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**Research Papers**

# The prediction of percutaneous absorption: I. Influence of the dermis on in vitro permeation models

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## Summary

In percutaneous penetration in vitro techniques, excised full-thickness skin with its stratum corneum, viable epidermis and dermis is often used. Since penetrants can be absorbed in vivo immediately below the viable epidermis, the dermal layer could act as an additional barrier in the in vitro experiments relative to the actual in vivo process. In the present paper, in vitro penetration studies through excised hairless rat skin devoid of its dermal layer are reported and compared with those previously carried out with the excised full-thickness skin of the same animal. A homologous series of 4-alkylanilines was used in all the studies, and the correlations found between permeability coefficients and *n*-octanol partition coefficients were analyzed. Correlations are bilinear in nature in both cases, but in the absence of the dermal layer the correlation line seems to tend to hyperbolicity, as assessed by a significant increment in permeability coefficients for the highly lipophilic compounds of the series and by a displacement of the optimal lipophilicity value (vertex of the correlation line) to a higher partition coefficient. It can be concluded that the heterogeneous nature of the skin, as far as absorption is concerned, may be due to the presence of the two anatomical hydrophilic layers, dermis and viable epidermis, rather than to the stratum corneum itself. A critical review of the results reported in the literature showed good agreement with these conclusions. The biophysical penetration model was identical and the optimal lipophilicity values very similar, so it may be that these features are independent of the type of epidermis used (rat, mouse or man) and also of the chemical composition of the penetrants.

## Introduction

Excised skin from common laboratory animals is often used in in vitro permeation experiments and the results then extrapolated to man. This research has been widely used to estimate the skin permeabilities of drugs, to explain and predict the in vivo absorption of chemicals, and to identify the ideal candidate from a chemically related series for transdermal delivery. The transdermal permeation of a chemical involves partitioning into and transport through the cutaneous layers, i.e., the stratum corneum, the viable epidermis and the upper dermis (Nabil El Tayar et al., 1991).

The basic strategy for understanding the penetration mechanisms of drugs through the differ-

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ent layers of the skin is based on establishing the correlations between permeability coefficients ($K_p$) of penetrants through the skin and partition coefficients ($P$) or other structural parameters for homologous series of xenobiotics. Several in vitro kinetic models for studying percutaneous penetration have been proposed. In some cases, linear relationships between log $K_p$ and log $P$ have been found (Scheuplein and Blank, 1973), but it is well known that linearity breaks down when the compounds reach high enough partition coefficients. In these cases, probabilistic dependencies were obtained from the human epidermis (Roberts et al., 1977) and other biological membranes (Díez-Sales et al., 1991a). For these membranes, parabolic or bilinear correlations may be attributed to their heterogeneous nature. This type of correlation may be envisaged as being provided by a series of alternative barriers, different in nature. The precise cause of this probabilistic behaviour has not yet been established. It would be very interesting to demonstrate whether this type of correlation could be attributed to the presence of dermis or if this behaviour is only a consequence of the epidermal layer itself, acting like a heterogeneous system.

In order to verify this, an experimental in vitro penetration study through the epidermis of the hairless rat was developed using a homologous series of anilines as test compounds. The permeability values were compared with in vitro data obtained with full-thickness skin of the same animal (Díez-Sales et al., 1991a) and the effect of the removal of the dermis on the diffusion model was tested.

On the other hand, the kinetic models established between permeability coefficients ($K_p$) obtained in this work and the octanol-water partition coefficients were compared with other kinetic models established on the basis of penetration values for phenolic (Roberts et al., 1977) and alkanol compounds (Durrheim et al., 1980) through epidermal membranes. Our purpose was to determine, if possible, the optimal lipophilicity value in order to identify the ideal candidate for transdermal delivery from a chemically related series.

