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A lamellar matrix model for stratum corneum intercellular lipids III. Effects of terpene penetration enhancers on the release of 5-fluorouracil and oestradiol from the matrix

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Abstract

Previously, we reported a lamellar lipid matrix capable of modelling the structural and barrier properties of the stratum corneum (SC) intercellular lipids and that oestradiol (OE) and 5-fluorouracil (5-FU) permeate the SC through intercellular channels. To investigate the ability of the matrix in modelling the effects of terpene penetration enhancers on the diffusion of OE and 5-FU through the SC, release of these drugs from cineole or limonene treated matrices were studied here. Results revealed that the matrix is able to model the effects of cineole and limonene on the diffusion coefficient of OE and the effect of cineole on the diffusivity of 5-FU through the SC. The matrix failed to model the enhancement effect of limonene toward diffusion of 5-FU through the SC which might indicate that limonene increases the permeation of 5-FU in part through interactions with SC proteins which are not modelled in the matrix. The effects of temperature on the enhancement efficiency of cineole toward diffusion of 5-FU through the matrix were investigated and results revealed that when the matrix lamellar structure is disrupted by temperature, little potential is left for further disruption and, therefore, penetration enhancement by cineole, is in good agreement with human epidermis data. Copyright © 1996 Elsevier Science B.V.

Keywords: 5-Fluorouracil; Oestradiol; Model human stratum corneum lipids; Terpene penetration enhancers; 1,8-Cineole; (+)-Limonene

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1. Introduction

The main barrier to transdermal delivery of most drugs is the outermost layer of the skin, the stratum corneum (Berenson and Burch, 1951). One of the major difficulties in transdermal drug delivery is the excellent barrier property of the stratum corneum (SC) which limits the range and amount of drugs that can be administered transdermally. A popular solution to this problem incorporates chemical penetration enhancers into dermatological products (Barry, 1991; Williams and Barry, 1992). It is widely accepted that the intercellular lipid domain, which arranges into bilayers, is the main pathway for permeation of most drugs through the SC (Elias and Friend, 1975; Elias et al., 1977, 1979; Albery and Hadgraft, 1979; Boddé et al., 1991; Moghimi et al., 1996a). The lipid-protein-partitioning theory of skin penetration enhancement (Barry, 1991) suggests that enhancers may increase the permeability of the SC by one or more of three mechanisms; (i) intercellular lipids disruption, (ii) interaction with proteins and (iii) improved partitioning of the drug, solvent or co-enhancer into the stratum corneum. Preparation of a model for the intercellular lipids provides opportunities to probe the barrier nature of the SC and the effects of skin penetration enhancers on the permeation of drugs through this membrane.

We prepared a simple lamellar mesomorphic structure (called matrix) consisting of 20% cholesterol, 25% water and 55% free fatty acids and their soaps (all w/w) as a model for the intercellular lipid domain of the stratum corneum. The matrix was characterised by X-ray diffraction, hot-stage polarised light microscopy and differential scanning calorimetry studies and showed good structural correlation with SC intercellular lipids (Moghimi et al., 1996b). Release and permeation studies using oestradiol (OE)—a model lipophilic drug—and 5-fluorouracil (5-FU)—a model hydrophilic drug—revealed that the matrix is also a good barrier model for the SC intercellular pathway and that both OE and 5-FU permeate the SC through the intercellular channels; we were able to predict the permeability coefficients of OE and 5-FU through human epidermis from model matrix data (Moghimi et al., 1996a). The studies presented in this paper aimed to investigate the effects of terpene penetration enhancers on the barrier performance of the model matrix toward OE and 5-FU and comparison with that of human SC.

