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Mechanisms of action of novel skin penetration enhancers: Phospholipid versus skin lipid liposomes

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Abstract

Employing thermal analysis, we investigated the mechanism of action of novel enhancers and probed phospholipid (PL) versus stratum corneum lipid (SCL) liposomes as model membranes. The enhancers included octyl salicylate (OS), padimate O (PADO) and 2-(1-nonyl)-1,3-dioxolane (ND). The negative controls were the empty liposomes. Positive controls employed dimethylsulfoxide (DMSO) and Azone™ (AZ). For PL liposomes, DMSO sharpened the transitions. AZ abolished the pre-transition, broadened the main transition and linearly reduced its transition temperature (T_m). OS or PADO reduced T_m and size of pre-transition, broadened the main transition and decreased its T_m (non-linearly). ND abolished the pre-transition but increased T_m of the main endotherm, suggesting retardation rather than enhancement. The results of SCL correlated with PL liposomes except for ND. In SCL liposomes, ND reduced T_m and broadened the peaks indicating lipid disruption, which indicated its enhancing effects. In conclusion, OS, PADO and ND can enhance drugs by disrupting intercellular lipid domain but they differ from AZ in terms of the relationship between efficacy and concentration. Although PL liposomes are simple model membranes with sharp transitions which give detailed information about the effects of enhancers, they can provide misleading results. Simultaneous use of other models like SCL liposomes is recommended.

Keywords: Skin penetration enhancers; Mechanisms; Model membranes; Stratum corneum lipid liposomes; High sensitivity differential scanning calorimetry

1. Introduction

Transdermal drug delivery has many advantages over other routs of drug administration. However, the barrier nature of the skin makes it difficult for most drugs to be delivered through skin (Barry, 1983). The

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use of penetration enhancers is one of the strategies employed to improve percutaneous absorption of drugs. New candidates for use as safe skin penetration enhancers have been developed. Examples of these enhancers include 2-(1-nonyl)-1,3-dioxolane (ND) (Diani et al., 1995), octyl salicylate (OS) and padimate O (PADO) (Morgan et al., 1998a,b,c). Knowledge of the mechanism of action of these enhancers will allow formulators to further optimise the formulation so as to maximise the efficacy of enhancers. Penetration enhancers may act by disrupting the packing of skin lipids and thus altering the barrier nature of the stratum corneum, by changing the partitioning behaviour of the drug at the stratum corneum-viable epidermis interface or by affecting the thermodynamic activity of the drug (Barry, 1987; Beastall et al., 1988).

Many techniques have been employed to investigate the mechanisms of action of enhancers. These included skin permeation, pre-treatment and partitioning studies, spectroscopic investigations and thermal analysis (e.g. Goodman and Barry, 1985, 1986; Barry, 1987; Golden et al., 1987; Bouwstra et al., 1989; Kia et al., 1990; Yamane et al., 1995; Lawson et al., 1998). Ideally, human skin should be employed throughout these investigations (Barry, 1983). However, the lack of supply of suitable human skin as well as the inherent variability has forced researchers to use animal skin or even model bilayer membranes. The model membranes used include liposomes of stratum corneum lipids, which have been mainly employed in partitioning, diffusion and fluorescent probe studies (e.g. Yoneto et al., 1996; De la Maza et al., 1998; Kirjavainen et al., 1999) with very few thermal analysis investigations (Kim et al., 1993). The simpler phospholipid liposomes have mainly been used as model membranes in transition temperature and thermal analysis studies (e.g. Beastall et al., 1988; Rolland et al., 1991; Bonina et al., 1994; Hadgraft et al., 1996; Inoue et al., 2001; El Maghraby et al., 2004; Auner et al., 2005). In the present study we employ high sensitivity differential scanning calorimetry (HSDSC) to probe the mechanism of action of novel penetration enhancers with the goal of comparing phospholipid liposomes (PL) with stratum corneum lipid liposomes (SCLL) as model membranes. The test enhancers included OS, PADO and 2-(1-nonyl)-1,3-dioxolane (ND, commercially known as SEPA^{TM}). Azone^{TM} (AZ) and dimethylsulfoxide (DMSO), which are believed to act by two different mechanisms (Barry, 1987), were employed as positive controls. As a negative control, the corresponding empty liposome formulation was adopted.

2. Materials and methods

2.1. Materials

Cholesterol (99%), dipalmitoylphosphatidylcholine (DPPC) (99%), palmitic acid (99%) and DMSO (99.5%) were from Sigma Chemical Company, St. Louis, MO, USA. Ceramide III (96%) and ceramide IIIB (>90%) were from Cosmoferm, The Netherlands. Cholesterol sulphate sodium salt was from Northern Lipids Inc., Canada. OS and PADO were from Bronson and Jacobs, Australia. AZ was obtained from NexMed, Inc., Lawrence, KS, USA. ND was synthesized by a method employing Dean and Stark technique for preparation of acetal (Samour and Daskalakis, 1989).

The chemical structures of the tested enhancers are presented in Fig. 1.

2.2. Preparation of liposomes

Phospholipid liposomes containing fixed DPPC concentration (3.33 mg/ml) and increasing concentrations of the required penetration enhancer (0–50 mol%,

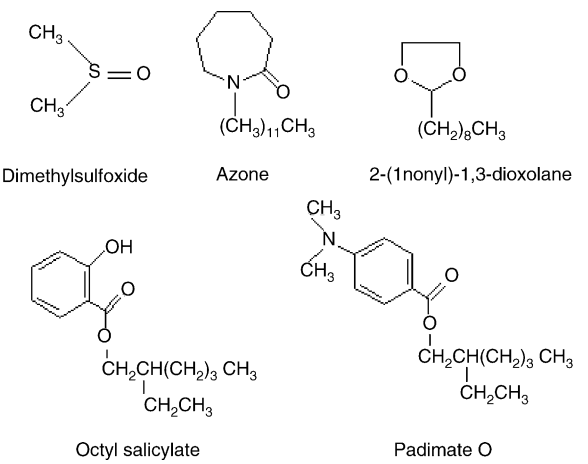

Fig. 1. Chemical structure of the skin penetration enhancers used in this study.

enhancer/lipid) were prepared by mechanical shaking. Briefly, DPPC and the required amount of enhancer were dissolved in chloroform, methanol; 2:1. The organic solvent was evaporated under a stream of nitrogen gas at 50 °C (above the lipid transition temperature). The deposited lipid film was hydrated with distilled water for 1 h by intermittent vortexing and heating at 50 °C. The liposome dispersion was left to swell at room temperature for 2 h. For complete hydration vesicles were stored at 4 °C overnight. These vesicles were degassed by bath sonication for 3 min before microcalorimetric analysis.

