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Solvent drag in ethanol/ethyl acetate enhanced skin permeation of *d*-norgestrel

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Summary

The relationship between the transdermal flux through hairless mouse skin of a solute (*d*-norgestrel) and two solvents (ethanol and ethyl acetate) at apparent steady state was studied using irreversible thermodynamics. To accomplish this, a model was developed based on the linear relationship between fluxes and forces related through a phenomenological coefficient. The model relates the flux of *d*-norgestrel to its uncoupled flux, the positive contributions of solvent (ethanol and ethyl acetate) flux, and the negative contribution of reverse water flux. The phenomenological coefficients relating drug and solvents flows were calculated from the permeation data through hairless mouse skin. It was found that the coefficients for solvent drag from ethanol and ethyl acetate were approximately equal; however, these coefficients were very small and hence insignificant. It was also found that the contribution of negative water flux from the receiver chamber to the donor vehicle leads to a relatively minor negative drag on the flux of *d*-norgestrel through hairless mouse skin. The implications of the model are discussed in terms of the existing knowledge about the function of skin and the inherent limitations of a phenomenological approach.

Introduction

The manner in which chemical solvents increase the permeability of biomembranes has been studied for a number of years. In this regard, the mechanism of skin penetration enhancement by certain solvents has also received considerable attention. A number of mathematical models have been developed to describe the process of percutaneous absorption (Flynn, 1989; Guy and Hadgraft, 1989). A pharmacokinetic model of Guy and Hadgraft (1982, 1985) has been expanded to include the effect of skin penetration enhancers (Guy and Hadgraft, 1987, 1988). However, the pharmacokinetic approach does not formally account for the flow of solvents, such as ethanol, through the skin when this solvent is used as a penetration enhancer. It is conceivable that, in addition to affecting the permeation properties of the skin and the partitioning characteristics of a drug between the skin and the

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formulation, the flow of solvent or solvents through the skin (in both the epidermal-dermal and the dermal-epidermal directions) could directly affect solute flux through the membrane.

Several investigators have measured solute (drug) and solvent flux through the skin in an attempt to correlate these two phenomena (Yum et al., 1987; Sato et al., 1988; Berner et al., 1989). While these analyses have provided some insight into the mechanism of skin penetration by solvents such as ethanol, none of these approaches have attempted to formally relate the contribution of solvent flow to solute flow. The relationship between solute and solvent flows, using irreversible thermodynamics, has been used to help interpret the permeability of solutes through biomembranes, e.g., gastrointestinal mucosa (Kedem and Katchalsky, 1958; Katchalsky and Curran, 1965; Friedman, 1986). However, irreversible (nonequilibrium) thermodynamics has apparently not been used in the analysis of transport of solutes through skin to help understand the effect of skin penetration enhancers. A series of equations based on thermodynamic principles are developed herein to describe the flux of a solute (*d*-norgestrel, a progestational contraceptive agent) and two solvents (ethanol and ethyl acetate) through a biomembrane (hairless mouse skin). The permeability data obtained from in vitro permeability experiments are used to calculate phenomenological coefficients. Back-diffusion of water from the dermal side of the skin (receiver phase) to the epidermal side (donor phase) is also considered.

Model Development

Ideally, a model describing solute and solvent fluxes through skin would account for the stratified nature of skin (the stratum corneum and the viable epidermis). Such a model would be relatively complex and a large number of assumptions would be required to derive useful information from it. The model derived herein is based on the grossly simplifying assumption that the skin can be regarded as a homogeneous isotropic membrane.

The model is based on a series of phenomenological equations as shown in Eqn 1 such that the there is a linear relationship between fluxes (J) and forces (X) and that these two are related through a phenomenological coefficient (L) (Kedem and Katchalsky, 1958):

$$J_N = -\sum_i L_{Ni} X_i \tag{1}$$

In this case, i represents the drug *d*-norgestrel (N), ethanol (E), ethyl acetate (A), or water (W). Eqn 1 can be expressed in terms of the diffusion coefficient, D, and the concentration gradient, dC_i/dx resulting in Eqn 2:

$$J_N = -\sum_i D_{Ni} \frac{dC_i}{dx} \tag{2}$$

Assuming linear concentration gradients within the skin results in Eqn 3:

$$J_N = \sum_i D_{Ni} \frac{K_i C_i^v}{\delta} \tag{3}$$

where K_i is the skin/vehicle partition coefficient of species i and C_i^v is the concentration of species i just outside the epidermal side of the membrane, i.e., in the vehicle; C_i on the dermal side of the membrane is assumed to be zero (sink conditions prevail). In the case of C_N^v, the saturation solubility of N in each vehicle was used; the concentration of the solvents in the donor and receptor phases were calculated from the known amounts of material used in each case.

