D. (n = 3 or 4).

Discussion

The occlusion simulation model allows for the investigation of drug penetration through SC in vitro under well-defined conditions; the water uptake by the SC inside the sandwich is well controlled (slow, zero-order), and does not change the SC permeability within the duration of a transport experiment (16 h). The SC water weight fraction increased from 46% to 55% during the 16 h of the permeation experiment. Within the indicated period of time the permeation of a drug through the SC and the effects of penetration enhancers can be investigated under conditions which may simulate the conditions in which they usually operate in vivo (e.g. when released from a patch): namely under occlusion, with an increasing water content, but without uncontrolled excessive water uptake. On the other hand it is shown that a 5 h submersion of the SC, without the protecting silicone membranes, in PBS already leads to a rather fast increase of the water content up to a weight fraction of 83%. This may lead to the physical deterioration of the SC as observed by Van der Merwe and Ackermann (1987).

The observation that the diffusional resistance of the chemically inert silicone membranes for NG was much lower than the resistance of the SC is supported by literature data which clearly show the high diffusivity of most non-ionic drugs in silicone polymers (e.g. Baker, 1974, 1987). Furthermore, with the help of appropriate controls the contribution of the silicone membranes to the total resistance of the sandwich can be separated from the contribution by the skin. The silicone membranes also provide mechanical strength to thin skin preparations and they prevent extraction of skin components by the donor and acceptor phases. Hence the determination of the drug in the acceptor phase is not hampered by the presence of impurities such as skin proteins and lipids.

Though NG is relatively lipophilic, having an oil/water partition coefficient of about 100 (Hansch and Leo, 1979) it is shown that the resulting flux of NG through hydrated skin is controlled by the SC which has a much lower permeability than the viable epidermis and the supporting silicone membranes. From the experi-

mental data it may also be concluded that the trypsin incubation, which was used to isolate the SC, does not significantly reduce its permeability for NG, unlike what has been suggested in the literature for other drugs (see e.g. Barry, 1983).

The sandwich model proved to be useful in studies concerning the effects of penetration enhancers, without the data being obscured by excessive hydration of the SC or extraction of any of its components. The major advantage of the use of SC is that the SC can easily be stored in a desiccator and rehydrated to any desired degree, which can be determined gravimetrically. The routine use of whole skin would necessitate its storage in a refrigerator or in liquid nitrogen which may lead to physical deterioration, and therefore to an increased permeability as has been revealed by Swarbrick et al. (1982) who also showed that dried and rehydrated epidermis had the same permeability for 6,7,8,9-tetrahydro-5-hydroxy-4-oxo-10-propyl-4H-naptho(2,3,-6)-pyran-2-carboxylic acid) as freshly prepared epidermis.

According to Fick's first law the steady-state flux of a drug from an infinite donor (containing only dissolved drug) through the SC, assuming 'perfect sink conditions' at the dermal side, can be formulated as follows:

$$J_s = \frac{D \cdot K}{h} \cdot C_d \qquad (3)$$

where J_s is the steady-state flux of the drug, D the diffusion coefficient of the drug in the SC, h the thickness of the SC, while C_d is the concentration of the drug in the vehicle and K is the partion coefficient of the drug between the SC and the vehicle.

Generally enhanced transdermal drug delivery in vivo from a vehicle containing a penetration enhancer may be considered to result from two jointly operating effects: the "push" and the "pull" effect (Kadir et al., 1987). The "pull" effect arises from interactions between the penetration enhancer and the SC: the penetration enhancer may e.g. enhance the SC diffusivity (D), or the solubility of the drug inside the SC (K). The "push" effect results from penetration enhancer/vehicle interactions, where a decrease in drug solubility would lead to a higher relative affinity of the drug for the SC (K) and therefore to an increased steady-state flux (Kadir et al., 1987; Poulsen, 1972).

The SC–silicone membrane sandwich model allows to quantify both the "push" and the "pull" effects separately. The ("pull") effects of penetration enhancers on skin permeability can be quantified by carrying out the experiments as described above: pretreating the skin with the enhancer before it is sandwiched between the inert silicone membranes and determining the skin permeabilities. Because the same donor solution was used in every experiment, the activity of the drug in the donor solution was the same in all experiments (constant "push"). The "push" effect is independent of the membrane used and can be determined by comparing the steady-state fluxes of the drug from non-saturated equimolar vehicles, with or without the penetration enhancer, through the inert silicone membranes.

The effects of the two penetration enhancers observed are due to an increased solubility and/or diffusivity of the NG inside the SC ("pull"). 10% Azone in PG, and an occlusively applied 50:50 Brij96/water mixture both enhance the NG permeability of the SC quite significantly. These findings can most likely be explained by the interaction of these enhancers with the intercellular lipid bilayers, as reflected in thermoanalytical measurements (Bouwstra et al., 1988) and freeze-fracture electron microscopy (Boddé et al., 1989). Optimal penetration enhancement by the non-ionic surfactant, Brij96, is observed when it is applied under occlusive conditions in a 50:50 mixture with water. Most likely under these conditions the SC is hydrated by the gel which gives the surfactant an optimal access to the SC. This increased availability enables the surfactant to act as a penetration enhancer.

In order to validate the application of the sandwich model to drugs other than NG one should compare the (enhanced) permeability of dermatomed skin and the silicone membrane for the particular drug of choice. For a successful use of this method the skin membrane in the sandwich should be rate-controlling, while the difference between the reciprocal sandwich permeability and

the reciprocal silicone membrane permeability should exceed the experimental error. Furthermore the sandwich model can be adapted to the finite dose technique by replacing the upper silicone membrane by a vehicle in which the drug is incorporated.

In a following paper an adaptation of the SC–silicone membrane sandwich model will be described which allows the measurement of the drug permeation through the SC at constant hydration levels (i.e. under thermodynamic equilibrium conditions) over a wide range of water contents down to water weight fractions as low as 18%.

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