Terpenes, naturally occurring substances, are the subject of recent interest as skin penetration enhancers and they may provide a series of relatively safe and clinically acceptable accelerants for lipophilic and hydrophilic drugs (Williams and Barry, 1992). In our laboratories, the enhancing abilities of a range of cyclic monoterpenes toward permeation of OE and 5-FU through human epidermis were studied (Williams and Barry, 1991a,b) and it was shown that they increase the permeation of 5-FU more than that of OE. Terpenes varied in their enhancement effect toward 5-FU. Among different classes of terpenes used at 32°C (the SC surface temperature), the maximum activity was obtained with 1,8-cineole (abbreviated to cineole here), a cyclic monoterpene of the ether class, which caused about a 95-fold increase in the permeability coefficient of 5-FU; whilst (+)-limonene (limonene), a cyclic hydrocarbon monoterpene, only doubled it (Williams and Barry, 1991a). These two enhancers showed almost the same activities toward the permeation of lipophilic oestradiol through human epidermis at 32°C (almost a four times increase in the permeability coefficient, Williams and Barry, 1991b). Therefore, these two terpenes which provide a wide range of skin enhancement activities were selected for the present investigation.

If the results of our experiments are to be compared with those performed previously using human SC, the amount of enhancer in both systems should ideally be the same. In our laboratories, most permeation and uptake studies used 12 h enhancer treatment (the duration of time in which the membrane was in contact with the enhancer before drug application). Therefore, it was necessary to prepare matrices with the same amount of enhancer which is taken up by the SC intercellular lipids after 12 h enhancer treatment. Uptake of limonene and cineole by the SC following 12 h treatment in neat terpenes is reported to be 9 and 26% (w/w) dry tissue weight respectively

(Cornwell et al., 1996). For calculation of the amount of terpenes in the SC intercellular domain, their partition coefficients between proteins of the corneocytes and intercellular lipids are needed. To the best of our knowledge, there are no such data available in the literature, but the log octanol/water partition coefficients of limonene and cineole are reported to be 4.54 and 3.53 respectively (Cornwell et al., 1996). Anderson et al. (1988) measured the octanol/water, SC protein domain/water and SC lipid domain/water partition coefficients of various hydrocortisone 21-esters. If we assume that the terpenes behave in a similar manner to hydrocortisone 21-esters and relate the octanol/water partition coefficients to those of lipid/protein, limonene and cineole would be expected to partition almost 25 and 5.5 fold more into the SC intercellular lipid domain relative to the intracellular proteins. Using these estimated lipid/protein partition coefficients, SC lipid content of almost 15% (Raykar et al., 1988) and the uptake of terpenes by the SC (Cornwell et al., 1996), the uptake of limonene and cineole by the intercellular lipids after 12 h terpene treatment was calculated to be 49 and 85% (w/w) of the original dry lipid weight respectively. These values equate approximately to 25% limonene and 40% cineole (w/w) in a mixture of terpenes and intercellular lipids containing 25% (w/w) water (water content of the model matrix).

Here, the effects of different concentrations of cineole and limonene on the diffusion coefficients of OE and 5-FU through the model matrix were studied through release experiments. Results were then compared with the effects of the same terpenes on the barrier performance of human SC toward OE and 5-FU. Because of difficulties with handling of the fragile SC, most permeability studies are performed on epidermal membranes. Permeation studies, performed at our laboratories (Williams and Barry, 1991b), indicated that the SC is the main barrier to transdermal permeation of 5-FU and OE. Therefore, the diffusion data of 5-FU and OE through epidermal membranes were used here as estimates of the SC values.

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). Unlabelled OE (99.6%), 1,8-cineole (99.5%) and (+)-limonene (99.2%) were supplied 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 and terpene loaded matrices

The preparative method of the model matrix was described previously (Moghimi et al., 1996b). Radiolabelled model drugs, OE and 5-FU, were added as solutions in methanol during the preparation of the lipid mixture (before hydration) to give final concentrations of 0.1% (w/w) in the model matrix. Cineole and limonene were added to matrices which originally contained 0.1% radiolabelled OE or 5-FU to give final concentrations of 5, 10, 15, 20 and 25% (w/w) terpenes in the model matrix.