Expanding Eqn 3 gives Eqn 4:

$$J_N = D_{NN} \frac{K_N C_N^v}{\delta} + D_{NE} \frac{K_E C_E^v}{\delta} + D_{NA} \frac{K_A C_A^v}{\delta} - D_{NW} \frac{K_W C_W^r}{\delta} \tag{4}$$

The first term on the right-hand side of Eqn 4 is the uncoupled flux of N through the skin (J_{NN}). The second and third terms are the contribution of solvent drag on the flux of N from E (J_{NE}) and A (J_{NA}), respectively. The fourth term (J_{NW}) is

the negative solvent drag from the reverse flux of water. The term for reverse water flux includes the concentration of water in the receptor phase, C_W^r. In this case, the influence of the back flux of water from the receptor into the donor is in the opposite direction and therefore given a negative value. Note we have assumed that the effect of the drug flux on solvent flux is negligible.

It is further assumed that the partition coefficient of N (K_N) between the skin and the vehicles is a linear function of E in the skin. This leads to Eqn 5 where α is the coefficient of linearity, and K_N^A the partition coefficient of N for pure A:

$$K_N = K_N^A + \alpha K_E C_E^v \tag{5}$$

Eqn 5 shows the relationship between the K_N and the addition of E to the donor vehicle. Therefore, Eqn 5 is substituted into Eqn 4 to give Eqn 6:

$$J_N = D_{NN}\frac{(K_N^A + \alpha K_E C_E^v)C_N^v}{\delta} + D_{NE}\frac{K_E C_E^v}{\delta} + D_{NA}\frac{K_A C_A^v}{\delta} - D_{NW}\frac{K_W C_W^v}{\delta} \tag{6}$$

Eqn 6 is the expression describing the flux of N through a membrane (skin) and the effect of solvent flux and the back diffusion of W. If A and E in the donor vehicle are replaced with W, Eqn 6 reduces to the common expression for the flux of a drug through a membrane (note that if pure water is placed on both sides of the membrane and the effect of dissolved drug on the donor phase is considered negligible, there is no driving force for water and hence no water flux). Additional cross terms can be considered as well. These terms would include coupling of the solvents to each other, viz., D_{AE}, D_{EA}, D_{EW}, and D_{AW}. While it is possible to obtain values for these terms, they would not necessarily provide further insight into the role of solute and solvent flows through the skin and it is expected that they will be insignificant.

To complete the model development, the partition coefficients for the solvents E and A were also calculated according to Eqn 7, which assumes there are no cross terms between E and A (an assumption that must be studied in future).

$$J_i = D_{ii}K_i C_i^v/\delta \tag{7}$$

Calculation of K_i requires a value for D_{ii}. The latter value was calculated from the lag time measurements, t_L and skin thickness, δ, according to Eqn 8 (Flynn, 1989):

$$D = \delta^2/6t_L \tag{8}$$

where t_L is the lag time estimated by extrapolation of the linear portion of the cumulative release curve to the axis where drug release = 0.

Materials and Methods

The permeability data of N, E, and A used to evaluate the model were obtained from experiments performed in a manner described previously (Catz and Friend, 1990). In these experiments, hairless mouse skin was used with an average measured thickness (δ) of 260×10^{-4} cm; this value was used as the permeation path length in the development of the model. In all cases, drug saturated vehicles were used to ensure a uniform thermodynamic activity in all the vehicles examined. N, E, and A permeating the hairless mouse skin over the first 24 h were measured in all cases by HPLC (Catz and Friend, 1990). The concentration of E and A in the vehicles, expressed as mol/cm^3, is shown in Table 1.