SCL liposomes having fixed concentration of 5 mg/ml were prepared by mechanical shaking. The lipid mixture (ceramide III, ceramide IIIB, cholesterol, palmitic acid, cholesterol sulfate; 20:20:25:25:10) was dissolved in chloroform, methanol (9:1). The required enhancer was added as methanolic solution in a quantity sufficient to produce an enhancer concentration of 5 mmol in the final liposome formulations. The solvent was evaporated and the lipid film was hydrated as before but at 80 °C. Complete hydration and degassing was achieved as for PL.

This method was shown to produce large multilamellar vesicles with penetration enhancers increasing the vesicle size (Auner et al., 2005). Our study concentrated on monitoring the effect of penetration enhancers on the phase behaviour of liposomes which is directly related to the mechanism of action of penetration enhancers.

2.3. High sensitivity differential scanning calorimetry (HSDSC)

The instrument used was Nano II Differential Scanning Calorimeter, model 6100, CSC, Spanish Forkes, Utah, USA. The system is controlled by NDSC data acquisition and Cpcalc software. The NDSC unit comprises two identical capillary cells, one for the reference and one for the sample. The cells are constructed of tantalum, which has excellent resistance to most common reagents. The cells are enclosed in a thermal jacket, which is heated and cooled at a constant rate. The cells have a volume of 0.3268 ml. The temperature difference between the two cells, measured by semiconductor thermoelectric battery and amplified by signal amplifier is maintained near zero by applying an appropriate power difference to the two cells using the electric heaters that are attached directly to the cell body and controlled by a feedback control algorithm and power compensation bridge. This power difference is also recorded as a signal by the computer. Degassed sample and reference (water) were loaded into the corresponding cell using an automatic pipette. Both reference and sample cells were filled completely to maintain equal volumes of reference and sample and to guarantee equal sample volume and thus similar amount of lipid from run to run. Measurements were performed at constant excess pressure (3 atm), which prevents formation of air bubbles and boiling of samples above 100 °C. The samples and reference were then heated from 10 to 70 °C at a rate of 1 °C/min (PL liposomes) or from 20 to 110 °C at a rate of 2 °C/min (SCL liposomes). A baseline was run before each determination (using the corresponding heating rate) by loading the reference solution (water) in both the sample and reference cells; this was subtracted from individual results on data analysis.

2.4. Data analysis

For each sample the reference was subtracted and the excess heat capacity function was normalised for phospholipid concentration (PL liposomes). The HSDSC trace is a plot of the excess heat capacity as a function of temperature. The following parameters are calculated (Lo and Rahman, 1995):

(i) The transition midpoint, T_m, is the temperature at which the transition is half completed (the peak point).
(ii) The transition enthalpy, ΔH, is the actual heat required for the entire transition, normalized per mol. This is calculated from the area under the transition peak.
(iii) The temperature width at half peak height, $\Delta T_{1/2}$, is the sharpness of the phase transition. This parameter is very sensitive to the presence of any impurities. It will be taken as a measure for the cooperativity of the transition.

Because the SCL liposomes were formulated from a mixture of lipids the excess molar heat capacity was not calculated and the data will be presented as the heat flow as function of temperature. Only the T_m will be calculated for individual transition peaks.

The Student's *t*-test was used for statistical analysis.

3. Results

3.1. The phase behaviour of pure PL and SCL liposomes

Pure liposomes of the corresponding lipid composition were used as negative controls in this study. Fig. 2(a and b, top trace) shows typical HSDSC trace of pure DPPC liposomes. In agreement with the published data (e.g. Mabrey-Gaud, 1981; Rolland et al., 1991; Lo and Rahman, 1995; El Maghraby et al., 2004), pure PL liposomes of DPPC showed typical thermotropic phase behaviour with a pre-transition endotherm between 35 and 36 °C and the main endotherm between 41 and 42 °C. The subtransition endotherm was not detected. This is expected as the subtransition can only be observed if the vesicles are incubated at low temperature for several days. This subtransition has been reported to have no analytical significance (Biltonen and Lichtenberg, 1993).

Fig. 2 (c and d, top trace) illustrates HSDSC trace of pure SCL liposomes (control) and Table 1 presents the obtained T_m value for SCL liposomes. Pure SCL liposomes showed three main transitions (Fig. 2, c and d, upper traces). The first transition (T1) had a T_m at 43.8 °C (Table 1). The second endotherm (T2)

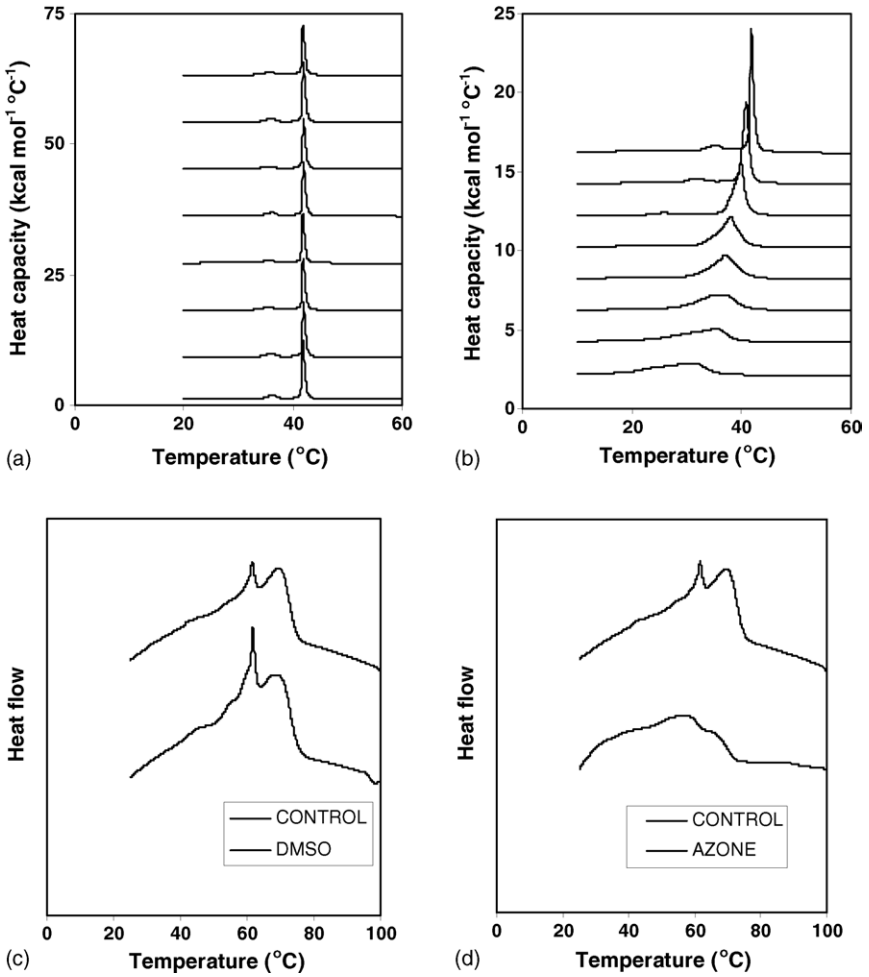

Fig. 2. Examples of the HSDSC traces of DPPC liposomes, containing (from top to bottom) 0, 5, 10, 16.7, 20, 25, 33.3 and 50 mol% of DMSO (a), or AZ (b) and traces of SCL liposomes containing 0 (top) or 5 mmol (bottom trace) of DMSO (c) or AZ (d).

