Effect of Receiver Fluid pH on *in Vitro* Skin Flux of Weakly Ionizable Drugs

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In *in vitro* skin permeation experiments, the pH of viable epidermis is readily conditioned by the receiver fluid. For weakly ionizable compounds, the flux determined experimentally thus depends on the receiver fluid pH. The purpose of the present work is to characterize this pH effect, since nonphysiological conditions have often been used in the receiver fluid to enhance the solubility of the subject compounds. A transport model was developed to analyze the above-mentioned pH effect of the receiver fluid on the steady state flux of weakly ionizable drugs. The results showed that the skin flux had a strong dependence on pH for those compounds with high intrinsic partition coefficients. Experimentally, this pH effect was observed with a model acid and a model base. The skin flux was found to have a profound dependence on the receiver fluid pH. This dependence also correlates with the octanol/water partition coefficient of the molecule. It was concluded that the use of a physiological receiver fluid would be crucial for a realistic estimation of transdermal potential. The results also suggested that, for weakly ionizable compounds with high partition coefficients, the viable epidermis could be a significant transport barrier for systemic absorption.

KEY WORDS: skin permeation; pH; diffusion; mass transport; ionization; partition coefficients.

INTRODUCTION

For transdermal drug delivery, permeation through stratum corneum was long known to be the transport barrier for systemic delivery. As such, the conventional approach was to focus on reducing the barrier property of this layer by methods such as using chemical enhancers. The transport resistance of viable epidermis/dermis was thus regarded as insignificant in general. In *in vitro* diffusion cell experiments, this assumption led to the use of any receiver fluid, either at a nonphysiological pH or with organic cosolvents, that offered a good solubility for the test compound so as to maintain a sink condition (1,2). It is to be emphasized that this approach is valid only in cases where the barrier property of viable epidermis is minimal. Otherwise, the receiver fluid can influence the permeation by conditioning the viable epidermis resulting in an unrealistic estimation of skin flux. Typical examples of compounds to which viable epidermis may present a significant barrier property are the ones with a high partition coefficient and limited solubility. For this category of molecules, viable epidermis offers a significant resistance to the overall diffusional transport. If the drug is also weakly ionizable, the *in vitro* skin flux will also depend on the pH used in the receiver fluid, which readily conditions the viable epidermis. Since there have not been any reports in the literature regarding the effect of receiver fluid pH on the *in vitro* skin flux, it appears that there is a need to establish the significance of this effect. In this paper, the results from both theoretical analysis and experimental investigation are presented. Some guidelines in anticipating this receiver pH effect are also discussed.

THEORY

Relevant modeling work was reported on the effect of pH on gastrointestinal absorption (4). To analyze the pH-buffer dependence of the transport of ionizable solutes, Suzuki *et al.* were the first to set up a two-phase model which describe solute diffusion from bulk medium to a lipid phase with a boundary layer in between. This model is the exact reverse in terms of the direction of transport of our interest. Taking a similar approach, Patel *et al.* developed a physical model for analyzing the steady state transport of alkyl amines across a silicone rubber membrane in a two-chamber diffusion cell (5). The model considered a boundary layer on each side of the membrane and also included the effect of buffer capacity. Because of the complexity of multiple equilibria of buffer and drug species, the equations were solved via a numerical procedure. Although these models contained interesting features, they were not constructed to address the issue of our present interest.

The model developed considered skin as a two-layer membrane composite. Figure 1 is the schematic of the model presented in the following discussions. Layer 1 is the stratum corneum and layer 2 is the viable epidermis/dermis. It is assumed that only the nonionized species permeate through the stratum corneum, while both ionized and nonionized species can permeate through the viable layer. For a weakly basic drug, the following equations govern the steady-state transport:

$$\frac{d^2C_{1B}}{dx^2} = 0, \qquad 0 \le x \le L_1 \tag{1}$$

$$\frac{d^2C_{2B}}{dx^2} = 0, \qquad L_1 \le x \le L_2 \tag{2}$$

$$\frac{d^2C_{2BH}}{dx^2} = 0, \qquad L_1 \le x \le L_2 \tag{3}$$

where the subscripts 1, 2, B, and BH refer to the stratum corneum, viable epidermis, free base, and ionized conjugated acid of the base, respectively. The related boundary conditions are

