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A lamellar matrix model for stratum corneum intercellular lipids. V. Effects of terpene penetration enhancers on the structure and thermal behaviour of the matrix

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Abstract

The principal barrier to transdermal delivery of most drugs is the lamellar intercellular lipid domain of the stratum corneum (SC). Previously, we reported a lamellar matrix capable of modelling the structural and barrier properties of the SC lipids and effects of terpene penetration enhancers on the permeation of oestradiol and, to some extent, that of 5-fluorouracil (5-FU) through the SC. The present work is aimed to investigate the interactions of two terpenes (1,8-cineole and (+) limonene) with the matrix. Terpenes (5–40%, w/w) were added to the matrix and their effects studied by hot-stage light microscopy (25–160°C) and differential scanning calorimetry (10–100°C). Results revealed that cineole breaks the matrix into a dispersed system of a lamellar mesomorphic structure in an isotropic liquid at 25 and 32°C (SC temperature), in good correlation with human SC data. Increasing concentrations of limonene caused initially a decrease in the lamellar structure, then a lamellar-to-cubic phase transition and finally, changed the matrix to a dispersed system of crystalline and mesomorphic phases in a continuous liquid phase at 25 and 32°C. The effects of limonene on the structure of the model matrix are in agreement with those reported for SC lipids derived from X-ray diffraction results. Effects of terpenes on the structure of the matrix were related to their actions on the barrier performance of this lamellar model.

Keywords: Differential scanning calorimetry; Model human stratum corneum lipids; Polarised light microscopy; Terpene penetration enhancers; 1,8-Cineole; (+)-Limonene

1. Introduction

The stratum corneum (SC), the main barrier to transdermal delivery of most drugs (Berenson and Burch, 1951), is a multilayered wall-like structure

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in which keratin-rich corneocytes embed in an intercellular lipid-rich matrix. It is widely accepted that the intercellular lipid domain is the major rate-determining pathway by which most drugs traverse the SC (Elias and Friend, 1975; Albery and Hadgraft, 1979; Tojo, 1987; Boddé et al., 1991; Moghimi et al., 1996a).

Intercellular lipids of the SC arrange into bilayers (Elias and Friend, 1975; Elias et al., 1977, 1979). Preparation of a model for the intercellular lipids of the SC provides opportunities to probe the barrier nature of the SC. We prepared a simple lamellar mesomorphic structure (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 and showed good structural correlation with SC intercellular lipids (Moghimi et al., 1996b). Release and permeation studies using 5-fluorouracil (5-FU) and oestradiol (OE) revealed that the matrix is also a good barrier model for the SC intercellular pathway (Moghimi et al., 1996a). The effects of two terpenes (1,8-cineole and (+)-limonene) on the barrier performance of the matrix toward 5-FU and OE were investigated through release and permeation experiments. Results showed that while cineole increases the permeation of both drugs through the matrix, limonene promotes only that of OE and decreases that of 5-FU (Moghimi et al., 1996c,d). The studies presented in this paper aimed to investigate the mechanisms by which these terpenes interact with the SC and the model matrix and affect their barrier performance.

2. Materials and methods

2.1. Materials

All materials were used as received. The sources and purities of fatty acids, cholesterol, antioxidants and terpenes were detailed previously (Moghimi et al., 1996b,c).

2.2. Preparation of terpene loaded matrices

The preparative method of the model matrix was described previously (Moghimi et al., 1996b). Neat terpenes were added to the matrix to give final concentrations of 5, 10, 20, 25 and 40% for cineole and 5, 10, 15, 20 and 25% for limonene (all w/w). Matrices containing 5–25% cineole and limonene were used in release and permeation studies (Moghimi et al., 1996c,d). Those containing 40% cineole or 25% limonene are equivalent to SC intercellular lipids after 12 h terpene treatment (Moghimi et al., 1996c), for which structural data are available in the literature.

2.3. Hot-stage light microscopy

Of the various techniques used to identify mesomorphic phases, the most widely used is polarised light microscopy (Tyle, 1989). Here, the structure of the matrices was assessed, in both normal and polarised light, using a Labophot-2A light microscope (Nikon, Japan) connected to a video cassette recorder (Mitsubishi, Japan) and a colour video copy processor (Mitsubishi, Japan). The thermal behaviour of matrices from 25 to 160°C was studied using a Stanton Redcroft heating-freezing stage (England) with a heating rate of 5°C/min. Results are presented as black and white pictures whereas the original photographs are coloured. Some pictures are omitted due to lack of contrast in black and white presentation.

2.4. Differential scanning calorimetry

Differential scanning calorimetry (DSC) is another technique which has been used extensively to study mesomorphic phases and stratum corneum structure and also to evaluate the effects of enhancers on these systems and their barrier properties (Golden et al., 1987; Ford and Timmins, 1989; Goodman and Barry, 1989; Barry, 1991; Schückler et al., 1993). Here, DSC studies were performed on matrix samples of 5–15 mg using a 7 Series Thermal Analysis System (Perkin Elmer, USA) from 10 to 120°C for the plain matrix and 10–100°C for terpene-treated matrices, at a heating rate of 5°C/min (Moghimi et

al., 1996b for more details). The effects of cineole and limonene on the thermal behaviour of the model matrix are presented as the mid-point transition temperatures, temperature shifts (ΔT), transition enthalpies and enthalpy ratios. Temperature shifts were calculated by subtracting the mid-point transition temperatures of untreated matrix from those of terpene-treated systems. Enthalpy ratios were calculated by dividing post-treatment enthalpies by control values. The changes in transition temperatures and enthalpies were analysed statistically using a two-tailed t-test, assuming that data were distributed normally and the populations had equal variances.

