Effect of Vehicle Pretreatment on the Flux, Retention, and Diffusion of Topically Applied Penetrants *in Vitro*

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Purpose. The flux of a topically applied drug depends on the activity in the skin and the interaction between the vehicle and skin. Permeation of vehicle into the skin can alter the activity of drug and the properties of the skin barrier. The aim of this *in vitro* study was to separate and quantify these effects.

Methods. The flux of four radiolabeled permeants (water, phenol, diflunisal, and diazepam) with log $K_{oct/water}$ values from 1.4 to 4.3 was measured over 4 h through heat-separated human epidermis pretreated for 30 min with vehicles having Hildebrand solubility parameters from 7.9 to 23.4 $(cal/cm^3)^{1/2}$.

Results. Enhancement was greatest after pretreatment with the more lipophilic vehicles. A synergistic enhancement was observed using binary mixtures. The flux of diazepam was not enhanced to the same extent as the other permeants, possibly because its partitioning into the epidermis is close to optimal (log K_{oct} 2.96).

Conclusion. An analysis of the permeant remaining in the epidermis revealed that the enhancement can be the result of either increased partitioning of permeant into the epidermis or an increasing diffusivity of permeants through the epidermis.

KEY WORDS: skin penetration enhancement; partition coefficient; diffusivity; solubility parameter.

INTRODUCTION

Vehicles used in topical formulations can affect the barrier properties of human skin and hence the rate of permeation of co- or subsequently administered drugs. Identification of the mechanisms involved in vehicle enhancement of permeation has been speculative and may vary with permeant properties such as lipophilicity. Because of the heterogeneity of the skin, different enhancement mechanisms may exist (1). In general, the steady-state flux of a permeant is a function of its partitioning from vehicle into the epidermis and its diffusion along the epidermal pathway. If the pathlength is assumed constant for a given donor, then *in vitro* flux can alter only if the vehicle has affected the barrier to change partitioning and/or diffusivity.

Transport through the intercellular lipid pathway involves a solute interacting with polar and lipid components (2). The ability of vehicles to affect, selectively, the permeation of either polar or lipophilic drugs differently therefore becomes an issue along this pathway. Other very polar solutes can diffuse between lipid polar head groups (3). This work reports the enhancement of penetration of lipophilic and moderately polar compounds by a range of vehicles. We further differentiate between alterations in epidermal partitioning and membrane diffusivity to elucidate the reason for changes in overall flux for a given solute.

Four model permeants (water, diazepam, diflunisal, and phenol) were used, covering a range of lipophilicity from log K_{oct} –1.38 to 4.32. Vehicles had Hildebrand solubility parameters (δ) from 8 to 23 $(cal/cm^3)^{1/2}$. Covehicle systems of propylene glycol with decanol and oleic acid were examined to determine whether the vehicles' effects were additive.

Heat-separated epidermal membranes were pretreated for 0.5 or 2 h with the chosen vehicle, and an aqueous solution of each permeant was then applied for 4 h. Flux and drug remaining in the epidermis after the experiment were measured and used to determine the separate effects of the vehicle on the partitioning and diffusion of the permeant.

MATERIALS AND METHODS

[^{3}H]Diazepam and [^{14}C]phenol were supplied by New England Nuclear (Boston, MA), [^{3}H]water by Amersham Australia (Sydney, NSW, Australia), and [^{14}C]diflunisal was a gift from the College of Pharmacy, University of Saskatchewan, Canada. Butyl acetate, octyl acetate, octanol, and decanol were supplied by Sigma Aldrich Co. (Dorset, UK); isopropyl myristate (IPM) by Croda Oleochemicals Ltd, UK; oleic acid by Thornton and Ross, UK; butanol, propylene glycol (PG), and polyethylene glycol 400 (PEG 400) by Fisher Scientific, UK.

Minitab release 13.32 (Minitab Inc., USA) was used for data analyses.