$$C_{1B} = C_0, \qquad x = 0 \tag{4}$$

$$-D_1\frac{dC_{1B}}{dx} = -D_{2B}\frac{dC_{2B}}{dx} - D_{2BH}\frac{dC_{2BH}}{dx}, \qquad x = L_1 \tag{5'}$$

$$C_{1B} = KC_{2B} \qquad x = L_1 \tag{6}$$

$$C_{2BH} = \frac{C_{2H}}{K_a}C_{2B}, \qquad L_1 \le x \le L_2 \tag{7}$$

$$C_{2B} = 0, \qquad x = L_2 \tag{8}$$

$$C_{2BH} = 0, \qquad x = L_2 \tag{9}$$

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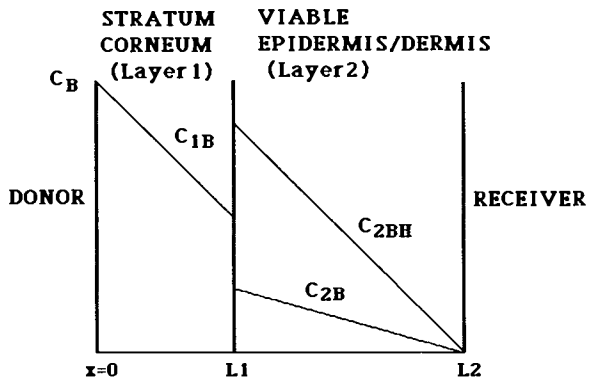

Fig. 1. A schematic for a physical model for analyzing the effect of ionization on skin permeation.

Table I. Physicochemical Properties and Experimental Donor Compositions of Model Compounds A and Nicardipine

Property	Compound A (free acid)	Nicardipine (free base)
Molecular weight	255	516
pK_a (water)	3.5[a]	7.33[b]
Solubility (intrinsic)[b]	50 μg/mL (25°C)	1.93 μg/mL (25°C)
Partition coefficient (octanol/water)	603[b]	6545[c]
Donor composition	35% IPA	50% IPA 2.1% IPM

[a] From Ref. 10.
[b] Determined by the Preformulation Group at Syntex Research.
[c] From Ref. 1.

where K is the partition coefficient, K_a is the dissociation constant of the conjugated acid, D_i is the diffusion coefficient of species i, C_{2H} is the hydrogen ion concentration in viable epidermis, and C_0 is the drug concentration at the stratum corneum/donor interface. Assuming that $D_{2B} = D_{2BH} = D_2$ and combining with Eq. (7), Eq. (5′) can be expressed by

$$D_1\frac{dC_{1B}}{dx} = D_2\left(1 + \frac{C_{2H}}{K_a}\right)\frac{dC_{2B}}{dx} \tag{5}$$

The steady-state concentration profiles for C_{1B}, C_{2B}, and C_{2BH} can thus be solved by the boundary conditions (4)–(9). From these profiles, the steady-state flux, J_{ss}, can be determined by

$$J_{ss} = -D_1\frac{dC_{1B}}{dx} \tag{10}$$

or by

$$J_{ss} = -D_2\left(1 + \frac{C_{2H}}{K_a}\right)\frac{dC_{2B}}{dx} \tag{11}$$

The model for a weak acid can be similarly set up by reinterpreting species B as the free acid (HA) and BH as the conjugate base (A) and substituting the following equation in place of Eq. (7):

$$C_{2A} = \frac{K_a}{C_{2H}}C_{2HA} \tag{12}$$

MATERIALS AND METHODS

Materials

The model weak acid, compound A, and weak base, nicardipine, selected are Syntex proprietary compounds. The relevant physicochemical properties of these two model drugs are listed in Table I. They were supplied as free acid and free base. Isopropyl alcohol, USP grade, was supplied by Mallinkrodt, and isopropyl myristate, NF grade, was supplied by Emery. The reagent grade citric acid and sodium dibasic phosphate were supplied from Sigma and Mallinkrodt, respectively. These excipients were used as supplied without further purification.