The flux of water from the receptor phase into the donor phase (back diffusion) was assessed

TABLE 1

Concentration (mol/cm^3) of E and A in the donor vehicles used in the permeability experiments

	E in A (volume fraction)				
	0	0.25	0.50	0.75	1.0
C_E^v	0	5.0×10^{-3}	8.5×10^{-3}	1.3×10^{-2}	1.75×10^{-2}
C_A^v	$1.0\times10_2$	7.1×10^{-3}	5.0×10^{-3}	2.9×10^{-3}	0

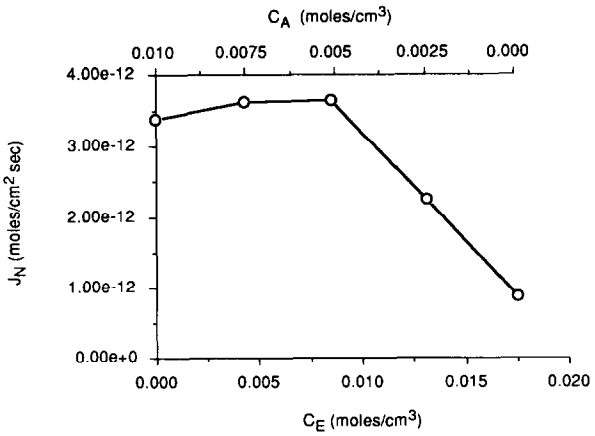

Fig. 1. J_N at apparent steady state through hairless mouse skin from the vehicles containing E and A ($n = 3$–4 for each vehicle). All vehicles were saturated with excess solid N.

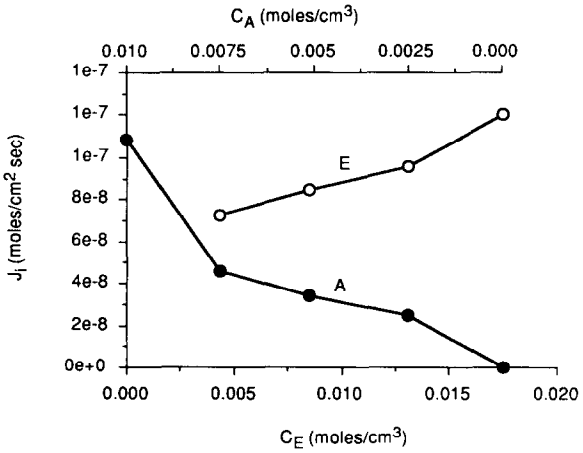

Fig. 2. J_i (i = E or A) at apparent steady state through hairless mouse skin from the vehicles containing E and A ($n = 3$–4 for each vehicle). All vehicles were saturated with excess solid N.

with ^3H_2O (Amersham, Arlington Heights, IL). The receptor solution was composed of saline and contained 0.125 μCi/ml of ^3H_2O. The receptor solution was passed under the skin and collected in a fraction collector. At various times, 100 μl of donor vehicle were withdrawn and added to a scintillation fluid (Phase Combining System, Amersham) and the radioactivity (dpm) measured on a Beckman LSC.

Results and Discussion

The J_N at apparent steady state from the various vehicles examined is shown in Fig. 1. J_N was greatest in the vehicles containing the highest concentration of A. As the amount of E in the vehicle was increased, J_N decreased. The measured values for J_A and J_E at apparent steady-state from the same vehicles are shown in Fig. 2. Both J_A and J_E were dependent on the concentration of each solvent in the vehicle: as the concentration of a solvent decreased, the flux of that solvent also decreased. J_W, measured from the dermal to the epidermal direction, is shown in Fig. 3. In this case, the reverse flux of water was greatest in the E/A solvent mixtures.

The calculated values for D_{NN} (uncoupled diffusion coefficient of N) from each the five vehicles examined are shown in Fig. 4. These values were calculated from the lag time data as indicated under Model Development. Likewise, the values for D_{ii} (uncoupled diffusion coefficients for the two solvents E and A) were calculated (see Fig. 5). Values for D_{ww}, also calculated from the lag time data for reverse water flux, are shown in Fig. 6. Values for K_E, K_A, and K_W were calculated using Eqns 7 and 8 for each of the vehicles tested. In order to calculate the partitioning coefficient and coupling constants

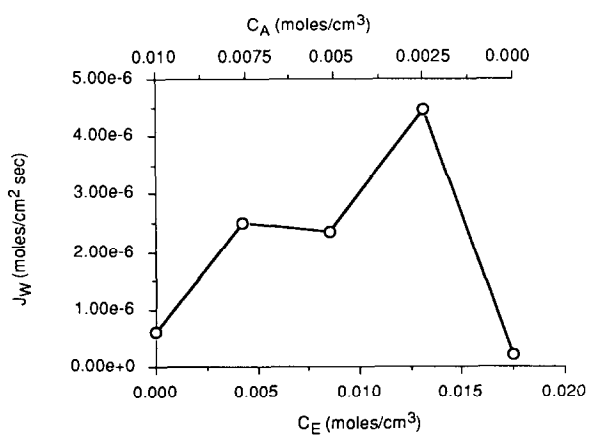

Fig. 3. J_W at apparent steady state measured from the dermal to epidermal direction using tritiated water. The vehicles were the E/A-containing donor systems, which were saturated with excess solid N ($n = 3$–4 for each of the vehicles).