Preparation of Isolated Human Epidermis

Human abdominal skin from white donors and obtained from cosmetic surgery was stored at –20°C. Storage of frozen skin tissue in a freezer for periods up to 3 months does not affect its barrier properties (4). Skin was thawed overnight, the adipose tissue removed by blunt dissection, and the skin immersed in water at 60°C for 1 min to separate the epidermal–dermal junction. The epidermal membrane was then peeled from the surface.

Diffusion Experiments

Experiments were conducted with a minimum of three replicates. Epidermal membranes were mounted, stratum corneum side uppermost, in horizontal Franz-type diffusion cells, surface area ~1.3 cm^2 and receptor volume ~3.5 ml. In the first set of experiments 0.5 ml of vehicle was placed in the donor compartment and left in contact with the membrane for either 30 min or 2 h. The flux of water and phenol was

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ABBREVIATIONS: *A*, diffusional area; *C*, permeant concentration; *D*, diffusion coefficient; *H*, diffusional pathlength; IPM, isopropyl myristate; *J*, steady-state flux; *K,* partition coefficient; M, membrane; PEG 400, polyethylene glycol 400; PG, propylene glycol; V, vehicle; X, mole fraction; δ, Hildebrand solubility parameter.

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Table I. Standardized Enhancement Ratios of Flux (J^*) and Partition (K^*)[a]

Vehicle	Hildebrand solubility parameter $(cal/cm^3)^{1/2}$ **Log P**	Enhancement ratios relative to control values							
		Flux J^*				Retention K^*			
		Water **−1.38**	Phenol **1.48**	Diazepam **2.96**	Diflunisal **4.32**	Water **−1.38**	Phenol **1.48**	Diazepam **2.96**	Diflunisal **4.32**
oleic acid	7.91	3.0	4.8	0.9	5.4	2.5	2.8	1.0	1.0
IPM	8.02	3.0	3.0	0.5	4.4	3.0	2.4	1.1	3.4
octyl acetate	8.67	4.1	6.5	1.0	4.3	4.4	3.7	1.3	2.2
butyl acetate	8.93	1.7	2.4	1.1	1.8	1.4	1.2	1.2	2.1
decanol	9.78	3.9	4.3	0.7	5.1	3.9	3.7	1.1	4.2
octanol	10.30	4.5	4.7	0.4	4.0	4.1	3.8	1.6	2.4
butanol	11.18	1.9	1.7	—	—	0.8	1.5	*	*
PEG 400	11.34	1.8	1.4	1.3	5.9	2.7	2.4	2.0	1.8
PG	14.00	1.4	1.7	0.6	0.6	2.1	1.7	1.7	2.5
water	23.40	1.3	1.4	—	—	1.2	1.2	*	*
PG:decanol 1:4	11.26	6.8	9.7	0.9	4.2	3.5	4.0	1.0	3.3
PG:decanol 1:1	12.74	4.9	7.2	1.5	13.5	3.1	3.4	0.9	1.3
PG:oleic acid 19:1	13.70	3.8	5.3	2.2	0.9	3.3	4.3	0.6	1.1

Note: Flux values are from regression of label (cpm) in receptor against time. Retention values are from label (cpm) in membrane at the end of the diffusion run. Both standardized with respect to donor cpm and control values. Standardized diffusion enhancments are calculated as $D^* = J^*/K^*$. Log K_{oct} values for permeants are shown in bold type beneath their names.

