Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 276 (2004) 143–161
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Interactions of surfactants (edge activators) and skin penetration enhancers with liposomes

G.M.M. El Maghraby, A.C. Williams, B.W. Barry*

Drug Delivery Group, The School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK

Received 14 July 2003; received in revised form 19 February 2004; accepted 21 February 2004

Abstract

Incorporating edge activators (surfactants) into liposomes was shown previously to improve estradiol vesicular skin delivery; this phenomenon was concentration dependent with low or high concentrations being less effective. Replacing surfactants with limonene produced similar behaviour, but oleic acid effects were linear with concentration up to 16% (w/w), beyond which it was incompatible with the phospholipid. This present study thus employed high sensitivity differential scanning calorimetry to probe interactions of additives with dipalmitoylphosphatidylcholine (DPPC) membranes to explain such results. Cholesterol was included as an example of a membrane stabiliser that removed the DPPC pre-transition and produced vesicles with a higher transition temperature (T_m). Surfactants also removed the lipid pre-transition but reduced T_m and co-operativity of the main peak. At higher concentrations, surfactants also formed new species, possibly mixed micelles with a lower T_m. The formation of mixed micelles may explain reduced skin delivery from liposomes containing high concentrations of surfactants. Limonene did not remove the pre-transition but reduced T_m and co-operativity of the main peak, apparently forming new species at high concentrations, again correlating with vesicular delivery of estradiol. Oleic acid obliterated the pre-transition. The T_m and the co-operativity of the main peak were reduced with oleic acid concentrations up to 33.2 mol%, above which there was no further change. At higher concentrations, phase separation was evident, confirming previous skin transport findings.

Keywords: Surfactants; Liposomes; High sensitivity differential scanning calorimetry; Skin; Penetration enhancer; Edge activators

1. Introduction

Skin delivery of estradiol (ES) from lipid vesicles containing surfactants (sodium cholate, Span 80 and Tween 80) or penetration enhancers (oleic acid and limonene) was studied previously (El Maghraby et al., 2000a,b). Too high or too low concentrations of surfactants in liposomes were less effective in delivering ES through human epidermis in vitro. Thus, increasing surfactant concentration in liposomes raised the maximum flux of ES up to a particular concentration, beyond which the flux decreased. This reflected the need to optimise individually the additive concentration in lipid vesicles. These additives, termed "edge activators", have been reported to confer elastic properties to lipid vesicles, allowing dramatically improved delivery of encapsulated agents to and through skin (Cevc et al., 1996).

Span 80 and Tween 80 were found to be as effective as sodium cholate as edge activators in phosphatidylcholine (PC) vesicles (El Maghraby et al., 2000a). Replacing surfactants with oleic acid produced a direct

* Corresponding author. Tel.: +44-1274-234760; fax: +44-1274-234769.
E-mail address: B.W.Barry@Bradford.ac.uk (B.W. Barry).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.02.024

relationship between the transepidermal flux of ES and oleic acid concentration. However, oleic acid concentrations above 16% (w/w) were difficult to investigate due to an incompatibility between oleic acid and PC. In contrast, replacing surfactants by limonene produced a trend similar to that obtained with surfactant-containing vesicles, where the maximum flux of ES increased on increasing limonene concentration in the vesicles to a optimal concentration, beyond which the flux decreased. This indicates that penetration enhancement is not the main factor in vesicular delivery of ES to skin, since a declining effect with concentration would not be expected, especially as the drug was used at saturation (maximum thermodynamic activity) in all systems. In addition, the results suggest that limonene may be a possible candidate as an effective edge activator (El Maghraby et al., 2000b).

Accordingly, the aim of this work is to investigate interactions of edge activators and penetration enhancers (oleic acid and limonene) with vesicular membranes and to correlate such interactions with edge activating properties. Cholesterol, a well-known membrane stabiliser, was included as a positive control to compare its effects with those obtained with the edge activators (surfactants) or with penetration enhancers.

Differential scanning calorimetry has been used to investigate the interactions of cholesterol, surfactants, penetration enhancers, drugs and proteins with lipid membranes (Papahadjopoulos et al., 1975; Ganesan et al., 1984; Rolland et al., 1991; Spink et al., 1991; McMullen et al., 1993, 1999; Lo and Rahman, 1995; Castile et al., 1998). Here, we have employed high sensitivity differential scanning calorimetry (HSDSC) to monitor the interactions of cholesterol, surfactants and penetration enhancers with phospholipid bilayers. Although phosphatidylcholine (PC) was used as the main vesicular lipid in skin permeation studies, especially those involving surfactants and penetration enhancers, dipalmitoylphosphatidylcholine (DPPC) was selected as a model phospholipid in the calorimetric studies. This choice was based on the fact that DPPC is one of the most widely used models for studying interactions with lipid bilayers. Its transition temperature (T_m) can be easily measured as it has a narrow main endothermic peak (Montenegro et al., 1996) and further structural information can be gleaned from effects on the pre-transition peak of the lipids. In addition, the distribution coefficients for sodium cholate in the two lipid systems are very similar; 0.05 for PC/water (Almog et al., 1986) and 0.049 for DPPC/water (Bayerl et al., 1989), suggesting similar interactions of the surfactant with both phospholipids. The concentrations of each additive tested included those used in the skin experiments, with additional concentrations included to obtain clearer profiles of additive/vesicle interactions.

2. Materials and methods

2.1. Materials

Dipalmitoylphosphatidylcholine (DPPC) (99%), (+)-limonene (97%), oleic acid (95%), sodium cholate (99%), sorbitan monooleate (Span 80) and polyoxyethylene sorbitan monooleate (Tween 80) were purchased from Sigma Chemical Co., St Louis, MO, USA. Cholesterol (99%) was from Aldrich Chemical Co., UK. Water was double distilled.

2.2. Preparation of liposomes

Lipid vesicles containing fixed concentration of DPPC (5 mg/ml) with increasing concentrations of the test additives were prepared by mechanical shaking. The phospholipid and the additive (cholesterol, surfactants or penetration enhancer) were dissolved in chloroform:methanol, 2:1 v/v. 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 swollen at room temperature for 2 h. These vesicles were stored at 4 °C overnight to ensure complete hydration, degassed by bath sonication for 3 min and analysed by HSDSC.

2.3. High sensitivity differential scanning calorimetry

A Micro Calorimetry System Differential Scanning Calorimeter (MCS DSC) from MicroCal Inc., Northampton, MA, USA, was used; the system was controlled by MicroCal observer data acquisition software linked to the MicroCal Origin data analysis software. The instrument was calibrated for heat capacity using electrical pulses of known power; temperature calibration employed sealed paraffin hydrocarbon standards of known melting transition temperatures.

Degassed sample and reference (water) were loaded into the corresponding cell using a needle syringe, which was introduced to just above the bottom of the DSC cell. Firm and rapid injection of the last 100–200 μl removed any trapped air bubbles. Both reference and sample cells were filled completely to maintain equal volumes and thus to standardise the amount of sample lipid from run to run. The samples and reference were then heated from 10 to 60 °C at 1 °C/min. A baseline, run before each determination by loading the reference solution (water) in both sample and reference cells, was subtracted from individual results on data analysis.

2.4. Data analysis

For each sample the reference data were subtracted and the excess heat capacity function was normalized for phospholipid concentration. The HSDSC trace is a plot of the excess heat capacity as a function of temperature. According to Lo and Rahman (1995), the following parameters were calculated:

(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 and can be taken as a measure for the co-operativity of the transition; $\Delta T_{1/2}$ is inversely proportional to co-operativity (co-operative units measure the number of phospholipid molecules undergoing simultaneous transition).

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

3. Results

3.1. Thermotropic phase behaviour of pure DPPC liposomes

Throughout this study, pure DPPC liposomes were used as the control (zero concentration of additives). Fig. 1 illustrates an HSDSC trace of pure DPPC vesicles (top trace). Pure DPPC vesicles showed a typical

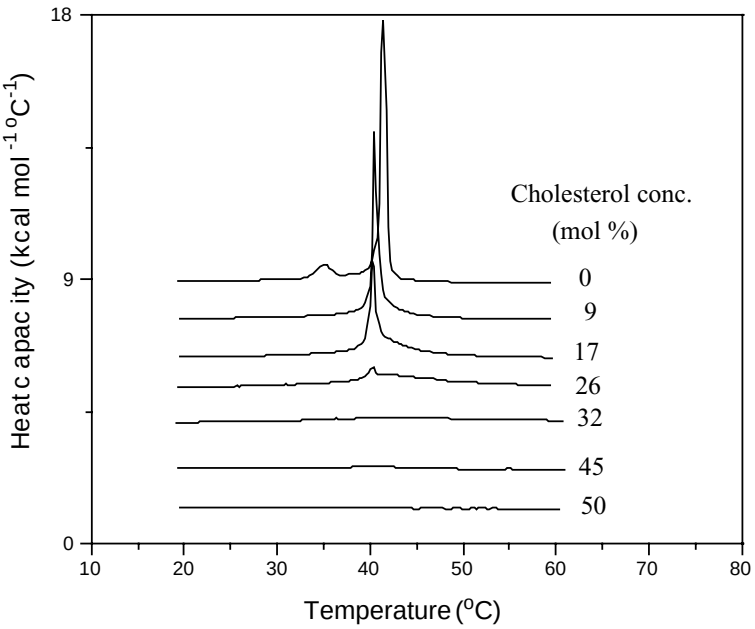

Fig. 1. Examples of the high sensitivity differential scanning calorimetric traces of dipalmitoylphosphatidylcholine liposomes, containing increasing concentrations of cholesterol.