Methods

The cadaver skin used in the permeation studies was harvested and supplied within 48 hr postmortum. An approximately 300-μm-thick layer was dermatomed without shaving or chemical treatment. All experiments were conducted with skin samples obtained from the same donor to minimize skin variation. A modified Franz diffusion cell system was used with a donor compartment of 2 mL, a receiver compartment of 22 mL, and a diffusion surface area of 2.0 cm^2 between compartments. The cells were thermostated at 32°C and the receiver compartment was stirred by a magnetic stir bar. After the skin was mounted and before adding the donor fluid, the skin was allowed to equilibrate for 30 min and a blank sample was taken to ensure that no impurities leached from the skin would interfere with the HPLC assay. At specified time intervals, 1-mL samples were taken and the receiver fluid was replaced with 1-mL portions of fresh buffer. The pH of the receiver fluid was monitored at every sampling to ensure that no pH drift occurred. The samples were assayed by reverse-phase HPLC methods. The donor composition was the same in all experiments for each compound (see Table I). The receiver fluids with different pH used in the experiments were prepared by mixing a stock citric acid solution and a disodium phosphate solution. For compound A experiments, the concentrations of citric acid and disodium phosphate were 0.1 and 0.2 M, respectively. For nicardipine experiments, the buffer concentrations used were 10-fold lower, i.e., 0.01 M for citric acid and 0.02 M for disodium phosphate, to minimize the shift in the chromatographic retention time of samples at various receiver fluid pH.

An experimental permeation curve was defined as the time profile, in hours, of the cumulative micrograms of drug permeated per square centimeter of skin. The slope of the steady-state portion of the permeation curve was then reported as the skin flux. The intersect of this slope with the time axis was termed lag time. Typically, each *in vitro* permeation experiment was conducted for 36 hr. Within this period a linear portion of the permeation curve was evidenced after an initial lag period.

RESULTS AND DISCUSSION

Model Analysis

Upon solving Eqs. (1)–(9), the steady-state flux for a weak base can be derived, according to Eq. (10) or (11), as

$$J_{ss} = C_0 \Big/ \left[\frac{L_1}{D_1} + \alpha\left(\frac{L_2 - L_1}{D_2}\right)\right] \tag{13}$$

where $\alpha = K[1 + (C_{2H}/K_a)]^{-1}$. A normalized flux, J^*, can be defined by taking the ratio of the steady-state flux at any particular pH, J_{ss}, and the steady-state flux at a reference pH, J_{ss}^{ref}, resulting in

$$J^* = \left[1 + \left(\frac{L_2}{L_1} - 1\right)\frac{D_1}{D_2}\alpha_{ref}\right] \Big/ \left[1 + \left(\frac{L_2}{L_1} - 1\right)\frac{D_1}{D_2}\alpha\right] \tag{14}$$

where α_{ref} is the α value determined at the reference pH. In analyzing the pH effect on J^*, several system parameters are readily identified from Eq. (14), namely, L_1/L_2, $K(D_1/D_2)$, and K_a. Here K and D_1/D_2 are considered as a combined parameter, $K(D_1/D_2)$. Figure 2 illustrates the effect of $K(D_1/D_2)$ on J^*, with pH 10 being the reference pH. The choice of pH 10 as the reference pH is strictly for convenience, because the flux is at minimum at this pH value. L_1 and L_2 are assumed to be 10 and 300 μm, respectively, giving a L_1/L_2 value of 0.033. The pK_a is arbitrarily assumed to be 7. Figure 2 shows that the pH dependence is significant above a $K(D_1/D_2)$ value of 0.01. Since the pH effect is mainly in the viable epidermis, the increase in the pH dependence indicates that the barrier property of the viable epidermis becomes significant when $K(D_1/D_2)$ increases. The denominator in Eq. (13) clearly represents the total resistance, and the permeability of each layer, P_i, can be readily derived as,

$$P_1 = \frac{L_1}{D_1} \tag{15}$$

$$P_2 = \alpha\frac{L_2 - L_1}{D_2} \tag{16}$$

The P_1/P_2 ratio thus indicates the relative importance of the layers in controlling the transport rate. If P_1/P_2 is less than unity, the stratum corneum is the major transport barrier; if P_1/P_2 is greater than unity, the viable epidermis becomes the rate limiting layer. Figure 3 plots P_1/P_2 versus pH at various $K(D_1/D_2)$ values with a pK_a of 7. When $K(D_1/D_2)$ is greater than 0.1, it is clear that the control is shifted from stratum corneum to viable epidermis when the pH is greater than 6.5. If one assumes a typical value of 0.01 for D_1/D_2, this means that the pH effect will exist at a K value above 10. Therefore, for most ionizable compounds suitable for transdermal delivery, this receiver fluid pH effect would exist. The effect of pK_a on the J^* versus pH plot is shown in Fig. 4. When only pK_a varies, a horizontal shift of the flux versus pH curve is evidenced with no change in the magnitude of the dependence.