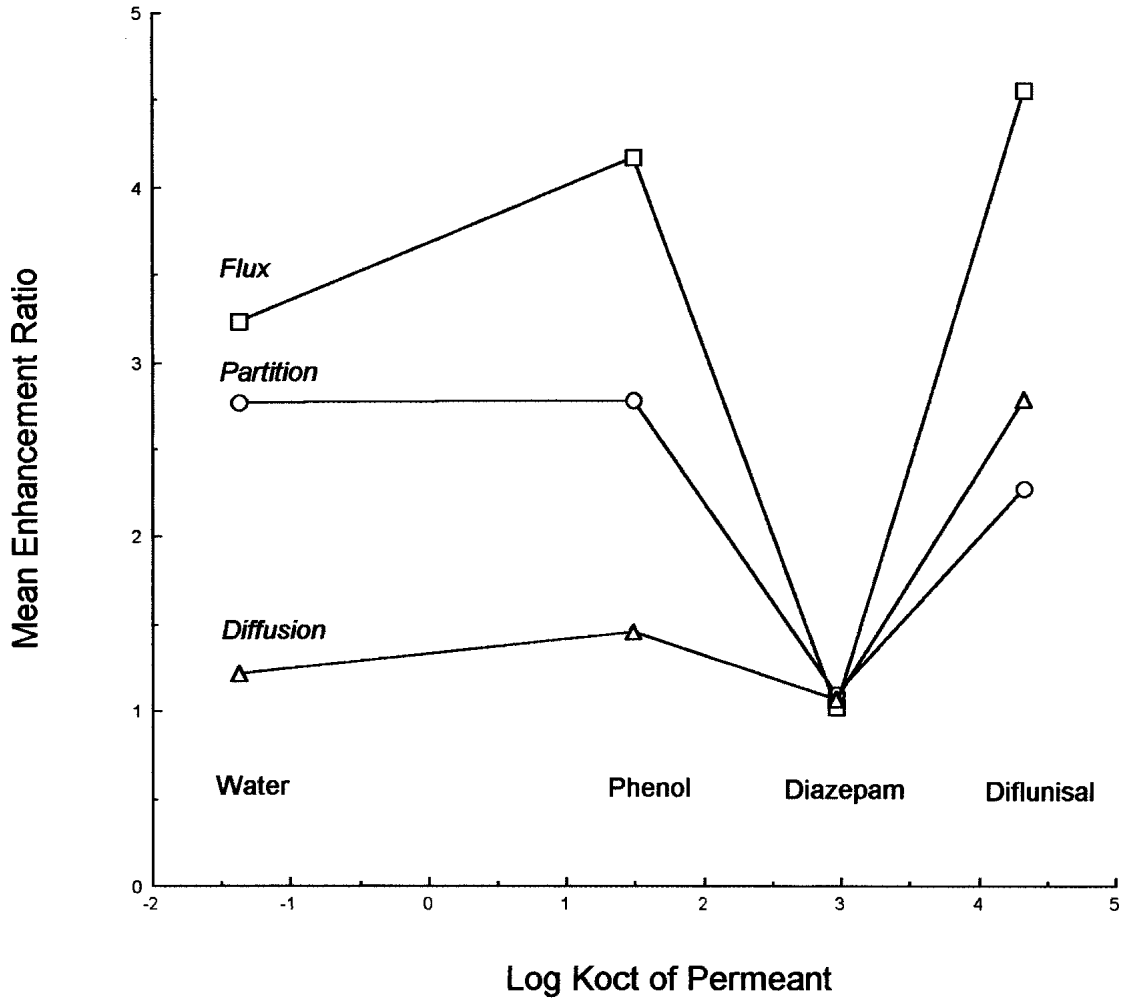

Fig. 1. Mean effects plot summarizing the effects of lipophilicity (log K_{oct}) of permeant on enhancement of flux, partition, and diffusion. Each point represents the average enhancement for all vehicle pretreatment.

measured to determine whether vehicle effects were consistent for both permeants at higher contact periods. Because the flux increase was consistent (~40%), only the 30-min pretreatment was used for diazepam and diflunisal. Before application of the permeant, the excess vehicle was removed with a Pasteur pipette, and the membrane surface washed three times with water before applying 0.7 ml of water containing standard trace amounts of radiolabeled permeant as donor solution; 400-μl samples of receptor phase (phosphate-buffered saline pH 7.4) taken at intervals over a 4-h period were analyzed by liquid scintillation counting. Drug flux across the membrane was estimated from the gradient of linear regression plots of (cumulative receptor dpm/donor dpm) against time. The fraction of applied drug remaining in the epidermis at the end of the experiment was also determined. This was achieved by removal of the donor phase, swabbing the epidermal surface, removal of remaining surface material by a single tape stripping across the surface, punching out the exposed area, and scintillation counting. Control values for flux and retention were found from identical experiments using untreated epidermis.

