Supersaturation: Enhancement of Skin Penetration and Permeation of a Lipophilic Drug

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Purpose. To increase the dermal delivery of a lipophilic model compound (LAP), and to deduce the underlying mechanism of enhanced absorption.

Methods. Penetration of LAP from mixtures of up to four degrees of saturation into the stratum corneum was evaluated using a tape-stripping method; epidermal permeation of the drug was measured in Franz diffusion cells. The relative diffusion and stratum corneum–vehicle partition coefficients of LAP were determined by fitting the results to the appropriate solutions to Fick's second law of diffusion.

Results. Both the skin permeation rate and the amount of LAP in the stratum corneum increased linearly with increasing degree of saturation. The apparent diffusivity and its partition coefficient deduced from the penetration experiments were independent of the degree of saturation of the drug in the applied formulation, and consistent with corresponding parameters derived from the permeation experiments.

Conclusions. Supersaturation can increase the skin penetration and permeation of lipophilic drugs. The diffusion and partition parameters deduced for LAP indicate that supersaturation acts exclusively via increased thermodynamic activity without apparent effect on the barrier function of the skin *per se.*

KEY WORDS: supersaturation; skin permeation; tape stripping; diffusion coefficient; partition coefficient; lipophilic drug.

INTRODUCTION

The clinical efficacy of topically administered therapeutic agents is often suboptimal because of their poor penetration into the skin. This has led to the development of an array of strategies for enhancing skin penetration including the use of chemical penetration enhancers and novel vehicle systems such as microemulsions, liposomal-based delivery systems, and supersaturated formulations (1).

In contrast to chemical penetration enhancers, which facilitate transport by perturbing stratum corneum (SC) barrier function, supersaturation is hypothesized to act directly on the drug and to increase skin permeation without alteration of SC architecture. The mechanism of enhancement via drug supersaturation would therefore be based simply on the increased thermodynamic activity of the drug in the vehicle, *i.e.,* an increased driving force for transit out of the formulation and into and across the SC (2). However, once drug concentration is increased above saturation (and the thermodynamic activity to greater than unity), the systems become thermodynamically unstable; indeed, supersaturated formulations are typically subject to recrystallization of the drug substance and concomitant loss of the enhancing effect. Even when the stability of supersaturated formulations is improved by the use of polymers (3–12), stability remains the limiting factor for achieving enhanced skin permeation, which can be observed only for as long as the formulations are supersaturated (5–9,11–18). *In vitro,* permeation through skin is usually measured in standard diffusion cells and is characterized by a steady-state flux (J_{ss}) and a formulation-specific permeability coefficient (K_p) equal to the ratio J_{ss}/c_v, where c_v is the drug concentration in the applied vehicle. But K_p is a composite term, specifically equal to $K*D/H$, where K is the drug's SC-to-vehicle partition coefficient and D is its apparent diffusivity across the SC of thickness H. Deconvolution of K_p to understand whether an enhancement technology has acted on D, K, or perhaps both is not straightforward and has met with limited success (19). A more useful approach has been an alternative experimental procedure, whereby the concentration profile of the drug across the SC is determined (using tape stripping and considering the SC as homogeneous membrane) and then analyzed mathematically to deduce the characteristic diffusion parameter (D/H^2) and the relevant partition coefficient (K) (20). This information can then be employed to "tease apart" the K_p measured in the conventional way and the effects of the enhancement strategy, in term, can be subsequently deduced.

Although detailed earlier work has shown clearly how the topical delivery (penetration and permeation) of oestradiol and piroxicam can be increased via supersaturation (7,9), less attention has been focussed on the use of this approach for more lipophilic drugs. Hence, we chose to investigate supersaturated solutions of the lipophilic lavendustin derivative, LAP (log [octanol-water partition coefficient] ~ 5 [21]), the structure of which is shown in Fig. 1. The experimental methods and the interpretation just discussed were then employed to aid understanding of the mechanism of enhancement.

