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A silicone membrane sandwich method to measure drug transport through isolated human stratum corneum having a fixed water content

Harry L.G.M. Tiemessen, Harry E. Boddé and Hans E. Junginger

Center for Bio-Pharmaceutical Sciences, Leiden University, Leiden (The Netherlands)

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Summary

A new and generally applicable in vitro method is introduced to study drug penetration through human stratum corneum having a fixed (low) water content. Hydration of the stratum corneum by aqueous donor or receptor fluids is controlled by sandwiching the stratum corneum between two silicone membranes which have a limited permeability for water. By selecting the appropriate salt solution for the donor and acceptor fluids, the relative humidity inside the silicone sandwich can be set and maintained at any desired level. Consequently, the stratum corneum inside the sandwich will adopt a water content which is in thermodynamic equilibrium with the salt solution outside. Furthermore, the silicone membranes provide mechanical strength to the stratum corneum, while their diffusional resistance is relatively small and can be eliminated mathematically. In this study the new method is used to quantify the influence of the water content of the stratum corneum on its permeability for nitroglycerin. A six-fold increase in stratum corneum permeability was observed as its water weight fraction was increased from 17.5 to 49%.

Introduction

The barrier of the skin resides mainly in the stratum corneum. The influence of hydration and occlusion on skin penetration has been the subject of many in vivo and in vitro studies (see e.g. Barry (1983) or Bronaugh and Maibach (1985)). There appears to be a positive relationship between the degree of hydration of the stratum corneum and the percutaneous absorption rate of both hydrophilic and lipophilic compounds.

A systematic in vitro simulation and study of this relationship for drugs of interest would require a versatile skin penetration setup in which the skin hydration should be actively controlled in such a way that the stratum corneum water content is kept at a 'preset' level throughout a permeation experiment. Such a method, which should be applicable to many different types of drugs, has not been available to date. Yet the literature provides a number of in vitro methods or 'penetration models' which are worth mentioning in this context, because they at least partially fulfill the requirements mentioned before. An outline of these methods is presented below.

Correspondence: H.L.G.M. Tiemessen, Center for Bio-Pharmaceutical Sciences, Section of Pharmaceutical Technology, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands.

A widely used method in which the stratum corneum can adopt realistic degrees of hydration is the finite dose technique to measure the drug transport through the skin (Franz, 1978; Akhter and Barry, 1986). The upper side of the skin is either occluded, or exposed to air with a known relative humidity; however, the actual degree of stratum corneum hydration remains unknown. Another disadvantage of this method is the fact that thin skin preparations (e.g. split epidermal slices or trypsinized stratum corneum) cannot be used because they are difficult to handle and because it is almost impossible to limit stratum corneum hydration if isolated sheets are in direct contact with an aqueous sink (Foreman and Clanachan, 1984). For those reasons, many authors have applied the finite dose technique to full thickness skin, even when the study focused on the stratum corneum. However, in vitro the dermis (which contains more than 85% water, and may be up to 800 μm thick) may present an unrealistic diffusional resistance for lipophilic drugs, so that the clearance from the lipophilic stratum corneum will be far less effective than in vivo, where the drug is quickly removed by the capillaries (Barry, 1983).

Blank et al. (1984) have developed an elegant in vitro method for measuring the flux of tritiated water vapour across a piece of isolated stratum corneum with a known, uniform water content. Stratum corneum is clamped between two closed chambers containing identical salt solutions and is only in contact with the vapour of the salt solutions. The actual water content of the stratum corneum is determined by the relative humidity in the chambers which in turn depends on the salt solution used. The relation between the ambient relative humidity and the stratum corneum water content has been determined by Spencer et al. (1975), among others. After equilibrium has been reached, tritiated water is introduced at the donor side and the stratum corneum permeability is calculated from the rate at which the tritiated water appears in the receptor solution. Blank has determined the permeability of the stratum corneum for water as a function of its water content.