3. Results and discussion

3.1. Effects of cineole on the structure of the matrix

3.1.1. Hot-stage polarised light microscopy

The textures of the model matrix in polarised light and its phase transformations from −30 to 160°C were discussed in detail previously (Moghimi et al., 1996b) and the events arising from 25 to 160°C (which are needed for comparison with those of terpene-treated systems) will be reviewed here only briefly. The matrix showed a mixture of oily streaks in the planar area (Fig. 1a) and mosaic textures, representative of lamellar mesomorphic structure (neat phase), at 25 and 32°C (SC temperature). During the heating process, positive units started to grow in planar areas around 35°C. This texture continued to extend in the system and gradually replaced the oily streaks and planar areas until around 95°C where all planar areas and oily streaks changed to a coarse mosaic texture (Fig. 1b). These textural changes were attributed to different gel-to-liquid crystalline transitions (Moghimi et al., 1996b). With further heating, the matrix showed fanlike and angular textures of hexagonal mesomorphic structure (middle phase) around 105°C (Fig. 1c). These textures faded with further temperature increase and the system formed an isotropic liquid around 140°C. Besides the growth of positive units, another minor textural change was also observed around 35°C which was disappearance of some fine birefringent grains from the system. This was attributed to one or both of solid-to-liquid and liquid crystalline-to-isotropic phase transitions.

Addition of cineole to the model matrix caused different structural changes which can be classified into three main groups: (i) effects of cineole on the structure of the model matrix at 25°C (standard laboratory temperature) and 32°C (SC temperature); (ii) effects of cineole on the final T_i, the temperature at which the matrix transforms to an isotropic liquid with no further phase transition noted until the end of experiment; and (iii) effects of cineole on the thermal behaviour of the model matrix from 32°C to T_i.

One of the most interesting changes after cineole-treatment was the replacement of most of the oily streaks of the untreated matrix by a network of positive and negative units in all cineole-treated systems (coarse mosaic texture) at 25°C (compare Fig. 1(a) and (d)), a transition which was induced by temperature in the untreated matrix (compare Fig. 1(a) and (b)). This phenomenon might be responsible for the effects of some enhancers on the permeation of drugs through SC intercellular lipids. A similar observation was reported by Engblom and Engström (1993) who showed that addition of Azone (a skin penetration enhancer) to a lecithin-water system induced a lamellar (L_α) to reversed hexagonal (H_{II}) phase transition at ambient temperature, a transition which occurred above 200°C in an Azone-free system. In matrices containing 25% cineole, the system further showed some large lamellar liquid crystalline (L_α) particles known as spherulites (picture not shown) and when the cineole concentration increased to 40%, the matrix provided some myelinic figures (Fig. 1(e)), representative of a lamellar liquid crystalline (L_α) structure (Benton et al., 1986; Small, 1986). As in the case of untreated matrix, there was no textural change between 25 and 32°C in cineole-treated systems.

Our light microscopy studies showed that besides the above mentioned mesomorphic structures, an isotropic liquid was also present in cineole-treated matrices, the proportion of which increased on raising the cineole concentration. This isotropic liquid was continuous in matrices

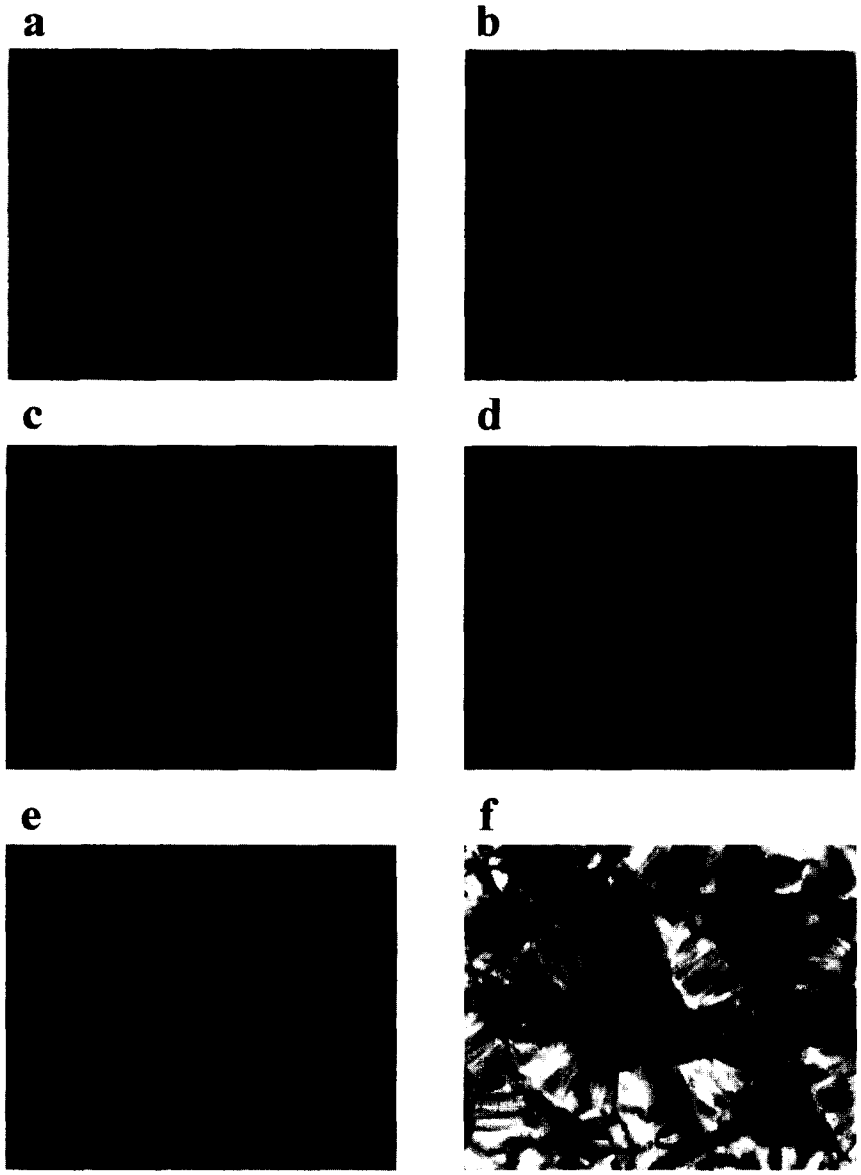

Fig. 1. Textural changes of the model matrix due to heating and cineole-treatment as studied by cross polarised light microscopy. (a) Oily streaks at 25°C; (b) coarse mosaic texture at 93°C; (c) fanlike and angular textures at 108°C in the plain matrix; (d) sample positive units and oily streaks in matrices containing 10% cineole at 25°C; (e) myelinic figures in matrices containing 40% cineole at 25°C; and (f) pinwheel texture in matrices containing 5% cineole at 70°C.