MATERIALS AND METHODS

Materials

LAP (SDZ LAP 977) was provided by Novartis Pharma AG (Basle, Switzerland). Propylene glycol p.a. and triethylamine puriss. p.a. were purchased from Fluka (Buchs, Switzerland). Acetonitrile (gradient grade) was from Merck KgaA (Darmstadt, Germany). Purified water was produced with a Milli-Q RG system from Millipore (Bedford, MA). GHP Acrodisc® 13 filters (pore size 0.45 μm) were obtained from Pall Gelman Sciences (Ann Arbor, MI). Nuclepore® polycarbonate membranes (pore size 1.0 μm, diameter 47 mm) were from Corning Separations Division (Acton, MA).

Preparation of Supersaturated Solutions

The solubility of LAP in a mixture of propylene glycol and water at a ratio of 7:3 (v/v) was determined previously to be 0.30 mg/ml at 25°C (21). Saturated and supersaturated

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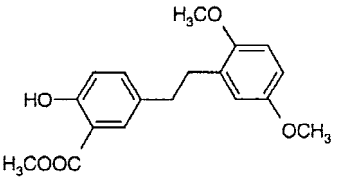

Fig. 1. Chemical structure of SDZ LAP 977 (LAP).

solutions at one, two, three and four degrees of saturation (DS; 1 DS = saturated) in mixtures of propylene glycol–water (7:3, v/v) were prepared using the method of mixed cosolvent systems. The drug was dissolved in propylene glycol and mixed with water by inverting the test tube several times. These supersaturated solutions were stable for 8 h (21).

Preparation of Skin

Fresh pig ears were obtained from a local abattoir. After cleaning with water, the skin was excised with a scalpel. For experiments with full-thickness skin, the samples were stored at –20°C for a maximum period of up to 1 month. Before use, the skin was allowed to thaw for 1 h. Epidermis was obtained by heat separation (22). Pieces of fresh, full-thickness skin were immersed in water at about 60°C for 2 min, after which the epidermis was carefully separated from the dermis with tweezers. The epidermis was washed with water and floated on Nuclepore polycarbonate membranes. After drying with soft paper, the epidermis was stored at –20°C for a maximum period of up to 1 month.

Epidermal Permeation of LAP

The permeation of LAP through pig ear epidermis was investigated in vertical (Franz-type) diffusion cells. The exposed skin surface area was 2.0 cm^2 and the receptor volume was 5.0 ml. Leak-proof seals between the skin and the two compartments of the diffusion cells were made with silicone grease (Dow Corning Corporation, Midland, MI). The receptor medium was a degassed 4% bovine serum albumin (BSA) solution in phosphate-buffered saline (PBS), pH 7.4, stirred at 300 rpm and maintained at 32°C. BSA increased LAP solubility in the buffer to 0.0025%. However, sink conditions were maintained in the permeation experiments, for which the maximum LAP concentration in the receptor never exceeded 0.0002%. After equilibration of the epidermis with buffer solution during 1 h, the donor compartment was filled with 4 ml of LAP solution in propylene glycol–water mixtures or in pure propylene glycol and covered with Parafilm®. At 1, 2, 3, 4, 5, 6, 7, and 8 h, 300 μl of receptor medium were removed and replaced with 300 μl of fresh medium. The samples were diluted with 450 μl of acetonitrile, mixed using a vortex agitator, filtered after 4–6 h through GHP Acrodisc syringe filters (pore size 0.45 μm), and analyzed by HPLC (method described below). The amount of LAP permeated $Q(t)$ was plotted as a function of time t. From the slope, the permeation rate J was calculated. The appropriate solution to Fick's second law of diffusion in a homogeneous membrane (23), *i.e.,*

$$Q(t) = (KH)C_v\left[\frac{D}{H^2}t - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(-n^2\pi^2\frac{D}{H^2}t\right)\right] \quad (1)$$

was fitted to the permeation curves and yielded values of K^*H and D/H^2, where H is the SC thickness, *i.e.,* parameters independently reflective of the partitioning and diffusivity behavior of the drug. The fitting was performed with n equal to 5.

