ELSEVIER

International Journal of Pharmaceutics 131 (1996) 117–129

international journal of pharmaceutics

A lamellar matrix model for stratum corneum intercellular lipids. II. Effect of geometry of the stratum corneum on permeation of model drugs 5-fluorouracil and oestradiol

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Received 26 May 1995; accepted 12 July 1995

Abstract

The principal barrier to transdermal delivery of most drugs is the lamellar intercellular lipid domain of the stratum corneum (SC). The low permeability of SC in comparison to other lipid barriers is in part due to its geometry. Here, effect of geometry of the SC on permeation of 5-fluorouracil (5-FU) — a model hydrophilic drug — and oestradiol (OE) — a model lipophilic drug — was investigated using a lamellar model matrix for SC intercellular lipids. Release studies at 32°C showed that the diffusion coefficients of 5-FU and OE in the matrix are approximately 6000–7000- and 60–260-times greater, respectively, than apparent human epidermis values, in good agreement with theoretical considerations. Release studies from 13 to 44°C revealed that 5-FU has a maximum diffusion coefficient around the main transition temperature of the matrix (35°C) in agreement with other lamellar systems reported in the literature. The diffusional activation energy of 5-FU in the matrix was 27.8 kcal mol^{-1} which correlates well with human epidermal data. Release experiments were then combined with permeation studies and the permeability of model drugs through the SC at 32°C was predicted from matrix data. The predicted permeability coefficients of 5-FU ($5.5–18 \times 10^{-5}$ cm h^{-1}) and OE ($0.07–0.24 \times 10^{-3}$ cm h^{-1}) were in agreement with human epidermis data. Our results show the effect of the SC morphology on the percutaneous absorption of drugs and illustrate that 5-FU and OE permeate the SC through intercellular lipids.

Keywords: 5-Fluorouracil; Oestradiol; Model human stratum corneum lipid; Diffusion coefficient; Permeability coefficient; Activation energy

1. Introduction

Before any topically applied drug can act either locally or systemically, it must permeate the barrier of the skin, which is usually located at its outermost layer, the stratum corneum (Berenson and Burch, 1951).

The stratum corneum (SC) is a multilayered wall-like structure in which keratin-rich corneocytes are embedded in an intercellular lipid-rich matrix (Fig. 1). In this two compartment

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SSDI 0378-5173(95)04307-V

system the only continuous phase is the intercellular domain in which the lipids arrange into bilayers (Michaels et al., 1975; Elias et al., 1979).

Fig. 1 illustrates the intercellular and transcellular routes of drug permeation through the intact SC. Because the intercellular space of the SC was originally assumed to comprise a tiny portion of its overall volume, this space traditionally had been discounted as a possible pathway. Freeze-fracture studies showed, however, that the intercellular volume may be a factor of 3–7-times greater than was previously appreciated and now it is believed to be between 5 and 30% of the total tissue volume (Elias and Friend, 1975; Elias, 1981). Tracer techniques demonstrated that this extracellular lipid material constitutes the morphological equivalent of the permeability barrier for most molecules (Elias and Friend, 1975). Albery and Hadgraft (1979) studied the percutaneous absorption of methyl nicotinate and showed that this molecule penetrates human skin through the intercellular and not via a transcellular pathway. These observations suggest that the intercellular lipid matrix might be the major rate-determining pathway by which many substances traverse the stratum corneum. One of the most direct pieces of evidence was found by Boddé et al. (1991) who visualised transport of Hg^{2+} mainly taking place through the intercellular bilayers with no significant contribution from transcellular transport.

It has been argued that as far as the barrier property of the SC intercellular lipids is concerned, the presence of a mesomorphic structure plays the major role in barrier performance and the differences in barrier property with specific lipid change may be negligible (Friberg and Kayali, 1989; Kayali et al., 1991). Additionally, the low permeability of SC in comparison to other lipid barriers is not only due to continuous bilayers and their compositions, but in part arises from its geometry, i.e., porosity and tortuosity (e.g., see Michaels et al., 1975; Albery and Hadgraft, 1979; Potts and Francoeur, 1991; Lange-Lieckfeldt and Lee, 1992; Lieckfeldt and Lee, 1995).

In the present work, an important feature is our complementary study of both lipophilic and hydrophilic drugs. Thus, the effects of geometry of the SC on the permeation of 5-fluorouracil (5-FU) — a model hydrophilic drug — and oestradiol (OE) — a model lipophilic drug — through human SC were investigated using a simple model (denoted matrix here) for stratum corneum intercellular lipids. The model matrix comprised 20% cholesterol, 25% water and 55% fatty acids and their soaps (w/w), and showed a lamellar structure at room temperature and 32°C (SC temperature) as determined by X-ray and polarised light microscopy studies (Moghimi et al., 1996). For more details about the structure of the model matrix, its thermal behaviour and comparison with the intercellular lipids of human and murine SC see our companion paper (Moghimi et al., 1995).

1.1. Theory

Chemicals penetrate the stratum corneum through a solution-diffusion process. The mathematical expressions for lag-time and steady-state flux of diffusion through an isotropic membrane are reviewed by Barry (1983) such that:

$$L = h^2/6D \quad (1)$$

$$J' = K_m DCA/h \quad (2)$$

in which L is the lag-time, h, the membrane thickness, D, the diffusion coefficient of the diffusant in the membrane, J', the flux (the mass of diffusant which permeates the whole membrane per unit time), K_m, the drug's membrane/vehicle partition coefficient, C, the dissolved concentration of the penetrant in the vehicle and A is the surface area of the membrane. Usually the flux is reported as the mass of diffusant which permeates unit area of the membrane per unit time (J), but here we separate the surface area from the flux to show the effect of porosity on the calculated diffusion parameters through the stratum corneum and define this flux as J'. However, we also use the flux in our permeability studies as usual (J).