containing 10% or more terpene. Cornwell et al. (1994, 1996) studied the effects of 12 h cineole-treatment on the intercellular lipids of the SC by X-ray diffraction and showed that some residual normal bilayers and a liquid phase co-exist in cineole-treated human SC at ambient temperature, in good correlation with our results. Such a phase separation was also reported by Walker and Hadgraft (1991) when oleic acid was added to extracted human epidermal lipids.

Another uniform change in cineole-treated matrices was a gradual reduction in the T_i (140°C in

the plain matrix). There was no change in the T_i of the matrix in systems containing 5 and 10% cineole, but the T_i decreased to around 100°C in matrices containing 20% cineole and then to around 90–95°C in systems containing 25 and 40% terpene.

We can also consider the thermal behaviour of the cineole-treated matrices in polarised light from 32°C to T_i. The matrix containing 5% cineole showed a network of positive and negative units (coarse mosaic texture) in an isotropic phase at 25 and 32°C (not shown). During the heating process, some more mosaic texture started to grow continuously in this system from about 35°C until around 70°C where some pinwheel textures, which are also representative of a lamellar mesomorphic structure, grew in the birefringent border adjoining planar areas (Fig. 1(f)). These planar areas are probably not truly isotropic, as the pinwheels fill them at higher temperatures (not shown); however, there were some areas which remained isotropic until the end of the experiment. Before the whole system changed to an isotropic liquid at around 140°C, a transition from lamellar to hexagonal was observed around 110°C in matrices containing 5% cineole (not shown).

In matrices containing 10% cineole, some positive units started to grow at around 35°C and continued to grow until around 70°C (pictures not shown). Above 70°C, the system started to become isotropic and before the whole system changed to an isotropic liquid around 140°C, a lamellar-to-hexagonal transition, represented by appearance of angular texture, occurred around 100°C. Like the untreated matrix, besides the mosaic texture growth, some birefringent areas changed to an isotropic phase around 35–40°C in matrices containing 5 and 10% cineole; however, the mosaic growth was dominant.

In systems containing 20 and 25% cineole, both growth and disappearance of the mosaic texture started around 35°C, while the disappearance of mosaic texture was dominant. From around 70°C, there was no more textural growth in matrices containing 20% cineole and all the birefringent areas started to disappear and, before complete disappearance of the neat phase around 100°C, a lamellar-to-hexagonal transition (represented by appearance of angular texture) was observed around 90°C. In matrices containing 25% cineole, the textural growth stopped around 50°C and all the birefringent areas continued to disappear until around 90°C when the system changed to an isotropic liquid. There was no lamellar-to-hexagonal transition in matrices containing 25% cineole.

In matrices containing 40% cineole, the system started to become more isotropic at around 35°C, while some mosaic textures changed to batonnets around 45°C before they disappeared (not shown). Batonnets are observed in both neat and middle phases (Rosevear, 1954; Winsor, 1968; Hartshorne, 1974). The mesomorphic-to-isotropic phase transition continued during heating to higher temperatures and meanwhile some lamellar fanlike units appeared in the system around 80°C (not shown). Finally, the whole system changed to an isotropic liquid around 90–95°C. There was no lamellar-to-hexagonal transition in matrices containing 40% cineole before the final T_i.

3.1.2. *Differential scanning calorimetry*

The DSC thermal behaviour of the model matrix from −30 to 120°C was detailed previously (Moghimi et al., 1996b). Briefly, the untreated matrix showed seven endothermic transitions ($T_1 - T_7$) over this window of which four ($T_1 - T_4$ at about −11, −2, 22 and 35°C) were seen in all samples. There were three other transitions (T_5, T_6 and T_7 at around 55, 80 and 105°C, respectively) which were absent in some matrix samples. Here, the same peak numbers will be used for both treated and untreated matrices.

Fig. 2 illustrates the thermograms of untreated and 5–40% (w/w) cineole-treated matrices in DSC and Tables 1 and 2 show the corresponding transition temperatures, enthalpies and their statistical comparisons. The T_3 transition was apparent in all cineole-treated matrices and the transition T_4 was obvious in all cineole-treated matrices except those containing 40% cineole (Fig. 2).

As illustrated by polarised light microscopy, the textural change started almost at the same temperature (around 35°C) in matrices containing 0–25% cineole, therefore, it is not possible to explain the increase in the T_4 transition tempera-

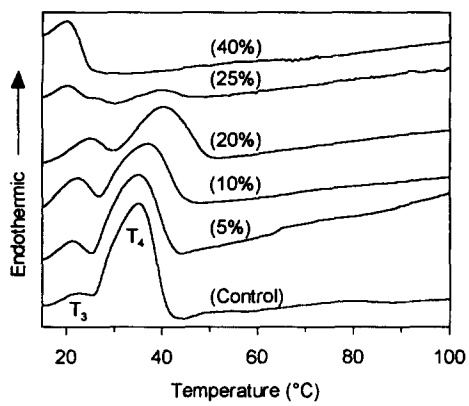

Fig. 2. Differential scanning calorimetry thermograms of the untreated model matrix (control) and matrices containing 5–40% (w/w) cineole.