Calculation of Apparent Partition Coefficient and Diffusivity

Steady-state flux, J, values were based on an approximation of Fick's first law:

$$J = \frac{DAKC}{h} \tag{1}$$

where D is the diffusion coefficient within the epidermis, A the diffusional area, K the epidermis/vehicle partition coefficient, C the permeant concentration in the vehicle, and h is the diffusional path length.

Because A, C, and h are constant for a given permeant, Eq. (1) can be rearranged to appear in the form (2):

$$D = (\text{constant})\frac{J}{K} \tag{2}$$

where

K = constant_K × (dpm in membrane at end of experiment)/(dpm in donor)

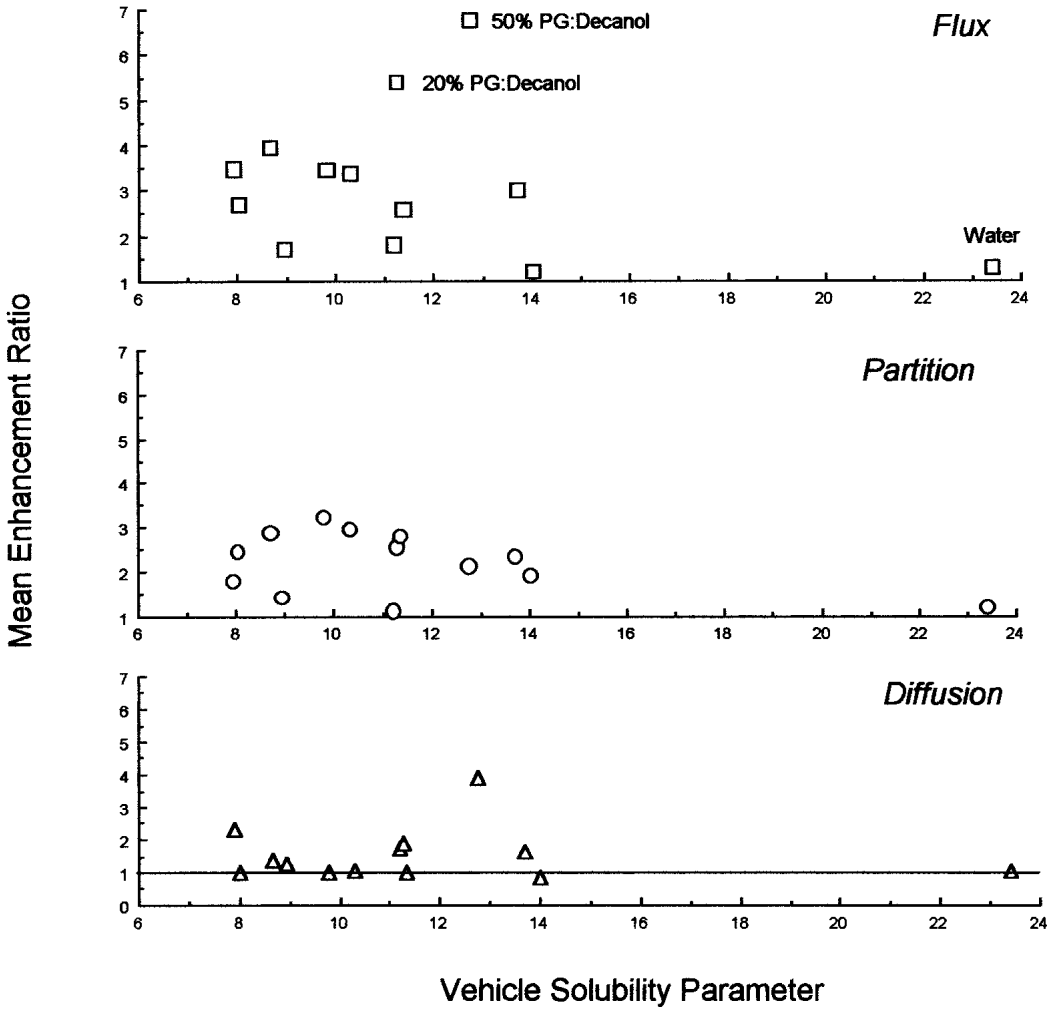

Fig. 2. Mean effects plot summarizing the effects of Hildebrand solubility parameter $(cal/cm^3)^{1/2}$, of vehicle on enhancement of flux, partition, and diffusion. Each point represents the average enhancement for the four permeants.

J = constant$_J$ × (cumulative dpm in receptor vs. time gradient)/(dpm in donor)

Incorporating the constants into J and K gives (2):

$$D_R = J_R/K_R$$

where D_R is the apparent diffusion coefficient within the epidermis.

K_R = (dpm in membrane at end of experiment)/(dpm in donor)

J_R = (cumulative dpm in receptor vs. time gradient)/(dpm in donor)

These three quantities can be further standardized by relating them to the corresponding control values, giving dimensionless quantities, J^*, K^*, and D^*.

Deviations of J^*, K^*, and D^* from unity enable both permeant–permeant and vehicle–vehicle comparisons to be made.