Table 1
Parameters of the main transition of dipalmitoylphosphatidylcholine liposomes containing cholesterol or sodium cholate

	R_e	T_m (°C)	Enthalpy (kcal/mol)	$\Delta T_{1/2}$ (°C)
Cholesterol concentration (mol%)				
0		41.43 (0.028, 5)	8.4240 (1.1, 5)	0.7242 (0.0030, 5)
9		40.42 (0.070, 3)	4.7720 (0.20, 3)	0.7210 (0.0017)
17		40.19 (0.0038, 3)	3.8600 (0.24, 3)	0.8435 (0.12, 3)
26		40.23 (0.083, 3)	1.9670 (0.34, 3)	2.8870 (0.49, 3)
32		43.06 (1.4, 3)	0.7203 (0.067, 3)	2.0500 (0.97, 3)
45		39.34 (0.036, 3)	0.5201 (0.14, 3)	6.0200 (1.7, 3)
50		No transition	–	–
Cholate (mol%)				
0	0	41.43 (0.028, 5)	8.424 (1.1, 5)	0.7242 (0.0030, 5)
8.2	0.022	41.15 (0.032, 3)	7.708 (1.1, 3)	0.7243 (0.0015, 3)
15.9	0.047	40.91 (0.013, 3)	7.710 (0.54, 3)	0.7253 (0.0055, 3)
21.7	0.069	40.67 (0.26, 3)	7.666 (0.28, 3)	0.7230 (0.0027, 3)
29.9	0.11	40.82 (0.052, 4)	8.053 (0.35, 4)	0.7858 (0.12, 4)
42.2	0.18	40.44 (0.19, 4)	7.646 (0.60, 4)	0.8350 (0.14, 4)
55.6	0.31	40.23 (0.14, 3)	7.379 (0.056, 3)	0.8113 (0.097, 3)
66.7	0.50	39.18 (0.62, 3)	5.295 (0.67, 3)	1.5300 (0.74, 3)
75.0	0.75	37.59 (0.34, 3)	4.790 (0.48, 3)	2.6600 (1.9, 3)
80.0	1.0	24.58 (0.78, 3)	4.467 (0.48, 3)	22.4200 (3.8, 3)

Cholate is expressed as total concentration (mol%) and as effective molar ratio (R_e). $\Delta T_{1/2}$ is the temperature width at half peak height and R_e is the effective molar ratio of cholate to DPPC. Values within parentheses are S.D. and number of replicates, respectively. Mean values to four significant figures to allow presentation of S.D.

profile with a pre-transition peak at 35.58 °C and the main transition peak at 41.43 °C, agreeing with published data (Mabrey-Gaud, 1981; Rolland et al., 1991; Lo and Rahman, 1995). No post-transition peaks were detected by this technique compared with those recorded by FT–Raman spectroscopy (Lawson et al., 1998).

3.2. Interactions of cholesterol with phospholipid membrane

HSDSC investigated the effects of cholesterol, a membrane stabiliser, on the thermotropic phase behaviour of DPPC bilayers to compare these effects with those produced by edge activators and enhancers. Fig. 1 shows examples of the HSDSC traces of DPPC liposomes containing increasing concentrations of cholesterol.

Incorporating cholesterol into DPPC liposomes at 9–50 mol% removed the pre-transition endotherm of pure DPPC vesicles (Fig. 1) agreeing with reports that cholesterol incorporated into bilayers above 5 mol% abolished the pre-transition endotherm (New, 1990; McMullen et al., 1993, 1994; McMullen and McElhaney, 1995).

Table 1 summarises the parameters calculated for the overall main transition endotherm of DPPC vesicles containing cholesterol. Fig. 1 shows that incorporating cholesterol into DPPC vesicles produced asymmetric endothermic peaks with a shoulder towards the higher temperature side of the transition.

Table 1 revealed that the transition temperature decreased with increasing cholesterol concentration up to 26 mol%, where it started to increase again. The enthalpy decreased with increasing cholesterol content and reached zero at 50 mol% where no endothermic transition was detected, possibly due to formation of species with no detectable transition at the temperature range studied. The temperature width at half peak height ($\Delta T_{1/2}$), which is inversely proportional to the co-operativity of the transition, increased with cholesterol up to 32 mol% then fell, but remained higher than the control before disappearance of the transition.

3.3. Interactions of edge activators (surfactants) with phospholipid membrane

Three surfactants—sodium cholate, Tween 80 and Span 80—were studied. Tween 80 and Span 80 are mixtures but our presentation here considers the main

species. Before dealing with the interactions of individual surfactant with the lipid bilayer, it is relevant to comment on the affinity of these surfactants for lipid and water. Accordingly, the HLB (hydrophilic/lipophilic balance), which gives a measure of the physicochemical properties of surfactants in terms of their affinity for, or solubility in, water or lipids, can be considered. HLB values are 4.3 and 15 for Span 80 and Tween 80, respectively (Attwood and Florence, 1983) and we calculated the HLB for sodium cholate to be 16.7. Based on these HLB values, the affinity for lipids is expected to be in the order of Span 80 > Tween 80 > sodium cholate. Considering the distribution of surfactant between lipid and aqueous components of the liposomes, there will be an effective molar ratio (R_e) of surfactant to lipid. This effective molar ratio describes the actual amount of surfactant in liposomes relative to the lipid concentration, and can be calculated (Almog et al., 1986); we aim to correlate our previous findings on transdermal estradiol delivery from PC vesicles containing different edge activator concentrations with interactions detected between DPPC and various edge activators at several concentrations. Since the permeation experiments used liposomes containing 50 mg/ml of lipids but the HSDSC interaction investigation employed 5 mg/ml lipid, it is important to compare systems in terms of the molar ratios of lipid to edge activator. The distribution coefficients for sodium cholate in the two lipid systems are very similar; 0.05 for PC/water (Almog et al., 1986) and 0.049 for DPPC/water (Bayerl et al., 1989). The latter value was used to calculate R_e for sodium cholate.

$$R_e = \frac{D_t}{L + 1/K}$$

where D_t is the total detergent concentration (mM), L is the total lipid concentration (mM) and K is the distribution coefficient of surfactant between lipid and water, which is governed by

$$K = \frac{D_b}{LD_w}$$

where D_b is the detergent concentration in the bilayer (mM) and D_w is detergent concentration in water (mM).

For Tween and Span we did not calculate the R_e as the K values were not available. Data are thus presented as total molar ratios (surfactant/lipid) with surfactants tested at higher concentration ranges compared with those tested in the skin work to ensure full coverage of the possible interactions between surfactants and vesicles.

3.3.1. Interactions of sodium cholate with lipid membrane

A wide range of concentrations of sodium cholate (from zero up to 80 mol%, total molar concentration of cholate to lipid) in DPPC liposomes was studied (Table 1). Fig. 2 shows the effects of (a) lower concentration of sodium cholate on the pre-transition peak (snapshot shown to emphasise pre-transition) and (b) high cholate concentration on the main transition endotherm.

Increasing sodium cholate concentration abolished the pre-transition endotherm (Fig. 2), which was only detected for the control (0 mol%) and with the lowest tested concentration (8.2 mol%, total cholate). At 8.2 mol%, the pre-transition endotherm had a T_m of $28.27 \pm 0.47\,^{\circ}C$, enthalpy of 0.13 ± 0.0090 kcal/mol and $\Delta T_{1/2}$ of $2.49 \pm 0.14\,^{\circ}C$ (S.D., $n = 3$), compared with T_m of $35.58 \pm 0.35\,^{\circ}C$, enthalpy of 0.7880 ± 0.24 kcal/mol and $\Delta T_{1/2}$ of $1.977 \pm 0.27\,^{\circ}C$ (S.D., $n = 5$), for the control. Above 8.2 mol% the pre-transition enthalpy was abolished completely (Fig. 2a). Thus, at low concentration of sodium cholate, the T_m of the pre-transition decreased, the enthalpy reduced and the transition broadened, compared with the control. Increasing cholate concentration removed the pre-transition endotherm between 8.2 and 15.9 mol%.