ture (Table 2) from microscopic observations. As explained previously (Moghimi et al., 1996b), dehydration increased the T_4 transition of the model matrix and its equivalent transition in human SC, therefore, a plausible reason for the increase in the T_4 transition temperature might be progressive replacement of water by cineole in the mesomorphic phase that undergoes transition. Alternately, as explained earlier, the T_4 transition (extending from 25 to 45°C in the plain matrix) is possibly a mixture of two events. Therefore, it is possible that selective extraction of lipids by cineole shifts the T_4 transition to higher temperatures, i.e. toward the end of the T_4 range. Finally, polarised light microscopy studies revealed that there was no mosaic texture growth around the T_4 transition in matrices containing 40% cineole, which correlates with the disappearance of T_4 in DSC. However, microscopic observations revealed a mesomorphic-to-isotropic phase transition in the range of T_4 in systems containing 40% cineole, the enthalpy of which would have been too low to be detectable by DSC.

The enthalpy of T_4 decreased significantly ($P > 0.01$) on addition of cineole to the matrix (Table 2). Two mechanisms might underlie this phenomenon. Firstly, as shown by polarised light microscopy, cineole progressively decreased the proportion of mesomorphic structures in the system. Secondly, as explained previously (Moghimi et al., 1996b), T_4 is mostly attributed to a transition from oily streaks and planar areas to mosaic texture, a phenomenon which was induced by cineole at room temperature. Therefore, there is less room left for temperature increase to cause such a transition in cineole-treated matrices.

Transition T_6 was seen in all samples of matrices containing 5 and 10% cineole. Control samples (untreated matrix) showed a mid-point temperature of 79.3 ± 1.3°C and enthalpy of 1.03 ± 0.69 J/g for T_6 transition (mean $\pm$ S.D.,

Table 1
The effects of cineole concentration (% w/w) on the transition temperature (T) and transition enthalpy (ΔH) of T_3 transition of the model matrix as studied by differential scanning calorimetry

Cineole concentration	Transition temperature			Transition enthalpy		
	T (°C)	ΔT (°C)[a]	P*	ΔH (J/g)	ΔH ratio[b]	P*
0 (Control)	21.5 ± 0.4	0.0	—	1.22 ± 0.41	1.00	—
5	21.7 ± 0.5	0.2	0.72	1.62 ± 0.18	1.33 ± 0.15	0.20
10	22.6 ± 0.5	1.1	0.05	3.00 ± 0.04	2.46 ± 0.03	0.00
20	24.5 ± 0.6	3.0	0.00	3.18 ± 0.36	2.61 ± 0.29	0.00
25[c]	22.3 ± 2.5	0.8	0.65	2.30 ± 1.22	1.88 ± 1.00	0.21
40	20.1 ± 0.1	−1.4	0.01	2.24 ± 0.87	1.84 ± 0.71	0.14

Data are mean $\pm$ S.D. ($n = 3$–4).
[a] Cineole treated − control.
[b] Cineole treated/control.
[c] Another transition was seen at 26.7 ± 1.6°C with enthalpy of 1.00 ± 1.40 J/g. Data are mean $\pm$ S.D., $n = 3$.
* P-values represent the smallest value for the level of significance for which the null hypothesis can be rejected in two-tailed t-test analysis. (Null hypothesis: values of terpene-treated and untreated matrices are the same).

Table 2
The effects of cineole concentration (% w/w) on the transition temperature (T) and transition enthalpy (ΔH) of T_4 transition of the model matrix as studied by differential scanning calorimetry

Cineole concentration	Transition temperature			Transition enthalpy		
	T (°C)	ΔT (°C)[a]	P-values	ΔH (J/g)	ΔH ratio[b]	P-values
0 (Control)	35.2 ± 0.4	0.0	—	15.6 ± 1.2	1.00	—
5	35.4 ± 0.1	0.2	0.45	11.7 ± 0.7	0.75 ± 0.04	0.01
10	37.3 ± 0.2	2.1	0.00	9.71 ± 0.83	0.62 ± 0.05	0.00
20	39.6 ± 0.8	4.4	0.00	6.69 ± 2.10	0.43 ± 0.13	0.00
25	39.5 ± 0.4	4.3	0.00	0.58 ± 0.48	0.04 ± 0.03	0.00
40	NO	—	—	NO	—	—

Data are mean ± S.D. ($n = 3$–4). See Table 1 for explanation of P-values. NO, transition not observed.
[a] Cineole treated − control.
[b] Cineole treated/control.

$n = 3$). Addition of 5 and 10% cineole decreased both temperature and enthalpy of T_6 transition. In matrices containing 5% cineole, T_6 showed transition temperature of 67.7 ± 4.0°C ($\Delta T = -11.6$°C) and enthalpy of 0.68 ± 0.01 J/g (enthalpy ratio = 0.66 ± 0.01). In matrices containing 10% cineole, the T_6 transition temperature and enthalpy were 71.8 ± 1.8°C ($\Delta T = -7.5$°C) and 0.38 ± 0.13 J/g (enthalpy ratio = 0.37 ± 0.13), respectively; data are mean ± S.D. ($n = 3$) in all cases. The decrease in transition temperature was significant in matrices containing 5 ($P > 0.01$) and 10% cineole ($P > 0.00$). However, the decrease in enthalpies became significant at higher P-values of 0.4 and 0.2 for matrices containing 5 and 10% cineole, respectively. Matrices containing 40% cineole showed the T_6 transition as a single peak in one sample at 60.2°C ($\Delta T = -19.1$°C) with enthalpy of 0.67 J/g (enthalpy ratio = 0.65). However, instead of a single broad T_6 transition, several peaks were observed between 60–75°C in most samples of matrices containing 40% cineole. These peaks correlated with appearance of fanlike units in the system as shown by polarised light microscopy and might be related to melting of individual fatty acids; myristic, palmitoleic and stearic acids melt around 54, 63 and 70°C, respectively. Transition T_6 was absent in matrices containing 20 and 25% cineole.