## Materials and Methods

*Xenobiotics*

Seven 4-alkyl anilines showing a perfect homology, from aniline to 4-*n*-hexylaniline, were used in the experiments. They are weakly basic compounds ($pK_a$ values ranging from 4.4 to 4.9), and should be considered highly lipophilic in nature, even at the working pH of 6.2 in the donor compartment (about 95–99% nonionized). The compounds were dissolved in a physiological solution buffered to pH 6.2 at a concentration of 0.2 mg/ml, except 4-*n*-butylaniline, 4-*n*-pentylaniline and 4-*n*-hexylaniline, which were prepared at 0.1, 0.03 and 0.0135 mg/ml, respectively, in order to avoid solubility problems during the in vitro experiments.

*Structural and lipophilicity indexes*

The number of carbons atoms in the alkyl 4-chain was used as an error-free structural index. In addition, the epidermis membrane-water partition coefficients of the anilines were also assessed. These values were obtained by equilibrating the aniline solutions buffered to pH 6.2 with a known mass of skin, 200–400 mg, weighed immediately after its removal from the animal. These epidermal sheets were immersed in 10 ml of saline solution containing the aniline. The samples were equilibrated in a shaker bath at 37°C overnight, which was sufficient to attain equilibrium. Distribution of the anilines between the aqueous phase and the tissue was estimated by the difference between the initial aqueous phase concentration and the aqueous phase concentration at equilibrium (Durrheim et al., 1980). The relationship used was:

$$P = \frac{(C_0 - C_e)/W_t}{C_0/V_a} \quad (1)$$

where $C_0$ is the initial amine solution concentration, $C_e$ the solution concentration at equilibrium, $W_t$ the tissue weight in grams and $V_a$ the solution phase volume (10 ml).

On the other hand, partition coefficients in *n*-octanol-water systems were chosen in order to

compare the permeability values obtained in this work with other results taken from the literature (Scheuplein and Blank, 1973; Roberts et al., 1977).

*In vitro diffusion procedure*

The study was performed in female hairless rats weighing 50–60 g. Each piece of full-thickness skin excised was immersed in water at 60°C for 70 s and the epidermis was carefully peeled away with the aid of a forceps (Scott et al., 1986).

The structural integrity of skin samples was assessed using hystological techniques. Epidermal membranes from microtomed sections were fixed in 5% formaldehyde and stained with thiazine buffered solution, for light microscopic observation, to confirm that the epidermal membranes were intact, without any dermal component.

All kinetic studies were performed with epidermal membranes. A six-cell battery system was used, as previously reported (Díez-Sales et al., 1991b). The membranes were placed in the diffusion cells with the desired section centered on the cell opening. The area available for diffusion was 3.14 $cm^2$, the receiver compartment capacity was 39 ml, and temperature was maintained at 37($\pm$0.5)°C by means of a surrounding jacket. The receptor cell was then completely filled with saline solution buffered to pH 7.4. Receptor solution was in close contact with the membrane sheet during the whole experiment and continuously and homogeneously stirred (150 rpm) by a rotating teflon-coated magnet placed inside the cell.

Then, at zero time, a 15 ml volume of the amine test solution, pH 6.2, was introduced into the donor cell. Samples of 1 ml were taken from the receptor compartment every 30 min. The volume withdrawn was always replaced with the same volume of the receptor solution. In order to achieve steady-state permeation and to determine permeability coefficients, donor cell content was entirely replaced with fresh test solution every 15 min for all of the compounds tested.

The amine concentration in samples was analyzed by HPLC, which provided an excellent separation and quantification technique. A Perkin-Elmer Model, Binary LC Pump 250, a Rheodyne P/N 7125-047 model injector, a Perkin-Elmer, LC 90 Bio Spectrophotometric UV detector set at 254 nm and a Model Perkin-Elmer, LCI-100 Laboratory Computing Integrator were utilized. Analytical Spherisorb S5 ODS2 columns (150/46 mm) with 5 mm Guardpack RCSS-C18 precolumns were employed.