Incorporating sodium cholate into DPPC liposomes at low levels did not change the shape of the main transition endotherm. At 42.2 mol% ($R_e = 0.18$) the transition started to broaden. It became asymmetric on the lower temperature side with 55.6 mol% cholate ($R_e = 0.31$). The broadening increased and the asymmetry became clearer at higher cholate levels, suggesting the formation of a new species that has a broad transition at lower T_m. Eventually at 80 mol% ($R_e = 1$), this new species became prominent (Fig. 2). Table 1 clarifies these effects. The T_m gradually decreased with increasing cholate concentration but fell sharply at 80 mol% where the new species was prominent. There was no significant change ($P > 0.05$) in the transition enthalpy until a cholate concentration of 66.7 mol% ($R_e = 0.5$) where the enthalpy signif-

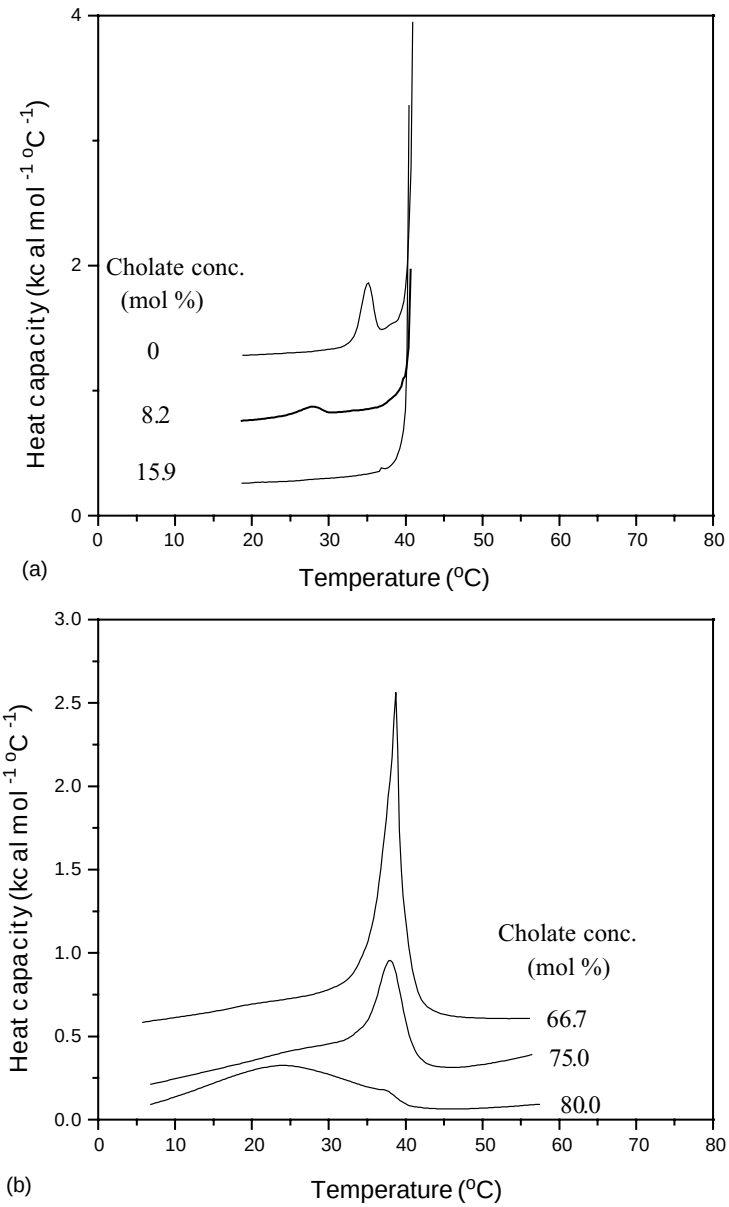

Fig. 2. Effect of sodium cholate at (a) low concentrations on the pre-transition endotherm of dipalmitoylphosphatidylcholine vesicles and (b) high concentrations on the main transition endotherm of DPPC liposomes.

icantly decreased ($P < 0.01$). This decrease continued with increasing cholate concentration. The temperature width at half peak height, which is inversely proportional to the co-operativity of the transition, increased along with decreasing enthalpy. This indicates reduced co-operativity of the transition, which can be attributed to the presence of more than one species. Bayerl et al. (1989) reported similar results.

3.3.2. Interactions of Tween 80 with lipid membrane

Tween 80, a nonionic surfactant with a large head group (containing about 20 polyoxyethylene units) and

Table 2
Effect of Tween 80 on the phase behaviour of dipalmitoylphosphatidylcholine liposomes

Tween concentration (mol%)	T_m (°C)	Enthalpy (kcal/mol)	$\Delta T_{1/2}$ (°C)
Pre-transition			
0	35.58 (0.35, 5)	0.7880 (0.24, 5)	1.977 (0.27, 5)
2.9	33.80 (0.11, 3)	0.9573 (0.034, 3)	3.005 (0.12, 3)
5.9	32.48 (0.016, 3)	0.6970 (0.076, 3)	2.852 (0.27, 3)
10.0	31.63 (1.3, 3)	0.5000 (0.076, 3)	3.055 (0.14, 3)
12.3	31.46 (1.4, 3)	0.5920 (0.0010, 3)	2.814 (0.14, 3)
19.4	31.36 (1.8, 3)	0.4953 (0.25, 3)	2.811 (0.37, 3)
27.2*	–	–	–
48.0*	–	–	–
Main transition			
0	41.43 (0.028, 5)	8.424 (1.1, 5)	0.7242 (0.0030, 5)
2.9	41.15 (0.0010, 3)	8.144 (0.22, 3)	0.7232 (0.0050, 3)
5.9	40.92 (0.012, 3)	8.006 (0.015, 3)	0.7253 (0.0038, 3)
10.0	40.74 (0.30, 3)	7.656 (0.090, 3)	0.8080 (0.13, 3)
12.3	40.65 (0.43, 3)	7.372 (0.38, 3)	0.8833 (0.28, 3)
19.4	40.50 (0.29, 3)	6.876 (0.76, 3)	1.2890 (0.97, 3)
27.2	40.71 (0.097, 3)	7.012 (0.56, 3)	2.7480 (0.13, 3)
48.0	40.46 (0.14, 3)	5.589 (0.43, 3)	2.9670 (0.36, 3)

$\Delta T_{1/2}$ is the temperature width at half peak height. (*): The pre-transition was not detected for 27.2 and 48.0 mol% Tween. Values within parentheses are S.D. and number of replicates, respectively. Mean values are to four significant figures to allow presentation of S.D.

HLB of 15, is miscible with water. Thus it is expected that Tween 80 will distribute between the lipid and water. Additionally, we included higher concentrations of Tween in the HSDSC work compared with those used in skin work. Tween concentrations will be presented as total concentrations.

Table 2 presents relevant data and Fig. 3 shows the effects of increasing concentrations of Tween 80 on the phase behaviour of DPPC vesicles.

The pre-transition endotherm was detected at low Tween concentrations (0–19.4 mol%). At 27.2 mol% the pre-transition peak was not seen. When apparent, the pre-transition gradually decreased in T_m, enthalpy and co-operativity with increasing Tween concentration before being abolished completely between 19.4 and 27.2 mol% (Table 2 and Fig. 3).

At low Tween concentrations, the shape of the main transition peak did not change (Fig. 3). Increasing Tween concentrations broadened the main peak, which became asymmetric towards the lower temperature side. This asymmetry became clearer at 19.4 mol% Tween, where it appeared as a shoulder, suggesting the occurrence of a new species. Eventually the main transition peak split into two at 48 mol%. This new peak had a T_m of about 35 °C. From Fig. 3, it may be thought that the new peak detected at 35 °C is the pre-transition. However, the pre-transition peak and the shoulder coexisted at 19.4 mol% Tween, then at 27.2 mol% the pre-transition disappeared and the shoulder became clearer, before separating at 48 mol%. Accordingly, it could be concluded that incorporating Tween 80 into DPPC liposomes produced more than one species, indicating the coexistence of vesicles and mixed micelles, most clearly seen at 48 mol% Tween.

3.3.3. Interactions of Span 80 with lipid membrane

Span 80 (sorbitan monooleate) has a relatively small head group compared with Tween 80, as it lacks the polyoxyethylene units. It has an HLB of 4.3, is lipophilic and immiscible with water and thus its distribution coefficient between lipid and water is high. Thus the effective molar ratio of Span 80 to DPPC in the vesicles is very close to the total molar ratio of Span to DPPC. Accordingly, Span concentrations will be presented as the total concentration (mol%).

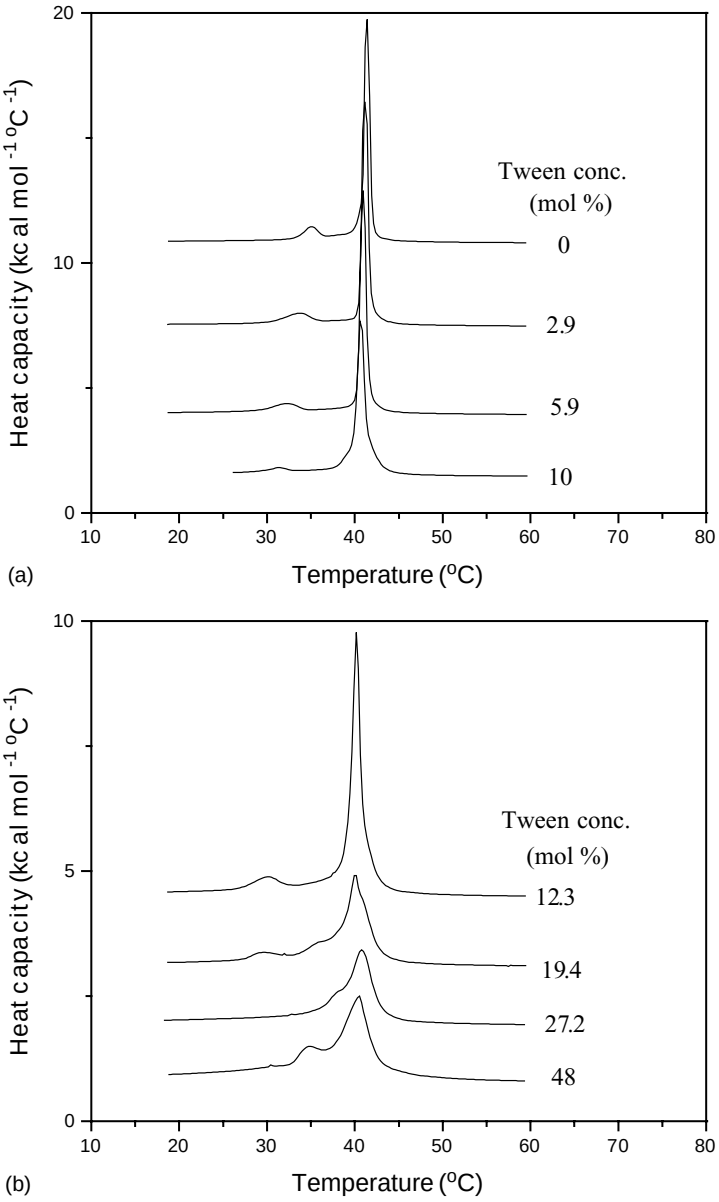

Fig. 3. Effect of Tween 80 on the phase behaviour of dipalmitoylphosphatidylcholine liposomes: (a) low concentrations and (b) higher concentrations.