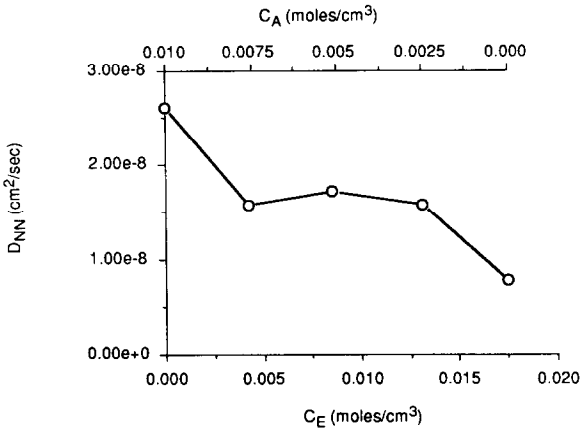

Fig. 4. D_{NN} for the diffusion of the N and solvents E and A through hairless mouse skin from the vehicles tested ($n = 3$–4 for each vehicle). All vehicles were saturated with excess solid N.

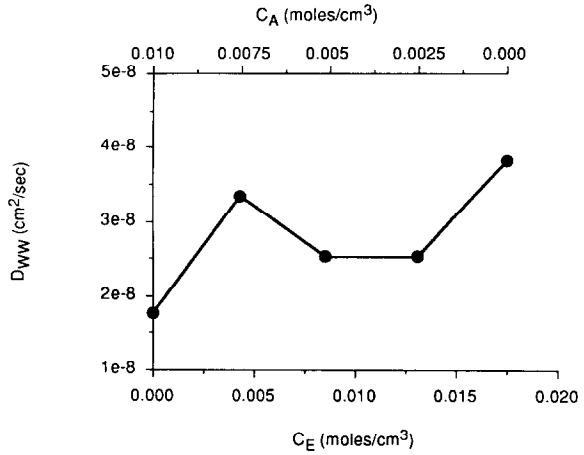

Fig. 6. D_{ww} for the diffusion of the N and solvents E and A through hairless mouse skin from the vehicles tested ($n = 3$–4 for each vehicle). All vehicles in the donor phase were saturated with excess solid N.

from each of the vehicles examined, the concentration, expressed in mol/cm^3 was used (see Fig. 7).

Using the data collected and calculated as described above, a series of simultaneous equations were solved to obtain values for the coefficients K_N^A, α, D_{NE}, D_{NA}, and D_{NW}. The values obtained in this manner were $K_N^A = 0.13$, $\alpha = -0.27$, $D_{NE} = 0.011 \times 10^{-12}$, $D_{NA} = 0.015 \times 10^{-12}$, and $D_{NW} = 0.005 \times 10^{-12}$. The implications of these coefficients with respect to the flux of N through hairless mouse skin are discussed below.

The calculated values for the partition coefficient in A (K_N^A) and α can be used to calculate the partition coefficient of N between the skin and the vehicles K_N. The values for K_N, as calculated in Eqn 5, are shown in Fig. 8. The data indicate that the addition of E to vehicle does not appreciably alter K_N. Also, K_N is essentially linear across the spectrum of vehicles tested. This finding is consistent with the assumption that partitioning of N between the skin and the vehi-

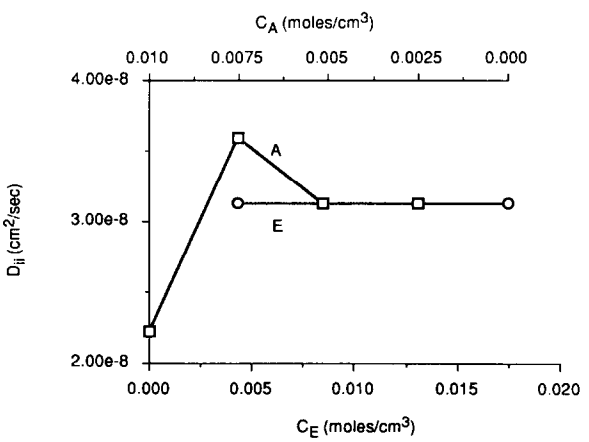

Fig. 5. D_{ii} (i = E or A) for the diffusion of the N and solvents E and A through hairless mouse skin from the vehicles tested ($n = 3$–4 for each vehicle). All vehicles were saturated with excess solid N.