Turning now to the comparison of the effects of cineole on the thermal behaviours of the model matrix and SC, we recall that SC shows four lipid-based endothermic transitions around 35, 55, 70 and 80°C (Goodman and Barry, 1989; Cornwell, 1993; Gay et al., 1994). Because of low enthalpies of the transitions at 35 and 55°C and their absence in some SC samples, most investigations regarding the effects of enhancers on the thermal behaviour of the SC have involved higher transitions which are absent in some model matrix samples.

One of the rare reports on the effects of enhancers on the 35°C transition of the SC (equivalent to T_4 in the model matrix) is by Goodman and Barry (1989) who showed that N-methyl-2-pyrrolidone, propylene glycol and dimethyl formamide increased the 35°C transition temperature by about 1–5°C. They revealed that the same penetration enhancers decreased the temperature of SC transitions of around 70 and 80°C by about 5–13°C. Yamane et al. (1995a) studied the effects of cineole on the SC transitions of 70 and 80°C and showed that cineole decreased the temperature of these two transitions by about 20 and 15°C, respectively. Considering the fact that cineole, N-methyl-2-pyrrolidone, propylene glycol and dimethyl formamide showed the same effect on the higher transitions (70 and 80°C), we may conclude that their effects on the transition at 35°C are possibly similar. If this conclusion is correct, cineole should, therefore, increase the transition temperature of 35°C in the SC, in good agreement with the matrix results.

To the best of our knowledge, there are no direct data available regarding the effects of cineole on the enthalpy of SC 35°C transition. However, using the effects of cineole on the enthalpies of SC transitions of around 70 and 80°C and classical thermodynamics calculations, Cornwell et al. (1996) showed that cineole is probably lipid disruptive around normal skin temperature (32°C) which might indicate that cineole decreases the enthalpy of 35°C transition of the SC, in correlation with the present results.

Using hydrated SC, Yamane et al. (1995a) showed that 1–12 h treatment of the SC with neat cineole decreased the temperatures of 70 and 80°C transitions (equivalent to matrix T_6 transition) by about 15–20°C. This observation correlates with our data which showed that cineole decreased the transition temperature of T_6 by about 8–19°C in matrices containing 5, 10 and 40% cineole. Cineole also decreased the enthalpies of T_6 equivalent SC transitions and produced enthalpy ratios of 0.94–0.45 for treatment times of 1–12 h (Yamane et al., 1995a). Such results correlate well with the matrix data which showed enthalpy ratios of 0.66, 0.37 and 0.65 in matrices containing 5, 10 and 40% cineole, respectively.

3.2. Effects of limonene on the structure of the matrix

3.2.1. Hot-stage light microscopy

Addition of limonene induced three general changes in the model matrix at room temperature as follows: (i) on increasing the limonene concentration, the oily streaks were gradually replaced by mosaic texture which itself decreased on increasing the terpene concentration; (ii) an apparently 'viscous isotropic' phase appeared in matrices containing 15–25% limonene; and (iii) contrary to the plain matrix, there was no lamellar-to-hexagonal phase transition before final T_i in limonene-treated systems studied from 25–160°C (matrices containing 15–25% limonene).

Matrices containing 5 and 10% limonene showed oily streaks and mosaic textures in polarised light (Fig. 3(a)), indicating the presence of a lamellar structure, at 25°C. There was no textural changes in these systems when samples were heated to 32°C.

Matrices containing 15% limonene showed a mosaic texture at 25 and 32°C in cross polarised light (not shown). In these systems, the proportion of mosaic texture was less than that of matrices containing 10% or less limonene and the oily streaks were not observed. During the heating process, the mosaic texture started to change to an isotropic liquid at around 40°C and at around 50°C little mosaic texture was left in an isotropic background. The background contained some non-liquid material which was isotropic under crossed polars but showed contrast in normal light (Fig. 3(b)). This isotropic phase was present in the system from 25°C. The gross character of this matrix was a moderately transparent and brittle semisolid with high consistency. Considering these features and its isotropic texture under polarised light, it is possible that the above mentioned non-liquid phase is a viscous isotropic (cubic) mesomorphic structure (Gilchrist et al., 1967; Winsor, 1968; Hartshorne, 1974). On increasing the temperature, while the viscous isotropic phase gradually liquefied, some mosaic texture appeared in the system around 60–70°C and after that, the whole system gradually changed to an isotropic fluid, a transition which completed around 120°C.

The matrices containing 20% limonene showed a mosaic texture at 25°C which started to become isotropic at around 30°C such that the proportion of mosaic texture in the system at 32°C was clearly less than that at room temperature (picture not shown). The mosaic texture continued to decrease on increasing the temperature and completely disappeared around 40°C. As was seen in matrices containing 15% limonene, the matrices containing 20% limonene also contained the viscous isotropic phase (Fig. 3(b)) at room temperature which started to liquefy around 95°C and melted completely around 105°C. Around 115°C, an apparently lamellar mesomorphic phase (Fig. 3(c)) appeared in the system which later changed to an isotropic phase around 125°C.