Fig. 4 shows the effects of increasing concentrations of Span 80 on the phase behaviour of DPPC liposomes.

Increasing the concentration of Span 80 in liposomes abolished the pre-transition endotherm (Fig. 4). This endotherm was only detected for the control (0 mol%) and with the lowest tested concentration of Span 80 (8.3 mol%). At 8.3 mol%, the pre-transition endotherm had a T_m of $31.28 \pm 0.0057\,^{\circ}C$, enthalpy of 0.2510 ± 0.0025 kcal/mol and $\Delta T_{1/2}$ of $2.533 \pm 0.12\,^{\circ}C$ (S.D., $n = 3$), compared with control values of T_m of $35.58 \pm 0.35\,^{\circ}C$, enthalpy of

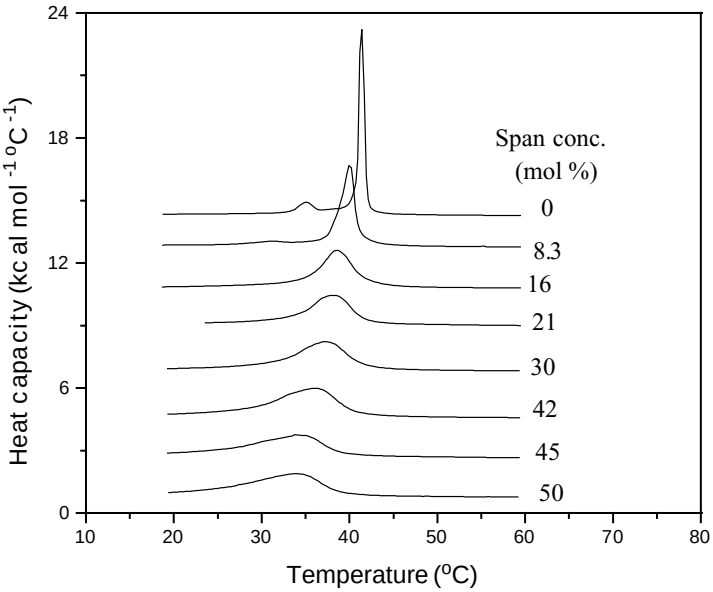

Fig. 4. Effects of Span 80 on the phase behaviour of dipalmitoylphosphatidylcholine liposomes.

0.7880 ± 0.24 kcal/mol and $\Delta T_{1/2}$ of 1.977 ± 0.27 °C (S.D., $n = 5$).

Table 3 presents the parameters calculated for the main transition endotherm of DPPC liposomes containing increasing concentrations of Span 80.

Raising the concentration of Span 80 in liposomes broadened the main transition peak and reduced T_m (Fig. 3). The temperature width at half peak height ($\Delta T_{1/2}$) increased with Span 80 concentration, reflecting decreased co-operativity of the main transition. These two parameters were very sensitive to changes in the Span concentration until 45 mol%, above which no change in T_m or $\Delta T_{1/2}$ was apparent (Table 3).

Table 3
Parameters of the main transition endotherm of dipalmitoylphosphatidylcholine liposomes containing Span 80 or oleic acid

	T_m (°C)	Enthalpy (kcal/mol)	$\Delta T_{1/2}$ (°C)
Span concentration (mol%)			
0	41.43 (0.028, 5)	8.424 (1.1, 5)	0.7242 (0.0030, 5)
8.3	40.07 (0.12, 3)	8.601 (1.4, 3)	1.5710 (0.12, 3)
16.0	39.05 (0.96, 3)	8.095 (1.1, 3)	3.2030 (0.38, 3)
21.0	38.19 (0.096, 3)	8.551 (0.68, 3)	4.6460 (0.13, 3)
30.0	37.13 (0.31, 3)	7.699 (0.58, 3)	5.9480 (0.37, 3)
42.0	35.74 (0.82, 3)	8.730 (1.9, 3)	7.3970 (0.50, 3)
45.0	34.10 (0.37, 3)	7.913 (0.012, 3)	7.9600 (0.011, 3)
50.0	34.13 (0.029, 3)	8.317 (1.0, 3)	7.8300 (0.12, 3)
Oleic acid (mol%)			
0	41.43 (0.028, 5)	8.424 (1.1, 5)	0.7242 (0.0030, 5)
12.0	39.65 (0.12, 3)	8.180 (0.18, 3)	0.9630 (0.0026, 3)
22.4	39.43 (0.036, 3)	8.779 (0.13, 3)	1.2090 (0.0010, 3)
33.2	39.19 (0.063, 3)	8.585 (0.20, 3)	1.0500 (0.14, 3)
39.4	39.19 (0.077, 3)	9.306 (1.1, 3)	1.0410 (0.14, 3)

$\Delta T_{1/2}$ is the temperature width at half peak height. Values within parentheses are S.D. and number of replicates, respectively. Mean values are to four significant figures to allow presentation of S.D.

Surprisingly, there was no significant change in the enthalpy of the main transition with increasing Span 80 concentration.

3.4. Interactions of penetration enhancers with phospholipid membrane

Penetration enhancers (oleic acid and limonene) were included in liposome formulations in increasing concentrations so as to deliver transdermal estradiol (El Maghraby et al., 2000b). The aim was to evaluate these enhancers as edge activators. For oleic acid, concentrations above 16% (w/w) in PC were difficult to prepare due to physical incompatibility. At the tested concentrations (0–16%, w/w) there was a direct relationship between drug flux and oleic acid concentration. For limonene, its incorporation into liposomes produced a similar trend to that obtained with the tested surfactants; increasing limonene concentration in liposomes raised the transepidermal drug flux up to a certain concentration, beyond which the flux decreased. Accordingly, we used HSDSC studies to attempt to explain these findings.

3.4.1. Interactions of oleic acid with phospholipid membrane

Fig. 5a shows the effects of increasing concentrations of oleic acid on the phase transition behaviour of DPPC liposomes.

Liposomes containing oleic acid at 12–39.4 mol% revealed no pre-transition endotherm (Fig. 5a). Thus, the lowest tested concentration of oleic acid was sufficient to abolish the pre-transition.

Table 3 presents the parameters calculated for the main transition endotherm of DPPC liposomes containing oleic acid.

Incorporating the acid into DPPC vesicles at 12 mol% broadened the main endotherm, with the peak showing asymmetry towards the lower temperature side. At 22.4 mol% the asymmetry became clearer, suggesting the formation of a new species. By 33.2 mol%, the asymmetry disappeared, implying that this new species might have been transformed into another type for which the transition was not detected in the experimental temperature range. However, this explanation can be disputed as the enthalpy was unchanged; possibly the formation of this new species is a reversible process. At a higher concentration of oleic acid (39.4 mol%), a peak identical to the previous concentration was detected.

Table 3 revealed that the T_m of the main transition initially fell at the lowest concentration of oleic acid (12 mol%). Further concentration increases produced smaller decreases in T_m up to 33.2 mol%, above which T_m remained constant. There was no significant change in the enthalpy of the transition with oleic acid concentration. The temperature width at half peak height ($\Delta T_{1/2}$) increased with acid concentration until a maximum at 22.4 mol%, after which it fell, but always remained higher than that of the control. This indicated reduced co-operativity of the transition. This trend can be explained on the basis of the presence of the asymmetry at the first two concentrations, which disappeared at 33.2 mol%, at which $\Delta T_{1/2}$ started to decrease.

The formulation containing oleic acid at 50 mol% phase-separated (detected visually) and thus was not tested. It was reported that in oleic acid/DPPC mixtures, the maximum molar fraction of oleic acid to be included is 0.4 (40 mol%) and concentrations above this produced unstable emulsions (Busquets et al., 1994).

3.4.2. Interactions of limonene with phospholipid membrane

Table 4 presents data and Fig. 5b shows thermographs of the effects of increasing limonene concentration on the phase transition behaviour of DPPC liposomes.