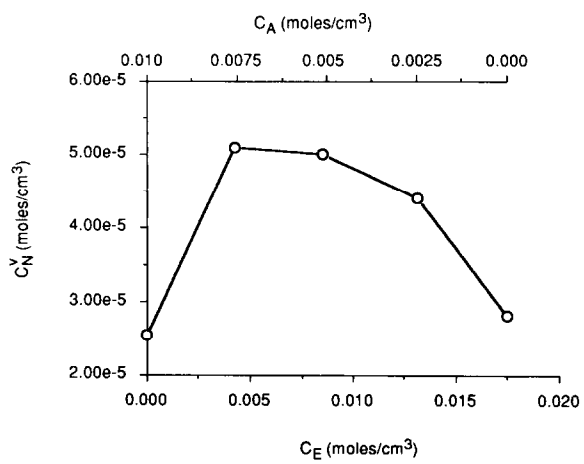

Fig. 7. Concentration (mol/cm^3) of N in the E/A vehicles.

cle is a linear function of the amount of E in the skin. Nonetheless, it should be noted that partitioning of solutes between mixed solvents can be non-ideal in nature and as such, the assumption of linearity represents at best an approximation of the partitioning phenomenon in the system described. It should also be noted that K_N is calculated for the entire thickness of the skin. Partitioning of N between the skin and the vehicle would be more accurately represented as a partitioning between the stratum corneum and the vehicle and partitioning between the viable epidermis and the stratum corneum. N is a very lipophilic compound with a $K_{octanol/water}$ of about 3.7 (Friend et al., 1988). Hence, the important partitioning step may be that between the viable epidermis and the stratum corneum. This step is buried in the value for K_N calculated from Eqn. 5.

The values for the cross terms for coupling of N with E (D_{NE}) and A (D_{NA}) were found to be about equal and relatively small ($D_{NE} = 0.011 \times 10^{-12}$ and $D_{NA} = 0.015 \times 10^{-12}$). Compared with D_{NN} the values of D_{NE} and D_{NA} were about 6 orders of magnitude smaller. The contribution of solvent drag of both E and A (J_{NE} and J_{NA}, respectively) can be calculated using the terms in Eqn 6. for each of the vehicles tested using the second and third terms in Eqn 6. These contributions are shown in Fig. 9 for each of the vehicles tested. Overall, the contribution of both J_{NE} and

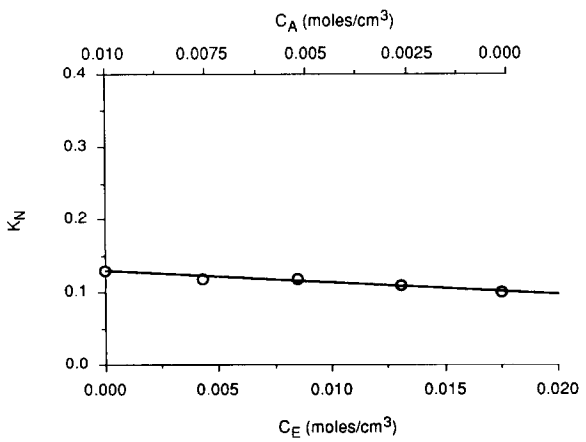

Fig. 8. Relationship between K_N and the concentration of A and E in each of the vehicles examined as calculated using Eqn 5 ($\alpha = -0.27$ and $K_N^A = 0.13$).