Fick's law usually holds well for permeation across the skin whether the penetrant is a gas, an ion, or a non-electrolyte (Scheuplein and Blank, 1971). For the determination of diffusion coefficients of drugs through the SC, this membrane

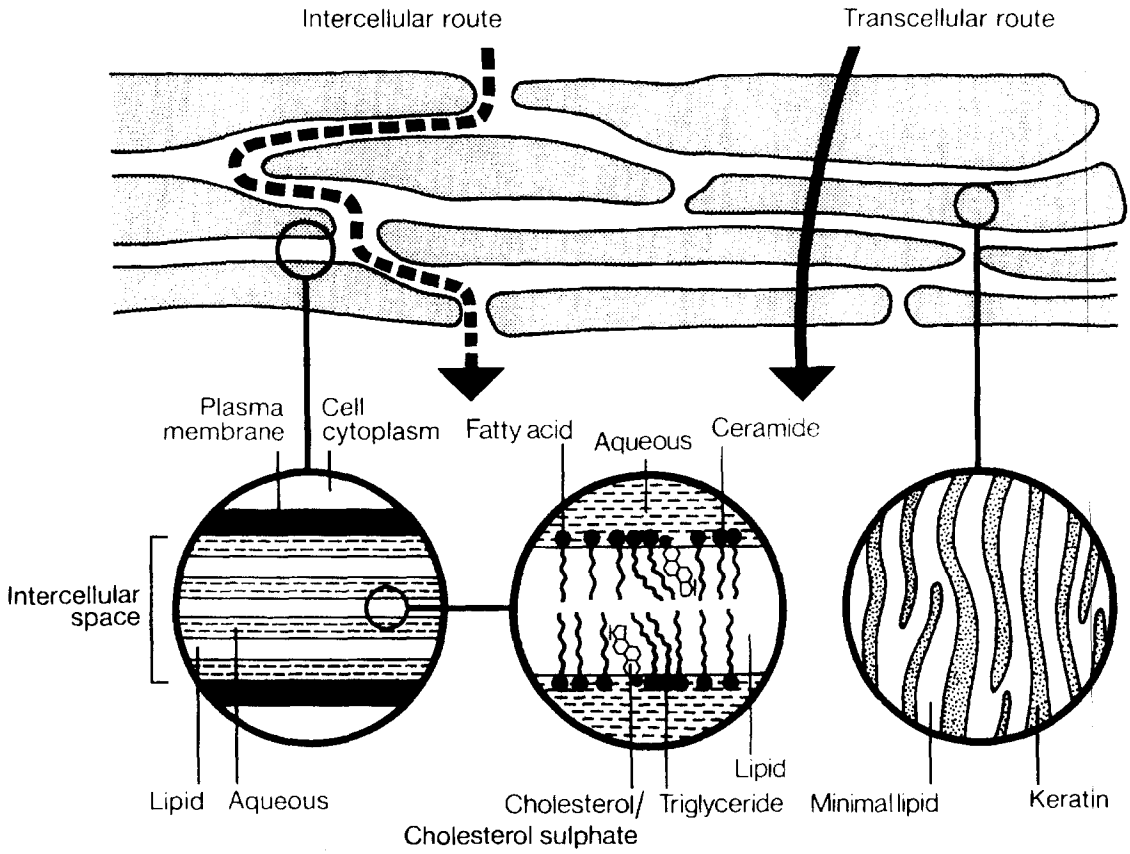

Fig. 1. Brick and mortar model of the stratum corneum, illustrating possible pathways of drug permeation through intact stratum corneum (transcellular and tortuous intercellular pathways), and the lamellar structure of intercellular lipids (after Michaels et al., 1975; Elias et al., 1979).

is normally considered isotropic (a gross simplification) and Eqs. (1) and (2) may be used. Lag-time and flux are measured practically from in vitro permeation data, the drug's stratum corneum/vehicle partition coefficient (K_{sc}) is typically determined by equilibrating a piece of the SC with the donor solution and analysing the concentration of the drug in both phases using the whole volume of the SC for calculation of the concentration of drug in the SC, h is set to be the SC thickness and A is the whole surface area of the SC used in the diffusion cell. As shown below, this approach fails to give correct values for true diffusion coefficients of drugs permeating the SC through intercellular lipids.

If we accept that for most drugs the intercellular pathway is the major rate-determining route for permeation, such molecules will however traverse a tortuous pathway (Fig. 1). Therefore, if diffusion coefficients are to be estimated, the SC cannot be treated mathematically as a simple isotropic barrier and its geometry must be taken into account.

There are two primary geometrical properties of importance related to the SC intercellular route; (i) the intercellular pathway is of greater length than the simple thickness of the SC, and (ii) the effective diffusional area, the cross-sectional area of the intercellular matrix normal to the flux, is smaller than the total cross-sectional area of the SC (Fig. 1) (Michaels et al., 1975; Lange-Lieckfeldt and Lee, 1992). A third geometrical property which should be considered and which is of great importance in the calculation of

partition coefficient but is usually neglected, is the volume fraction of intercellular lipids in the SC, which is less than one (Raykar et al., 1988).