2.3. Release experiments

Release 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 matrix. In each release experiment, a PTFE filter was placed between the donor and receptor chambers of each diffusion cell to hold the matrix. Prior to

Table 1
Effect of cineole concentration on the release of oestradiol from the model matrix (containing 0.1% w/w drug before addition of cineole) at 32°C

Cineole concentration (% w/w)	Release rate ($\mu g\ cm^{-2}\ h^{-1/2}$)	Diffusion coefficient ($cm^2\ h^{-1} \times 10^5$)	Diffusivity ratio[a]
0 (Control)	4.35 ± 0.27	1.74 ± 0.21	1.00
5	5.41 ± 1.02	3.08 ± 1.03	1.78 ± 0.59
10	5.05 ± 0.88	2.83 ± 0.97	1.63 ± 0.56
15	4.55 ± 0.92	2.75 ± 1.00	1.58 ± 0.57
20	4.70 ± 0.99	3.44 ± 1.38	1.98 ± 0.79
25	4.81 ± 0.85	4.16 ± 1.50	2.40 ± 0.86

Data are mean ± S.D., $n = 3$–4.
[a] Cineole-treated/control.

use, the PTFE filters were wetted with methanol and stored in distilled water overnight. Matrix samples were placed in the donor chambers of the diffusion cells (diameter and height of 4 mm) and covered with glass beads and parafilm.

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} and samples collected in scintillation vials over 1–1.5 h periods for at least 16 h. The activity of the released radiolabelled drugs was determined as counts per minute using a TRI-CARB liquid scintillation analyser (model 1600 TR, Packard, USA) and was then converted to the weight of drug using a standard.

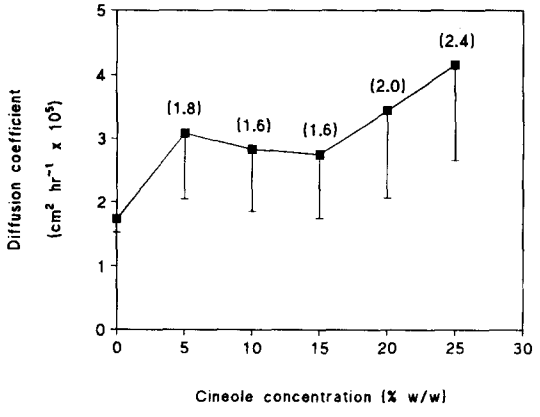

Fig. 1. Effect of cineole concentration on the diffusivity of oestradiol in the model matrix at 32°C. Numbers in parentheses are the diffusivity ratios. Data are mean ± S.D., $n = 3$–4.

To assess the effects of terpenes on the diffusion coefficients of OE and 5-FU in the model matrix, release of these drugs from untreated matrix and matrices containing 5, 10, 15, 20 and 25% (w/w) cineole or limonene was studied at 32°C (SC surface temperature). As explained in Section 1, the intercellular lipids take up equivalent to 40% cineole in the matrix. Because of liquefaction of the model matrix, we were unable to perform diffusion experiments with matrices containing more than 25% terpenes. Therefore, the diffusion coefficients of OE and 5-FU in a model matrix containing 40% cineole were estimated by extrapolation of diffusion data versus cineole concentration profiles.

The model matrix undergoes an endothermic thermal transition at about 35°C with a range of 25–45°C (Moghimi et al., 1996b) and the diffusion coefficient of 5-FU through this lamellar system varies with increasing temperature and shows a maximum value around 32°C (Moghimi et al., 1996a). However, cineole performs its enhancement effect through disruption of the lamellar structure and by introducing liquid pools into the model matrix (Moghimi et al., 1996c). Because the matrix is already disrupted (to some extent) and shows a maximum value for the diffusion coefficient of 5-FU around the transition temperature, it was expected to be less susceptible to the disruption and, therefore, decrease in the barrier performance by cineole at the transition temperature than at higher or lower temperatures. To assess this feature, release of 5-FU from matrices

Table 2
Effect of limonene concentration on the release of oestradiol from the model matrix (containing 0.1% w/w drug before addition of limonene) at 32°C

Limonene concentration (% w/w)	Release rate ($\mu g\ cm^{-2}\ h^{-1/2}$)	Diffusion coefficient ($cm^2\ h^{-1} \times 10^5$)	Diffusivity ratio[a]
0 (Control)	3.22 ± 0.43	0.990 ± 0.265	1.00
5	4.00 ± 0.82	1.56 ± 0.63	1.58 ± 0.64
10	2.65 ± 0.46	0.857 ± 0.315	0.866 ± 0.318
15	3.04 ± 0.62	1.19 ± 0.47	1.20 ± 0.47
20	2.83 ± 0.44	1.34 ± 0.43	1.36 ± 0.43
25	4.60 ± 0.96	3.95 ± 1.41	3.99 ± 1.43

Data are mean ± S.D., $n = 4$.
[a] Limonene-treated/control.

containing 10% (w/w) cineole was studied at 20, 25, 32, 38 and 44°C.