RESULTS AND DISCUSSION

Log K_{oct} values of the four permeants, predicted using ACD/LogP, version 1.0 software as –1.38 (water), 1.48 (phenol), 2.96 (diazepam), 4.32 (diflunisal), spanned a wide rang of lipophilicities. Solubility parameters of vehicles calculated using Fedors' group additivity method (5) are given in Table I.

Mean effects plots (Minitab software) simplify masses of data arising from several variables and are useful for identifying trends. For example, the standardized flux values, J^*, for diazepam are averaged for all the vehicles used and plotted on Fig. 1 as ~3. More detailed analyses such as ANOVA can then be applied to refine particular points of interest.

The effect of permeant lipophilicity, plotted as log K_{oct}, on flux is summarized in the mean effects plot (Fig. 1). Although there is significant enhancement of flux for water, phenol, and diflunisal, insignificant enhancement occurs for diazepam. It has been suggested (6) that log K_{oct} of 2 to 3 is optimal for transdermal drug delivery, so the partition of diazepam into the intercellular skin lipids is already high, and the vehicle pretreatment did not significantly improve it**.** Flux of the other three permeants was enhanced on average by a factor of 3 to 4, and no relationship between flux and lipophilicity could be discerned.

The effect of vehicle on flux is summarized in the mean effects plot (Fig. 2a and Table I). Enhancement is generally higher for oleic acid and the long-chain alcohols. It is note-