In a previous paper (Tiemessen et al., 1989a) we have described a so-called 'occlusion simulation model' based on sandwiching the stratum corneum between sticky silicone membranes (see Fig. 1). The sandwich model provided a means of simulating skin penetration under occlusion, in that the sandwiched stratum corneum was allowed to take up water very slowly (45–54% in 16 h) from its environment during the penetration experiment. The silicone membranes were shown to have a nearly negligible resistance for a number of drugs (Baker, 1974) and provided a useful mechanical support for the stratum corneum. The initial studies on the sandwich model (Tiemessen et al., 1989a) showed that it provided an elegant means to *control* the water *uptake* by the stratum corneum *kinetically*. However, if the stratum corneum water content should be *fixed* at a certain level, *thermodynamic equilibration conditions* are required. These cannot be created with the sandwich model alone. By using salt solutions instead of water as the donor and receptor fluids the relative humidity inside the sandwich can be set and maintained at any level, and any desired degree of stratum corneum hydration can be achieved, in principle. In this study the use of salt solutions as described by Blank et al. (1984) is *combined* with the stratum corneum sandwich model (Tiemessen et al., 1989b) to meet the aforementioned requirements.

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

The adapted stratum corneum–silicone membrane sandwich method is used to determine the influence of the stratum corneum water content on its permeability for nitroglycerin.

Materials and Methods

Skin preparation

All experiments described in this report are performed with stratum corneum prepared from a

single sample of full-thickness human abdominal skin obtained from cosmetic surgical correction.

The skin was stored less than 18 h at 4°C before it was dermatomed (Padgett electro dermatome, model B) to a thickness of about 120 μm, in order to isolate the epidermis mechanically. The epidermal slices were incubated (stratum corneum side up) on filter paper soaked in a 0.2% solution of trypsin (bovine pancreas type III, Sigma) in isotonic phosphate-buffered saline, pH 7.4 at 37°C. After a 24 h incubation the stratum corneum was peeled off (avoiding shear) and carefully rinsed with distilled water several times. From the obtained hydrated stratum corneum 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) 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) to achieve perfect contact with the stratum corneum. The stratum corneum–silicone membrane was dried and stored in a desiccator for less than 1 month. In a penetration experiment the same fluid was used as the donor and receptor. The two aqueous salt solutions used contained 16 and 27 %w/w of sodium bromide (Merck) respectively. Prior to the permeation experiment the stratum corneum–silicone membrane was placed in a closed chamber over the salt solution to be used as the donor and acceptor fluid at 32°C for 48 h, to 'preset' the stratum corneum water content at a desired level. Following the pre-equilibration the stratum corneum was immediately covered with another sticky silicone membrane and placed in the diffusion cell to start the permeation experiment. As described previously (for the 'occlusion simulation model') (Tiemessen et al., 1989a), in the experiments in which water was used as the donor and acceptor fluid, the stratum corneum was prehydrated in a constant relative humidity chamber (over a saturated Na_2HPO_4 solution, relative humidity = 95%) at 20°C for 48 h allowing the stratum corneum to adopt a water weight fraction of 46 ± 2%.

Hydration measurements

The water content of the stratum corneum after a 3 days equilibration over the salt solutions (containing either 16 or 27% w/w sodium bromide) was determined gravimetrically using a microbalance (Mettler TG50 thermobalance). To compare the water content of stratum corneum equilibrated *over* salt solutions with that of stratum corneum located inside the silicone sandwich *submerged in* salt solutions, a submersion experiment was also carried out. Dry reference samples of stratum corneum were sandwiched between silicone membranes, sealed with the adhesive along the edges only, and submerged in the salt solutions at 32°C. After 3 days submersion the sandwiches were removed, dried and cut open, and the stratum corneum water weight fraction was determined gravimetrically.