When the limonene content increased to 25%, the matrix showed a mixture of crystalline and mesomorphic phases dispersed in an isotropic liquid at room temperature (Fig. 3(d)). The meso-

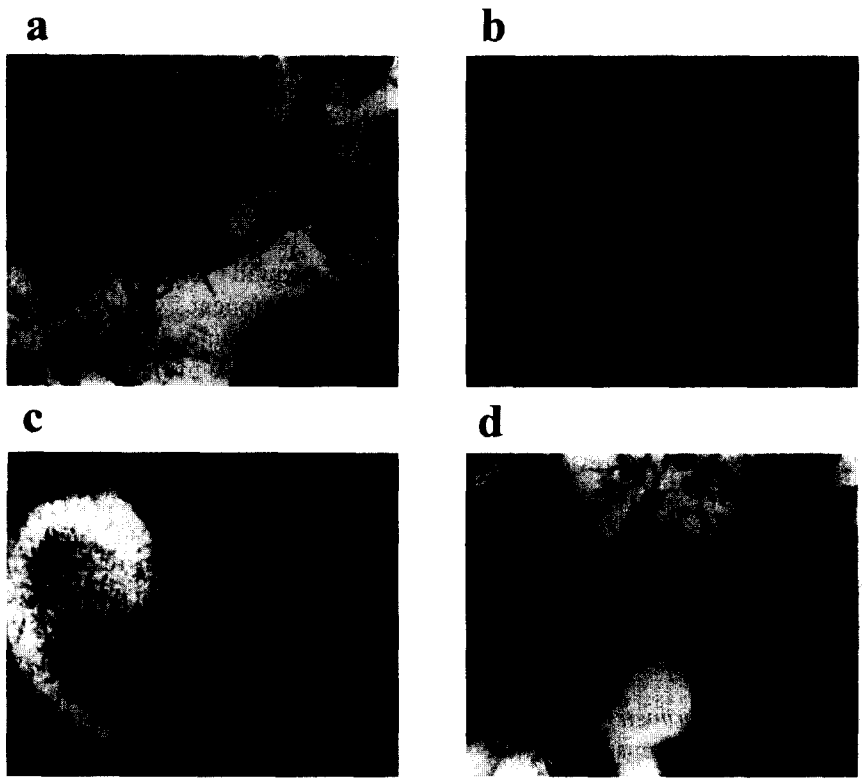

Fig. 3. Sample textures of limonene-treated matrices under cross polarised (a, c, d) and normal (b) light. (a) Oily streaks and mosaic textures in matrices containing 5% limonene at 25°C; (b) the apparently cubic phase in matrices containing 20% limonene; (c) a sample at 122°C illustrating the apparently lamellar structure appearing in matrices containing 20% and 25% limonene at high temperatures; and (d) mesomorphic and crystalline phases in matrices containing 25% limonene at 25°C.

morphic phase seems to be a mixture of hexagonal and lamellar structures, because it illustrates both batonnets and non-geometric textures (Fig. 3(d)). When the temperature increased, the birefringent areas started to disappear around 30°C and around 50°C only an apparently viscous isotropic phase was left in the system. Around 90°C the apparently cubic phase started to change to an isotropic fluid, a phenomenon which completed around 110°C. As was seen in the case of systems containing 20% limonene (Fig. 3(c)), an apparently lamellar mesomorphic phase appeared in the system containing 25% limonene around 115°C which later changed to an isotropic liquid around 125°C.

X-ray diffraction studies showed that after 12 h limonene-treatment, both lipid bilayers and areas of liquid enhancer co-exist in the SC. However, some SC samples did not show the small angle reflections attributed to lipid bilayers (Cornwell, 1993, Cornwell et al., 1994, 1996). As explained earlier, SC takes up the equivalent of 25% limonene in the model matrix after 12 h terpene treatment (Moghimi et al., 1996c). Our studies showed that matrices containing 25% limonene show crystalline lipids, a hexagonal mesophase and possibly a lamellar structure dispersed in a liquid phase, in reasonable agreement with the SC data.

As explained above, the addition of 15% or more limonene caused a lamellar-to-viscous isotropic phase transition in the matrix and when the limonene concentration increased to 25%, some hexagonal phases were observed. The same phenomenon was reported by Engblom and Engström (1993) who showed that increasing the

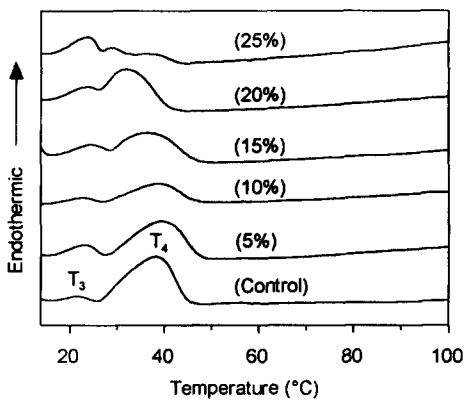

Fig. 4. Differential scanning calorimetry thermograms of the untreated model matrix (control) and matrices containing 5–25% (w/w) limonene.

concentration of Azone (a skin penetration enhance) in monoolein-water system induced a lamellar-to-cubic and then a cubic-to-hexagonal phase transition.

3.2.2. *Differential scanning calorimetry*

Fig. 4 illustrates the thermograms of matrices containing 0–25% limonene and Table 3Table 4 provide the related transition temperatures and enthalpies and their statistical comparisons. The limonene-treated matrices showed both T_3 and T_4 transitions, but higher temperature transitions (T_5 and T_6) were not observed in these systems. In matrices containing 25% limonene, the T_4 transition split into two peaks with mid-point temperatures of around 29.7 and 36.4°C. The transitions T_5 and T_6 arise from a change in the lamellar structure of the model matrix (growing of more mosaic texture) between 50 and 90°C. As shown by polarised light microscopy, there is no such change in matrices containing 20–25% limonene.

As shown by hot-stage light microscopy, the textural changes in limonene-treated matrices started around 30°C in matrices containing 20 and 25% limonene, while in untreated matrices, they began around 35°C. This change correlates with the decrease in the T_4 transition temperature in matrices containing 20 and 25% limonene (Table 4). The decrease in the enthalpy of T_4 transition after limonene treatment should be mostly due to replacement of the lamellar structure by a viscous isotropic phase which does not undergo a transition in the T_4 range.