In the absence of such geometric consideration, the calculated diffusion coefficients from lag-time, Eq. (1), or from flux, Eq. (2), are actually apparent diffusion coefficients (D_{app}) which we can specify as $D_{app(L)}$ and $D_{app(J)}$, respectively. Therefore Eqs. (1) and (2) become Eqs. (3) and (4) for calculation of the diffusion coefficient of drugs through the stratum corneum:

$$D_{app(L)} = h^2/6L \tag{3}$$

$$D_{app(J)} = J'h/K_{sc}CA \tag{4}$$

However if we take into account the geometry of the SC and assume that the intercellular channels are uniform in cross-section, but tortuous, for drugs that permeate the SC only through intercellular lipids, Eqs. (3) and (4) will change to Eqs. (5) and (6):

$$D_{lip} = (\tau h)^2/6L \tag{5}$$

$$D_{lip} = J'\tau h/K_{lip}CA\epsilon \tag{6}$$

where D_{lip} is the penetrant diffusion coefficient in the intercellular lipids (the true diffusion coefficient), τ, the tortuosity factor and τh the effective path length for transport via tortuous lipid channels, K_{lip}, the drug's intercellular lipids/vehicle partition coefficient, and ϵ, the porosity factor, the fraction of the total area normal to the flow direction which is lipid.

As we can see from Eqs. (3) and (5) for the lag-time method, the only geometrical property which directly affects the diffusion coefficient is the tortuosity factor, by increasing the diffusional pathlength. However, if we use Eq. (4) to calculate the apparent diffusion coefficient from flux data, $D_{app(J)}$, the porosity and, as explained later, the lipid volume fraction (f_v, fraction of stratum corneum volume which is lipid) will also indirectly affect the calculated apparent diffusion coefficient. This means that the value of the apparent diffusion coefficient calculated from lag-time will be different from that calculated from flux data.

Here we define a diffusivity ratio, the ratio of true to apparent diffusion coefficient (D_{lip}/D_{app}), to represent the error in calculation of true diffusion coefficient of drug in intercellular lipids. As shown below, diffusivity ratios depend on the physicochemical properties of the penetrant and the method used to determine the diffusion coefficient.

If we use lag-time to calculate the apparent diffusion coefficient, $D_{app(L)}$, the diffusivity ratio can be derived from Eqs. (3) and (5) as:

$$D_{lip}/D_{app} = D_{lip}/D_{app(L)} = \tau^2 \tag{7}$$

If we use the steady-state flux to calculate the apparent diffusion coefficient, $D_{app(J)}$, Eqs. (4) and (6) give a diffusivity ratio of:

$$D_{lip}/D_{app} = D_{lip}/D_{app(J)} = \tau K_{sc}/\epsilon K_{lip} \tag{8}$$

As mentioned above, K_{sc} is normally measured using whole SC. The protein domain in the SC is much more polar than the lipid domain and depending on the lipophilicity of the penetrant, its uptake and therefore its partition coefficient may be governed by either the protein or the lipid domain or both of them (Raykar et al., 1988). Therefore there are three different situations: (i) $K_{sc} > K_{lip}$, which happens when the drug partitions mainly into the corneocytes; (ii) $K_{sc} = K_{lip}$, which is the situation when drug partitions equally into corneocytes and intercellular lipids; and (iii) $K_{sc} < K_{lip}$, where uptake by the intercellular lipids is greater than by corneocytes. According to these situations, the D_{lip}/D_{app} ratios in Eq. (8) will be:

$K_{sc} > K_{lip}$:

$$D_{lip}/D_{app} = D_{lip}/D_{app(J)} > \tau/\epsilon \tag{9}$$

$K_{sc} = K_{lip}$:

$$D_{lip}/D_{app} = D_{lip}/D_{app(J)} = \tau/\epsilon \tag{10}$$

$K_{sc} < K_{lip}$:

$$D_{lip}/D_{app} = D_{lip}/D_{app(J)} < \tau/\epsilon \tag{11}$$

Eq. (10), which defines the situation where $K_{sc} = K_{lip}$, is also mentioned by other investigators (Lange-Lieckfeldt and Lee, 1992) and recently has been used to calculate the value of τ/ϵ through permeation of tiamenidine from a buffer donor solution across the buffer-filled intercellular channels of lipid-free stratum corneum (Lieckfeldt and Lee, 1995).

In the special case where the drug is taken up only by the intercellular lipids and instead of intercellular lipids volume ($f_v V$) the whole SC volume (V) is used in the calculation of partition coefficient, K_{sc}/K_{lip} will be equal to f_v. On the other hand if we assume that the intercellular channels of the stratum corneum are uniform in cross-section, we can consider these channels as narrow tubes with total surface area of $A\epsilon$ and length of τh. Thus the volume of intercellular lipids will be $A\epsilon\tau h$. Since the total volume of the stratum corneum (V) is equal to Ah, the volume fraction of lipids in the stratum corneum (f_v) will be $A\epsilon\tau h/Ah$ or $\tau\epsilon$. If in Eq. (8) use $\tau\epsilon$ instead of K_{sc}/K_{lip}, the diffusivity ratio then will be:

$$D_{lip}/D_{app} = D_{lip}/D_{app(J)} = \tau^2 \quad (12)$$

The diffusivity ratios calculated from lag-time, Eq. (7), and special case calculation from flux, Eq. (12), both equal τ^2. This shows that for very lipophilic drugs which partition essentially into the intercellular lipids, the lag-time and flux methods should give the same diffusivity ratios and thus apparent diffusion coefficients.

Another parameter of permeation of molecules across a membrane which is normally measured and reported is the permeability coefficient (K_p) which is:

$$K_p = J/C = J'/CA = K_m D/h \quad (13)$$

Combination of Eqs. (6) and (13) gives Eq. (14) from which the permeability coefficients of drugs in the SC ($K_{p(sc)}$) can be calculated from the partition coefficient of drugs between intercellular lipids of the SC and donor, their diffusion coefficient in the stratum corneum intercellular lipids and geometrical parameters of the SC:

$$K_{p(sc)} = K_{lip} D_{lip} \epsilon/\tau h \quad (14)$$

In this project, to study the importance of intercellular pathway and SC geometry in permeation of drugs, the diffusion and permeability coefficients of 5-FU and OE through a model matrix for the intercellular lipids of the SC were measured and results were then related to the SC in vitro data using the above-mentioned equations and the geometrical dimensions of the SC.