SC Penetration of LAP

The penetration of LAP into pig ear stratum corneum was determined *ex vivo* following a 3-h application of saturated and supersaturated solutions of the drug in propylene glycol–water to full thickness skin positioned on an inert support. The duration of the application was chosen to allow sufficient LAP penetration into the SC so that a nonsteady-state concntration profile, amenable to the data analysis, could be established. A donor compartment (area = 32 cm^2) containing 30 ml of vehicle was fixed to the skin with silicone grease (Dow Corning Corp., Midland, MI). After the 3-h application, the solution was removed and the skin carefully dried with soft tissue. The skin was equilibrated to environmental conditions for 15 min; then the skin was stripped 20 times with Scotch Book Tape No. 845 (3M, St. Paul, MN). Tapes were equilibrated at ambient conditions overnight and then weighed before and after stripping. From the weight difference, and assuming a density of 1 g cm^{-3} (24), the thickness of SC removed was calculated.

Before stripping and after every second strip, transepidermal water loss (TEWL) was measured (TEWAMETER™ 210 from Courage+Khazaka electronic GmbH, Koln, Germany). Then, 1/TEWL was plotted against the SC thickness removed; the linear plot enabled the intact thickness of the SC to be determined from the x-axis intercept (25). The relative thickness of SC removed by each tape was calculated by dividing the absolute thickness (x) of SC stripped by each tape by the thickness of the intact SC (H).

The tapes were extracted in 11 ml acetonitrile for 3 h with stirring (recovery = 96±3%). The extracts were filtered through GHP Acrodisc syringe filters (pore size 0.45 μm) and analyzed by HPLC. The concentration (C_x) of LAP in each tape was calculated and plotted against the relative SC thickness (x/H) removed. The data from each experiment were fitted separately to the appropriate solution of Fick's unsteady state diffusion equation:

$$C_x = C_{x=0}\left\{\left(1 - \frac{x}{H}\right) - \frac{2}{\pi}\sum_{n=1}^{\infty}\frac{1}{n}\sin\left(\frac{n\pi x}{H}\right)\exp\left(\frac{-Dn^2\pi^2 t}{H^2}\right)\right\} \quad (2)$$

to obtain the concentration of the drug in the outermost layer of the stratum corneum $C_{x=0}$ and the diffusion parameter D/H^2 (20). These parameters were used to reconstruct the concentration profiles of LAP in the stratum corneum for each experiment. The area under the concentration profiles was then calculated to yield the total amount of LAP in the entire SC, *i.e.,* to provide an assessment of an approximate LAP "bioavailability" in the SC. The mean values of $C_{x=0}$ and D/H^2 at each degree of saturation were calculated and the "average" concentration profile for each degree of saturation was reconstructed. From D/H^2, the diffusion coefficient was calculated assuming a diffusion path length equal to the thickness (H) of the SC. The partition coefficient K was calculated by dividing the concentration of the drug in the outermost

layer of the stratum corneum $C_{x=0}$ by the concentration in the vehicle $C_{vehicle}$ (Equation 3).

$$K = \frac{C_{x=0}}{C_{vehicle}} \quad (3)$$

HPLC Analysis

The HPLC-system consisted of a Kontron autosampler 460, Kontron pumps 420 and a Jasco UV-975 detector.

For the receptor phase samples from the epidermal permeation experiments, a LiChrospher 60-5 Select B 125 × 4 mm column with a CC 8/4 LiChrospher 100-5 RP-18 precolumn (Machery-Nagel GmbH, Düren, Germany) maintained at 50°C was used. The mobile phase, with a flow-rate of 1 ml/min, comprised 40% ammonium sulfate (10 mM), adjusted to pH 6.0 with triethylamine, and 60% acetonitrile. Samples of 20 µl were injected and LAP was detected using its UV-absorbance at 230 nm. The retention time was approximately 5 min.