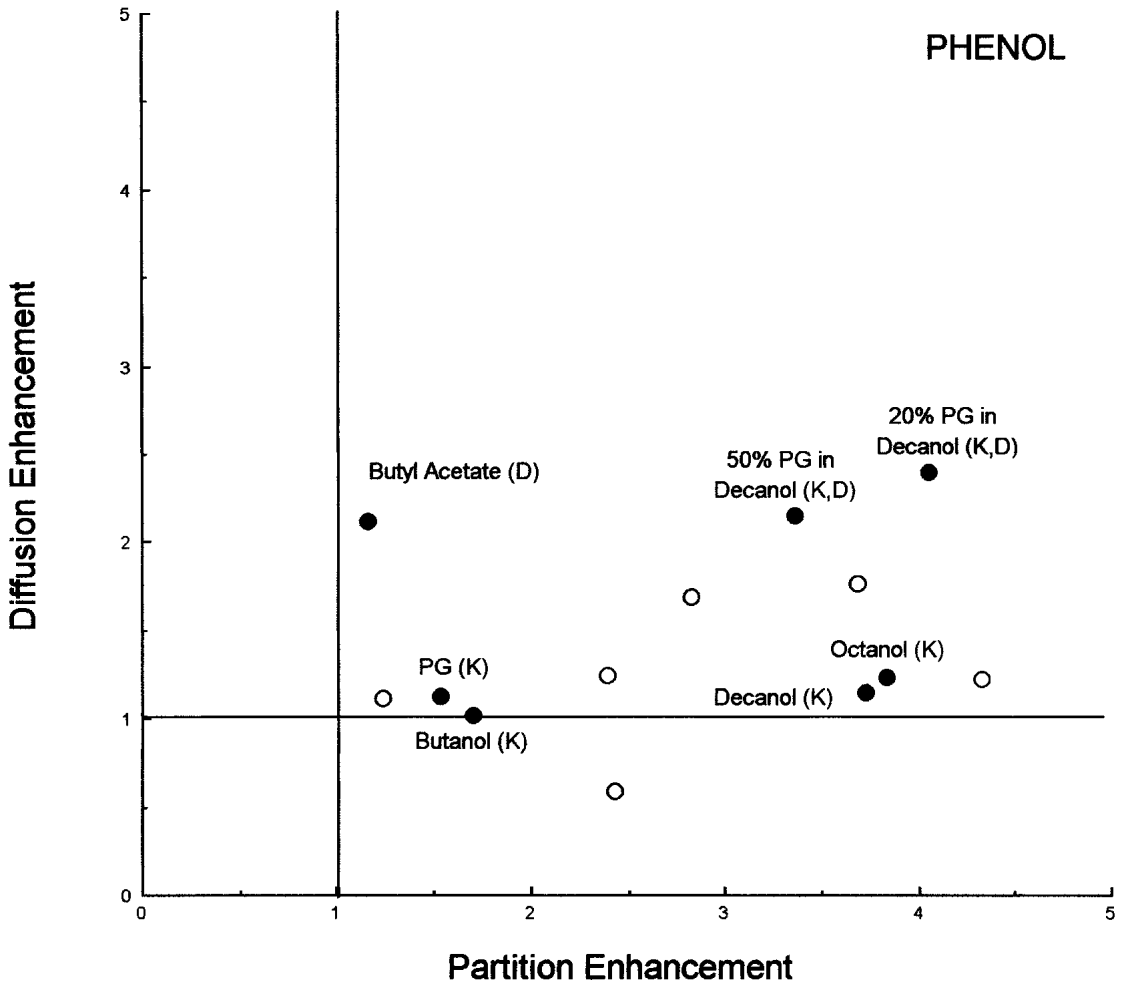

Fig. 3. Comparison of the effects of vehicle pretreatment on diffusion and partition of phenol. Dotted lines indicate no change from control in diffusion (horizontal) and partition (vertical). Points that differ at $p < 0.05$ are filled. Bracketed terms show whether the significance applies to diffusion and/or partition.