In all release experiments, after an initial short lag-phase (approximately 1 $h^{1/2}$), the release of OE and 5-FU from matrices reached a steady-state phase and the profiles of cumulative amount released versus square root of time became linear. The slopes of the linear part of these profiles are equal to the release rates from which the diffusion coefficients were calculated using Higuchi's equation (Higuchi, 1962) and the activities of terpenes were expressed as the diffusivity ratios (DR, Eq. (1)).

$$\mathrm{DR} = \frac{\text{Diffusion coefficient after terpene treatment}}{\text{Diffusion coefficient before terpene treatment}} \quad (1)$$

The changes in the diffusion coefficients of drugs in the model matrix due to terpenes were analysed statistically using a two-tailed t-test, assuming that data are distributed normally and the populations have equal variances.

3. Results and discussion

3.1. Effects of cineole on the release of oestradiol from the model matrix

Table 1 and Fig. 1 summarise the effects of cineole on the release of OE from the model matrix at 32°C. The diffusion coefficient of OE in the model matrix containing 5% cineole was 1.8 fold greater than its control value and further increased on increasing the cineole concentration to 25% where the diffusivity ratio reached 2.4 (Table 1 and Fig. 1). Analysis of data by t-test ($P = 0.05$) showed that the increase in the diffusion coefficient of OE due to cineole is only significant for matrices containing 25% terpene.

Cineole caused a 2.1-fold increase in the diffusion coefficient of oestradiol through human epidermis at 32°C after 12 h enhancer treatment (Williams and Barry, 1991b). As discussed earlier, if the results of the cineole-treated matrix are to be compared with the human epidermis data for 12 h enhancer treatment, the diffusivity ratio for a

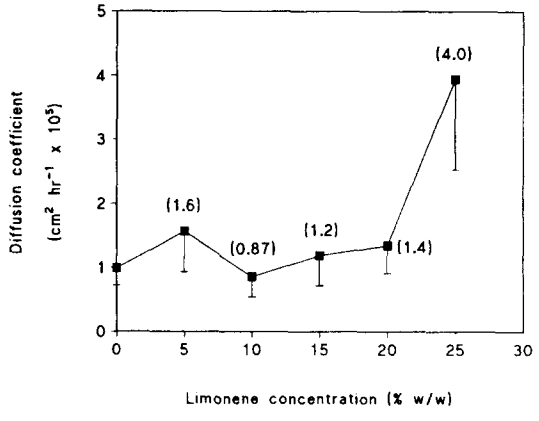

Fig. 2. Effect of limonene concentration on the diffusivity of oestradiol in the model matrix at 32°C. Numbers in parentheses are the diffusivity ratios. Data are mean ± S.D., $n = 4$.

Table 3
Effect of cineole concentration on the release of 5-fluorouracil from the model matrix (containing 0.1% w/w drug before addition of cineole) at 32°C

Cineole concentration (% w/w)	Release rate ($\mu g\ cm^{-2}\ h^{-1/2}$)	Diffusion coefficient ($cm^2\ h^{-1} \times 10^4$)	Diffusivity ratio[a]
0 (Control)	27.3 ± 2.5	6.46 ± 1.17	1.00
5[b]	32.4 ± 4.2	9.33 ± 2.21	1.47 ± 0.35
10[c]	32.8 ± 4.6	13.3 ± 2.2	2.09 ± 0.35
15	42.8 ± 1.7	22.4 ± 1.7	3.47 ± 0.27
20	55.5 ± 5.9	43.2 ± 8.9	6.69 ± 1.37
25	72.6 ± 12.0	83.5 ± 26.2	12.9 ± 4.1

Data are mean ± S.D., $n = 3$–6.
[a] Cineole-treated/control.
[b] Control values: release rate = $25.4 \pm 3.1\ \mu g\ cm^{-2}\ h^{-1/2}$, diffusion coefficient = $6.32 \pm 1.43 \times 10^{-4}\ cm^2\ h^{-1}$ (mean ± S.D., $n = 4$).
[c] Control values: release rate = $26.2 \pm 2.8\ \mu g\ cm^{-2}\ hr^{-1/2}$, diffusion coefficient = $6.40 \pm 1.22 \times 10^{-4}\ cm^2\ h^{-1}$ (mean ± S.D., $n = 7$).