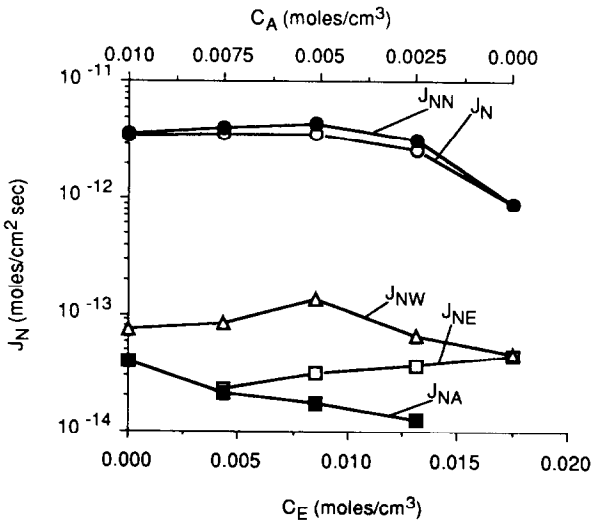

Fig. 9. The flux of N (J_N) through hairless mouse skin from the various vehicles tested along with the contribution of the coupled flow of solvents (E, A and W) with N (J_{NE}, J_{NA}, and J_{NW}). The contribution of J_{NW} is negative in relationship to J_N. The uncoupled flux of N (J_{NN}) through the skin is also shown.

J_{NA} are negligible despite the relatively large amounts of E and A permeating through the skin compared with that of N (see Figs 1 and 2). The relative contribution from permeation of both E and A appears to depend on the amount of each in the vehicles, e.g., J_{NE} decreases as the amount of E permeating through the skin decreases. In terms of solvent drag, the effects of E and A are about equal.

Fig. 9 also shows the relationship between J_N, the uncoupled flux of N (J_{NN}), and the contribution of reverse flow of W (J_{NW}) through the skin for each of the vehicles tested. While the value of D_{NW} is very small (0.005×10^{-12}), the effect, in terms of negative drag, i.e., resistance due to reverse water flux, was somewhat greater than the counterbalanced coupling of N with E and A. This was true for the vehicles containing higher amounts of A. In the higher E containing vehicles, the effect of flows through the skin were approximately counterbalanced. For instance, in the pure E vehicle (0.017 mol/cm^3 of E), $J_{NE} = J_{NW}$.

Reduced solute flow through a biomembrane resulting from movement against the bulk flow

was first demonstrated by Anderson and Ussing (1957). While the values for J_{NW} were relatively small compared with J_N, it should be recalled that the flow of E and A through the skin against the flow of water probably reduces the reverse flux of water. In the absence of the flows of E and A, it is conceivable that J_{NW} is larger than calculated herein.

It is known that the rate of drug penetration through skin is increased in many cases when the application site is occluded (Schaefer et al., 1982). Occlusion normally increases the water content of the stratum corneum leading to a disruption of the membrane's physical structure. This disruption is thought to lead to an increase in surface area and reduced diffusional resistance to the permeation of certain solutes. Another factor that should be considered is the reduction in water flux from the viable tissues across the stratum corneum, i.e., transepidermal water loss, under occlusion. Transepidermal water loss has been measured at approx. 500 mg/cm^2 per h (5×10^{-4} mol/cm^2 per s) across cadaver skin and in vivo (Wilson and Maibach, 1989). Compared with the flux of solutes across the skin from the epidermis to the dermis, the outward (dermis to epidermis) flux of water is many orders of magnitude larger. Limiting reverse water flux by occlusion may contribute to a reduction in the negative drag effect, as calculated to occur in this report, and as a result lead to an overall increase in drug delivery into and through the skin. Clearly, reduced reverse solvent drag is only one of several factors involved in alteration of drug permeation under occlusion.

The enhanced delivery of N from the E/A solvent system appears to be related primarily to the effect of these vehicles on the D_{NN}, K_N, and C_N^v. Examination of Fig. 4 (D_{NN} from the various vehicles), Fig. 7 (C_N^v in the various vehicles), and Fig. 8 (K_N in the various vehicles) suggests that the observed flux (J_N) is controlled primarily by the interplay between D_{NN} and C_N^v.

As a final note, the effect of solvent drag on transmembrane diffusion has been used to quantitate fluxes through porous membranes (Van Bruggen et al., 1982). The lack of a solvent drag effect suggests that the skin is a nonporous membrane considerably different from the gastrointestinal mucosa. Therefore, these initial studies using irreversible thermodynamics do not support the existence of pores in mammalian skin as hypothesized to be a salient feature of skin by Higuchi and co-workers (Higuchi et al., 1987; Ghanem et al., 1992). Overall, the general utility of the model developed herein must be explored further using other solutes and solvents as well as other skin types.

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References

Anderson, B. and Ussing, H.H., Solvent drag on non-electrolytes during osmotic flow through isolated toad skin and its response to antidiuretic hormone. *Acta Physiol. Scand.*, 39 (1957) 228–239.