2. Materials and methods

2.1. Materials

All materials were used as received. Tritium-labelled 5-fluorouracil (5-[6-^{3}H]-FU) and oestradiol ([2,4,6,7-^{3}H(N)]-OE), both with radiochemical purity of 99%, were supplied by NEN (Dupont) Research Products (Dreiech, Germany). Unlabelled 5-FU (99%) was supplied by Aldrich Chemical Company (Dorset, England) and unlabelled OE (99.6%) by Sigma Chemical Company (Dorset, England). PTFE filters (pore size 200 nm, thickness 60 μm and 80% porosity) were purchased from Sartorius (Germany). All other solvents and reagents were of analytical grade.

2.2. Preparation of drug-loaded matrices

The preparative method of the model matrix is explained in the companion paper (Moghimi et al., 1996). Radiolabelled model drugs, 5-FU and OE, were added as solutions in methanol during the preparation of the lipid mixture (before hydration) to give final concentrations of 0.1 and 1% for 5-FU and 0.1% for OE (all w/w) in the model matrix.

2.3. Release and permeation experiments

Release and permeation experiments employed an automated diffusion system equipped with 24 stainless-steel diffusion cells with flow-through receptor compartments as described by Akhter et al. (1984). The cells provided a diffusional area of 0.126 cm^2 and were mounted on four copper arms of the diffusion apparatus through which temperature controlled water was circulated to maintain a desired temperature on the surface of the membrane. HCl solution (pH 1) was used as the receptor fluid to minimise the extraction of the matrix lipids (Lange-Lieckfeldt and Lee, 1992). The receptor solution was pumped through the cells at 2 ml h^{-1} in order to achieve sink conditions for both 5-FU and OE.

2.3.1. Release experiments

Release of drugs from matrices containing 0.1 and 1% 5-FU and 0.1% OE (w/w) was studied at 32°C (SC temperature). To investigate the effect of temperature on the barrier property of the model matrix, release of 5-FU from matrices containing 0.1% drug was studied at 13, 20, 25, 32, 38 and 44°C. In each experiment, a PTFE filter was placed between the donor and receptor chambers of each diffusion cell to hold the matrix. Prior to use, the PTFE filters were wetted with methanol and stored in distilled water overnight. Matrix samples containing radiolabelled 5-FU or OE were then placed in the donor chambers of the diffusion cells (diameter and height of 4 mm) and covered with glass beads and parafilm. Receptor solution was then collected over 1–1.5-h periods for at least 16 h.

2.3.2. Permeation experiments

For permeation experiments, matrix samples were placed in a circular hole (4 mm in diameter) of a PTFE matrix spacer and covered from both sides with PTFE filters to provide membranes of approximately 1.35 mm in thickness. The PTFE filters were wetted with methanol and stored in distilled water overnight prior to use. The matrix membranes were then placed between the receptor and donor chambers of the diffusion cells and 150 μl donor solutions were dispensed into each of the cell donor compartments. Receptor solution was then collected over 1–2-h intervals for a minimum of 30 h. Drug donor solutions were saturated aqueous solutions of radiolabelled 5-FU and OE at 32°C. Aqueous solubility of 5-FU and OE at 32°C are reported to be 14.3 mg ml^{-1} and 3.6 μg ml^{-1}, respectively (Cornwell, 1993). Since the solubility of OE in water is very low, donor depletion was expected. To prevent such change, OE donor solutions were replenished every 8 h.

The activity of the released or permeated drugs was determined as counts per minute using a TRI-CARB Liquid Scintillation Analyser (model 1600 TR, Packard, USA) and was then converted to amount of drug using a standard.

3. Results and discussion

3.1. Release studies

Release of 5-FU from matrices containing 0.1 and 1% (w/w) drug were studied at 32°C. Fig. 2a and b shows sample release profiles of 5-FU from matrices containing 0.1 and 1% drug, respectively. The curves do not start from the origin but show a short lag-phase. This lag-phase arises from the drug initially partitioning into and diffusing through the receptor-filled pores of the PTFE filter and the stagnant hydrodynamic layer adjacent to the membrane. The linearity of plots after the initial lag-phase was taken to indicate the establishment of the matrix-release process. The slopes of the linear part of these graphs provide

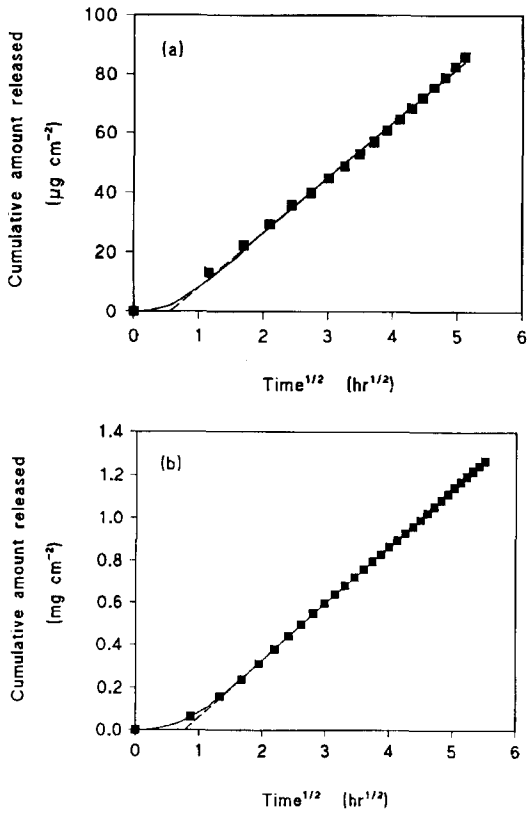

Fig. 2. Sample profiles of 5-fluorouracil release from the model matrix containing (a) 0.1% and (b) 1% (w/w) diffusant at 32°C.