To the best of our knowledge, there are no DSC data available on the effects of limonene on the thermal behaviour of the SC lipids around 35°C transition (equivalent to matrix T_4 transition). However, using the effects of limonene on the higher SC transitions (around 70 and 80°C) and classical thermodynamic analysis, Yamane et al. (1995b) and Cornwell et al. (1996) concluded that limonene does not interact with the SC lipids to disorganise them around 35°C. This finding contradicts both the stratum corneum X-ray diffraction studies (Cornwell et al., 1994, 1996) and our matrix results; the reason is not clear and requires further investigation.

Table 3
The effects of limonene concentration (% w/w) on the transition temperature (T) and transition enthalpy (ΔH) of T_3 transition of the model matrix as studied by differential scanning calorimetry

Limonene concentration	Transition temperature			Transition enthalpy		
	T (°C)	ΔT (°C)[a]	P-values	ΔH (J/g)	ΔH ratio[b]	P
0 (Control)	21.6 ± 0.4	0.0	—	1.12 ± 0.47	1.00	—
5	22.3 ± 0.2	0.7	0.04	1.89 ± 0.14	1.69 ± 0.12	0.05
10	23.2 ± 0.6	1.6	0.01	2.44 ± 0.13	2.18 ± 0.12	0.01
15	23.5 ± 0.3	1.9	0.00	3.07 ± 0.21	2.74 ± 0.19	0.00
20	23.7 ± 1.6	2.1	0.04	3.48 ± 0.40	3.11 ± 0.36	0.00
25	23.6 ± 1.8	2.0	0.11	5.20 ± 1.59	4.64 ± 1.42	0.01

Data are mean ± S.D. ($n = 3$–4). See Table 1 for explanation of P-values.
[a] Limonene treated − control.
[b] Limonene treated/control.

Table 4
The effects of limonene concentration (% w/w) on the transition temperature (T) and transition enthalpy (ΔH) of T_4 transition of the model matrix as studied by differential scanning calorimetry

Limonene concentration	Transition temperature			Transition enthalpy		
	T (°C)	ΔT (°C)[a]	P-values	ΔH (J/g)	ΔH ratio[b]	P-values
0 (Control)	38.2 ± 0.3	0.0	—	18.6 ± 1.1	1.00	—
5	38.5 ± 0.3	0.3	0.30	16.3 ± 0.5	0.88 ± 0.03	0.03
10	39.3 ± 0.3	1.1	0.02	14.9 ± 0.1	0.80 ± 0.00	0.00
15	38.1 ± 0.4	−0.1	0.70	13.0 ± 0.5	0.70 ± 0.03	0.00
20	32.5 ± 1.2	−5.7	0.00	10.9 ± 2.4	0.59 ± 0.13	0.00
25[c]	36.4 ± 0.5	−1.8	0.00	3.30 ± 3.42	0.18 ± 0.18	0.00

Data are mean ± S.D. ($n = 3$–4). See Table 1 for explanation of P-values.
[a] Limonene treated − control.
[b] Limonene treated/control.
[c] Another endothermic transition with a temperature of 29.7 ± 1.4°C ($\Delta T = -8.5$°C) and enthalpy of 2.50 ± 0.99 J/g (ΔH ratio = 0.13 ± 0.05) was observed in two out of four matrix samples containing 25% limonene. Data are mean ± S.D.

Finally, a new transition was observed between T_3 and T_4 in matrices containing 25% cineole (Table 1 and Fig. 2) or 25% limonene (Table 4 and Fig. 4). However, the polarised light microscopy studies revealed that the T_4 transition arises from two events in the untreated matrix, represented by the disappearance of some birefringent grains (minor change) and growth of a mosaic texture (major change). DSC experiments, even at a heating rate of 1°C/min, did not resolve the T_4 transition into two peaks, indicating that either these events are very close with respect to temperature or one of them is of very low enthalpy. The appearance of a new transition between T_3 and T_4 in matrices containing 25% terpenes might, therefore, be due to the separation of the above mentioned events.

3.3. Structure-diffusivity relationship

The effects of cineole and limonene on the barrier performance of the model matrix toward 5-FU and OE were investigated at 32°C (SC temperature) through release studies (Moghimi et al., 1996c) and the diffusivity ratio ((DR) the ratio of treated/untreated diffusion coefficients) were calculated. The release experiment temperature (32°C) is within the range of the T_4 transition. However, it has been suggested that the enthalpy ratio is a better indicator for the effects of enhancers on the SC lipids than is the transition temperature shift (Yamane et al., 1995a). Therefore, the T_4 enthalpy ratios will be used here for comparison of the matrix DSC results with release data.

3.3.1. Effects of cineole on the structure of the matrix-relation to enhancement

Addition of 5–25% cineole to the model matrix yielded diffusivity ratios of 1.5–13 for 5-FU and 1.6–2.4 for OE at 32°C and revealed that as the cineole concentration increases, the diffusivity ratios of 5-FU and OE increase (Fig. 5, see Moghimi et al., 1996c for more details). However,

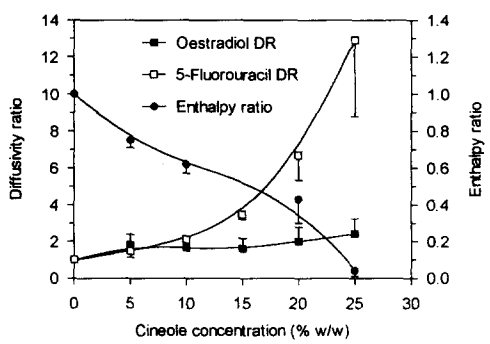

Fig. 5. Comparison of the effects of cineole on the structure of the model matrix (represented as T_4 enthalpy ratio) and its barrier properties (shown as diffusivity ratio DR). Ratios are cineole treated/untreated values. Data are mean and S.D., $n = 3$–6.