The pre-transition was detected with liposomes containing limonene at all concentrations. The T_m of the pre-transition decreased gradually with increasing limonene up to 50 mol%; at 66.7 mol% an abrupt fall in T_m was seen. The enthalpy of the pre-transition showed no clear trend, but generally decreased until 66.7 mol%, when it started to increase. The width of the peak at half height increased initially before reducing again to be close to that of the control. Thus limonene was unable to abolish the pre-transition endotherm of DPPC liposomes.

Fig. 5b reveals that the main transition endotherm was affected by limonene incorporation. The peak width gradually increased up to 50 mol% of limonene, above which the tested concentrations abruptly changed the peak shape; it broadened and became asymmetric.

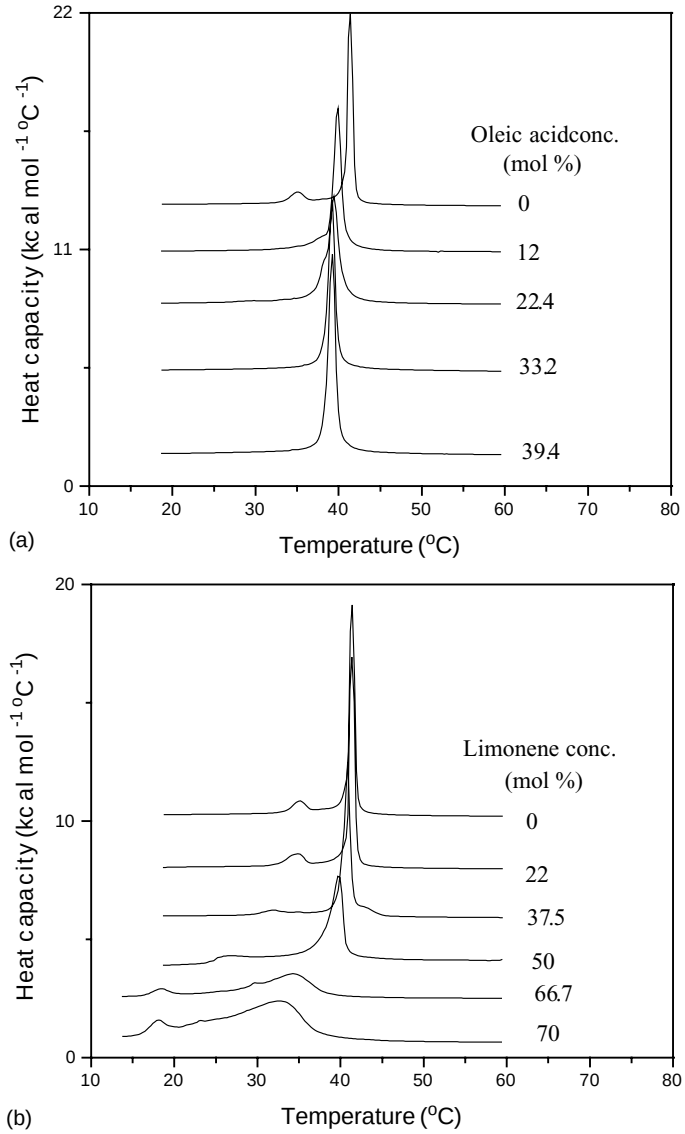

Fig. 5. Effect of (a) oleic acid and (b) limonene on the phase transition behaviour of dipalmitoylphosphatidylcholine liposomes.

The T_m of the main transition decreased gradually with increasing limonene concentration up to 50 mol%, above which T_m abruptly decreased (Table 4). The enthalpy showed a trend of slight reduction with limonene concentrations except that of 70 mol%, which increased. The $\Delta T_{1/2}$ of the transition rose gradually up to 50 mol%, above which it abruptly increased, indicating reduced co-operativity.

4. Discussion

In this section we report on the thermotropic behaviour of DPPC vesicle (control) so as to understand the origin of the transitions. This helps to explain the effects of additives on the thermograph. Fig. 6 illustrates the conformational changes in the phospholipid bilayer structure along with its phase transition.

Table 4
Effect of limonene on the phase behaviour of dipalmitoylphosphatidylcholine liposomes

Limonene (mol%)	T_m (°C)	Enthalpy (kcal/mol)	$\Delta T_{1/2}$ (°C)
Pre-transition			
0	35.58 (0.35, 5)	0.7880 (0.24, 5)	1.977 (0.27, 5)
22.0	34.97 (0.14, 3)	0.9796 (0.033, 3)	2.170 (0.0047, 3)
37.5	32.46 (0.85, 3)	0.4200 (0.074, 3)	2.491 (0.55, 3)
50.0	31.26 (4.4, 3)	0.5866 (0.089, 3)	2.107 (1.1, 3)
66.7	18.44 (0.12, 3)	0.5936 (0.039, 3)	2.052 (0.12, 3)
70.0	18.11 (0.015, 3)	0.8850 (0.083, 3)	1.920 (0.0073, 3)
Main transition			
0	41.43 (0.028, 5)	8.424 (1.1, 5)	0.7242 (0.0030, 5)
22.0	41.36 (0.049, 3)	7.526 (0.0050, 3)	0.7197 (0.0032, 3)
37.5	40.90 (0.013, 3)	7.555 (0.36, 3)	0.8046 (0.14, 3)
50.0	40.66 (0.85, 3)	7.341 (0.13, 3)	1.0450 (0.56, 3)
66.7	34.14 (0.14, 3)	7.250 (0.20, 3)	7.2790 (0.18, 3)
70.0	32.83 (0.017, 3)	11.780 (0.43, 3)	7.8810 (0.29, 3)

$\Delta T_{1/2}$ is the temperature width at half peak height. Values within parentheses are S.D. and number of replicates, respectively. Mean values are to four significant figures to allow presentation of S.D.

Below the DPPC pre-transition temperature, bilayer lipids are in the highly ordered gel state (L_β) with the hydrocarbon chains in an all-*trans* configuration (tilted one-dimensional arrangement). This arrangement is favourable because the head groups of the phospholipids are relatively bulky, taking more space compared with the hydrocarbon chains. The 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 to two-dimensional assemblies with periodic undulations (rippled gel phase, P_β). 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). It is thus clear that the presence of the bulky head group of the phospholipid causes the tilted one-dimensional arrangement of the hydrocarbon chains. Conversion of this highly ordered gel state (L_β) to the rippled gel phase (P_β) is the origin of the pre-transition. Thus,

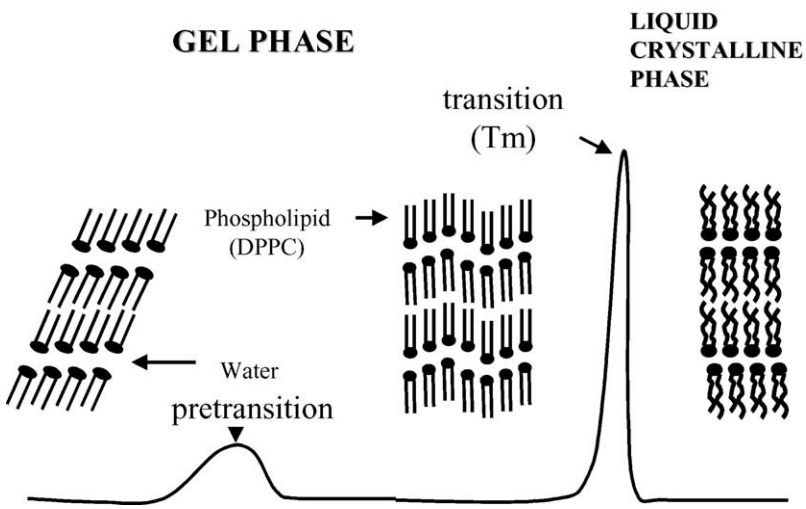

Fig. 6. Conformational changes in the phospholipid bilayer structure along with its phase transition (modified from Rolland et al., 1991).

anything that could compensate for the relative bulkiness of the head group can abolish the pre-transition.

It has been reported that, at equal to or less than 5 mol%, cholesterol progressively decreased the temperature and enthalpy with no effect on the co-operativity of the pre-transition (McMullen et al., 1993, 1994; McMullen and McElhaney, 1995). In our study, no pre-transition was detected as we tested cholesterol at concentrations of 9 mol% or more. As shown in Fig. 7a, cholesterol inserts into the membrane with its hydroxyl group oriented towards the aqueous surface and the aliphatic side chain positioned parallel to the acyl chains of the phospholipid. The 3β-hydroxyl group orientates level with the carboxyl residues of the phospholipid ester linkages, with limited freedom of vertical movement. The rigid steroidal nucleus thus occupies the places alongside the first 10 carbon atoms in the acyl chains of the phospholipid. This reduces the freedom of motion of these carbons but creates a space for movement for the remaining carbons towards the end of chains. This could lead to changes in the main transition (see below) (New, 1990). At a certain cholesterol concentration, the volume occupied by acyl chains and cholesterol combined will be equal to or greater than that of the head groups. This leads to the absence of chain tilt in the gel phase, which means that the origin of the pre-transition is abolished. This process explains the removal of the pre-transition endotherm after incorporation of cholesterol into the liposomes.