Table 1
Release parameters of 5-fluorouracil from the model matrix containing 0.1 and 1% (w/w) drug at 32°C (data are mean ± SD, n = 11–13)

Concentration (% w/w)	Release rate (μg cm^{-2} h$^{-1/2}$)	Lag-phase (h$^{1/2}$)	Diffusion coefficient (cm^2 h^{-1} × 10^4)
0.1	23.4 ± 5.0	0.33 ± 0.20	5.97 ± 1.75
1.0	246 ± 27	1.1 ± 0.2	5.31 ± 1.26

the release rate. Using the density of the model matrix (0.95 g cm^{-3}), the w/w concentration of 5-FU in the matrix was changed to w/v and the diffusion coefficients of 5-FU through matrices (D_{mx}) were calculated from release rates and drug concentration in the matrix using Higuchi's equation (Higuchi, 1962):

$$Q = 2C_o(D_{mx}t/\pi)^{1/2} \quad (15)$$

where Q is the cumulative amount of drug released per unit area of the diffusion cell, C_o, initial concentration of drug in the matrix and t is the time.

Table 1 illustrates release rates, lag-phases and diffusion coefficients of 5-FU releasing from matrices containing 0.1 and 1% drug. The diffusion coefficients of 5-FU through matrices (D_{mx}) were calculated to be 5.97 ± 1.75 × 10^{-4} cm^2 h^{-1} (mean ± S.D., n = 11) for matrices containing 0.1% and 5.31 ± 1.26 × 10^{-4} cm^2 h^{-1} (mean ± S.D., n = 13) for matrices with 1% drug. There was no significant difference between diffusion coefficients of 5-FU in matrices containing 0.1 and 1% drug as analysed by t-test (P = 0.05). All subsequent experiments were performed using 5-FU concentration of 0.1%.

The apparent diffusion coefficient of 5-FU through human epidermis at 32°C as calculated from flux data on the assumption of an isotropic structure for the SC ($D_{app(J)}$) is reported to be 0.95 × 10^{-7} cm^2 h^{-1} (Cornwell and Barry, 1994) and 0.81 × 10^{-7} cm^2 h^{-1} (Yamane, 1994). From these results and diffusion coefficient of 5-FU through the model matrix, the diffusivity ratio ($D_{mx}/D_{app(J)}$) was calculated to be approximately 6–7 × 10^3 (Table 2).

The stratum corneum intercellular geometrical dimensions are summarised in Table 3. Stratum corneum thickness (h), the effective pathlength for transport via tortuous lipid channels (τh) and porosity factor (ϵ) were abstracted from literature (Michaels et al., 1975; Albery and Hadgraft, 1979; Potts and Guy, 1993) and from these data, τ (the tortuosity factor) was calculated to be 23–33, τ^2, 500–1100, τ/ϵ, 1400–4800, and volume fraction of SC intercellular lipids ($f_v = \tau\epsilon$) to be 0.16–0.53 (Table 3). A value of almost 900 for τ/ϵ is also measured from permeation of tiamenidine through buffer-filled intercellular channels of lipid-free stratum corneum (Lieckfeldt and Lee, 1995).

The diffusivity ratio ($D_{mx}/D_{app(J)}$) of 5-FU (6–7 × 10^3) is greater than τ/ϵ (0.9–4.8 × 10^3) and correlates well with Eq. (9) in which a $D_{lip}/D_{app(J)}$ of greater than τ/ϵ is predicted for some drugs. This is reasonable, because the assumption underlying Eq. (9) favours the partitioning of drug into corneocytes. 5-FU is a hydrophilic drug and should thus have more affinity for corneocytes than for intercellular lipids. Good correlation of the diffusivity ratio ($D_{mx}/D_{app(J)}$) with theoretical expectation provides evidence indicating that 5-FU permeates the SC through the intercellular route. Such a difference (7–29 × 10^3) between the diffusion coefficient of drug in model matrices and human SC is also reported for tiamenidine (Lange-Lieckfeldt and Lee, 1992) and correlates well with the present results.

We should bear in mind that the domain with the higher uptake does not necessarily correlate with the rate-controlling route for permeation. It was shown that corneocytes and intercellular lipids will take up Hg^{2+} while corneocytes do not participate in the ion's permeation control (Boddé et al., 1991).

Release of 5-FU from the model matrix containing 0.1% drug was studied at 13, 20, 25, 32, 38 and 44°C and diffusion coefficients were calcu-

Table 2
Diffusion coefficients of 5-fluorouracil and oestradiol in the model matrix at 32°C and comparison with human stratum corneum (SC) data

Diffusant	Concentration (% w/w)	Diffusion coefficient ($cm^2\ h^{-1}$)		Diffusivity ratio (matrix/SC)
		In matrix[a] ($\times 10^4$)	In SC[b] ($\times 10^7$)	
5-Fluorouracil	0.1	5.97 ± 1.75	0.81-0.95[c]	6300-7400
	1.0	5.31 ± 1.26	0.81-0.95[c]	5600-6500
Oestradiol	0.1	0.219 ± 0.109	0.85-3.4[d]	60-260

[a]Data are mean ± SD, n = 11-13.[b]Apparent values calculated from flux data.[c]From Cornwell and Barry (1994) and Yamane (1994).[d]Extracted from Scheuplein et al. (1969), Goodman and Barry (1988), Williams and Barry (1991a), Williams et al. (1992) and Megrab (1994).

lated (Table 4 and Fig. 3). The diffusion coefficient increased with temperature from 13 up to 32°C and then decreased from 5.97 ± 1.75 × 10^{-4} $cm^2\ h^{-1}$ (mean ± S.D., n = 11) at 32°C to 4.61 ± 0.70 × 10^{-4} $cm^2\ h^{-1}$ (mean ± S.D., n = 4) at 38°C, and 1.37 ± 0.86 × 10^{-4} $cm^2\ h^{-1}$ (mean ± S.D., n = 3) at 44°C. One-tailed t-test analysis showed that the differences between diffusion coefficients at 32°C and higher temperatures become significant at P = 0.08 for 38°C and P = 0.002 for 44°C.