For the SC penetration study, a Superspher 100 RP-18 endcapped 250 × 4 mm column (particle size 4 µm, Merck KGaA, Darmstadt, Germany) with a LiChrospher 100 RP-18 4 × 4 mm precolumn (particle size 5 µm, Merck KGaA, Darmstadt, Germany) at a temperature of 50°C was used. The mobile phase, with a flow-rate of 1 ml/min, consisted of (a) phase A composed of acetonitrile–water 85:15, and (b) phase B composed of acetonitrile–water 95:5. After 4 min with 0% B, the mobile phase was changed to 100% B for 2 min. Then, after 12 min, the mobile phase was changed back to 0% B for 2 min and finally equilibrated for 7 min before injection of the next sample. Samples of 20 µl were injected and LAP was detected using its UV-absorbance at 209 nm. The retention time was approximately 4 min.

Statistics

The results were compared using analysis of variance (ANOVA) at $\alpha = 0.05$.

RESULTS

Epidermal Permeation of LAP

The cumulative permeation of LAP from propylene glycol–water (7:3) mixtures at one, two, three, and four degrees of saturation through pig ear epidermis is shown in Fig. 2. From the slopes of these curves, the steady-state permeation rates were calculated (Table I). Permeation rate was directly related to the degree of saturation ($r = 0.96$). The permeation profiles were fitted to Equation 1 and the parameters K^*H and D/H^2 were found (Table I). Both parameters were independent of the degree of saturation (ANOVA, $p > 0.05$).

Table I. Parameters Derived from the Permeation Experiments Measuring the Transport of LAP from Propylene Glycol–Water Mixtures (7:3) at 1, 2, 3, and 4 Degrees of Saturation (DS) Through Pig Ear Epidermis (Mean ± SD, n = 4–6)

	DS = 1	DS = 2	DS = 3	DS = 4
Permeation rate [µg cm^{-2} h^{-1}]	0.12 ± 0.02	0.21 ± 0.08	0.26 ± 0.12	0.44 ± 0.14
$K^*H^a \times 10^3$ [cm]	3.2 ± 1.4	3.4 ± 2.4	2.0 ± 0.9	3.5 ± 1.4
$D/H^{2b} \times 10^5$ [s^{-1}]	4.2 ± 1.3	3.8 ± 1.6	4.3 ± 0.9	3.3 ± 0.6

[a] Partition coefficient multiplied by the diffusion length estimated by fitting the permeation data to Equation 2.
[b] Diffusion coefficient divided by the diffusion length squared estimated by fitting the permeation data to Equation 2.

To investigate the influence of absolute LAP concentration in the vehicle, the permeation of the drug from a more concentrated but not supersaturated vehicle was investigated. In Fig. 3 the permeation of LAP from saturated and supersaturated propylene glycol–water mixtures in a ratio of 7:3 (0.3 mg/ml for DS = 1 and 1.2 mg/ml for DS = 4) is compared with that from a saturated solution in pure propylene glycol (4.0 mg/ml). Independent of the different concentrations of LAP in the propylene glycol–water mixture and in pure propylene glycol, the saturated solutions yielded similar permeation rates, whereas the supersaturated solution (containing a lower absolute concentration of LAP) showed a significantly higher permeation rate (ANOVA, $\alpha = 0.05$).

SC Penetration of LAP

The individual results ($n = 3$) for the penetration of LAP into the SC following a 3-h application of a vehicle containing the drug at DS = 2 are shown in Fig. 4. These profiles present

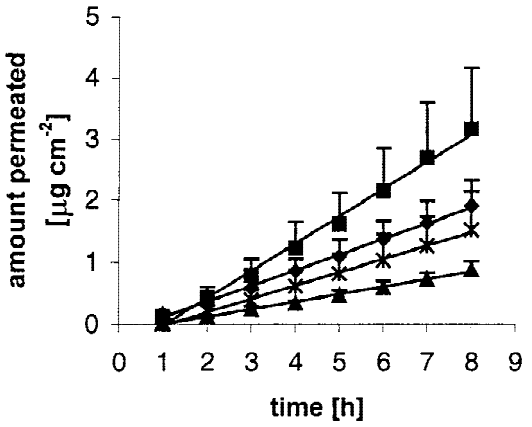

Fig. 2. Cumulative permeation of LAP through pig ear epidermis as a function of time. Solutions in propylene glycol–water 7:3 at 1 (▲), 2 (X), 3 (♦), and 4 (■) degrees of saturation were investigated. (Mean + SD, n = 4–6).