matrix containing 40% cineole should be calculated by extrapolation of the plot of diffusion coefficient against cineole concentration. Such an extrapolation based on an assumed linear relationship between the diffusivity values of OE in the model matrix and cineole concentration in the range of 15–25% (Fig. 1) gave a diffusivity ratio of 3.6 which is in good agreement with the human epidermal membrane data and which shows that the matrix is able to model the effect of cineole on the diffusion of oestradiol through the stratum corneum.

It is worth noting that, although there is a good correlation between the diffusivity ratio of OE in the cineole-treated human epidermis (2.1) and the model matrix after extrapolation of the matrix data to the cineole content of 40% (DR = 3.6), a better correlation exists without extrapolation (DR = 2.4 for the matrix cineole content of 25%). This might suggest that the enhancement efficiency of cineole toward OE through the model matrix and human epidermis reaches a plateau at an equivalent of 25% cineole in the model matrix. It is interesting in this context to note that the enhancement efficiency of azone toward permeation of diazepam across human SC was reported to reach a plateau at azone loading of 12% (Schückler and Lee, 1992).

3.2. Effects of limonene on the release of oestradiol from the model matrix

The release rates, diffusion coefficients and diffusivity ratios of OE in limonene-treated matrices at 32°C are shown in Table 2. Limonene increased the diffusion coefficient of OE almost 1–2-fold in matrices containing 5–20% terpene (Table 2 and Fig. 2), which is not significant as analysed by t-test ($P = 0.05$). On increasing the limonene content of the model matrix to 25%, which resulted in the liquefaction of the matrix,

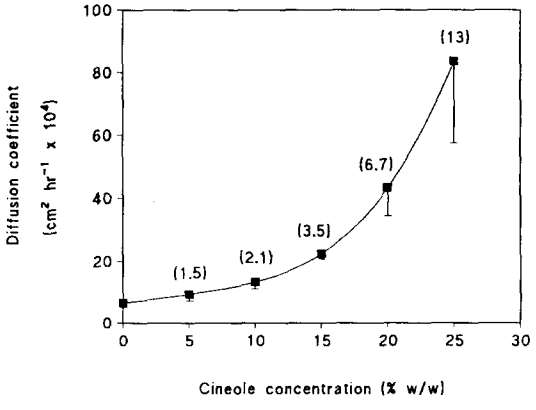

Fig. 3. Effect of cineole concentration on the diffusivity of 5-fluorouracil in the model matrix at 32°C. Numbers in parentheses are the diffusivity ratios. Data are mean ± S.D., $n = 3$–6. Error bar of the first data point is within the size of symbol.

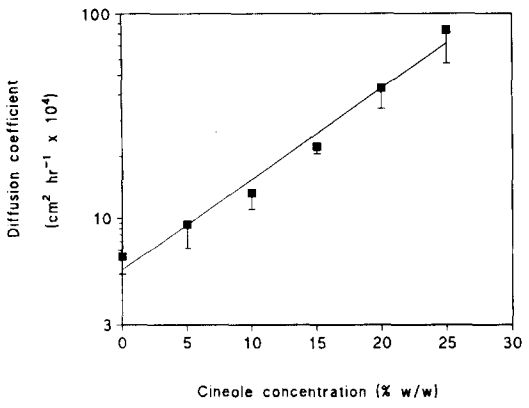

Fig. 4. Semilogarithmic plot of diffusion coefficient of 5-fluorouracil in the model matrix versus cineole concentration at 32°C. Data are mean ± S.D., $n = 3–6$.

the diffusivity ratio increased significantly to 4.0 ($P = 0.05$) which is in good agreement with the reported diffusivity ratio of 3.8 for human epidermal membrane at 32°C after 12 h enhancer treatment (Williams and Barry, 1991b). Good correlation of our results with the human epidermis data indicates that the matrix is able to model the effects of limonene on the diffusivity of OE through human epidermis.