Fig. 3 and Table 4 illustrate that 5-FU shows a maximum diffusion coefficient in the model matrix somewhere between 25 and 38°C. This maximum corresponds with the matrix transition revealed in differential scanning calorimetry and hot-stage microscopy where the model matrix showed a mixture of gel-to-liquid crystalline and solid-to-fluid mesomorphic-to-isotopic phase transitions from 25 to 45°C with a mid-point temperature of almost 35°C (Moghimi et al., 1995).

It is reported that the diffusion rate of $^{22}Na^+$ through phospholipid vesicles reaches a maximal value at the transition temperature. This phenomenon is attributed to the formation of local microscopic regions of disorder which are formed during the phase transitions with a maximum fractional area at the mid-point of the phase transition (Papahadjopoulos et al., 1973). The same phenomenon may be attributed to our model matrix. Our model matrix is heterogeneous and consists of more than one mesomorphic phase and possibly some crystalline lipids (Moghimi et al., 1995). Therefore, it is possible that some part of the matrix (which may not be the same phase which is undergoing the gel-to-liquid crystalline transition) melts from solid to liquid around the transition temperature and thus increases the diffusion coefficient. At higher temperatures, however, the same liquid part may incorporate into the lamellar structure and increase the proportion of the ordered structure in the system and therefore decrease the diffusion coefficient.

Diffusional activation energy for 5-FU was calculated using the Arrhenius equation (see Barry, 1983):

$$\log D = \log D_o - E_a/(2.303RT) \quad (16)$$

where D is the diffusion coefficient, D_o, the hypothetical diffusion coefficient at infinite temperature, E_a, the activation energy of diffusion, R, the gas constant (1.987 cal deg^{-1} mol^{-1}) and T, the absolute temperature (°K).

Because the main transition in our model starts at 25°C (Moghimi et al., 1995), the diffusion coefficients at temperatures of higher than 25°C were not used in the calculation of activation energy. Our model matrix also shows a minor pretransition at around 20°C (Moghimi et al., 1995) which was assumed to cause no significant structural alteration in the model matrix. With this assumption, the diffusional activation energy of 5-FU through the model matrix was estimated from the diffusion coefficients of 5-FU at 13, 20 and 25°C.

Fig. 4 shows the plot of log$D$ versus reciprocal absolute temperature. The plot is linear over the

Table 3
Geometrical dimensions of stratum corneum intercellular lipid domain of stratum corneum

Thickness (h, μm)	Tortuous pathlength (τh, μm)	Tortuosity factor (τ)	τ^2	Porosity factor (ϵ)	τ/ϵ	Volume fraction ($f_v = \tau\epsilon$)
15[a]	340[a]	22.7	500[c]	0.007	3200	0.16
				0.016[b]	1400	0.36
	500[c]	33.3	1100	0.007[a]	4800	0.23
				0.016[b]	2100	0.53
					900[d]	

[a] From Albery and Hadgraft (1979).[b] From Michaels et al. (1975).[c] From Potts and Guy (1993).[d] From Lieckfeldt and Lee (1995).

temperature range selected ($r = 0.994$) indicating that no significant structural alterations had occurred within the matrix. According to Eq. (16), the slope of the derived line is equal to $-E_a/(2.303R)$ from which the activation energy of diffusion of 5-FU through the model matrix was calculated to be 27.8 kcal mol^{-1}. Our result compares well with the activation energy of permeation of the same drug through human epidermal membrane (21.6–24.5 kcal mol^{-1}) which is calculated with the assumption that the minor transition in the SC does not cause significant structural change (Cornwell and Barry, 1993; Yamane, 1994), and again shows that 5 – FU permeates the SC through intercellular lipids.

The activation energy of diffusion of 5-FU through the matrix is comparable to the activation energy of penetration of non-electrolytes into bovine erythrocytes and is higher than that of diffusion in relatively unstructured systems such as water, 4–6 kcal mol^{-1} (Stein, 1967; Stein, 1981). This implies that the rate-limiting barrier for permeation of 5-FU through the model matrix and the intercellular route of the SC is a structured rather than an unstructured region like the aqueous domain between the lipid bilayers.

Table 4
Effect of temperature on the diffusion coefficient of 5-fluorouracil through the model matrix containing 0.1% (w/w) drug: data are mean ± SD; n represents the number of replicates

Temperature (°C)	Diffusion coefficient (cm^2 h^{-1} × 10^4)	n
13	0.163 ± 0.030	3
20	0.437 ± 0.413	3
25	1.18 ± 0.10	3
32	5.97 ± 1.75	11
38	4.61 ± 0.70	4
44	1.37 ± 0.86	3

Fig. 5 shows a sample profile of release of oestradiol from the model matrix containing 0.1% (w/w) drug at 32°C. The curve does not start from the origin and show a short lag-phase (0.68 ± 0.18 h$^{1/2}$, mean ± S.D., $n = 13$) for the same reason as explained earlier. The release rate of OE from the matrix was 4.80 ± 1.36 μg cm^{-2} h$^{-1/2}$ (mean ± S.D., $n = 13$) and from this value, the diffusion coefficient of OE through the model matrix (D_{mx}) was calculated, as explained earlier, to be 2.19 ± 1.09 × 10^{-5} cm^2 h^{-1} (mean ± S.D., $n = 13$).