Fig. 1 shows that incorporating cholesterol into DPPC vesicles produced asymmetric endothermic peaks with a shoulder towards the higher temperature side of the transition. The literature considers this to be due to superimposed sharp and broad components and some workers have deconvoluted the endotherm into those two components. The sharp element is due to melting of cholesterol-poor domains and the broad component arises from melting of cholesterol-rich regions. The presence of both sharp and broad components in the main transition peak of cholesterol-containing vesicles indicates that cholesterol forms a new species with DPPC. This new species exhibits an extremely broad transition and is converted by addition of cholesterol above 20 mol% to one or more additional forms, which show no detectable phase transition at the temperature range studied (Mabrey et al., 1978; McMullen et al., 1993, 1994; McMullen and McElhaney, 1995). In our investigation, a very small part of the sharp component was detected at 26 mol% cholesterol, but the broad peak was lost at 50 mol% (Fig. 1). In our study, the main transition enthalpy decreased with increasing cholesterol concentration and approached zero at 50 mol% concentration (where there was no detectable peak). This finding supports the formation of a new species with no transition in the studied temperature range. Another possible explanation for the complete disappearance of the transition endotherm at 50 mol% (1:1 molar ratio) is that the rigid cholesterol molecules between DPPC molecules in the orientation presented in Fig. 7 could limit the freedom of movement of acyl chains of DPPC. This could restrict the rotational isomerisation along the chains, which occurs at the transition temperature.

For sodium cholate at concentrations above 8.2 mol%, the pre-transition endotherm was abolished completely. This indicates again that at this concentration the combined space occupied by the lipophilic part of the cholate and the acyl chains equals that occupied by the head groups. Thus the tilted one-dimensional arrangement no longer existed and hence the pre-transition was lost.

Almog et al. (1986) studied the micellar–lamellar phase transformations of a phosphatidylcholine–cholate mixed dispersion. They concluded that at cholate concentrations below an R_e of 0.3 the dispersion is in vesicular form, between 0.3 and 0.4 both vesicles and micelles coexist, and above 0.4 only mixed micelles develop. Applying this finding to our data suggests that at 55.6 mol% cholate ($R_e = 0.31$) the appearance of asymmetry in the transition towards the lower temperature side of the peak, which suggests the formation of a new species, could indicate the presence of some micelles along with the vesicles, as this concentration lies on the border where micelles and vesicles coexist. At 66.7 mol% ($R_e = 0.5$), in the region of mixed micelles, the transition became broader with clearer asymmetry and T_m reduced. Increasing cholate concentration to 80 mol% ($R_e = 1$), again in the micellar region, resulted in a broader transition with an abrupt decrease in T_m. If the micellar–lamellar phase transformations with cholate reported by Almog et al. (1986) apply to our data, it can be concluded that the phase transition changes, even within the micellar region. This may suggest a possible alteration in the micellar structure, as we still have transition variations

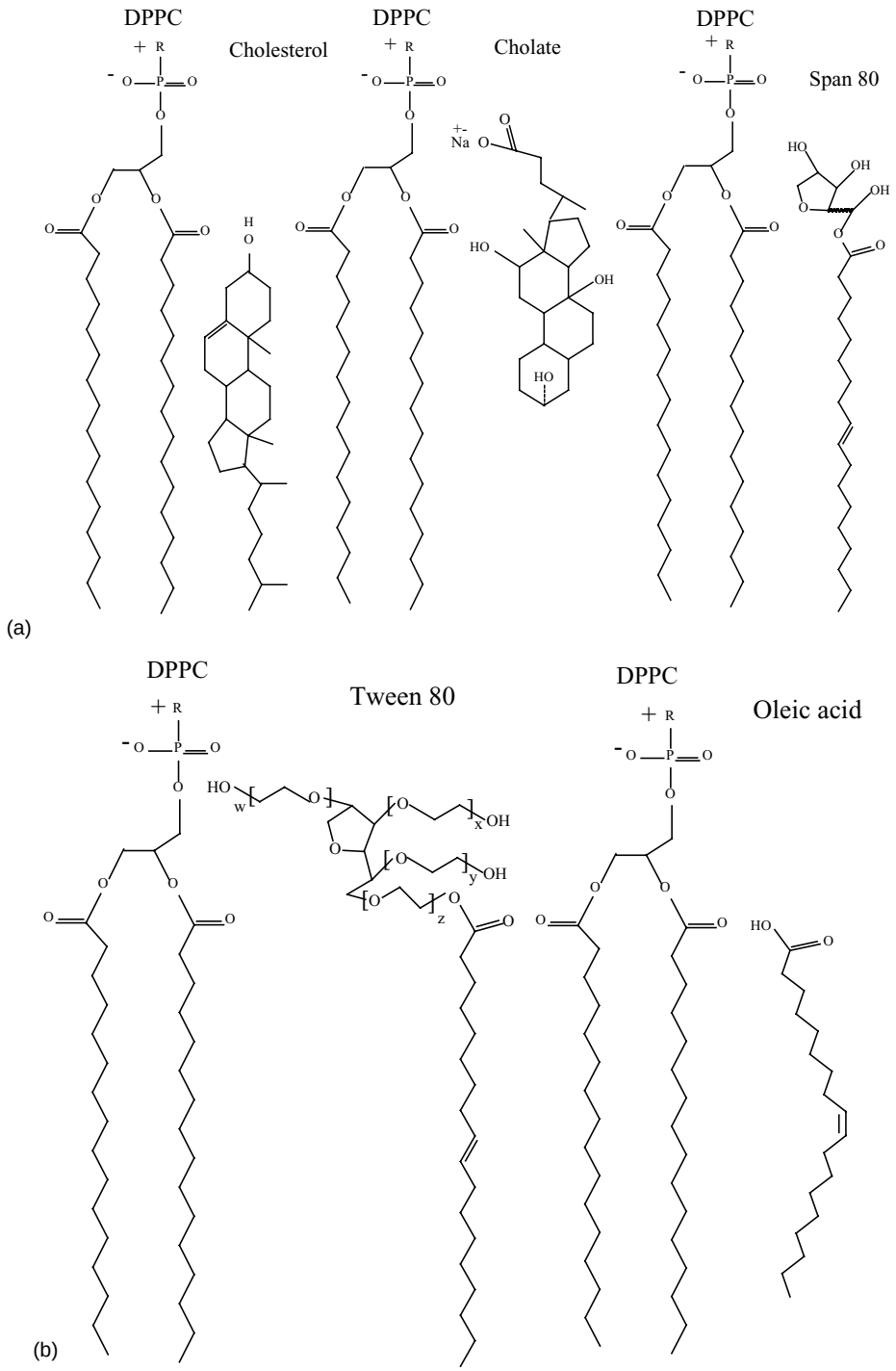

Fig. 7. Position occupied by cholesterol in the membrane bilayer (modified from New, 1990) and tentative suggestions for the interactions of sodium cholate, Span 80 (USP, 1995), Tween 80 (USP, 1995) and oleic acid with the dipalmitoylphosphatidylcholine bilayer. $w+x+y+z = 20$. R is choline ($C_5H_{13}N$). (N.B.: Our suggestions are tentative as they are based on DSC data only.)

even after increasing cholate concentration from R_e = 0.5–1 (i.e. above the 0.4 reported as the mixed micelle region).

Two sites of interaction for cholate with DPPC vesicles have been suggested (Bayerl et al., 1989). Site 1 is an interaction at the interface region, which changes the transition temperature but not the enthalpy. Site 2 is an interaction with the hydrocarbon region, and this is expected to reduce the enthalpy. On this basis, they concluded that at lower cholate concentrations, where there was no alteration in enthalpy, cholate should interact at the interface region (with the head group of phospholipid). At high concentrations (66.7 mol%, $R_e = 0.5$ in our study) where the enthalpy started to reduce, cholate is thought to interact at the hydrocarbon region. This concentration is said to be above the saturation concentration.

However, if we explain the removal of the pre-transition endotherm on the basis of the absence of a tilted one-dimensional arrangement when the space occupied by the lipophilic portion of cholate and acyl chains (of DPPC) combined was equal to or more than that occupied by the head group, we can conclude that cholate can interact with site 2 at a lower concentration. This is because the pre-transition was abolished at low concentration (15.9 mol%, R_e = 0.047). It could be thus suggested that cholate positions with the carboxylic group interacting with the head group of DPPC and the steroidal nucleus parallel to the acyl chains at the first few carbons of these chains (Fig. 7a). This gives some freedom for mobility to the rest of the chain (i.e. provides a more flexible structure), and may be responsible for the so-called edge activating effect. Although cholate has a steroidal nucleus similar to cholesterol, the presence of the carboxylic group in the hydrocarbon chain and the hydroxyl groups on the steroid produced different positioning and orientation of cholate in the lipid membrane, compared with that of cholesterol (Fig. 7). The difference in orientation may be responsible for the alteration in effect, from membrane stabilising action for cholesterol to an edge activating operation for sodium cholate. However, this scheme requires further investigation to establish the exact positioning and orientation of cholate in the bilayer.

Tween 80 may position in the bilayer with the head group oriented towards the head group of the phospholipid and the oleate residue (lipophilic part) aligned parallel to the acyl chains of the phospholipid (Fig. 7). If so, it could be concluded that at a Tween concentration of 27.2 mol%, the tilted one-dimensional arrangement (origin of pre-transition) will not exist, leading to absence of the pre-transition endotherm. The concentration required to abolish the pre-transition endotherm for Tween 80 was higher than that for sodium cholate. This is because Tween has a larger head group compared with cholate, which can add to the bulkiness of the phospholipid head group.