Berner, B., Mazzenga, G.C., Otte, J.H., Steffens, R.J., Juang, R.-H. and Ebert, C.D., Ethanol:water mutually enhanced transdermal therapeutic system II: Skin permeation of ethanol and nitroglycerin. *J. Pharm. Sci.*, 78 (1989) 402–407.

Catz, P. and Friend, D.R., Effect of cosolvents on ethyl acetate enhanced percutaneous absorption. *J. Controlled Release*, 12 (1990) 171–180.

Flynn, G.L., Mechanism of percutaneous absorption from physicochemical evidence. In Bronaugh, R.L. and Maibach, H.I. (Eds), *Percutaneous Absorption: Mechanisms – Methodology – Drug Delivery*, 2nd Edn, Dekker, New York, 1989, pp. 27–51.

Friedman, M.H., *Principles and Models of Biological Transport*, Springer, Berlin, 1986, pp. 105–133.

Friend, D.R., Catz, P., Heller, J., Reid, J. and Baker, R., Transdermal delivery of levonorgestrel: II. Effect of prodrug structure on skin permeability in vitro. *J. Controlled Release*, 7 (1988) 251–261.

Ghanem, A.-H., Mahmoud, H., Higuchi, W.I., Liu, P. and Good, W.R., The effects of ethanol on the transport of lipophilic and polar permeants across hairless mouse skin: Methods/validation of a novel approach. *Int. J. Pharm.*, 78 (1992) 137–156.

Guy, R.H. and Hadgraft, J., A pharmacokinetic model for percutaneous absorption. *Int. J. Pharm.*, 11 (1982) 119–129.

Guy, R.H., Hadgraft, J. and Maibach, H., Percutaneous absorption in man: a kinetic approach. *Toxicol. Appl. Pharmacol.*, 79 (1985) 123–129.

Guy, R.H. and Hadgraft, J., The effect of penetration enhancers on kinetics of percutaneous absorption. *J. Controlled Release,* 5 (1987) 43–51.

Guy, R.H. and Hadgraft, J., Physicochemical aspects of percutaneous absorption and its enhancement. *Pharm. Res.,* 5 (1988) 753–758.

Guy, R.H. and Hadgraft, J., Mathematical models of percutaneous absorption. In Bronaugh, R.L. and Maibach, H.I. (Eds), *Percutaneous Absorption: Mechanisms – Methodology – Drug Delivery,* 2nd Edn, Dekker, New York, 1989, pp. 13–26.

Higuchi, W.I., Rohr, U.D., Burton, S.A., Liu, P., Fox, J.L., Ghanem, A.H., Mahmoud, H., Borsadia, S. and Good, W.R., Effects of ethanol on the transport of β-estradiol in hairless mouse skin. In Lee, P.I. and Good, W.R. (Eds), *Controlled Release Technology – Pharmaceutical Applications,* ACS Symposium 348, American Chemical Society, Washington, DC, 1987, pp. 232–240.

Katchalsky, A. and Curran, P.F., *Nonequilbrium Thermodynamics in Biophysics,* Harvard University Press, Cambridge, 1965, pp. 114–132.

Kedem, O. and Katchalsky, A., Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. *Biochim. Biophys. Acta,* 27 (1958) 229–246.

Sato, K., Sugibyashi, K. and Morimoto, Y., Effect and mode of action of aliphatic esters on the in vitro skin permeation of nicorandil. *Int. J. Pharm.,* 43 (1988) 31–40.

Schaefer, H., Zesch, A. and Stüttgen, G., *Skin Permeability,* Springer, Berlin, 1982, pp. 733–735.

Van Bruggen, J.T., Chalmers, B. and Muller, M., Effects of solvent and solute drag on transmembrane diffusion. *J. Gen. Physiol.,* 79 (1982) 507–528.

Wilson, D.R. and Maibach, H., Transepidermal water loss: A review. In Leveque, J.-L. (Ed), *Cutaneous Investigation in Health and Disease, Noninvasive Methods and Instrumentation,* Dekker, New York, 1989, pp. 113–133.

Yum, S.I., Lee, E.S., Taskovich, L. and Theeuwes, F., Mechanism of action of ethanol as flux enhancer for drug permeation through human skin. *Proc. Int. Symp. Controlled Release Bioact. Mater.,* 14 (1987) 103–104.