3.3. Effects of cineole on the release of 5-fluorouracil from the model matrix

Table 3 and Fig. 3 summarise the effects of cineole on the release of 5-FU from the model matrix at 32°C. Cineole increased the diffusion coefficient of 5-FU by about 1.5–13 times in matrices containing 5–25% enhancer relative to the untreated matrix (Table 3 and Fig. 3). Analysis of data by t-test ($P = 0.05$) showed that the increased diffusion coefficient due to cineole is significant for all cineole concentrations used here. The diffusivity ratio of 5-FU in 12 h cineole-treated human epidermal membrane is reported to be 39 at 32°C (Yamane, 1994). As explained earlier, if the matrix results are to be compared with that of 12 h cineole-treated human epidermal membrane, the diffusivity ratio in a model matrix containing 40% cineole should be calculated through an extrapolation of diffusion coefficient vs cineole concentration profile. Fig. 3 shows that the relationship between the cineole concentration and the diffusion coefficient of 5-FU in the model matrix at 32°C is not linear for the whole concentration range and seems to be exponential. Fig. 4 shows that log D versus cineole concentration is linear ($r = 0.99$) and extrapolation of this line to the cineole concentration of 40% gives a diffusivity ratio of 52, which is in agreement with the human epidermal data (DR = 39) and reveals that cineole might show its enhancement effect toward 5-FU through interacting with the intercellular lipids of the SC.

Table 4 summarises the effects of temperature on the enhancement efficiency of cineole toward release of 5-fluorouracil from the model matrix. The diffusivity ratios show that the enhancement efficiency of cineole around the mid-point of the matrix thermal transition (around 35°C) is lower than that at the higher or lower temperatures and clearly illustrates that when the matrix is disrupted due to temperature, not much potential is left for the disruption by the enhancer (Table 4 and Fig. 5). Human SC shows a low enthalpy thermal transition around 35–38°C (Goodman and Barry, 1989; Gay et al., 1994). The effect of temperature on the enhancement action of cineole toward permeation of 5-FU through human epidermis was investigated by Yamane (1994) where it was shown that the enhancement ratio decreased from 94 at 29°C to 57 at 35°C, in correlation with our results.

3.4. Effects of limonene on the release of 5-fluorouracil from the model matrix

Table 5 summarises the effects of limonene on the release of 5-FU from the model matrix at 32°C. Limonene did not increase the diffusivity of 5-FU in the model matrix at 32°C. The differences between the diffusion coefficient of 5-FU in the untreated matrix and those of matrices containing 5 and 10% limonene (DR = 0.97 and 0.62 respectively) were not significant as analysed by t-test ($P = 0.05$). As the limonene concentration increased, the diffusivity ratio decreased significantly ($P = 0.05$) and reached values of 0.170, 0.015 and 0.066 in matrices containing 15, 20 and 25% limonene respectively (Table 5 and Fig. 6).

Table 4
Effect of temperature on the release of 5-fluorouracil from the model matrix containing 10% (w/w) cineole

Temperature (°C)	Release rate ($\mu g\ cm^{-2}\ h^{-1/2}$)		Diffusion coefficient ($cm^2\ h^{-1} \times 10^4$)		Diffusivity ratio[a]
	Untreated	Cineole-treated	Untreated	Cineole-treated	
20	6.21 ± 3.23	8.77 ± 0.71	0.437 ± 0.413	1.07 ± 0.16	2.45 ± 0.37
25	11.6 ± 0.5	25.6 ± 5.5	1.18 ± 0.10	7.16 ± 2.87	6.07 ± 2.43
32	26.2 ± 2.8	32.8 ± 4.6	6.40 ± 1.22	13.3 ± 2.2	2.09 ± 0.35
38	20.2 ± 1.6	31.2 ± 3.5	4.61 ± 0.70	16.9 ± 4.6	3.67 ± 1.00
44	10.2 ± 3.4	28.6 ± 7.1	1.37 ± 0.86	19.3 ± 9.7	14.1 ± 7.1

Data are summarised as mean ± S.D., $n = 3–7$.
[a] Cineole-treated/untreated.