Mixtures of methanol and phosphate-buffered solution (pH 6.2) in variable proportions, depending of the lipophilicities on the tested solutes, were used at a flow rate of 1 ml/min, at room temperature.

A calibration line was employed with each determination. Excellent linearity between peak area and concentration was observed for every compound over the entire range of concentrations assayed.

The linear steady-state expresion (Eqn 2) was used to fit experimental data (Scheuplein, 1967).

$$Q(t) = A \cdot P \cdot h \cdot C \cdot \left[ D \cdot \frac{t}{h^2} - \frac{1}{6} \right] \qquad (2)$$

where $Q(t)$ is the quantity which passes through the membrane and reaches the receptor solution at a given time ($t$), $A$ represents the actual surface diffusion area (3.14 $cm^2$ in our particular case), $P$ the partition coefficient of the permeant between membrane and donor vehicle, $h$ the membrane thickness, $D$ the diffusion coefficient of the permeant across the membrane, and $C$ the actual concentration of the permeant in the donor solution.

The products $P \cdot h$ and $D/h^2$ were replaced, in Eqn 1, by $P_1$ and $P_2$, respectively, and calculated through fitting theoretical equation to individual in vitro permeation data using a computerized nonlinear least-squares method, PCNONLIN. Then, permeability coefficients, $K_p$ ($= P_1 \cdot P_2$) were calculated and used as representative permeation parameters.

*Fitting of models to data*

Correlations between permeability coefficients ($K_p$) and lipophilicity indexes for homologous series of xenobiotics were used as the most suitable source of information about the passive permeation mechanisms and the role of hydrophilic-

lipophilic alternate barriers in penetration. Since there is a perfect homology in the tested series, at first, $K_p$ values and 4-alkyl chain lengths, $N$, were correlated through the three classical model equations: hyperbolic (as representative of the compartmental approaches), parabolic and bilinear as representative of the probabilistic approaches). This was merely a theoretical approach. In order to place the experiences within a practical context, in a second phase, $K_p$ values were correlated with partition constants (*n*-octanol and the epidermis/water partitioning) which were used as lipophilicity values to replace the structural index ($N$). The fitting operations were developed in an IBM-PC computer, with the aid of the nonlinear least-squares regression program, PCNONLIN.

In the first place, the hyperbolic equation was used as representative of the general compartmental approach.

$$K_p = \frac{K_m \cdot P_0^a}{B + P_0^a} \tag{3}$$

In the second place bilinear and parabolic models – as representatives of the probabilistic approaches – were employed:

Bilinear

$$K_p = \frac{B \cdot P_0^a}{1 + B' \cdot P_0^{a'}} \tag{4}$$

Parabolic

$$\log K_p = a \cdot \log(P_0)^2 + b \cdot \log(P_0) + c \tag{5}$$

In these equations, $a$, $b$ and $B$ ($= 10^{-b}$), $a$, $c$ and $B$ are constants which can be experimentally calculated, depending on the technique, and $K_m$ is the limiting asymptotic $K_p$ value for the series.

The statistical criteria for assessing the quality of the fits were the Akaike information criterion, AIC (Akaike, 1976), and the correlation coefficient found between experimentally observed and model-predicted $K_p$ values.

## Results and Discussion

As shown in Fig. 1, in percutaneous in vitro penetration studies, the drug has to permeate several skin layers different in nature. A partition process ($K_1$) is first established for the drug between the donor solution and the stratum corneum, followed by diffusion through the lat-