In Vitro Permeation

To test the theory, the *in vitro* skin fluxes of compound A and nicardipine were both determined at various receiver fluid pH's. In the experiments, care was taken to ensure that the sink condition in the receiver fluid was maintained, especially for pH's in the vicinity of the pK_a. This was particularly important for nicardipine, which has a fairly low intrinsic solubility. The dimensionless J^*'s versus receiver fluid pH's are plotted in Fig. 5. The reference pH's used in obtaining J^* were pH 1.2 for compound A and pH 6.6 for nicardipine, where the fluxes were the smallest (see Table II for experimental flux values). It is readily seen that there is a 2.5-fold increase in flux for compound A when the pH increases from 1 to 5, while the nicardipine flux increases by more than an order of magnitude when the pH decreases from 7 to 4. The impact of the receiver fluid pH on the *in vitro* skin flux is obvious.

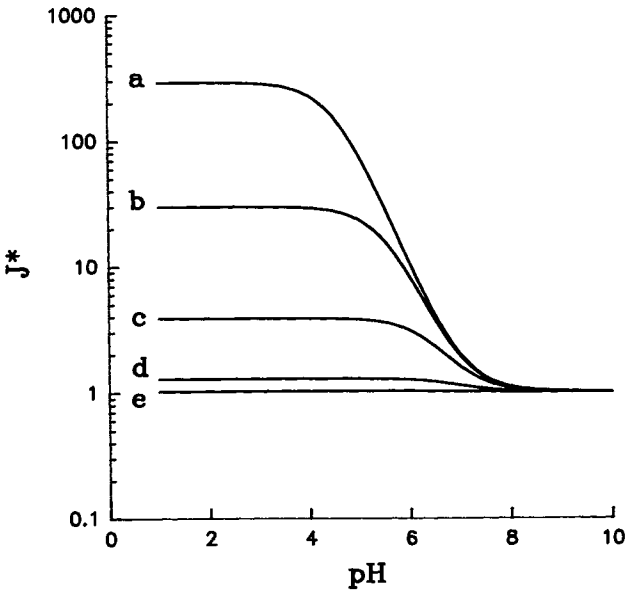

Fig. 2. Effect of pH in layer 2 on J^* of a weak base at $K(D_1/D_2)$ values of 10 (a), 1 (b), 0.1 (c), 0.01 (d), and 0.001 (e), assuming L_1/L_2 = 0.033 and pK_a = 7.

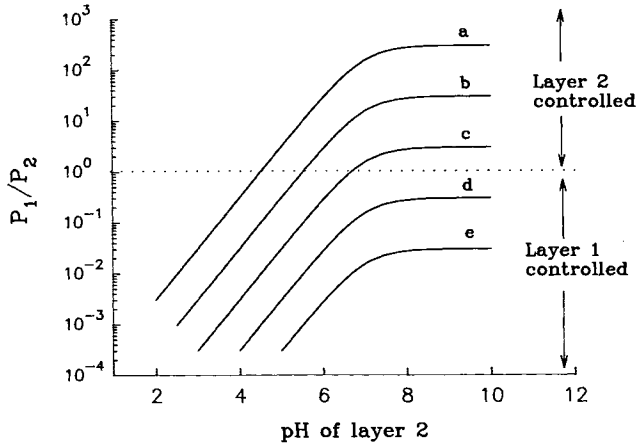

Fig. 3. Plots of P_1/P_2 vs pH of layer 2 for a weak base showing the effect of $K(D_1/D_2)$ values of 10 (a), 1 (b), 0.1 (c), 0.01 (d), and 0.001 (e) on the relative control of layer 1 and 2 on overall transport, assuming pK_a = 7 and L_1/L_2 = 0.033.