The effects of limonene on the structure of the model matrix are discussed in detail in our companion paper (Moghimi et al., 1996c). Briefly, limonene causes lamellar-to-viscous isotropic and lamellar-to-hexagonal phase transitions in the model matrix and apparently introduces a continuous lipophilic domain and a hydrophilic dispersed phase into the system. In such a condition, 5-FU (a hydrophilic drug) would be expected to accumulate in the dispersed hydrophilic phase and favourable partitioning toward this internal phase would render the drug relatively unavailable to the continuous lipophilic phase. Therefore, the main limiting step for the release of 5-FU from the limonene-treated matrices is possibly partitioning from an internal hydrophilic to an external lipophilic phase. This problem does not exists for oestradiol, because this lipophilic drug prefers the lipid phase.

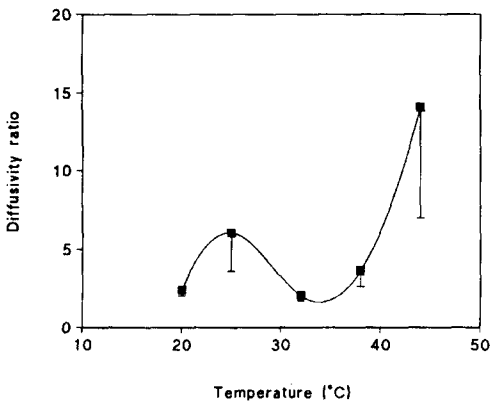

Fig. 5. Effect of temperature on the enhancement efficiency of cineole toward 5-fluorouracil releasing from the model matrix containing 10% (w/w) enhancer. Data are mean ± S.D., $n = 3–7$.

Limonene is not a good penetration enhancer toward 5-FU permeating through human epidermal membrane. The DR of 5-FU through human epidermis after 12 h limonene treatment is reported to be only 1.3 (Williams and Barry, 1991a) and 3.8 (Yamane, 1994). Our results, however, show that limonene is not an enhancer toward 5-FU releasing from the model matrix at low concentrations and is a retardant at concentrations of higher than 10% and clearly indicate that the matrix does not model well the effect of limonene on the diffusion of 5-FU (a hydrophilic drug) through the SC. As was shown in Section 3.2, there was no such problem for OE (a lipophilic drug) and the matrix was able to model the effects of limonene on the diffusivity of OE through the SC. Miyajima et al. (1994) also showed that the enhancement efficiencies of azone, decylmethylsulphoxide, oleic acid, lauric acid and capric acid toward permeation of a hydrophilic drug (cyclobarbital) through a lamellar model matrix membrane are up to seven times less than the effects of the same enhancers toward the permeation of the same drug through the skin. However, the difference between the effects of the same enhancers toward permeation of a lipophilic drug (ibuprofen) in the model matrix and skin was around 2 fold (Miyajima et al., 1994). A plausible reason for the underestimation of the effect of limonene on the permeation of 5-FU through human epidermis by our model matrix

Table 5
Effect of limonene concentration on the release of 5-fluorouracil from the model matrix (containing 0.1% w/w drug before addition of limonene) at 32°C

Limonene concentration (% w/w)	Release rate ($\mu g\ cm^{-2}\ h^{-1/2}$)	Diffusion coefficient ($cm^2\ h^{-1} \times 10^4$)	Diffusivity ratio[a]
0 (Control)	18.4 ± 4.2	5.23 ± 2.46	1.00
5	17.0 ± 1.8	5.08 ± 1.09	0.972 ± 0.209
10	12.6 ± 1.2	3.23 ± 0.61	0.618 ± 0.117
15	6.36 ± 1.10	0.888 ± 0.309	0.170 ± 0.059
20	1.81 ± 0.32	0.081 ± 0.028	0.015 ± 0.005
25	2.85 ± 0.67	0.343 ± 0.160	0.066 ± 0.031

Data are mean ± S.D., $n = 3$–4.[a] Limonene-treated/control.

and up to seven times underestimation of the effects of different enhancers on the skin permeability of hydrophilic cyclobarbital by a lamellar model matrix (Miyajima et al., 1994) might be the importance of the transcellular route for the permeation of hydrophilic drugs through enhancer-treated SC as discussed below.