Table 1
Effects of different enhancers on the transition temperature of stratum corneum lipid liposomes

	T_m (°C)		
	T1	T2	T3
Control	43.8 (1.9)	61.7 (0.058)	70.1 (0.38)
DMSO	44.3 (0.60)	61.7 (0.058)	70.2 (0.25)
AZ	Very broad[a]	56.4 (0.10)***	65.6 (0.25)***
OS	44.1 (3.4)	61.2 (0.21)*	66.0 (1.2)*
PADO	43.3 (0.97)	60.9 (0.26)*	67.7 (0.36)**
ND	Disappeared	56.7 (3.1)	66.7 (0.26)**

All enhancers were included at 5 mM concentration. Values between brackets are S.D., $n = 3$.
[a] The T1 in case of AZ was so flat that it was difficult to determine its T_m.
* $P < 0.05$ (significantly different from the control).
** $P < 0.01$ (significantly different from the control).
*** $P < 0.001$ (significantly different from the control).

showed a T_m at 61.7 °C. The third peak revealed a T_m at 70.1 °C.

3.2. Effects of DMSO on the phase behaviour of PL and SCL liposomes

DMSO was used as the first positive control. Fig. 2(a and c) shows the effects of DMSO on the phase behaviour of DPPC and SCL liposomes. Fig. 3a–c shows the parameters calculated for the main transition endotherm of DPPC liposomes relative to DMSO concentration in these vesicles. DMSO (Fig. 2a) produced no significant changes in the transition behaviour of DPPC liposomes even when its concentration was increased to 50 mol% (enhancer/lipid). However there was a trend of increasing the sharpness of the main transition as indicated from the slight reduction in $\Delta T_{1/2}$ (Fig. 3c). This is in agreement with the published reports (Rolland et al., 1991).

In SCLL, DMSO produced a change in the shape of T2 with the transition becoming sharper although there was no significant ($P > 0.05$) effect on the T_m of all transitions, compared with the corresponding empty liposomes (Fig. 2c and Table 1).

3.3. Effects of AZ on the phase behaviour of PL and SCL liposomes

Incorporation of AZ in DPPC liposomes changed the phase behaviour (Fig. 2b). At low concentrations, AZ reduced the T_m and the size of the pre-transition, before abolishing it completely at concentrations equal to or more than 20 mol%.

Fig. 3d–f shows the parameters calculated for the main transition endotherm of DPPC liposomes containing increasing concentrations of AZ. AZ reduced the T_m of the main transition significantly ($P < 0.001$), compared with the empty liposomes. There was a linear relationship between this T_m and AZ concentration (Fig. 3d). There were no significant changes ($P > 0.05$) in the enthalpy of the main transition. AZ also broadened the main endotherm as indicated by the increased $\Delta T_{1/2}$, but this effect was not linear with AZ concentration. The main transition showed asymmetry towards the lower temperature side.

Incorporation of AZ into SCL liposomes broadened and reduced the sizes of all transitions with T1 nearly disappearing (Fig. 2d). The T_m values of T2 and T3 were significantly reduced ($P < 0.001$) after incorporation of AZ (Table 1).

3.4. Effects of OS on the phase behaviour of PL and SCL liposomes

Fig. 4a shows examples of HSDSC traces of DPPC liposomes containing increasing concentrations of OS. Fig. 5a–c illustrates the effects of OS on the transition parameters calculated for the main transition endotherm of DPPC vesicles. Incorporation of OS in DPPC liposomes reduced the T_m and the size of the pre-transition (Fig. 4a). OS also significantly reduced ($P < 0.01$) the T_m and broadened the main endothermic peak. As for AZ, the main transition showed an asymmetry towards the lower temperature. It should be noted that there was no linear relationship between the reduction in the T_m and the OS concentration (Fig. 5). Thus increasing OS concentration in liposomes from 0 to 10 mol% produced a gradual decrease in the T_m. Above 10 mol% there was sharp decrease in the T_m up to 20 mol%, above which the decrease in the T_m was marginal. Similarly, the increase in $\Delta T_{1/2}$ was not linear with OS concentration. There was a trend of reduced enthalpy of the main transition with OS concentration (Fig. 5b).

The effect of OS on the phase transitions of SCL liposomes is presented in Fig. 4c. Incorporation of OS in SCL liposomes broadened T2 and reduced the T_m of