Diffusion experiments

The steady-state flux of nitroglycerin through the sandwich containing the stratum corneum was measured at 32°C using a two-chambered flow-through diffusion cell (Tiemessen et al., 1988) which had a diffusion area of 0.63 cm^2 while the volume of each compartment was 16 μl. The aqueous donor solutions, prepared by a 20-fold dilution of a 1% ethanolic solution of nitroglycerin (Merck), finally contained 0.05% nitroglycerin and 5% ethanol. Depending on the desired hydration level of the stratum corneum both the donor and the acceptor contained 0, 16 or 27% w/w sodium bromide. The donor solution was pumped through the donor compartment at a rate of 35 ml/h. The acceptor perfusate was pumped through the acceptor chamber at a flow rate of 5 ml/h and collected by a fraction collector at 1 h intervals. Control experiments were carried out using the silicone double membrane only, without stratum corneum.

Nitroglycerin detection

The nitroglycerin concentrations in the acceptor perfusates not containing sodium bromide 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 con-

sisting of methanol/water (50 : 50 v/v) at a flow rate of 1.1 ml/min (Spectroflow 400, Kratos). The absorbance of the column effluent was monitored at 201 nm (Waters Model 450 variable wavelength detector). The retention time of nitroglycerin was 1.9 min. The nitroglycerin concentrations in the acceptor perfusate containing sodium bromide were determined using a gas chromatographic-electron-capture (GC-ECD) detection method, slightly modified from Lee et al. (1988). Sample preparation: after the addition of the internal standard butane-1,2,4 triyl trinitrate (BTN) (Settlage et al., 1983), 1 ml of the acceptor fluid was extracted once with 5 ml of methylene chloride-pentane solvent (5 : 5, v/v). The samples were vigorously shaken for 2 min with a vortex. After separation the organic phases were evaporated under nitrogen at room temperature, and the residue was redissolved in 0.1 ml *n*-butyl acetate. One μl from the obtained sample was injected into the gas chromatograph. A Packard GC (model 439) equipped with an ^{63}Ni electron capture detector was used with a Hewlett-Packard fused silica capillary column (OV 101, 25 m × 0.31 mm i.d., 1 μm film thickness). Nitrogen (high purity carrier gas, nitrogen content > 99.994 vol%, Hoekloos, The Netherlands) at a flow rate of 1.8 ml/min was used as a carrier gas, while nitrogen at a flow rate of 44 ml/min was used as the make-up gas. The temperature of the injection-port and the detector were both 200 °C. The column temperature program was set at 150 °C initially, held for 6 min and then increased to 170 °C at 10 °C/min. The retention times of nitroglycerin and BTN were 5.8 and 9.3 min, respectively.

Calculations

The permeabilities of the sandwich and the stratum corneum were calculated using equations 1 and 2 (Flynn et al., 1974)

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

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

where J is the flux of the drug through the sandwich; P, the permeability; C_d, the drug concentration in the donor solution (this equation is valid if it is assumed that the drug concentration in the acceptor solution will be close to zero: "perfect sink" conditions); s, denotes sandwich; m, silicone doublemembrane; sc, stratum corneum.

The permeability of the silicone double-membrane, P_m, was determined for each donor/acceptor fluid to account for the possibility that the membrane permeability depends on the donor fluid used.

Results

Table 1 shows the results of experiments in which the water content of stratum corneum inside the silicone sandwich, submerged in a salt solution, is compared with the water content of the stratum corneum in contact with the vapour of the same salt solution after an equilibration of 3 days. There is clearly no difference between the two. This proves that, when the proper salt solutions are used for the donor and acceptor, the pre-set water content of the stratum corneum can be maintained throughout a penetration study.