The apparent diffusion coefficient of OE in human epidermis ($D_{app(J)}$) at 32°C was calculated

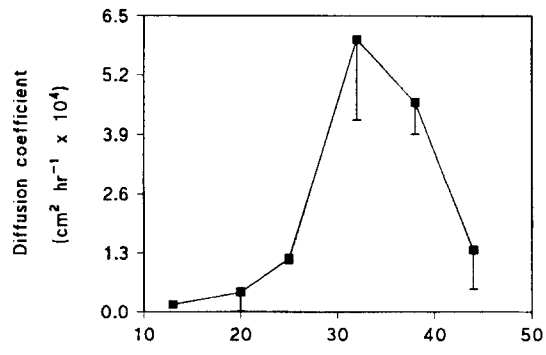

Fig. 3. The effect of temperature on the diffusion coefficient of 5-fluorouracil in the model matrix containing 0.1% (w/w) diffusant. Data are mean ± SD, $n = 3$–11. Error bar lines are within the symbol size where not shown.

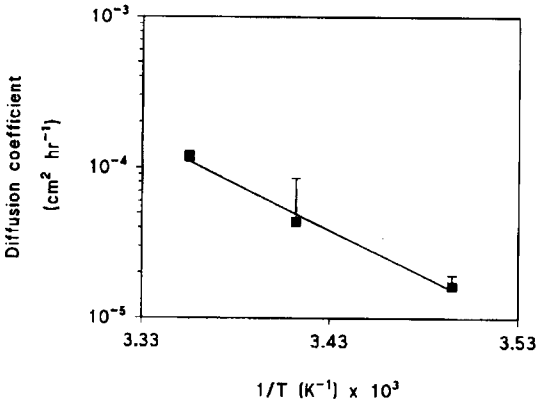

Fig. 4. Arrhenius plot of the diffusion coefficient of 5-fluorouracil in the model matrix containing 0.1% (w/w) drug. Data are mean ± SD, n = 3–4. Error bar line of the first data point is within the size of the symbol.

from published permeability and partitioning data. The permeability coefficient of OE across human epidermis from its aqueous saturated solution at 32°C is reported to be 1.30–5.23 × 10^{-3} cm h^{-1} at 32°C (Goodman and Barry, 1988; Williams and Barry, 1991a; Williams et al., 1992; Megrab, 1994). Using these values, SC/water partition coefficient of 46 (Scheuplein et al., 1969) and Eq. (13), the apparent diffusion coefficient of OE through human epidermal membrane ($D_{app(J)}$) was calculated to be 8.48–34.1 × 10^{-8} cm^2 h^{-1}.

Using the calculated apparent diffusion coefficient of oestradiol in the epidermal membrane and its diffusion coefficient through the model matrix (2.19 × 10^{-5} cm^2 h^{-1}), the diffusivity ratio ($D_{mx}/D_{app(J)}$) was estimated to be approximately 60–260. These values are less than τ/ϵ (900–4800) and are close to the lower range of τ^2 (500) and correlate well with Eq. (11) which predicts a $D_{lip}/D_{app(J)}$ of less than τ/ϵ, and Eq. (12) which predicts a $D_{lip}/D_{app(J)}$ of equal to τ^2. An assumption underlying the derivation of Eqs. (11) and (12) is the favourable partitioning of the penetrant into the intercellular lipids which applies to lipophilic drugs. Oestradiol is such a drug and good agreement of the diffusivity ratio ($D_{mx}/D_{app(J)}$) with theoretical expectation provides evidence indicating that OE permeates the SC through the intercellular route.

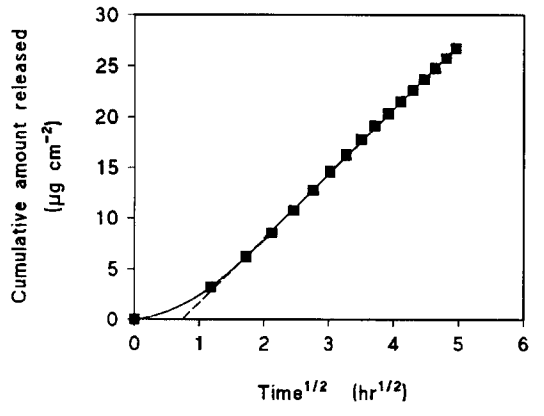

Fig. 5. Sample profile of oestradiol release from the model matrix containing 0.1% (w/w) diffusant at 32°C.

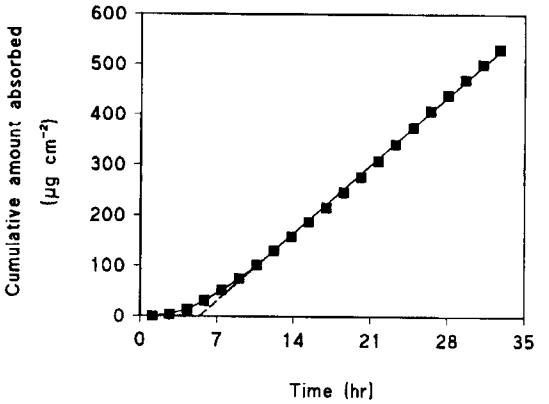

Fig. 6. Typical profile of permeation of 5-fluorouracil across the model matrix at 32°C.

3.2. Permeation studies

Figs. 6 and 7 show sample permeation profiles of 5-FU and OE penetrating across the model membrane at 32°C, respectively. The permeation parameters of these drugs through the model matrix, i.e., flux (J), permeability coefficient ($K_{p(mx)}$) and lag-time (L) are illustrated in Table 5.