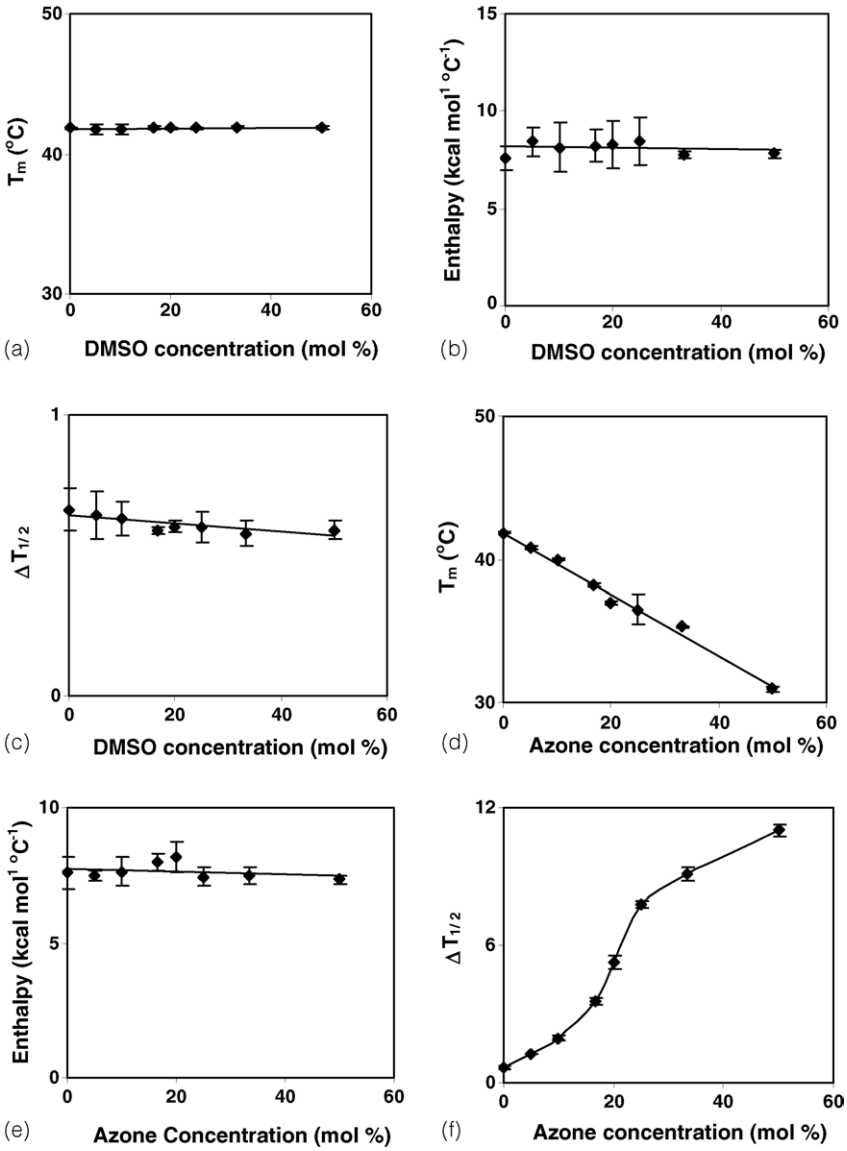

Fig. 3. Effects of different concentrations of DMSO (a–c) or AZ (d–f) on the transition parameters of the main endotherm of DPPC liposomes. $\Delta T_{1/2}$ is the temperature width at half peak height.

both T2 and T3 (Fig. 4c and Table 1). Reduction in the size of T3 was also noted.

3.5. Effects PADO on the phase behaviour of PL and SCL liposomes

Fig. 4b shows examples of HSDSC traces of DPPC liposomes containing increasing concentrations of PADO. Fig. 5d–f illustrates the effects of PADO on the transition parameters of the main transition endotherm of DPPC vesicles. As for OS, incorporation of PADO in DPPC vesicles reduced the T_m and the size of the pre-transition with the pre-transition becoming very weak at high enhancer concentrations. The main transition showed a reduction in the T_m with the transition becoming significantly broader ($P < 0.01$).

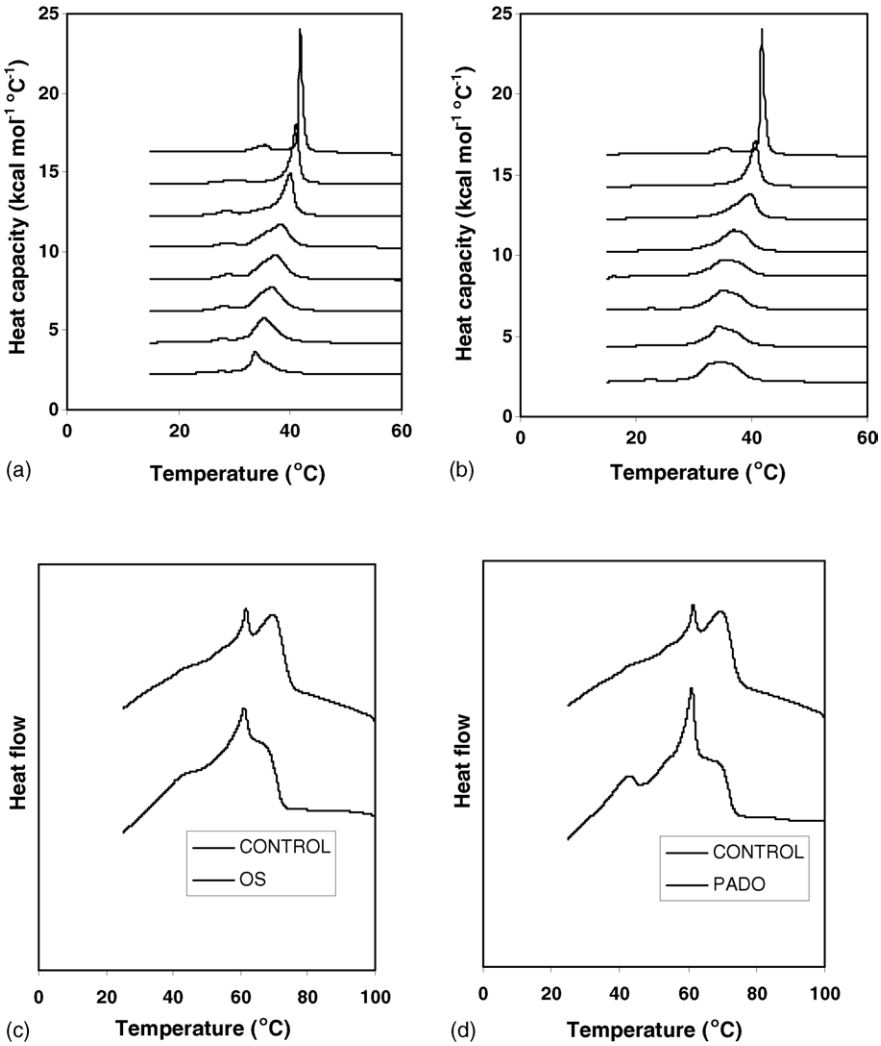

Fig. 4. Examples of the HSDSC traces of DPPC liposomes, containing (from top to bottom) 0, 5, 10, 16.7, 20, 25, 33.3 and 50 mol% of OS (a), or PADO (b) and traces of SCL liposomes containing 0 (top) or 5 mmol (bottom trace) of OS (c) or PADO (d).

The main transition also became asymmetric towards the lower temperature (Fig. 4b). Again there were no linear relationship between the T_m and PADO concentration (Fig. 5d). Thus increasing PADO concentration from 0 to 10 mol% showed a slight reduction in the T_m. Increasing PADO concentration above 10 mol% resulted in sharp decrease in the T_m up to 25 mol% above which the reduction in T_m was marginal. The increase in the peak width showed a similar trend (Fig. 5f). There was a trend of reduction in the enthalpy with increasing PADO concentration.