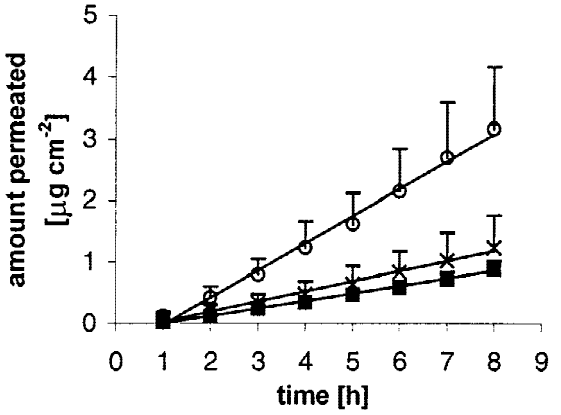

Fig. 3. Cumulative permeation of LAP through pig ear epidermis as a function of time. Saturated solutions in propylene glycol–water 7:3 (0.3 mg/ml, ■), in pure propylene glycol (4.0 mg/ml, x), and supersaturated solutions in propylene glycol–water 7:3 (DS = 4, 1.2 mg/ml, o) were compared. (Mean + SD, n = 4–6).

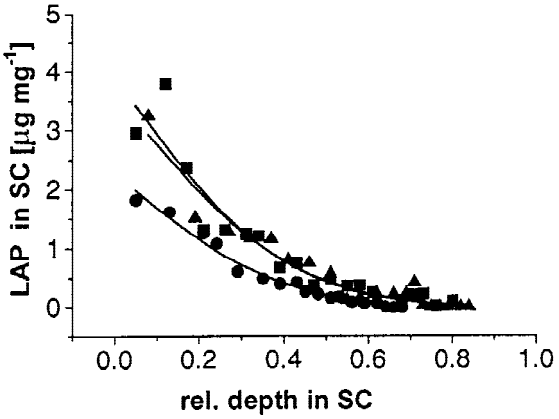

Fig. 4. Concentration profile of LAP in pig ear SC after application of a solution at two degrees of saturation in propylene glycol–water 7:3. The concentration of LAP measured in three experiments (different symbols) is plotted against relative position (depth) within the SC. The lines drawn through the data represent the best fit for each skin sample.

Table II. Parameters Derived from Penetration Experiments Measuring the Uptake of LAP from Propylene Glycol–Water Mixtures (7:3) at 1, 2, 3, and 4 Degrees of Saturation (DS) into Pig Ear SC (Mean ± SD, $n = 3$)

	DS = 1	DS = 2	DS = 3	DS = 4
Amount in SC [µg cm^{-2}]	0.34 ± 0.17	0.89 ± 0.20	1.25 ± 0.25	2.41 ± 0.93
K[a]	3.8 ± 1.7	5.3 ± 1.4	3.9 ± 0.5	6.3 ± 1.7
D/H^{2}[b] × 10^{6} [s^{-1}]	4.3 ± 1.8	4.5 ± 0.4	6.5 ± 1.9	6.1 ± 3.5
H[c] [µm]	12.3 ± 2.5	11.1 ± 1.0	12.0 ± 3.0	12.2 ± 4.8
D_H^d × 10^{12} [cm^2 s^{-1}]	6.0 ± 0.8	5.5 ± 0.7	8.8 ± 2.2	7.7 ± 5.2

[a] Partition coefficient estimated by fitting the concentration versus SC depth profile to Equation 3.
[b] Diffusion coefficient divided by the diffusion length squared estimated by fitting the concentration versus SC depth profile to Equation 3.
[c] Thickness of SC calculated from TEWL measurements.
[d] Apparent diffusion coefficient calculated assuming that the path length is equal to the membrane thickness, H.

the concentration of LAP as a function of relative SC depth. The average profiles from the experiments at each DS (1 through 4) are in Fig. 5. Integration of these profiles to give the corresponding area-under-the-curve (*i.e.,* topical "bioavailabilities") yields a linear relationship ($r = 0.97$) between the total amount of LAP in the SC (Table II) and the DS of the drug in the applied vehicle.