5-FU and OE provided permeability coefficients of 0.28 ± 0.14 × 10^{-2} cm h^{-1} (mean ± S.D., n = 21) and 0.38 ± 0.24 × 10^{-2} cm h^{-1} (mean S.D., n = 10) through the matrix, respectively. Using the above-mentioned permeability co-

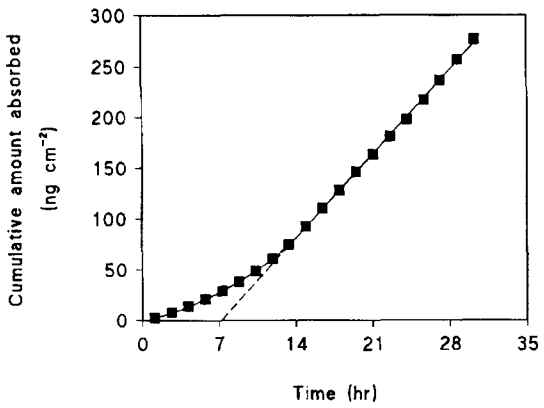

Fig. 7. Typical profile of permeation of oestradiol across the model matrix at 32°C.

efficients ($K_{p(mx)}$), diffusion coefficient of drugs in the model matrix (D_{mx}), thickness of the model membrane used in permeation studies (1.35 mm) and Eq. (13), the partition coefficients of 5-FU and OE between the model matrix and water were calculated to be 0.64 ± 0.33 (mean ± S.D., n = 21) and 23 ± 15 (mean ± S.D., n = 10), respectively.

As explained above, the permeability coefficient of a drug through the SC ($K_{p(sc)}$) can be calculated from geometrical dimensions of the SC (ϵ and τh), partition coefficient of drug between the intercellular lipids of SC and donor (K_{lip}) and diffusion coefficient of drug through the SC intercellular pathway (D_{lip}) using Eq. (14). Here, we used the matrix data for the SC intercellular lipids and for different reported geometrical dimensions of the SC, the permeability coefficients of 5-FU and OE through the SC at 32°C were predicted (Table 6).

The predicted permeability coefficient for 5-FU was 5.5–18 × 10^{-5} cm h^{-1} which correlates well with reported average human epidermis data of 3.06–12.0 × 10^{-5} cm h^{-1} (Kadir and Barry, 1991; Williams and Barry, 1991b; Williams et al., 1992; Cornwell and Barry, 1994; Yamane, 1994) at 32°C.

For OE, the predicted permeability coefficient was 0.07–0.24 × 10^{-3} cm h^{-1} which is somewhat lower than the mean reported values of 1.30–5.23 × 10^{-3} cm h^{-1} at 32°C (Goodman and Barry, 1988; Williams and Barry, 1991a; Williams et al., 1992; Megrab, 1994). However, Williams et al. (1992) have mentioned that permeability coefficients of as low as 0.1 × 10^{-3} cm h^{-1} are seen for permeation of OE across human epidermal membrane at 32°C, which is well correlated with the model matrix data. The correlation of predicted permeability coefficients and observed values again show that 5-FU and OE possibly permeate the SC through intercellular lipids.

In conclusion, the present results emphasise the effect and importance of the geometry of the SC in the percutaneous absorption of drugs. These data provide evidence indicating that the intercellular route is the major rate-determining pathway for permeation of 5-FU and OE and thus presumably for other drugs, in good agreement with the literature (Albery and Hadgraft, 1979; Potts and Francoeur, 1991; Lange-Lieckfeldt and Lee, 1992). However, in spite of these results and recent concentration on the intercellular pathway, we should not dismiss the transcellular route, which seems to be the major pathway for permeation of some drugs across human stratum corneum (Tojo, 1987).

Our model matrix is simple and does not contain all the components of the SC. However, it has been argued that the presence of a mesomorphic structure plays the major role in barrier performance of the SC intercellular lipids and the differences in barrier property with specific lipid

Table 5
Permeation parameters of 5-fluorouracil and oestradiol penetrating through 1.35 mm thick membrane of the model matrix at 32°C: data are mean ± SD; n = 21 for 5-fluorouracil and 10 for oestradiol

Permeant	Flux (μg cm^{-2} h^{-1})	Permeability coefficient (cm h^{-1} × 10^2)	Lag-time (h)	Partition coefficient
5-Fluorouracil	40.6 ± 20.8	0.28 ± 0.14	4.1 ± 2.4	0.64 ± 0.33
Oestradiol	0.014 ± 0.009	0.38 ± 0.24	7.0 ± 2.8	23 ± 15

Table 6
Predicted permeability coefficients of 5-fluorouracil and oestradiol through human stratum corneum (SC) from SC geometrical dimensions and model matrix data at 32°C

SC porosity factor	SC tortuous pathlength (μm)	Predicted permeability coefficient	
		5-Fluorouracil[a] (cm h^{-1} × 10^{5})	Oestradiol[b] (cm h^{-1} × 10^{3})
0.016[c]	340[c]	18	0.24
	500[c]	13	0.17
0.007[c]	340[c]	7.9	0.11
	500[c]	5.5	0.07

[a]Observed values: averages of 3.06–12.0 × 10^{-5} cm h^{-1} (Kadir and Barry, 1991; Williams and Barry, 1991b; Williams et al., 1992; Cornwell and Barry, 1994; Yamane, 1994).[b] Observed values: averages of 1.30–5.23 × 10^{-3} cm h^{-1} with values of as low as 0.1 × 10^{-3} cm h^{-1} (Goodman and Barry, 1988; Williams and Barry, 1991a; Williams et al., 1992; Megrab, 1994).[c] See Table 3 for references.

change may be negligible (Friberg and Kayali, 1989; Kayali et al., 1991). Our results support this argument.

This model matrix should be useful in probing enhancer interactions with stratum corneum intercellular lipids. Further work is in progress on the effect of established skin penetration enhancers on the structure and barrier properties of the matrix towards the model drugs, 5-FU and OE.

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