The effect of PADO on the phase transitions of SCL liposomes is presented in Fig. 4d. PADO reduced the T_m of T2 and T3 of the SCL liposomes (Table 1). There was an increase of the sizes of T1 and T2 and a decrease in the size of T3 (Fig. 4d).

3.6. Effects of ND on the phase behaviour of PL and SCL liposomes

Fig. 6a shows examples of the HSDSC traces of DPPC liposomes containing increasing concentrations

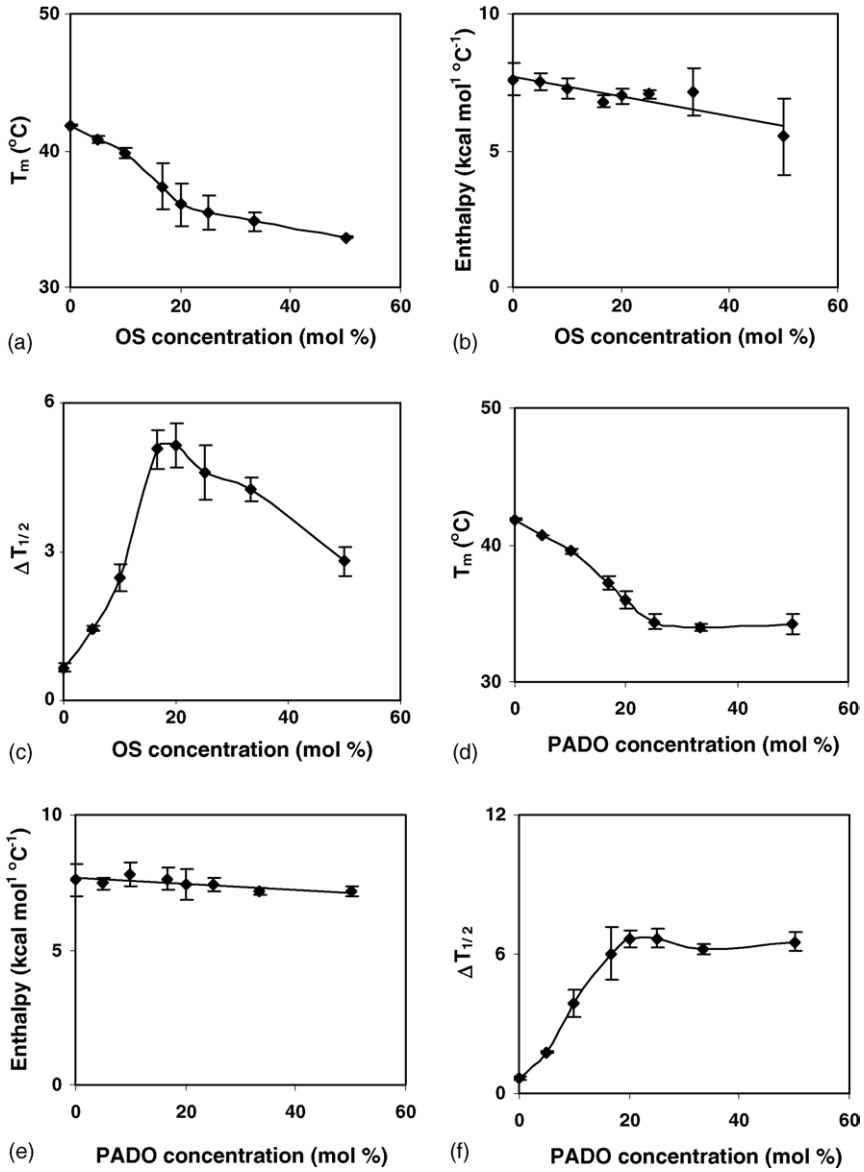

Fig. 5. Effects of different concentrations of OS (a–c) or PADO (d–f) on the transition parameters of the main endotherm of DPPC liposomes. $\Delta T_{1/2}$ is the temperature width at half peak height.

of ND. Fig. 7 illustrates the effects of ND on the transition parameters of the main transition endotherm of DPPC vesicles. In DPPC liposomes, increasing ND concentration reduced the size of the pre-transition endotherm but increased its T_m, before eventually removing the pre-transition at concentrations equal to or more than 25 mol% (Fig. 6a). For the main transition there were no significant changes in the T_m up to 25 mol% where there was a significant increase in the T_m ($P < 0.001$). There was a trend of increased enthalpy with increasing ND concentration (Fig. 7). The $\Delta T_{1/2}$ was increased along with the T_m up to 25 mol% then decreased again but remained higher than that of the control.

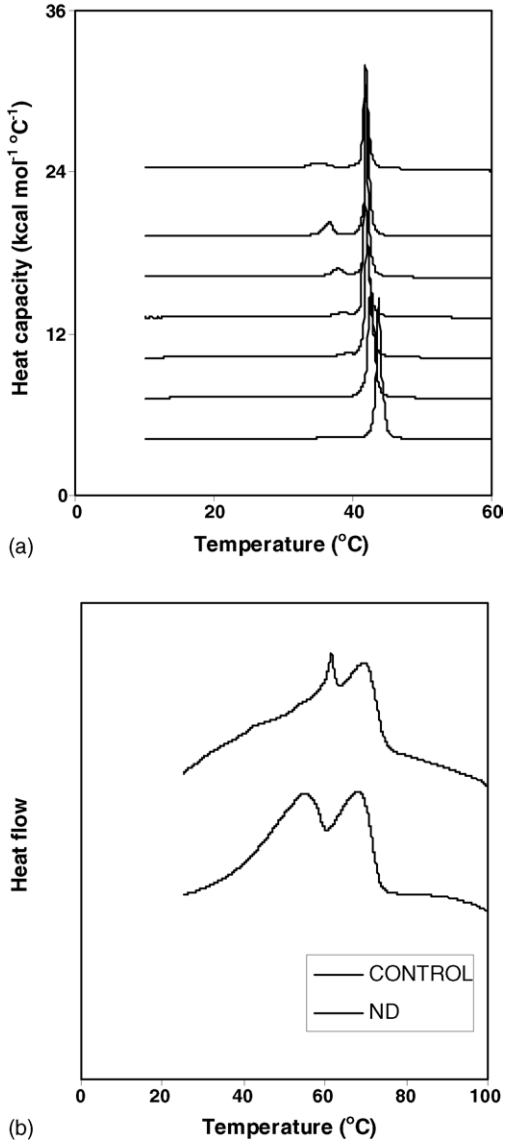

Fig. 6. Examples of the HSDSC traces of DPPC liposomes, containing (from top to bottom) 0, 5, 10, 16.7, 20, 25, and 33.3 mol% of ND (a) and traces of SCL liposomes containing 0 (top) or 5 mmol (bottom trace) of ND (b).