In spite of recent concentration on the intercellular route of the SC as the rate-determining pathway for drug permeation, the transcellular route should not be dismissed and the diffusivity in lipids and proteins and the partitioning of drugs between these domains may play important roles in the permeation of drugs and the effects of enhancers on this process (Michaels et al., 1975; Albery and Hadgraft, 1979; Tojo, 1987; Barry, 1991). For a hydrophilic drug, partitioning into the corneocytes should not be a rate limiting step and if such drugs do not permeate the SC through the transcellular route, the limiting step should be a diffusional barrier. Suppose that an enhancer decreases the diffusivity barrier of the corneocytes and/or their envelopes. This reduction in the diffusivity barrier of the transcellular pathway will improve the permeation of hydrophilic drugs but may not be useful for lipophilic drugs as they find the intercellular domain favourable and do not, therefore, partition easily into the more hydrophilic corneocytes. Such a mechanism may explain why the model lipid systems underestimate the effects of enhancers on the barrier performance of the SC toward hydrophilic (and not lipophilic) drugs.

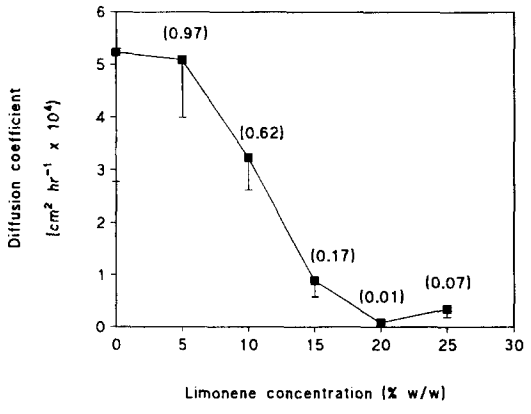

Fig. 6. Effect of limonene concentration on the diffusivity of 5-fluorouracil in the model matrix at 32°C. Numbers in parentheses are the diffusivity ratios. Data are mean ± S.D., $n = 3$–4. Error bar of the fifth data point is within the size of symbol.

Differential scanning calorimetry studies suggested that limonene does not interact with the SC lipids to disorganise them at skin temperature, but it may interact with the SC proteins (Yamane, 1994; Cornwell et al., 1996). However, small-angle X-ray diffraction studies showed that limonene reduces lipid bilayer periodicity in the SC which contradicts differential scanning calorimetry findings (Cornwell et al., 1996). On the other hand, our differential scanning calorimetry and polarised light microscopy studies showed that limonene causes phase transformation in the model matrix around physiologic temperature (Moghimi et al., 1996c). The reason for this discrepancy is not clear and requires further investigation. These data show that limonene might affect both lipids and proteins of the SC and may

increase the diffusivity of 5-FU in the SC through protein interactions, which is not, of course, modelled in the simple lipid matrix.

In summary, a lamellar mesomorphic matrix was used as a model for the intercellular lipids of human stratum corneum. Release and permeation experiments suggested that model drugs, OE and 5-FU, permeate the SC through the intercellular pathway (Moghimi et al., 1996a). In the present paper the effects of established skin penetration enhancers (cineole and limonene) toward diffusion of OE and 5-FU through the model matrix were investigated. The effects of cineole and limonene on the structure of the model matrix and the mechanism of action of these terpenes on the diffusion of model drugs through the matrix are discussed in our companion paper (Moghimi et al., 1996c). Briefly, cineole creates a liquid pool in the model matrix and limonene first increases the consistency of the matrix up to a terpene concentration of 20% and then fluidises the model matrix when limonene concentration increases to 25%. Limonene also introduces a continuous lipophilic phase into the system.

The effects of limonene and cineole on the diffusion coefficient of oestradiol through the model matrix were in good correlation with human epidermal membrane data and revealed that the matrix is able to model the effects of enhancers on the diffusion of OE and possibly other lipophilic drugs through human epidermis.

Although the matrix was able to model reasonably the effects of cineole on the diffusivity of 5-FU in the SC, it failed to model the effects of limonene on the diffusion of the same drug through the SC. This might indicate that limonene increases the diffusivity of 5-FU through protein interactions in human SC, a mechanism which is supported by differential scanning calorimetry (Yamane, 1994; Cornwell et al., 1996) and requires further investigation.

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