The pre-equilibration of stratum corneum over a saturated disodium hydrogen phosphate solution yielded a water weight fraction of 46 ± 2%. According to a previous report (Tiemessen et al., 1989a) during penetration experiments, pieces of stratum corneum, having an initial water weight fraction of 46%, sandwiched between silicone

TABLE 1

A comparison between a direct exposure of stratum corneum to the vapour of the donor/acceptor medium and an indirect contact of the stratum corneum with the donor/acceptor medium via the silicone membrane

Medium	Water weight fractions of the stratum corneum upon 3 days equilibration at 32 °C	
	In vapour	Submerged in sandwich
16% aqueous NaBr solution	34.0 ± 0.5%	33.5 ± 0.5%
27% aqueous NaBr solution	17.5 ± 1.5%	17.5 ± 1.5%

The data are presented as the mean ± S.D. (n = 3).

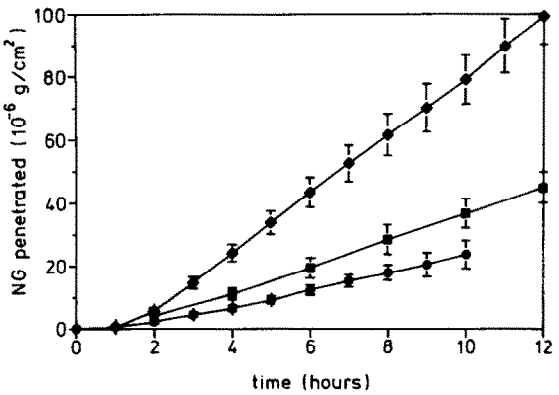

Fig. 2. The influence of water on nitroglycerin penetration through isolated stratum corneum. The stratum corneum water weight fraction is (◆) 49%, (■) 34% and (●) 17.5% respectively. The data points are presented as the mean ± S.E.M. ($n = 4$).

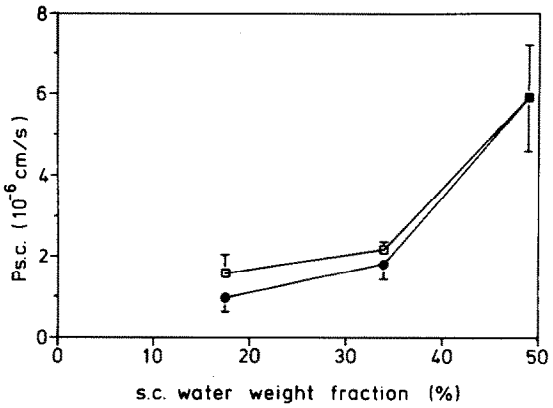

Fig. 3. The stratum corneum permeability coefficients for nitroglycerin as a function of its water content. The open squares indicate the permeability coefficients directly calculated from the nitroglycerin flux ($P_{sc} = D_{sc} \cdot K_{sc/donor} / h_{sc}$). The solid data points show the permeability coefficients after correction for the different "push" effects by the three donor solutions ($P'_{sc} = D_{sc} \cdot K_{sc/water} / h_{sc}$). The data points are presented as the mean ± S.D. ($n = 4$).

membranes and submerged in pure water, take up water very slowly. During a 12 h penetration experiment the stratum corneum water weight fraction will increase near linearly to 52 ± 2%. The average water weight fraction of the stratum corneum disks *for the duration of the transport experiments* is therefore taken as 49 ± 2%, the arithmetic mean of 46 and 52%.

The cumulative amounts of nitroglycerin penetrated through the stratum corneum sandwiches at the 3 different hydration levels are given in Fig. 2. The observed lag times are mainly due to the void volume of the tube to and from the diffusion cells and the small contribution of the silicone membranes (together about 0.75 h); therefore the effective lagtime of nitroglycerin penetration through the stratum corneum may be estimated at about 15 min for the 3 cases. The mean fluxes of the nitroglycerin through the stratum corneum sandwiches are 2.8, 3.7 and 9.3 μg/cm^2/h for the stratum corneum water weight fractions of 17.5%, 34% and 49%, respectively. Since the permeability coefficients of the silicone membranes are known (Table 2), the permeability coefficients of the stratum corneum at the different degrees of hydration can be calculated. The obtained stratum corneum permeability coefficients for nitroglycerin are plotted versus the stratum corneum hydration levels in Fig. 3. These results clearly show that the stratum corneum permeability varies non-linearly with its level of hydration, and that only a 3-fold increase in its water content can result in a 6-fold increased permeability.