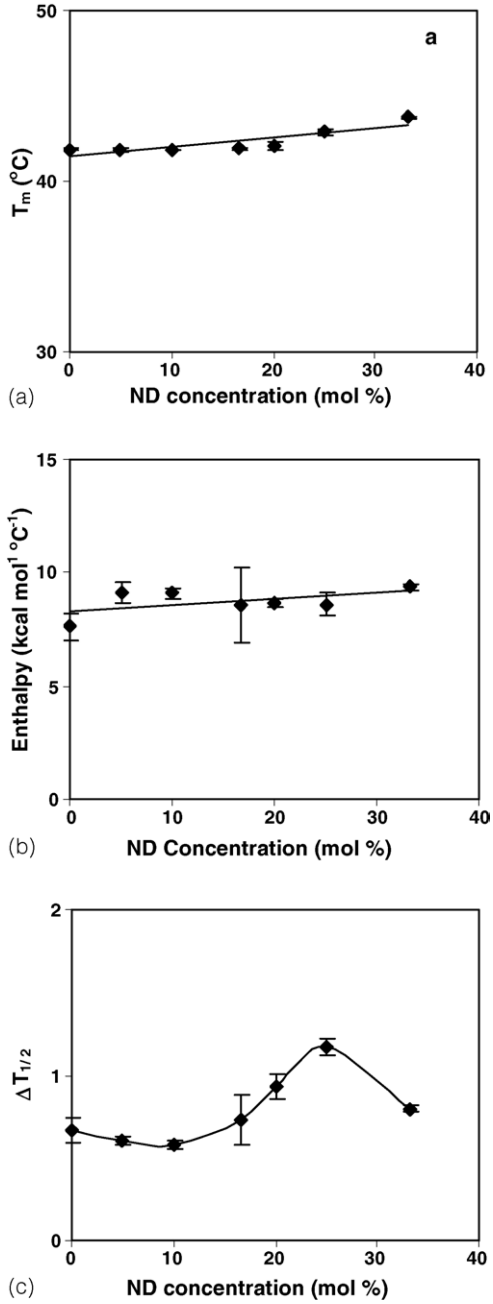

Fig. 7. Effects of different concentrations of ND on the transition parameters of the main endotherm of DPPC liposomes. $\Delta T_{1/2}$ is the temperature width at half peak height.

Fig. 6b presents the effect of ND on the phase transition of SCL liposomes. The behaviour observed with ND incorporated into SCL liposomes was different from that obtained with the PL liposomes (Fig. 6b). In SCL liposomes, ND abolished T1 completely. It also reduced the T_m of T2 and T3 with the peaks becom-

ing broader compared with the corresponding control (pure SCL liposomes). This effect observed with the SCL liposomes is consistent with the reported interaction of ND with human skin (Morganti et al., 1999).

4. Discussion

Before discussing the interactions of enhancers with the model membranes it is important to understand the origin of transitions obtained with the negative controls (pure DPPC or SCL liposomes). Pure DPPC liposomes showed a pre-transition endotherm followed by the main transition (see e.g. Fig. 2b upper trace). Fig. 8a shows the conformational changes in the phospholipid bilayer structure along with its phase transition. The origin of the pre-transition could be the rotation of the phospholipid head groups or the conformational changes in the bilayer. The main transition is due to chain melting (Janiak et al., 1976). Pure DPPC vesicles undergo distinct structural changes at the phase transition temperature (Fig. 8a). Below the pre-transition temperature, the bilayer lipids are in the highly ordered gel state ($L_{\beta'}$) with the hydrocarbon chains in an all-trans configuration (tilted one-dimensional arrangement). The reason for this arrangement is that the head groups of the phospholipids are relatively bulky taking more space compared with the hydrocarbon chains. The hydrocarbon chains thus tilt relative to the plane of the membrane to fill the extra space created by the head groups (New, 1990). At the pre-transition temperature, lipids change from the tilted one-dimensional arrangements to two-dimensional arrangements with periodic undulations (rippled gel phase, $P_{\beta'}$). Above T_m, trans/gauche rotational isomerisation along the chains laterally expands and decreases the thickness of the bilayer and the system reverts to one-dimensional arrangements. Thus the lipids become more fluid (liquid crystalline phase L_α) (Janiak et al., 1976; Rolland et al., 1991).

A simple model for the lipid bilayer structure within the intercellular spaces of human stratum corneum has been proposed (Fig. 8b) (Barry, 1987). The model revealed a bilayer structure with ceramides being infiltrated with cholesterol, fatty acids, cholesterol esters and triglycerides. This arrangement is different from that of the simple DPPC liposomes in which lipid molecules are arranged sequentially (Fig. 8a). This suggests that liposomes made of SCL with a lipid arrangement more like that in human skin might be a better model.

DSC has been employed to investigate the thermal phase transition of human stratum corneum by Goodman and Barry (1985, 1986) who showed four endothermic transitions. The first one (around 40 °C) has been attributed to melting of sebaceous lipids or cholesterol side-chain motion. The second transition (around 70 °C) is due to melting of the lipid chain portion buried within the bilayer. The third one (around 80 °C) arises from the break-up of associations between the lipid polar head group together with disruption of cholesterol-stiffened regions. The fourth transition (around 100 °C) results from denaturation of the intracellular protein. It should be noted that the position of these transitions was affected by the degree of skin hydration (Barry, 1987). For our SCL liposomes we obtained three main transitions (see e.g. Fig. 2c and Table 1). The first (T1) was at 43.8 °C and can be attributed to movement of cholesterol side-chain. The second transition (T2), which was detected at 61.7 °C, can be attributed to melting of the cholesterol free regions. The last one (at 70.1 °C) can be linked to the break-up of the association of the head groups together with melting of cholesterol-rich regions. Three transitions were similarly reported for SCL liposomes but at different temperatures with the transition at about 100 °C being attributed to evaporation of bound water (Kim et al., 1993). The difference between our results and those of Kim et al. (1993) can be attributed to the difference in the heating rate used as they employed a higher heating rate (5 °C/min). As the results reported here employed a technique that allowed scanning under pressure, the evaporation of water was prevented.