Fits of Equation 2 to the individual concentration profiles at each DS studied were of acceptable quality ($r \geq 0.86$) and provided values of K and D/H^2 (Table II). Neither parameter showed any dependence on the DS of LAP (ANOVA, $p > 0.05$). From the deduced values of H from the TEWL measurements, apparent diffusivities (D) of LAP across the SC were calculated (Table II).

Comparison of Permeation and Penetration Results

Figure 6 evaluates the SC "bioavailabilities" of LAP determined in the penetration experiments with the corresponding permeation fluxes for all DS. The correlation coefficient of the linear regression is 0.99. Table III compares the partitioning and diffusion parameters (K^*H and D/H^2, respectively) obtained from the permeation and penetration experiments. Although the apparent partition coefficients derived differed by less than a factor of two, the diffusion parameters were markedly (and highly significantly) different ($p < 0.001$; unpaired t-test).

DISCUSSION

Skin penetration of the drug is often the limiting factor in dermal delivery. Supersaturation is one method to improve the delivery of dermally applied drug substances. In this study the effect of supersaturation on the penetration of LAP into and the permeation through pig ear skin is investigated.

The permeation of LAP from saturated and supersaturated solutions in propylene glycol–water mixtures through pig ear epidermis was measured. The permeation rate of LAP increased linearly with increasing degree of saturation of the applied solution. This finding is in good agreement with both published data for the skin permeation of piroxicam and oes-

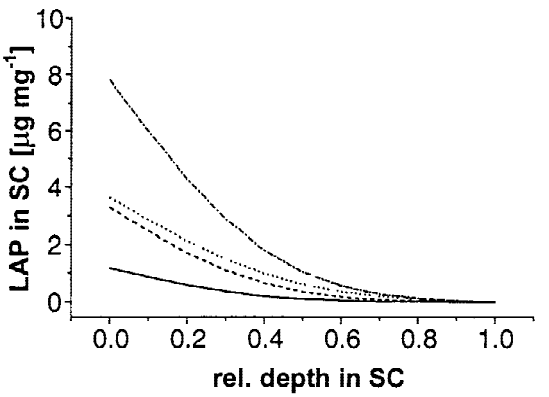

Fig. 5. Concentration profile of LAP in pig ear SC after application of solutions at one (——), two (– – –), three (· · ·), and four (–· – · –) degrees of saturation in propylene glycol–water 7:3. Each curve represents an average profile obtained using the mean values for D/L^2 and K derived from three different experiments each of which yielded 20 data points.

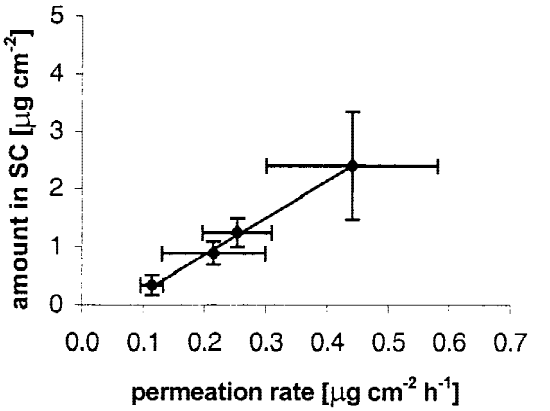

Fig. 6. Correlation of the uptake of LAP into the SC with its permeation rate through epidermis following application of vehicles containing the drug at one, two, three, and four degrees of saturation. (Mean ± SD, $n = 3$–6).