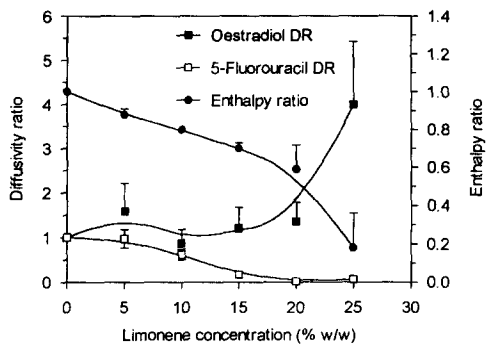

Fig. 6. Comparison of the effects of limonene on the structure of the model matrix (shown as T_4 enthalpy ratio) and its barrier performance (represented as diffusivity ratio DR). Ratios are limonene treated over untreated values. Data represent mean and S.D., $n = 3–4$.

as was shown earlier (Table 2), as the cineole concentration increases, the enthalpy of T_4 decreases. Polarised light microscopy studies showed that cineole disrupted the lipid bilayers and created a lipid pool in cineole-treated systems; both phenomena are responsible for the decrease in the enthalpy of the T_4 transition. Microscopic observations also revealed that the lipid pools are continuous in matrices containing 10% or more cineole. These observations suggest that 5-FU and OE transverse the matrix through the lipid pools and/or disturbed bilayers and/or interfacial regions, all of which have less resistance to diffusion than organised bilayers. From X-ray diffraction data (Cornwell et al., 1994, 1996) and differential scanning calorimetry studies (Yamane et al., 1995a), the same mechanisms are suggested for the effects of cineole on the permeation of drugs through the SC. The progressive increase in the diffusion coefficient on raising the cineole concentration should arise from the increase in the proportion of these more permeable structures.

3.3.2. Effects of limonene on the structure of the model matrix-relation to enhancement

As shown previously (Moghimi et al., 1996c), limonene decreased the diffusion coefficient of 5-FU successively in matrices containing 5–20% terpene (DR = 0.972–0.015, Fig. 6) and then the diffusion coefficient slightly increased when limonene concentration increased to 25% (DR = 0.066, Fig. 6). The decrease in the diffusion coefficient was significant ($P = 0.05$) for matrices containing 15–25% limonene. The effects of 5–20% limonene on the diffusion coefficient of OE through the model matrix (DR = 0.866–1.58, Fig. 6) was not significant ($P = 0.05$), but when the limonene concentration increased to 25%, the diffusion coefficient of oestradiol increased significantly (DR = 3.99, Fig. 6).

When the concentration of limonene in the matrix increased, the consistency of the system also rose up to a limonene concentration of 20% and then suddenly decreased in matrices containing 25% limonene where the matrix was partially liquefied. Note that the rheological behaviour of the matrices was not measured instrumentally and the consistency trend of limonene-treated matrices was estimated simply by manipulation with a spatula. The changes in the consistency may suggest why the diffusivity of 5-FU in the matrix decreased up to 20% and then increased when the limonene concentration increased to 25%, but cannot explain why the diffusivity of 5-FU in matrices containing 25% limonene, which is liquefied, is still around 15 times less than that of the ordered plain matrix. However, the diffusivity of OE in the model matrix did not fall as drastically as the limonene concentration increased. Therefore, another mechanism rather than simple consistency change could be involved.

As shown by light microscopy, the lamellar structure was replaced by an apparently viscous isotropic phase in limonene-treated matrices. In systems containing 25% limonene, the matrix showed also some crystalline lipids and hexagonal liquid crystalline phases dispersed in a liquid phase. If these mesomorphic structures are of reversed type (i.e. the matrices contain a continuous lipophilic phase), 5-FU (a hydrophilic molecule) would be expected to accumulate in the dispersed hydrophilic phase of the system and favourable partitioning toward this internal phase would render the drug almost unavailable to the continuous lipophilic phase. After liquefaction of the matrix, the diffusivity is expected to increase. The diffusivity ratio of 5-FU increased almost four times after matrix liquefaction relative to matrices containing 20% limonene but still was 15

times less than that of untreated matrix. This shows that the rate-limiting step for the release of 5-FU from the limonene-treated matrices (partitioning from an internal hydrophilic to an external lipophilic phase) cannot be compensated completely by liquefaction of the model matrix.

The diffusivity of OE in the matrix is almost constant for matrices containing 5–20% limonene which may be due to two opposing phenomena; (i) the increase in the matrix consistency which would decrease the diffusion coefficient and (ii) replacement of lamellar with a reversed viscous isotropic phase which creates a continuous lipophilic medium and consequently removes the OE partitioning step between hydrophilic and lipophilic layers of the lamellar structure. When the matrix liquefied at 25% limonene content, OE diffusivity ratio suddenly increased three times presumably due to decreased viscosity of the system.

Fig. 6 illustrates the effects of limonene on the enthalpy of the T_4 transition and diffusion coefficients of 5-FU and OE and illustrates that as the limonene concentration increases, the enthalpy of T_4 decreases and the diffusion coefficient of OE increases and that of 5-FU decreases. The decrease in the enthalpy of T_4 is mostly related to a decrease in the proportion of the lamellar structure and its replacement with a viscous isotropic phase which does not undergo a transition around T_4.

In summary, the effects of cineole and limonene on the structure of the model matrix were investigated by hot-stage microscopy and differential scanning calorimetry. Results showed that cineole breaks the model matrix into a dispersed system in which a lamellar mesomorphic structure is in equilibrium with an isotropic liquid at 25 and 32°C, in good correlation with the effects of cineole on the intercellular lipids of the SC. Increasing the concentration of limonene caused initially a decrease in the oily streaks, then a lamellar-to-viscous isotropic phase transition and finally, changed the matrix to a dispersed system of crystalline and mesomorphic phases in a continuous liquid phase at 25 and 32°C. The effects of limonene on the structure of the model matrix is in good agreement with that reported for intercellular lipids of SC from X-ray diffraction results, but contradict DSC studies of the SC.

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