DMSO is a polar penetration enhancer ($\log P = -1.35$). Accordingly it will not be expected to partition in high amounts into the lipid domains of the bilayer. Its effective molar ratio (enhancer/lipid) in the lipid domains will be far less than the total molar ratio as it will mostly reside in the aqueous regions of the vesicles. DMSO is believed to enhance skin penetration of drugs due to many factors. It may displace bound protein–water substituting a looser structure. Being a powerful solvent that can mix isothermally with water, it can displace water from the lipid head groups creating larger solvation shells around these head groups. Moreover due to

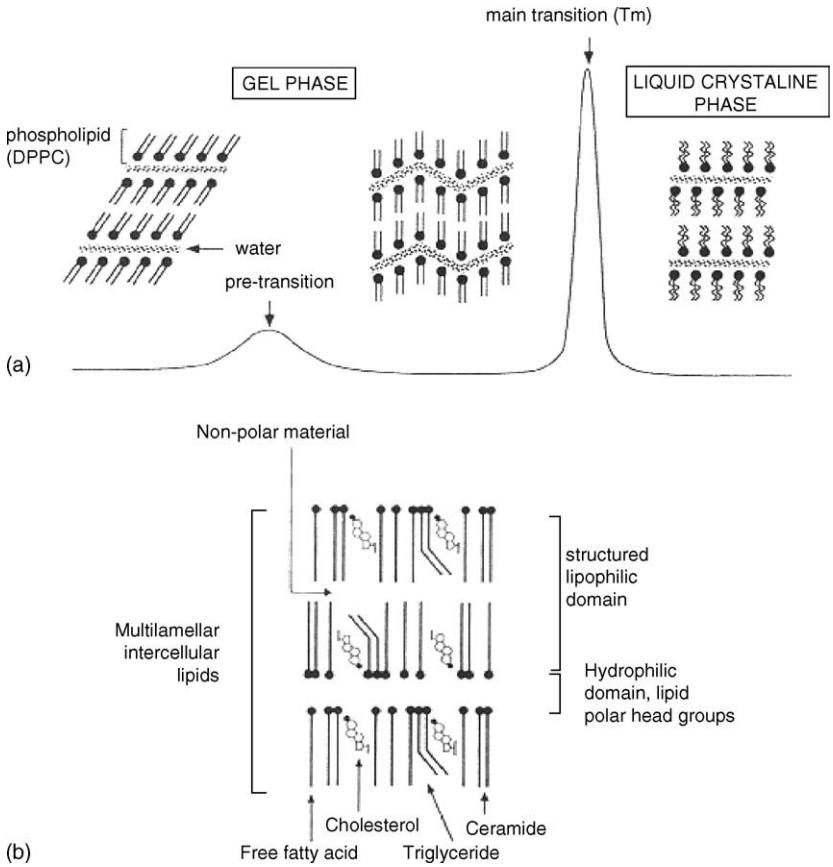

Fig. 8. Conformational changes in the phospholipid bilayer structure along with its phase transition (a) (after Rolland et al., 1991) and the proposed model for the lipid bilayer structure within the intercellular spaces of human skin (b) (after Barry, 1987).

its solvent power, high levels of sulfoxide within the membrane can improve drug partitioning and thus increase the flux (Barry, 1987). In terms of interactions with membranes, an interaction was reported with human skin only at high DMSO concentrations (Barry, 1987). However, there were no interactions with DPPC liposomes even at 60 mol% DMSO (Rolland et al., 1991). In our study, we were able to show only a trend of increased cooperativity of the main transition (increased sharpness i.e. reduced $\Delta T_{1/2}$) of DPPC liposomes with increasing DMSO concentration. For the SCL liposomes there was a noticeable change in the peak shape, particularly T2, which became sharper and more intense after incorporation of DMSO. Sharpening of the endothermic peaks was linked with increased hydration of SC (Lee and Tojo, 1998). Based on our results we should be able to draw similar conclusion to that reported earlier (Barry, 1987), particularly the formation of larger solvation shells around the head group, which is evidenced here by peak sharpening (increased cooperativity of transition) in both model membranes.

AZ is a lipophilic penetration enhancer ($\log P$ = 6.28). Accordingly it will be expected to partition into the lipophilic domains of the bilayers. In DPPC liposomes AZ reduced the T_m and size of the pre-transition before removing the whole pre-transition completely at a concentration of 20 mol%. This indicates that AZ was able to compensate for the difference in bulkiness between the head groups and the acyl chains of the phospholipid. Accordingly, the tilted one-dimensional arrangement, which is responsible for the pre-transition

will no longer exist. This explains the absence of the pre-transition. The effect on the pre-transition is dependent on the molecular volume of the enhancer as well as its ability to intercalate into the bilayer structure. Accordingly, OS and PADO which have a relatively smaller molecular volume reduced the T_m and size of the pre-transition but failed to abolish it. ND removed the pre-transition at 25 mol% but its effect at lower concentrations was different (see below). AZ can fit into the phospholipid bilayer with its oxygen level with the head group and the rest of the molecule inserted between the acyl chains. This can produce significant perturbation in the packing characteristics of the phospholipid membrane. Accordingly, AZ was able to reduce the T_m of the main transition. This effect was linear with AZ concentration. A similar relationship was reported after monitoring the T_m of DPPC liposomes with different AZ concentrations, by turbidimetry (Beastall et al., 1988). AZ broadened the main transition with the endotherm becoming asymmetric towards the lower temperature side. This effect is due reduction of the cooperativity of the transition (Lo and Rahman, 1995). The broadness increased with concentration but this was not linear. In SCL liposomes AZ showed significant effects on the transition compared with the control. Again AZ reduced the T_m of T2 and T3, broadened the transitions and reduced their sizes with T1 nearly disappearing. This indicates that AZ affected both cholesterol rich and cholesterol free domains in the SCL liposomes.

These effects of AZ strongly indicate that AZ exerts a significant disruption in the packing characteristics of the model lipid membranes. This will produce a looser structure. A correlation between such effect and the increase of drug flux was reported (Golden et al., 1987). Accordingly, lipid disruption can be taken as a mechanism of action of AZ as skin penetration enhancer. A similar conclusion has been reported after thermal analysis of human SC, which was treated with AZ (Goodman and Barry, 1985; Barry, 1987; Bouwstra et al., 1989). Similar conclusions have been drawn from experiments using DPPC liposomes and monitoring the effect of AZ on the T_m with either turbidimetry or DSC (Beastall et al., 1988; Rolland et al., 1991).