Table III. Comparison of LAP Diffusion and Partition Parameters from Saturated and Supersaturated Propylene Glycol–Water Mixtures (7:3) in Pig Ear Skin as Estimated from the Permeation and Penetration Experiments (Mean ± SD)

	Permeation (n = 19)	Penetration (n = 12)
$K*H^a \times 10^3$ [cm]	3.1 ± 1.6	5.7 ± 1.9
$D/H^{2b} \times 10^6$ [s^{-1}]	39.0 ± 11.5	5.3 ± 2.1

[a] Product of partition coefficient and diffusion path length.
[b] Diffusion coefficient divided by the diffusion length squared.

tradiol (7–9), and with our recent work on the permeation of LAP through silicone membranes (again demonstrating a linear increase with increasing degree of saturation [21]). The individual partition ($K*H$) and diffusion (D/H^2) parameters derived were independent of the degree of saturation.

In the solutions with DS > 1, the absolute concentration of the drug in the vehicle is obviously greater than its saturation solubility. To differentiate thermodynamic activity (as measured by the degree of saturation) from absolute drug concentration, the skin permeation of LAP from a saturated solution in propylene glycol was measured. This vehicle was chosen for its similarity to the propylene glycol–water mixtures (7:3) used to achieve different DS and because it allowed a higher absolute concentration of LAP to be studied. In fact, the saturation concentration of LAP in propylene glycol was 13 times greater than in propylene glycol–water (7:3), yet the drug permeation rate was only slightly increased. This increase in skin permeation is most probably a result of the penetration enhancing properties of propylene glycol (7); however, the difference in permeation rates was not statistically significant. A propylene glycol–water (7:3) supersaturated vehicle (DS = 4), on the other hand, resulted in a significantly higher flux (Fig. 3). Therefore, the degree of saturation and not the absolute concentration in the vehicle determines the permeation rate of LAP through pig ear epidermis.

The area under the LAP concentration versus SC depth profile increased with increasing degree of saturation. Although there is only a minor difference between the vehicles at two and three degrees of saturation, it should be noted that differences in barrier function of different skin samples can be sufficiently high to sometimes blur the differences between the individual results. Comparison of the amount of LAP penetrated into the SC with its permeation through the epidermis yielded a linear relationship; in other words, LAP permeation through the skin increased proportionately with uptake of the drug into the SC.

From the LAP concentration profiles across the SC, the drug's SC-vehicle partition coefficients and apparent diffusivities across the SC were derived. These values of each parameter were statistically equivalent and were independent of the degree of saturation (in agreement with results from the permeation experiments). This finding implies that supersaturation did not change the barrier properties of the SC and that the increased flux was exclusively the result of increased thermodynamic activity of the drug in the formulation. The diffusion parameter D/H^2 derived from the permeation experiments was about seven times higher than the value from the penetration measurements. Two possible reasons for this difference are (i) the skin barrier function of the epidermis used for the permeation experiment was altered by the skin preparation procedure (including, for example, exposure of the tissue to elevated temperature, or trauma due to physical separation of epidermis and dermis), and/or (ii) in the permeation experiments, the skin was more hydrated and therefore offered a diminished diffusional resistance to LAP transport (26). A similar difference between permeation and penetration has been observed previously (27). Therefore, it is most likely that the level of hydration was responsible for the differences in the diffusion coefficient. This conclusion is consistent with the fact that, in the permeation experiments, the epidermis was in complete contact with aqueous buffer (permitting full hydration of the entire tissue, including the SC) whereas, in the penetration study there was no receptor phase in the conventional sense, only the natural level of hydration present in the pig ear skin. We also estimated a somewhat lower partition coefficient from the permeation data, consistent once more with increased SC hydration (*i.e.,* decreased solubility of the lipophilic LAP in the barrier and hence a smaller partition parameter).

CONCLUSION

Supersaturation leads to increased permeation of the lipophilic model compound LAP through pig ear epidermis. This increase in permeation was shown to be a result of a higher thermodynamic activity of the drug in the vehicle. The amount of LAP in the SC increased linearly with the degree of saturation and correlated well with the permeation through epidermis. The data analysis, which "dissects" out the diffusion and partitioning components contributing to the observed penetration and permeation results, indicates that supersaturation increases skin transport of LAP exclusively via an effect on the drug's thermodynamic activity, whereas its intrinsic diffusivity across the stratum corneum remains unaltered.

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