TABLE 1

*Permeability coefficients ($k_p$) found through the epidermis and full-thickness hairless rat skin and the partition coefficients in n-octanol and epidermis hairless rat for tested compounds*

| Tested amines | Epidermis partition coefficient ($cm^3/g$) | *n*-Octanol partition coefficient [a] (pH 6.2) | Permeability coefficients ($K_p$) ($\times 10^3$) ($cm\ h^{-1}$) | |
|---|---|---|---|---|
| | | | Epidermis | Full-thickness [c] |
| Aniline | 5.33 ± 0.38 | 9.56 ± 0.27 | 66.63 ± 1.29 | 43.61 ± 1.85 |
| 4-Methylaniline | 4.12 ± 0.14 | 22.49 ± 0.18 | 85.49 ± 3.94 | 66.44 ± 2.11 |
| 4-Ethylaniline | 9.19 ± 0.48 | 86.52 ± 6.20 | 115.17 ± 10.69 | 102.19 ± 6.34 |
| 4-*n*-Propylaniline | 19.82 ± 2.25 | 248.68 ± 5.71 | 169.25 ± 5.49 | 166.97 + 4.43 |
| 4-*n*-Butylaniline | 48.07 ± 3.05 | 852.52 ± 53.86 | 230.89 ± 8.96 | 252.74 + 7.38 |
| 4-*n*-Pentylaniline | 107.12 ± 8.91 | 2525.96 [b] | 247.93 ± 9.54 | 100.18 + 1.49 |
| 4-*n*-Hexylaniline | 197.07 ± 13.65 | 7885.69 [b] | 229.53 ± 8.28 | 46.03 ± 4.17 |

[a] From Martín-Villodre et al. (1986).
[b] Theoretical value.
[c] From Díez-Sales et al. (1991a).

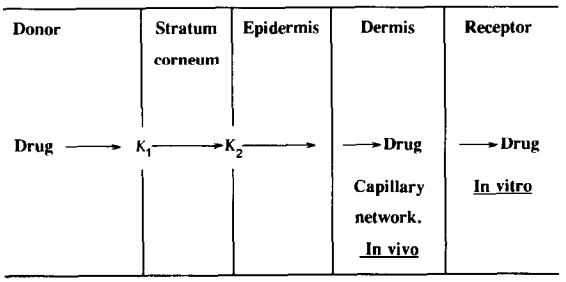


Fig. 1. A schematic diagram of the different physicochemical processes involved in drug absorption through the skin.

ter. When the drug reaches the interface between the stratum corneum and the living epidermis, a second partition process is established ($K_2$), followed by diffusion from the epidermis to the dermis, which must also be passed through by the drug in order for it to reach the receptor compartment.

The drugs may be absorbed in vivo at the capillary level, immediately below the living epidermis, in which case they do not necessarily have to go through the dermis in order to reach the bloodstream. As a consequence, the presence of the dermal layer in the in vitro experiments might act as an additional barrier, mainly for low hydrophilicity compounds, relative to their in vivo absorption process.

In order to quantify the importance of the presence of the dermis in the in vitro penetration models, the permeability coefficients of the selected alkylanilines were determined through hairless rat epidermis, and compared with the previously determined permeability coefficients through the full-thickness skin of the same animal (Díez-Sales et al., 1991a), as shown in Table 1. It can be seen that, with the exception of 4-*n*-butylaniline, the permeation values found were higher for epidermis; there are, however, considerable quantitative differences between the compounds, which seem to be related to lipophilicity. For example, highly lipophilic elements show much higher increments, so the diffusion of such compounds through the dermal layer can be considered the limiting step for penetration. For the hydrophilic compounds, the influence of the dermis is greatly reduced.

The data for the partitioning of the amines ($n = 5$) tested at 37°C between epidermis hairless rat and saline solution are shown in Table 1. The