OS, a commonly used sunscreen was reported to have a penetration enhancing effect (Morgan et al., 1998a). It is a lipophilic ester, which is expected to have high affinity to the lipid domains in the SC. When incorporated in DPPC vesicles, OS reduced the T_m and size of the pre-transition endotherm. It was not able to eliminate the pre-transition completely. Accordingly, the tilted one-dimensional arrangement did exist but the effect on the pre-transition indicates that this arrangement was highly perturbed. OS can fit into the lipid bilayer with its hydrophilic part level with the PL head groups and the rest of the molecule parallel to the acyl chain. This could give more freedom to the acyl chains. Its effect on the main transition endotherm revealed significant reduction in the T_m, broadening of the transition with asymmetry towards the lower temperature. This can be attributed to reduction in the cooperativity of the main transition. The effect on the T_m and $\Delta T_{1/2}$ was not linear with OS concentration (Fig. 5). Self-association was reported for OS with increasing concentration in liquid paraffin (Jiang et al., 1997). The process of self-association can be used here to explain the non-linear relationship between the T_m and OS concentration. Thus it may be possible that OS molecules started to associate together at high concentration with the result that the reduction in T_m changed from significant to marginal at high concentration. It should be noted that the obtained trend of reduced enthalpy with increasing OS concentration could be due to the formation of a new species, which has a T_m outside our range of study. This can explain further the initial increase of $\Delta T_{1/2}$ followed by decrease.

In SCL liposomes, OS reduced the T_m of both T2 and T3. There was a noticeable reduction in the size of T3 with T1 becoming clearer. Reduction of the size of T3 again indicates the formation of a new species, which melts at a temperature close to that of T1 and hence the increase in the size of T1. These results suggest that OS is able to insert within the lipid bilayers of both DPPC and SCL liposomes exerting a significant perturbation for the bilayer structure rendering it disordered. This membrane disrupting effect can be taken as a mechanism of action of OS as skin penetration enhancer.

PADO is another lipophilic sunscreen for which a penetration enhancing effect has been reported (Morgan et al., 1998a,b,c). It is a lipophilic ester with a slightly different structure from OS, whereby the phenolic hydroxyl group of OS is absent and there is a dimethylamino group in the *para*-position of the aromatic ring (Fig. 1). It is also expected to partition into the lipid domains of the bilayer structure of liposomes. In DPPC liposomes PADO showed a pattern of interac-

tion close to that of OS with some variation. There was a reduction in T_m and size of the pre-transition. The main transition showed significant reduction in the T_m up to certain concentration above which there was no further effect. Along with this the $\Delta T_{1/2}$ increased with PADO concentration before reaching a plateau. These results suggest a possible self-association or pooling process for PADO in DPPC liposomes at high concentration. It could be postulated that PADO can fit into the bilayer with its ester residue level with the head groups and the aliphatic chain as well as the aromatic ring, (probably kinked so that both can fit simultaneously) parallel with the acyl chains. This can significantly disrupt the bilayer structure increasing its fluidity. In SCL liposomes the T_m of T2 and T3 was reduced. Also there was a reduction in the size of T3, with T1 and T2 being increased in size. This again suggests the formation of new species with a T_m at lower temperature. Also the results indicate interaction of PADO with both cholesterol-stiffened and cholesterol-free regions.

ND is another lipophilic compound, which is expected to preferentially partition into the lipid domains of the bilayer structure. Incorporation of ND into the DPPC liposomes produced non-expected results for a penetration enhancer. At low concentrations the size of the pre-transition was significantly reduced but its T_m was increased before being abolished completely at 25 mol%. For the main transition there was no significant effect below 25 mol%. At concentrations of 25 mol% or more the T_m of the main endotherm was increased with the peak becoming broader. ND can fit into the bilayer with its head (dioxolane ring) level with the head groups and the aliphatic chain parallel with the acyl chains of the phospholipids. It was able to add bulkiness to the acyl chain and thus compensated for the bulkiness of the head groups. Thus the chains will no longer tilt which can explain the absence of the pre-transition. ND, being a dioxolane derivative with two oxygen atoms in 1,3 position, can create hydrogen bonds with each of two adjacent DPPC molecules. This can immobilise the phospholipids and thus add more structure to the bilayer. This can explain the increase in the T_m and the apparent increase in the enthalpy of the main transition. The behaviour of ND in DPPC liposomes suggested that ND could work as penetration retarder rather than penetration enhancer. Similar behaviour was reported for N-0915 (a compound having oxygen atoms in a *trans*-position) when incorporated in DPPC liposomes and it was considered as a possible penetration retarder (Hadgraft et al., 1996). However, it is difficult to classify ND as a penetration retarder when other researchers have reported an enhancing effect, which was proved further using thermal and spectroscopic analysis of human SC (Diani et al., 1995; Morganti et al., 1999). It should also be noted that insignificant enhancing ability was reported for ND in propylene glycol. This was attributed to low concentration, vehicle combination or inability to alter the thermodynamic activity of hydrocortisone (Fuhrman et al., 1997).

In contrast to the above finding, employing the SCL liposomes, ND produced a noticeable reduction in the T_m values and broadened the peaks of T2 and T3, compared with empty SCL liposomes. T1 was also abolished. These effects suggested that ND is able to penetrate into the lipid domains of the SCL liposomes disrupting the packing characteristics of the bilayer structure resulting in a looser structure. It was able to affect both cholesterol-stiffened and cholesterol-free regions. These results agree with the published data on the interaction of ND with human SC (Morganti et al., 1999).

The results obtained here with ND with both DPPC and SCL liposomes highlight the importance of proper selection of model membranes with SCL liposomes being a more realistic model for human SC. It is useful to understand the reasons behind the different results obtained with DPPC and SCL liposomes. As mentioned early in this section, the arrangement of the lipid molecules in SCL liposomes is closer to that proposed for human skin whereby the ceramides are infiltrated with cholesterol, cholesterol esters and fatty acids. This is different from that of DPPC liposomes where phospholipid molecules are packed sequentially and can be immobilised by a compound like ND. Another important factor to consider is that the chain interdigitation of ceramides (in human SC as well as in SCL liposomes), which play an important role in the rigidity of the bilayer structure, can be easily disrupted if any compound is intercalated between the ceramide molecules. This can provide a satisfactory explanation for the difference between DPPC and SCL liposomes as models for human SC. It also explains the obtained effects of ND on both cholesterol free and cholesterol-stiffened domains of SCL liposomes.

5. Conclusions

OS, PADO and ND can enhance skin penetration of drugs at least partly via disrupting the intercellular lipid domains. This will decrease the barrier nature of the SC and reduce the diffusional resistance. Enhancers like these can enhance the skin delivery of compounds with balanced hydrophilic–lipophilic properties (Beastall et al., 1988). For enhancing the skin delivery of lipophilic molecules combination with appropriate solvents is essential.

Although DPPC liposomes are the most extensively used model membrane due to simplicity and ability to produce detailed information about interactions of xenobiotics with biological membranes, our study highlighted the possibility for misleading results in some cases. Accordingly, for skin studies, simultaneous use of another model like SCL liposomes is highly recommended.

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