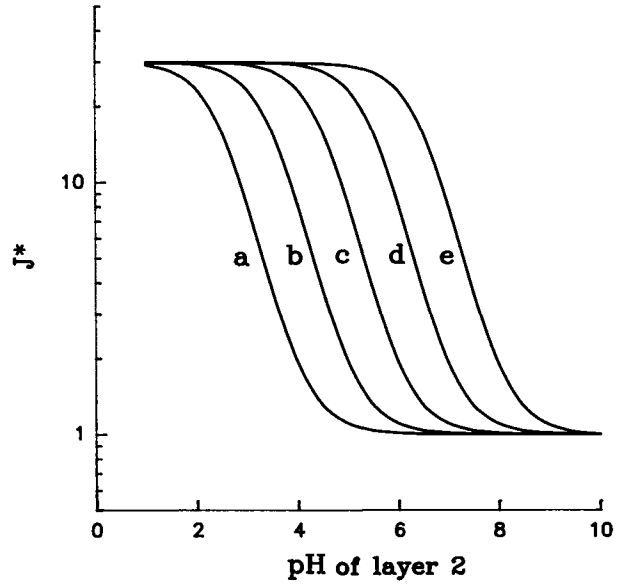

Fig. 4. Effect of pK_a on J^* of a weak base at various pH's of layer 2 at pK_a values of 4 (a), 5 (b), 6 (c), 7 (d), and 8 (e), assuming $L_1/L_2 = 0.033$ and $K(D_1/D_2) = 1$.

Also plotted in Fig. 5 are the best-fitted curves according to Eq. (14) with pK_a and $K(D_1/D_2)$ being the adjustable parameters. The L_1/L_2 is set equal to 0.033 based on a stratum corneum thickness of 10 μm and a viable epidermis/dermis thickness of 300 μm. The fitted values and associated statistics are shown in Table III. The fit for compound A appears to be excellent; however, the fit is less satisfactory for nicardipine. As shown in Table III, the pK_a values are somewhat lower than the values in water (see Table I). It is generally known that the presence of organic solvent shifts the pK_a higher for weak acids and lower for weak bases (6). Because isopropyl alcohol (IPA) was used in the donor solution and the IPA skin flux, previously determined in our laboratory to be 4.2 mg/cm²/hr (9), was quite high, the ionization equilibrium of compound A and nicardipine in the viable epidermis/dermis was expected to be modified by the presence of IPA in the aqueous stratum. The result for

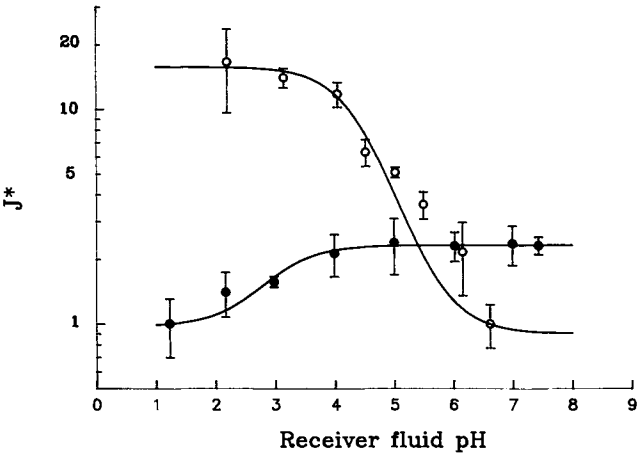

Fig. 5. The effect of receiver fluid pH on J^* of compound A (•) and nicardipine (○).

Table II. *In Vitro* Steady-State Skin Fluxes of Compound A and Nicardipine Determined at Various Receiver Fluid pH's at 32°C

Compound A		Nicardipine	
Receiver pH	Flux (μg/cm²/hr)[a]	Receiver pH	Flux (μg/cm²/hr)[a]
1.2	3.29 (± 1.00)	2.2	12.3 (± 5.20)
2.2	4.62 (± 1.09)	3.2	10.4 (± 1.10)
3.0	5.17 (± 0.30)	4.1	8.72 (± 1.17)
4.0	7.01 (± 1.57)	4.5	4.67 (± 0.67)
5.0	7.87 (± 2.31)	5.0	3.76 (± 0.21)
6.0	7.59 (± 1.19)	5.5	2.66 (± 0.39)
7.0	7.77 (± 1.63)	6.2	1.60 (± 0.60)
7.4	7.63 (± 0.723)	6.6	0.74 (± 0.17)

[a] Each value is the mean ± SD of three diffusion experiments.

nicardipine seems to be consistent with this explanation. Although the reason for the lower pK_a value observed for compound A is not apparent, a pK_a value of 3.5 in water is within the 95% confidence interval of the estimated pK_a in Table III.