The data in Table 2 reveal that the T_m of the main transition decreases with increasing Tween concentration. The T_m increased slightly at 27.2 mol% compared with the previous concentration. There was a trend of decreasing enthalpy of the main transition with increasing Tween concentration, with the fall being significant ($P < 0.01$) at 48 mol%, compared with the control (pure DPPC vesicles). This again confirms the formation of a new species with a separate peak at lower T_m. The co-operativity of the transition decreased with increasing Tween concentration, as evidenced by the increase in the temperature width at half peak height ($\Delta T_{1/2}$), especially at high concentrations. This again indicates the presence of more than one species undergoing transitions.

Removal of the pre-transition peak with increasing Span concentration can be explained as before. Accordingly, we expect that Span 80 positions in the membrane with its carboxyl head group level with that of DPPC and the oleate residue aligned parallel to the acyl chains (Fig. 7).

Span 80 abolished the pre-transition endotherm at a lower concentration than Tween 80. This is expected as Tween has a relatively larger head group and thus higher concentrations are required to compensate for the head moiety.

The main transitions broaden upon increasing Span concentration in liposomes, but there were no significant changes in the enthalpy. Bayerl et al. (1989) reported that compounds that interact with site 2 (hydrocarbon region of the vesicles) are expected to decrease the enthalpy. On this basis, if enthalpy does not fall, there will be no interaction with site 2. However, it is difficult to apply this concept with a detergent like Span 80. This is because Span 80 is a lipophilic surfactant (HLB = 4.3), and has a structure that will fit with its oleate hydrocarbon chain aligned between the acyl chains of the phospholipid.

In addition, incorporating Span 80 in DPPC vesicles not only reduced the T_m but increased $\Delta T_{1/2}$. For these reasons, Span 80 is expected to interact with the hydrocarbon region of the membrane (site 2).

Comparing the three surfactants in terms of their effects on the phase behaviour of DPPC liposomes, it could be concluded that both the tenside structure and its HLB are important factors. For Tween 80, with an HLB of 15 (between sodium cholate, 16.7 and Span 80, 4.3), higher concentrations were required to abolish the pre-transition. This can be due to the presence of a relatively large head group, and thus a higher concentration of Tween was required to compensate for the bulky head group of DPPC; it is not because of the water solubility as Tween is not the extreme of the tested surfactants. The effect of HLB is clear if we compare Span with Tween and cholate. The T_m and $\Delta T_{1/2}$ were very sensitive to lower concentrations of the more lipophilic Span 80. It could be thus concluded that detergents disrupted the packing characteristics of DPPC vesicles before they were gradually transformed into mixed micelles, depending on the surfactant and its concentration.

We can correlate the HSDSC data obtained for the effect of different concentrations of surfactants on the phase behaviour of DPPC liposomes, with the trends obtained for the transepidermal flux of estradiol from vesicles containing increasing concentrations of surfactants (El Maghraby et al., 2000a). The three surfactants showed similar trends; flux increased with increasing surfactant concentration in liposomes up to an optimum value, above which the flux decreased. Sodium cholate, for which we calculated the effective molar ratios, will be considered first. The concentrations in the skin permeation experiments (El Maghraby et al., 2000a) were 0, 10, 16, 20 and 30% (w/w) (sodium cholate/phosphatidylcholine, PC). These concentrations produced effective molar ratios (R_e) of 0, 0.15, 0.250, 0.33 and 0.54, respectively. In skin permeation studies, increasing sodium cholate concentration in liposomes raised the transepidermal ES flux up to a concentration of 16% (w/w), above which the flux decreased.

Almog et al. (1986) concluded that at cholate concentrations below 0.3 (R_e) the dispersion is in vesicular form, at a concentration range of 0.3–0.4 (R_e) both vesicles and micelles coexist, and above R_e of 0.4 only mixed micelles develop. Our experiments revealed that at 55.6 mol% cholate ($R_e = 0.31$) the appearance of asymmetry in the transition towards the lower temperature side of the peak (suggesting the formation of a new species) could be due to the presence of some micelles along with the vesicles. At 66.7 mol% ($R_e = 0.5$), which is in the region for mixed micelles, the transition became broader with clearer asymmetry and T_m reduced. Accordingly, in the skin permeation studies, a formulation of 20% (w/w) cholate/PC ($R_e = 0.33$) contained both vesicles and mixed micelles and that at 30% (w/w) cholate/PC ($R_e = 0.54$), contained only mixed micelles. Mixed micelles are believed to be less effective as skin delivery systems compared with ultradeformable vesicles (Cevc et al., 1993, 1996). This explains the reduced estradiol skin delivery from formulations containing cholate concentrations of 20 and 30% (w/w).

Applying the same principles to Tween 80 and Span 80, the reduced skin delivery of estradiol from formulations containing high concentrations of these surfactants can be similarly explained.

With penetration enhancers, oleic acid abolished the pre-transition. Oleic acid can orientate with its hydrocarbon chain parallel to the acyl chains of the phospholipid and its carboxylic group level with that of the lipid head group (Fig. 7). Due to its kinked conformation, oleic acid can add to the space occupied by the acyl chains of the phospholipid so that the space occupied by oleic acid and acyl chains combined will be equal to, or more than, that occupied by the head groups. This explains the disappearance of the pre-transition.

The action of oleic acid on the main transition of DPPC vesicles, with an asymmetry appearing at lower concentrations and disappearing with high levels, suggests the formation of a new species. In the light of the invariant enthalpy of the main transition, the process may be considered reversible. Rolland et al. (1991) monitored the effect of oleic acid on the phase transition of DPPC liposomes using DSC, but the maximum concentration was 25 mol%. In the tested concentration range, T_m and $\Delta T_{1/2}$ showed similar trends to our results. On the basis of these data they suggested that oleic acid perturbed the lipid bilayer arrangement. They explained this on the basis that oleic acid can intercalate between adjacent DPPC molecules. Because oleic acid has a *cis* double bond, it has a kinked structure (Fig. 7b). Thus, when interposed between DPPC

molecules, it disrupts the ordered packing of the phospholipid molecules.

Oleic acid can produce a skin penetration enhancing effect alone or when combined with a solvent such as propylene glycol (e.g. Goodman and Barry, 1988; Yamane et al., 1995a; Murakami et al., 1998). Its enhancing effect was attributed to the reduction of the phase transition temperatures of skin lipids, with the result that the fluidity of these structures increased (Golden et al., 1987; Francoeur et al., 1990; Yamane et al., 1995a). The possibility that oleic acid exists as a separate phase (pool) within the stratum corneum lipids was also proposed (Ongpipattanakul et al., 1991).

Thus, it could be concluded that oleic acid can intercalate between the DPPC molecules but it distributes heterogeneously as indicated by the reduced co-operativity. Also, a phase separation may occur with possible pooling of oleic acid within the bilayer.

Incorporating oleic acid in liposomes increased the transepidermal flux of estradiol from these liposomes. There was a direct relationship between oleic acid concentration and the drug flux (El Maghraby et al., 2000b). This may be explained on the basis of the enhancing effect of oleic acid, as considered above. However, in the light of the DSC results, the presence of oleic acid in liposomes could produce a more fluid structure (i.e. more flexible vesicles). These flexible liposomes are believed to be more efficient than rigid vesicles in skin drug delivery.

Limonene did not abolish the pre-transition peak, indicating that even at high concentrations the space occupied by limonene and the acyl chains of the phospholipid (combined) was less than that inhabited by the head groups of the phospholipid.

The effects of limonene on the main transition of the DPPC vesicles strongly suggest the formation of one or more new species at 66.7 mol% limonene. These new species showed very broad transition endotherms at significantly lower temperatures.

The penetration enhancing effect of limonene has been reported. Limonene interaction with skin lipids was probed using DSC, which reflected a shift in the lipid transition endotherms (Yamane et al., 1995a,b). Cornwell et al. (1996) employed an uptake study and calculated the molar ratio of the limonene to the total skin lipids as 3.5:1. Based on this calculation, they proposed that higher enhancer uptake might occur alongside the maintenance of the bilayer structures, if the enhancers pool together. In our study, a limonene concentration of 66.7 mol% (2:1 molar ratio, limonene/DPPC) showed an abrupt change in the phase transition behaviour of DPPC vesicles. Increasing the concentration above this value further changed transition characteristics. This could indicate that two molecules or more can locate between adjacent DPPC molecules. It may also suggest the arrangement of limonene molecules as pools. Further investigations are necessary to confirm these possibilities. Incorporating limonene into PC liposomes increased the transepidermal flux of estradiol from these vesicles, with increasing limonene concentration, to a value beyond which the flux tended to decrease (El Maghraby et al., 2000b). The results obtained with limonene, where one or more new species formed at high concentrations as evidenced by the broad peaks, may explain the reduced transepidermal flux of estradiol from liposomes containing high concentrations of limonene.

5. Conclusions

Although sodium cholate has a steroidal backbone similar to that of the membrane stabiliser cholesterol, the presence of the carboxylate and hydroxyl groups in cholate produced an edge activating effect rather than a membrane stabilising effect.