TABLE 2

Permeabilities of two sticky silicone membranes put together: the influence from the donor/acceptor medium

Donor/acceptor	Permeability coefficient (10^{-6} cm/s)	"Push" factor F (water as reference)
Water	41 ± 7	1
16% NaBr in water	50 ± 4	1.21 ± 0.24
27% NaBr in water	67 ± 4	1.63 ± 0.31

The data are presented as the mean ± S.E.M. ($n = 4$). "Push" factor $F = P_m$ (with NaBr)/P_m (with water)

Discussion

First the equilibration studies compiled in Table 1 will be considered. Because there is no difference between the water contents of the pieces of isolated stratum corneum equilibrated in the two different ways described, we may conclude, assuming the silicone membrane is impermeable

for sodium bromide, that the relative humidity in the sandwich is the same as in the vapour over the salt solution. In other words the water activity inside the sandwich equals that in the salt solution.

Obviously in any penetration study, the water weight fraction of the stratum corneum inside the sandwich will depend on the thermodynamic activity of water in the donor and receptor solutions in contact with the sandwich. The results obtained with the equilibration experiments most convincingly show that by choosing the proper donor and acceptor solution in combination with the silicone sandwich model, any preset stratum corneum hydration level can be maintained for the duration of the permeation experiment. In principle any desired degree of stratum corneum hydration between 0% and saturation can be obtained experimentally. From the work of Spencer et al. (1975) it is possible to find the relative humidity needed to obtain a particular equilibrium hydration value of the stratum corneum. The salt solutions needed to produce these relative humidities can be found in the literature (see e.g. Young, 1967; Weast, 1977). For practical reasons (avoidance of salt precipitation) only subsaturated salt solutions were used in this study. An estimation of the relative humidity above a subsaturated salt solution can be made by using Raoult's Law.

The observation that the diffusional resistance for nitroglycerin of the chemically inert silicone membranes was much lower than the resistance of the stratum corneum is supported by literature data which underscore the high permeability of silicone polymers for most non-ionic drugs (Baker, 1974). 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.

From Table 2 the conclusion may be drawn that the silicone permeability for nitroglycerin varies somewhat with the concentration of sodium bromide in the donor and acceptor solutions. The membrane permeability P_m appearing in Eqn. 2 may be expressed as (Flynn, 1974):

$$P_m = \frac{D_m \cdot K_{m/d}}{h_m} \tag{3}$$

where D_m is the diffusion coefficient of the drug in the silicone double membrane, h the thickness of the silicone double membrane, while $K_{m/d}$ is the partition coefficient of the drug between the silicone double membrane and the donor solution.

Generally, the enhancement of drug transport from a vehicle in which the drug is dissolved through a membrane by an additive, may be considered to be the result of two cooperative effects: the so-called "pull" and "push" effects respectively, as formulated by Kadir et al. (1987). The "pull" effect arises from interactions between the additives and the membrane; enhancement of the diffusivity (D), or the solubility of the drug inside the membrane (K). The "push" effect results from vehicle–drug interactions, e.g. whereby a decreased drug solubility leads to a higher relative affinity of the drug for the membrane (K) and therefore to an increased steady state flux (Kadir, 1987). Table 2 shows that addition of sodium bromide to an aqueous donor and acceptor causes an increase in the permeability of the silicone membrane for nitroglycerin which is probably due to a decreased solubility of the nitroglycerin in the aqueous phase; direct interactions between sodium bromide and the silicone membrane are unlikely. In the third column in Table 2 the "push" effect is quantified by the "push" factor F, defined as the ratio between the silicone permeability coefficients with and without sodium bromide, respectively:

$$F = \frac{P_m \text{ (with NaBr)}}{P_m \text{ (with water)}} = \frac{K_{m/d}}{K_{m/water}} \tag{4}$$