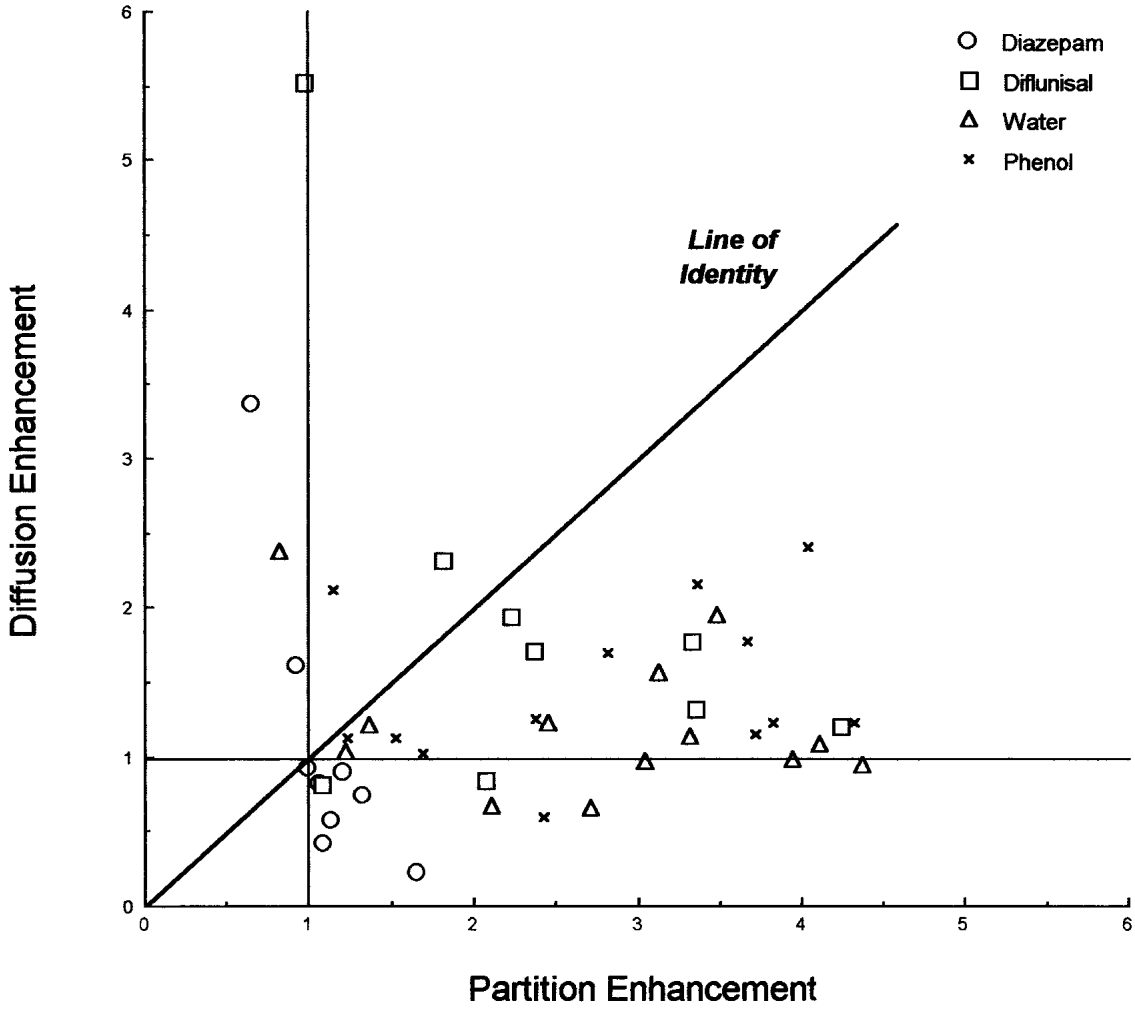

Fig. 4. Composite of the effects of vehicle pretreatment on diffusion and partition of water, phenol, diazepam, and diflunisal. The scatter of points around the oblique line of identity suggests that the primary enhancement mechanism is an increase in partition into the epidermis.

worthy that Liron and Cohen (7) considered the stratum corneum to have a δ value of about 10 $(cal/cm^3)^{1/2}$, implying that vehicles with a value close to this should mix freely with the stratum corneum lipids and have maximal enhancement properties. This is not seen in the present study, where no peak is discernible in this region. It is likely that the δ value of 10 for SC is an oversimplification. It must be remembered that the stratum corneum barrier is a bipolar, anisotropic structure with outer polar groups essentially COOH with δ values of about 10 [acetic acid = 10.4 (8)] and a lipid core that is essentially hydrocarbon with δ about 7 [mineral oil = 7.1 (8)]. It may be anticipated that there will be graded values between the two extreme regions. It seems likely, then, that any vehicle in the range 7–11 will penetrate the barrier effectively. This hypothesis is in accordance with the similarity in enhancement values for the more lipophilic vehicles. PG, which is commonly used in formulations on the basis of a supposed enhancement effect, showed no enhancement when used alone. It might be relevant that Cross *et al.* (2) noted a similar nonspecificity in the effect of vehicle on silicone membranes, where the effect on D depended simply on the volume increase in membrane cause by absorbed vehicle.