TABLE 2

*Equation parameters describing the correlations established between permeability coefficients ($k_p$, $cm \cdot h^{-1}$), obtained through epidermal membranes of hairless rat, and lipophilicity for the amines tested*

| Model equations | Equation parameters (values ± SD) | | | |
|---|---|---|---|---|
| | Symbol | $P_{n\text{-octanol}}$ | $P_{\text{epidermis}}$ | $N$ |
| Parabolic | $a$ | $-0.096 \pm 0.006$ | $-0.289 \pm 0.011$ | $-0.024 \pm 0.009$ |
| | $b$ | $0.613 \pm 0.021$ | $1.167 \pm 0.019$ | $0.259 \pm 0.018$ |
| | $c$ | $-1.859 \pm 0.061$ | $-1.787 \pm 0.032$ | $-1.308 \pm 0.078$ |
| | AIC | −43.99 | −45.59 | −43.56 |
| | $r$ | 0.992 | 0.991 | 0.988 |
| Bilinear | $B$ | $0.030 \pm 0.006$ | $0.007 \pm 0.001$ | $0.060 \pm 0.006$ |
| | $a$ | $0.322 \pm 0.025$ | $0.500 \pm 0.045$ | $0.161 \pm 0.002$ |
| | $B$ | $(0.442 \pm 0.030) \cdot 10^{-3}$ | $0.007 \pm 0.001$ | $0.003 \pm 0.005$ |
| | $a$ | $0.898 \pm 0.088$ | $1.332 \pm 0.098$ | $0.444 \pm 0.098$ |
| | AIC | −52.28 | −55.58 | −51.67 |
| | $r$ | 0.998 | 0.997 | 0.998 |
| Hyperbolic | $k_m$ | $0.263 \pm 0.040$ | $0.252 \pm 0.053$ | $0.263 \pm 0.025$ |
| | $a$ | $0.613 \pm 0.032$ | $1.198 \pm 0.012$ | $0.307 \pm 0.027$ |
| | $b$ | $14.881 \pm 0.101$ | $15.672 \pm 0.120$ | $4.114 \pm 0.071$ |
| | AIC | −40.12 | −41.26 | −39.78 |
| | $r$ | 0.991 | 0.983 | 0.975 |

relationship obtained between these values of partition coefficients, $P$, and alkyl chain lengths, $N$, are linear and semilogarithmic ($r > 0.98$). The $\pi$ value ($= 0.295 \pm 0.003$) is consistent with those reported in the specialized literature (Yalkowsky and Flynn, 1973). The correlation coefficient is better without aniline ($r > 0.99$) and this behaviour has been attributed to the so-called first-element effect (Bermejo et al., 1991).

Correlations between epidermal permeability coefficients and the number, $N$, of methylene groups in the 4-aliphatic chain were clearly bilinear (Table 2), i.e., probabilistic in nature, as were those found for the complete skin permeability coefficients. The absence of dermis produces, however, a dramatic increase in the $K_p$ values for lipophilic compounds; a consequence of this effect is that the vertex of the curve appears at 5 carbon atoms instead of 4 as occurs for the values found in the presence of dermis, as shown in Fig. 2. In other words, the nature of the correlation does not change but its features are completely different, thus indicating that the heterogeneous system constituted by the full-thickness skin tends to become almost homogeneous as the dermis is removed. The presence of the living epidermis in the epidermal layer experiments could prevent the existence of a clearly defined homogeneous barrier system. Therefore, the correlation found remains probabilistic in nature and not hyperbolic.

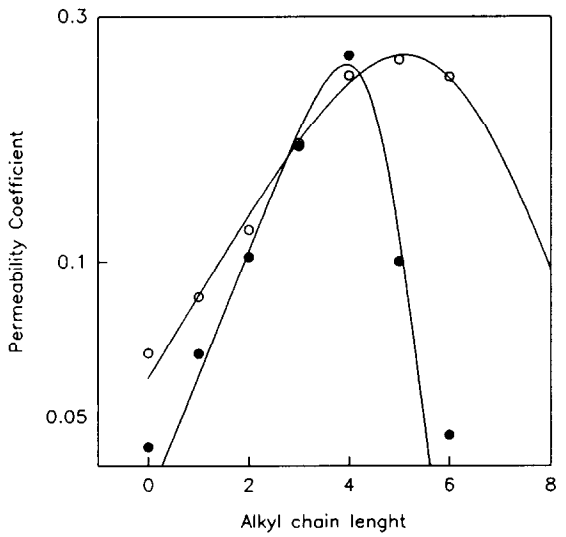


Fig. 2. Plots of permeability coefficients, $k_p$, found for the tested amines through the epidermis (○) and full-thickness hairless rat skin (●), and structural constants, $N$, according to the best fitting model equation (Bilinear).