The stratum corneum permeability coefficients calculated directly from the nitroglycerin fluxes through the sandwiches (see Fig. 3, open squares) are not exactly comparable because they contain

partition coefficients ($K_{sc/d}$), each referring to a different donor solution having a different "push" effect. In order to obtain comparable parameters, we may simply divide the stratum corneum permeability coefficients directly calculated from the nitroglycerin fluxes, by the "push" factor F obtained from the silicone membrane permeabilities for nitroglycerin, since the "push" effect only depends on the donor used. In so doing, the stratum corneum permeability coefficients are normalized with respect to water as the reference donor, and may in fact be written as:

$$P'_{sc} = \frac{D_{sc} \cdot K_{sc/water}}{h_{sc}}$$

They are given as the solid points in Fig. 3. It is clear that the stratum corneum permeability for a rather lipophilic compound such as nitroglycerin ($K_{oil/water} \approx 100$, Hansch and Leo, 1979) correlates positively and non-linearly with its water content. The increase of the stratum corneum water weight fraction from 17.5 to 35% leads to an increase in its permeability of 87%, while a further increase of the stratum corneum water weight fraction to 49% leads to a 500% increase of the stratum corneum permeability. These results reflect what happens in vivo if nitroglycerin is applied under occlusive conditions; namely a local increase in stratum corneum permeability due to the accumulation of endogenous water underneath the occlusive vehicle. The considerable enhancement ("pull") effect of water on the nitroglycerin permeation through the stratum corneum obtained under 'occlusion' is most likely due to a strongly increased diffusion coefficient of the drug in the s.c. (D_{sc}), because other effects water may have on the stratum corneum would probably lead to a decreased permeability: water will decrease the solubility of the lipophilic nitroglycerin inside the stratum corneum ($K_{sc/water}$ decreases) and it will increase the thickness (h_{sc}) of the stratum corneum due to swelling. Both of the final two effects mentioned would tend to *decrease* the overall permeability. Hence the effect of the water on the diffusion coefficient of nitroglycerin in the stratum corneum is most likely even larger than 500%. Since lipophilic compounds such as nitroglycerin are thought to penetrate the stratum corneum via the intercellular lipophilic pathway (Elias, 1981), the strong enhancement effect of water may be the result of water-induced changes in the lipid bilayers, possibly fluidization (Barry, 1987), or lipid head group disorganization.

The introduced stratum corneum–silicone sandwich method is very well suited for studying effects of penetration enhancers under both occlusive (Tiemessen et al., 1989a) and non-occlusive conditions because it enables the investigator to perform the in vitro drug transport studies while maintaining the stratum corneum in a realistic hydration state. With this method the ("pull") effects of penetration enhancers on skin permeability can be studied separately from vehicle effects by pretreating the skin with the enhancer before it is sandwiched between the inert silicone membranes (Tiemessen et al., 1989b).

Furthermore, the sandwich model can easily be adapted to the finite dose technique by replacing the silicone membrane at the donor side by a vehicle in which the drug is incorporated. Particularly for hydrophilic vehicles (e.g. occlusive hydrogel patches (Boddé et al., 1989)) which would swell fast if the stratum corneum or the skin is in direct contact with the aqueous acceptor fluid, a closer simulation of the in vivo conditions may be achieved, when drug release from the vehicle through stratum corneum or other skin preparations is measured under the experimental circumstances described. In a following paper the methods developed in this study will also be used for in vitro evaluation of the effects of hydrophilic moisturizing creams on the stratum corneum permeability (Tiemessen et al., 1989a).

In order to test the applicability of the sandwich model to a wider range of drugs one should compare the permeability of the silicone double membrane with and without the stratum corneum for the particular drug of choice. For this method to be successful, the stratum corneum inside the sandwich should be rate controlling.

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