With surfactants, the packing characteristics of the lipids in the liposome bilayer resulting in more fluid liposomes (more efficient in skin delivery of drugs) before transformation to mixed micelles (less efficient in delivery) at high surfactant concentrations. This behaviour explained the transepidermal estradiol flux profiles obtained with increasing surfactants concentrations in vesicles, where the flux increased with surfactant up to an optimum concentration, above which the flux decreased.

Oleic acid can intercalate between the phospholipid molecules in liposomes, but with a hetergeneous distribution probably allowing pooling. The presence of oleic acid can lead to more flexible liposomes. This can explain the improved estradiol skin delivery from liposomes containing oleic acid.

Limonene can fit between the acyl groups of the phospholipid in the liposome membrane, with the possibility that more than one molecule inserts between

each two adjacent phospholipid molecules, suggesting possible pooling. The transformation from one species to another, on increasing limonene concentration in liposomes, again explained the transepidermal estradiol flux profile produced by liposomes containing higher concentrations of limonene.

References

Almog, S., Kushnir, T., Nir, S., Lichtenberg, D., 1986. Kinetic and structural aspects of reconstitution of phosphatidylcholine vesicles by dilution of phosphatidylcholine–sodium cholate mixed micelles. Biochemistry 25, 2597–2605.

Attwood, D., Florence, A.T., 1983. Surfactant Systems: Their Chemistry, Pharmacy and Biology. Chapman and Hall, London, New York.

Bayerl, T.M., Werner, G.D., Sackmann, E., 1989. Solubilization of DMPC and DPPC vesicles by detergents below their critical micellization concentration: high-sensitivity differential scanning calorimetry, Fourier transform infrared spectroscopy and freeze-fracture electron microscopy reveal two interaction sites of detergents in vesicles. Biochim. Biophys. Acta 984, 214–224.

Busquets, M.A., Mestres, C., Alsina, M.A., Garcia Anton, J.M., Reig, F., 1994. Miscibility of dipalmitoylphosphatidylcholine, oleic acid and cholesterol measured by DSC and compression isotherm of monolayers. Thermochimica Acta 232, 261–269.

Castile, J.D., Taylor, K.M.G., Buckton, G., 1998. The influence of polaxamer surfactants on the thermal pretransition of DMPC and DPPC liposomes. J. Pharm. Pharmacol. 50, 147.

Cevc, G., Schätzlein, A., Gebauer, D., Blume, G., 1993. Ultra-high efficiency of drug and peptide transfer through the intact skin by means of novel drug-carriers, transfersomes. In: Brain, K.R., James, V.A., Walters, K.A. (Eds.), Prediction of Percutaneous Penetration, 3rd International Conference, vol. 3b, 14–16 April 1993. STS Publishing, Cardiff, pp. 226–236.

Cevc, G., Blume, G., Schätzlein, A., Gebauer, D., Paul, A., 1996. The skin: a pathway for systemic treatment with patches and lipid-based agent carriers. Adv. Drug Deliv. Rev. 18, 349–378.

Cornwell, P.A., Barry, B.W., Bouwstra, J.A., Gooris, G.S., 1996. Mode of action of terpene penetration enhancers in human skin: differential scanning calorimetry, small-angle X-ray diffraction and enhancer uptake studies. Int. J. Pharm. 127, 9–26.

El Maghraby, G.M.M., Williams, A.C., Barry, B.W., 2000a. Oestradiol skin delivery from ultradeformable liposomes: refinement of surfactant concentration. Int. J. Pharm. 196, 63–74.

El Maghraby, G.M.M., Williams, A.C., Barry, B.W., 2000b. Penetration enhancers in liposomes for skin delivery of oestradiol. Can they replace surfactants? In: Brain, K.R., James, V.A., Walters, K.A. (Eds.), Prediction of Percutaneous Penetration, 7th International Conference, vol. 7B. STS Publishing, Cardiff (in press).

Francoeur, M.L., Golden, G.M., Potts, R.O., 1990. Oleic acid: its effects on stratum corneum in relation to (trans)dermal drug delivery. Pharm. Res. 7, 621–627.

Ganesan, M.G., Weiner, N.D., Flynn, G.L., Ho, N.F.H., 1984. Influence of liposomal drug entrapment on percutaneous absorption. Int. J. Pharm. 20, 139–154.

Golden, G.M., McKie, J.E., Potts, R.O., 1987. Role of stratum corneum lipid fluidity in transdermal drug flux. J. Pharm. Sci. 76, 25–28.

Goodman, M., Barry, B.W., 1988. Action of penetration enhancers on human skin as assessed by permeation of model drugs 5-fluorouracil and estradiol. I. Infinite dose technique. J. Invest. Dermatol. 91, 323–327.

Janiak, M.J., Small, D.M., Shipley, G.G., 1976. Nature of the thermal pretransition of synthetic phospholipids: dimyristoyl- and dipalmitoyllecithin. Biochemistry 15, 4575–4580.

Lawson, E.E., Anigbogu, A.N.C., Williams, A.C., Barry, B.W., Edwards, H.G.M., 1998. Thermally induced molecular disorder in human stratum corneum lipids compared with a model phospholipid system; FT–Raman spectroscopy. Spectrochimica Acta Part A 54, 543–558.

Lo, Y.L., Rahman, Y.E., 1995. Protein location in liposomes, a drug carrier: a prediction by differential scanning calorimetry. J. Pharm. Sci. 84, 805–814.

Mabrey-Gaud, S., 1981. Differential scanning calorimetry of liposomes. In: Knight, C.G. (Ed.), Liposomes: From Physical Structure to Therapeutic Applications. Elsevier, North-Holland, Amsterdam, pp. 105–138.

Mabrey, S., Mateo, P.L., Sturtevant, J.M., 1978. High-sensitivity scanning calorimetric study of mixtures of cholesterol with dimyristoyl- and dipalmitoylphosphatidylcholines. Biochemistry 17, 2464–2468.

McMullen, T.P.W., McElhaney, R.N., 1995. New aspects of the interaction of cholesterol with dipalmitoylphosphatidylcholine bilayers as revealed by high-sensitivity differential scanning calorimetry. Biochim. Biophys. Acta 1234, 90–98.

McMullen, T.P.W., Lewis, R.N.A.H., McElhaney, R.N., 1993. Differential scanning calorimetric study of the effect of cholesterol on the thermotropic phase behavior of a homologous series of linear saturated phosphatidylcholines. Biochemistry 32, 516–522.

McMullen, T.P.W., Lewis, R.N.A.H., McElhaney, R.N., 1994. Comparative differential scanning calorimetric and FTIR and ^{31}P-NMR spectroscopic studies of the effects of cholesterol and androstenol on the thermotropic phase behavior and organization of phosphatidylcholine bilayers. Biophys. J. 66, 741–752.

McMullen, T.P.W., Lewis, R.N.A.H., McElhaney, R.N., 1999. Calorimetric and spectroscopic studies of the effects of cholesterol on the thermotropic phase behavior and organization of a homologous series of linear saturated phosphatidylethanolamine bilayers. Biochim. Biophys. Acta 1416, 119–134.

Montenegro, L., Panico, A.M., Bonina, F., 1996. Quantitative determination of hydrophobic compound entrapment in dipalmitoylphosphatidylcholine liposomes by differential scanning calorimetry. Int. J. Pharm. 138, 191–197.

Murakami, T., Yoshioka, M., Yumoto, R., Higashi, Y., Shigeki, S., Ikuta, Y., Yata, N., 1998. Topical delivery of keloid therapeutic drug, tranilast, by combined use of oleic acid and

propylene glycol as a penetration enhancer: evaluation by skin microdialysis in rats. J. Pharm. Pharmacol. 50, 49–54.

New, R.R.C., 1990. Liposomes: A Practical Approach. Oxford University Press, Oxford.

Ongpipattanakul, B., Burnette, R.R., Potts, R.O., Francoeur, M.L., 1991. Evidence that oleic acid exists in a separate phase within stratum corneum lipids. Pharm. Res. 8, 350–354.

Papahadjopoulos, D., Moscarello, M., Eylar, E.H., Isac, T., 1975. Effects of proteins on thermotropic phase transition of phospholipid membranes. Biochim. Biophys. Acta 401, 317–335.

Rolland, A., Brzokewicz, A., Shroot, B., Jamoulle, J.C., 1991. Effect of penetration enhancers on the phase transition of multilamellar liposomes of dipalmitoylphosphatidylcholine. A study by differential scanning calorimetry. Int. J. Pharm. 76, 217–224.

Spink, C.H., Lieto, V., Mereand, E., Pruden, C., 1991. Micelle-vesicle transition in phospholipid-bile salt mixtures. A study by precision scanning calorimetry. Biochemistry 30, 5104–5112.

USP (The United States Pharmacopoeia), 1995. The United States Pharmacopoeia, vol. 23. United States Pharmacopeial Convention, Inc., Twinbrook Parkway, Rockville, MD.

Yamane, M.A., Williams, A.C., Barry, B.W., 1995a. Effects of terpenes and oleic acid as skin penetration enhancers towards 5-fluorouracil as assessed with time; permeation, partitioning and differential scanning calorimetry. Int. J. Pharm. 116, 237–251.

Yamane, M.A., Williams, A.C., Barry, B.W., 1995b. Terpene penetration enhancers in propylene glycol/water co-solvent systems: effectiveness and mechanism of action. J. Pharm. Pharmacol. 47, 978–989.