Having established that flux (J^*) was increased by vehicle pretreatment, we proceeded to separate it into its partition and diffusion components.

Vehicles can potentially influence permeation through the epidermal barrier by altering the partition coefficient between the vehicle and the membrane (K^*) and/or permeant diffusion (D^*) within the membrane. The first is a thermodynamic effect, whereas the second is a kinetic effect that might be related to structural effects such as changes in intermolecular binding to lipids or lipid loss from the stratum corneum.

When vehicle has no effect on the epidermis, the flux of a given permeant appears to be independent of vehicle for saturated solutions; i.e., a constant chemical potential exists. This has been observed experimentally (9,10) for many vehicles, but others are known to enhance flux and are therefore themselves either entering the epidermis to alter partitioning and diffusion or removing epidermal components.

The results were analyzed with an approach that enables the amount of solute in the epidermis and the flux to be used to separate and quantify the effect of vehicle on partitioning and diffusion (2).

The apparent partition coefficient, K^*, was determined experimentally as described, so that the apparent diffusivity,

D^*, could be found indirectly from J^*/K^*. The main effects plots for K^* and D^* are in Fig. 2b,c.

Generally the effects of vehicle on K^* seem to be larger than those on D^*, but no trend with respect to δ of the vehicle can be seen. Water, as might be expected, had no effect on any of the three factors J^*, K^*, and D^*.

A representative plot of D^* against K^* for phenol is shown in Fig. 3. The dotted lines indicated no enhancement, so that the relative enhancements in D^* and K^* can be easily seen. One-way ANOVA (Dunnett *post hoc* comparison with control) was used to estimate the significance of the enhancements. For example, the diffusion of phenol is enhanced ($p < 0.05$) by butyl acetate, but no effect is seen on its partition into the epidermis. The partition and diffusion properties are both enhanced ($p < 0.05$) by 50% PG in decanol. Figure 4 is a composite plot for all four permeants. The oblique line is the line of identity, so it is immediately apparent that in most cases the effect of vehicle on partition is greater than that on diffusion, suggesting that the enhancement mechanism involves a greater increase in the thermodynamic (partition) than in the kinetic (diffusion) term.

CONCLUSIONS

There was no enhancement of diazepam flux following vehicle pretreatment of human heat separated epidermis. This might be because its log K_{oct} value (2.96) is already close to optimal. Vehicle treatment increased the flux of water, phenol, and diflunisal by factors up to about 5. Water had no effect. Enhancement could not be related to the Hildebrand solubility parameter of the vehicle. Standardization of the permeant flux and epidermal retention data enabled a simple, novel plotting procedure that clearly separated and quantified the effects of vehicle on permeant partition and diffusion. These plots indicate that generally, enhancement is mainly related to an increase in partition into the skin with an increase in diffusivity playing a minor role.

Evidence in the literature generally considers that vehicles act by promoting partition into the SC. For example, Alberti *et al.* (11) studied terbinafine (TBF) in formulations containing isopropyl myristate and ethanol. The concentration profile of TBF in the SC was fitted to Fick's second law to find the drug's SC/vehicle partition coefficient (K) and characteristic diffusion parameter (D/L^2). D/L^2 was essentially constant, but K was significantly ($p < 0.05$) higher from formulations containing ethanol. However, there was no comparison of results with those from an "inert" vehicle such as water. Curve-fitting techniques are associated with large errors, and their failure to show differences in D/L^2 at $p < 0.05$ could be caused by the high coefficients of variation in their results. Bach and Lippold (12) state that the diffusion coefficient in lipids (10^{-8} cm^2s^{-1} for a lamellar gel phase containing cholesterol, ceramides, and fatty acids) can never exceed that in water (~10^{-5} cm^2s^{-1}), but changes in diffusion should be measurable for some vehicles. The present study shows that it is possible to use simple *in vitro* experimental data to produce estimates of diffusivity changes, and although the majority of vehicles do appear to influence partitioning more than diffusion, significant changes in diffusivity can be clearly seen in some cases.

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