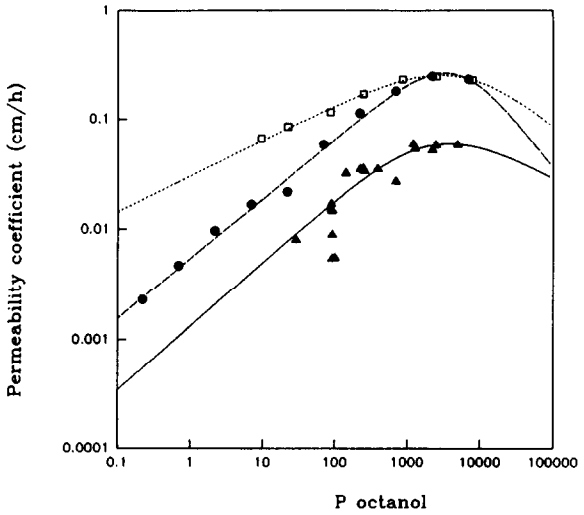


Fig. 3. Plots of permeability coefficients, $K_p$, for the amines (□), alkanols (●) and phenolic compounds (▲) obtained through the epidermis membranes, according to bilinear model.

When $n$-octanol partition coefficients are used for correlation instead of $N$, the same results are obtained, as shown in Table 2 and in Fig. 3 (open squares). The partition coefficients in $n$-octanol are illustrative for comparative purposes. Similar results were obtained by using partition coefficients determined using epidermis as the lipoidal phase, as demonstrated by the statistical figures found for correlation (Table 2).

From these results, it becomes clear that the existence of anatomical hydrophilic layers such as the dermis and, to a lesser extent, the living epidermis, confers on the skin the character of a heterogeneous barrier system which it actually exhibits in vivo.

Similar conclusions can be deduced from some results reported in the literature. Flynn et al. (1981), with hairless mouse skin, found an optimal theoretical lipophilicity value for alkanols which corresponds to $n$-octanol in full-thickness skin and to $n$-nonanol in epidermis alone. On the other hand, the work of Durrheim et al. (1980) on

alkanols with hairless mouse skin with and without dermis and that of Roberts et al. (1977) on phenols with human epidermis, based on correlations between *n*-octanol partition coefficients and permeability coefficients, clearly show that the bilinear model is better in all cases. Fig. 3 gives the plots of the correlations found in our work for alkylanilines, alkanols and phenols on epidermis. Note that the optimal lipophilicity values are 2900, 3200 and 3400, respectively. In terms of log *P* (*n*-octanol), they would be 3.46, 3.51 and 3.53, respectively. In light of these results, one can conclude that the model which defines the in vitro penetration through the epidermis is bilinear with very similar optimal lipophilicity values; these behaviors would be, for practical purposes, independent of the nature of the skin used and that of the penetrants. If these features were further confirmed, they would be of great interest in the design of better drugs intended for percutaneous administration.

As far as the in vivo results reported in the literature are concerned, there is a good concordance with the kinetic permeation model, which is probabilistic (parabolic or bilinear). Optimal lipophilicity values, however, do not coincide since for nicotinic acid esters (Hadgraft, 1991) the optimal *P* value, as deduced from the correlations found with in vivo erithema production was about 20, and for nonsteroidal anti-inflammatory agents (Yano et al., 1986), a value of approx. 316 was obtained in function of the amounts of drug remaining on the skin surface after a 4 h contact period. A possible explanation for these discrepancies is that the values could not necessarily reflect the percutaneous absorption rate of the substances or that they have been obtained from a finite-dose technique, contrary to what occurs in the in vitro experiments.

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