The fitted value of $K(D_1/D_2)$, as shown in Table III, for nicardipine is approximately 12-fold higher than that for compound A. We suggest that this is due mainly to the difference in K rather than D_1/D_2 because the molecular sizes of these compounds are not sufficiently dissimilar to give a significantly different diffusional mobility. For example, using the method of Hayduk and Laudie, the aqueous diffusion coefficients of compound A and nicardipine are estimated to be 6.2×10^{-6} and 4.4×10^{-6} cm²/sec, respectively (7). These values are similar because of the closeness of the molecular weights. Further, as shown in Table I, the octanol/water partition coefficient of nicardipine base is approximately 10-fold larger than that of compound A. These findings suggest that K is likely to be the main factor contributing to the observed difference in $K(D_1/D_2)$. Further evidence which supports the above-mentioned hypothesis comes from the results of the skin permeation studies of nicardipine using ethanol as cosolvent in the receiver fluid. As shown in Table IV, incorporating ethanol in the receiver fluid increases the nicardipine flux by approximately 10-fold. This is presumably due to the reduction in the partition coefficient between the stratum corneum and the viable epidermis. The effect of ethanol on diffusion mobility of nicardipine in stratum corneum should be minimal since the stratum corneum was readily conditioned by 50% IPA in the donor solution.

Table III. The Parameters Fitted According to Eq. (14) and Associated Statistics[a]

Parameter	Statistic	Compound A	Nicardipine
pK_a	estimate	2.60	5.68
	(95% c.i.)[b]	(2.34,3.35)	(5.47,6.12)
$K(D_1/D_2)$	Estimate	0.0477	0.571
	(95% c.i.)[b]	(0.0431,0.0524)	(0.495,0.647)
r^2		0.997	0.988

[a] Nonlinear regression was performed using MINSQ v. 4.03 by Micromath Inc., Utah.
[b] c.i., confidence interval.

Table IV. The *in Vitro* Skin Permeation Rates of Nicardipine Showing the Effect of Alcohol as Cosolvent in the Receiver Fluid

Receiver fluid composition	Flux ($\mu g/cm^2/hr$)	SD	n^a
pH 4	10.6	0.70	3
pH 6.8	1.07	0.26	3
pH 6.8 in 50% ethanol	9.93	1.9	2

[a] Number of experiments.

This finding also shows that the conclusion drawn in Ref. 1 is erroneous. Their *in vitro* skin flux data using 50% ethanol in the receiver fluid apparently overestimated the *in vivo* transdermal potential.

Another implication from the transport analysis is that the viable epidermis can become a transdermal transport barrier for weakly ionizable compounds such as nicardipine, which has a pK_a in the neutral range and a high intrinsic partition coefficient for the nonionized species. This is an important but often overlooked aspect, since the drug molecules have to traverse both a 10- to 15-μm stratum corneum layer and a 150- to 200-μm aqueous viable layer before reaching the systemic circulation. It will be useful to have an indicator which can predict whether the epidermis will assume a significant barrier property. One can certainly determine P_1/P_2 experimentally by a stripping technique as proposed by Flynn *et al.* (7). An estimation, however, can be made. For example, one can assume that $L_1/L_2 = 0.033$ for a 300-μm-thick skin, and with estimated D_1/D_2, P_1/P_2 can be calculated at pH 7 by substituting the K and pK_a values. If P_1/P_2 is greater than 1, caution should be exercised in approaching the transdermal formulation design. In this case, the strategy should be focused on reducing the partition coefficient of the molecule rather than enhancing the permeation through the stratum corneum.

CONCLUSIONS

The theoretical analysis and experimental evidence presented have unambiguously demonstrated the effect of receiver fluid pH on *in vitro* skin flux for weakly ionizable drugs. It is thus cautioned that an undiscriminating use of nonphysiological pHs in the receiver fluid will result in an unrealistic estimation of the transdermal permeation. The results indicate that this pH dependence is more significant for compounds having high partition coefficients. Our data also caution the use of organic cosolvents in the receiver fluid for a similar reason. Because the viable epidermis can be a significant barrier to this category of compounds, the strategy for transdermal formulation should be properly targeted to reduce the barrier property of the viable epidermis rather than the stratum corneum.

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