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Terpene Penetration Enhancers in Propylene Glycol/water Co-solvent Systems: Effectiveness and Mechanism of Action

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Abstract

The effects of propylene glycol/water co-solvent systems and terpene penetration enhancers (1,8-cineole, menthone, (+)-limonene and nerolidol) on the absorption rate of the model hydrophilic permeant, 5-fluorouracil, were investigated using excised human skin.

Similar fluxes for 5-fluorouracil were obtained from saturated enhancer-free co-solvent systems. Co-application of each terpene with the drug, both at saturation, in propylene glycol co-solvent systems increased drug flux significantly. Terpene activity depended on the propylene glycol content in the vehicles. Maximum fluxes were obtained from formulations containing the terpenes in 80% propylene glycol systems (highest concentration used), which when normalized to the flux from the pure vehicles yielded enhancement ratios of about 24, 21, 4 and 18, with 1,8-cineole, menthone, (+)-limonene and nerolidol, respectively. Combining the permeation studies with differential scanning calorimetry (DSC) and partitioning experiments revealed that increased lipid disruption is probably an important mechanism involved in the enhancing ability of formulations containing 1,8-cineole, menthone and nerolidol. This was clearly demonstrated by applying thermodynamic principles to interpret DSC results.

This approach has indicated that these terpenes are probably able to disrupt stratum corneum lipids at physiological temperature as manifested by reductions in the entropy changes associated with the lipid-related transitions, particularly T2, the first major lipid transition. Additionally, increased drug partitioning contributed to the effect of the high propylene glycol content formulations. (+)-Limonene, as interpreted from DSC results, produced a freezing point-depression effect on stratum corneum lipids, suggesting little interaction with lipids at skin temperature; its small enhancement effect may involve phase separation of the oil in stratum corneum lipids.

Terpenes in co-solvent systems such as propylene glycol/water at appropriate propylene glycol content might thus be useful vehicles for the delivery of drugs from topical formulations.

Drug delivery across the skin is a substantial challenge. The stratum corneum has evolved primarily as a barrier to water loss and to inhibit the entry of microorganisms and molecules into the body (Blank 1964). This barrier function creates difficulties for formulators aiming to deliver drugs via the skin in therapeutic quantities. The search for solutions to this problem led investigators to employ several enhancement techniques (Barry 1992), such as the inclusion of penetration enhancers in the formulation. Ideally, a penetration enhancer is a chemical which reduces reversibly the barrier resistance of the stratum corneum without damaging the viable cells (Barry 1983). The list of substances that have been evaluated as penetration enhancers is long (Walters 1989; Williams & Barry 1992) and research is extending with the growing need for safe and effective accelerants.

Previous studies reported the effectiveness of monoterpenes and sesquiterpenes as penetration enhancers towards 5-fluorouracil (Williams & Barry 1989, 1991; Cornwell & Barry 1994; Yamane et al 1995). These investigations evaluated the enhancers for maximum effect by pretreating human skin samples with pure terpenes and then measuring the absorption of the drug from its saturated aqueous solutions before and after enhancer treatment. The mechanism of action of these terpenes was shown to be mainly by increasing the diffusivity of the drug through the stratum corneum.

In view of the generally low irritancy, good toxicological profiles (Opdyke 1973–1979) and excellent enhancement ability, the terpenes appear to be promising candidates for clinically acceptable enhancers. Propylene glycol is a widely used solvent in topical formulations and in addition provides a synergistic effect on the promoting ability of the terpenes (Barry & Williams 1989). Hence, this study aimed to evaluate the enhancing activity of selected terpenes, the chemical structures of which are illustrated in Fig. 1, by applying them concomitantly with the drug in propylene glycol/water formulations, and to investigate the contributions of the solvent system (propylene glycol/water) and the accelerants to the observed enhancement.

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Materials and Methods

Materials

5-[6^{3}H]Fluorouracil was purchased from NEN (Dupont) Research Products (Dreich, Germany). Unlabelled 5-fluorouracil, menthone (monoterpene) and nerolidol (sesquiterpene) were obtained from Aldrich Chemical Co. (Poole, UK), and 1,8-cineole, (+)-limonene (monoterpenes)

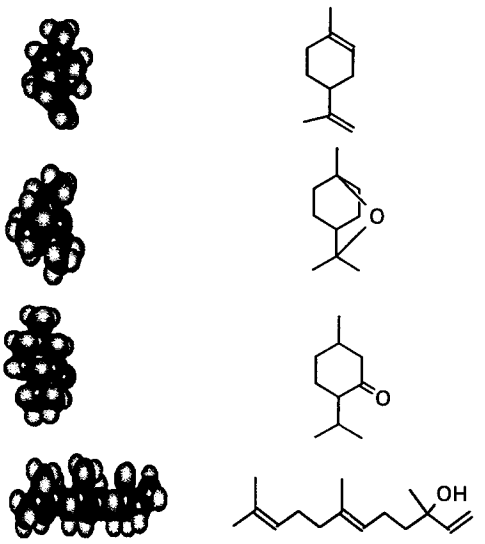

FIG. 1. Molecular structures and space filling models of the enhancers (from top to bottom) (+)-limonene, 1,8-cineole, menthone and nerolidol. Space filling models were produced using the Hyperchem computational chemistry approach. The option used for minimizing the charge distribution was the AM1 semi-empirical method; geometry optimization employed the MM+ approach.

and propylene glycol from Sigma Chemical Co. (Poole, UK). All were used as received.

Terpene purity was assessed using a Perkin Elmer 8320B Capillary Gas Chromatograph installed with an AS-8300 autosampler and a 25-m fused silica capillary column (SGE, Australia) with a stationary phase of a cross-linked polymer of diphenyldimethylsiloxane. The carrier gas (helium) was set at 285°C, 8 psi and 60 mL min^{-1}. The eluted samples were analysed by a hydrogen flame-ionization detector at 285°C. Integrated peak areas were used for purity determinations. Menthone met the purity specified by the manufacturers (85%), whereas 1,8-cineole, (+)-limonene and nerolidol were 91–94% pure, which was lower than the claimed purity. However, no single impurity was found at greater than 2% and at such low thermodynamic activity each was considered to have an insignificant effect on human skin.

Preparation of epidermal membranes

Human abdominal skin was obtained post-mortem, sealed in evacuated polyethylene bags and stored at −20°C (Harrison et al 1984). Epidermal membranes were prepared using a heat separation technique (Kligman & Christophers 1963). The epidermal membranes were floated, epidermal side down, on aqueous 0·002% w/v sodium azide solution for three days to ensure essentially full hydration of the stratum corneum.

Preparation of stratum corneum

Stratum corneum samples were prepared by floating freshly prepared epidermal membranes, stratum corneum side up, on 0·0001% w/v trypsin solution containing 0·5% w/v sodium hydrogen carbonate for 12 h. Stratum corneum sheets were cleaned from the digested material with water and dried on wire meshes under ambient conditions for 12 h. The membranes were rinsed with ice-cold acetone for 10 s and stored under vacuum over silica gel.

Solubility studies

The solubilities of 5-fluorouracil in propylene glycol/water systems ranging from 0 to 100% w/v were determined at 32°C. Excess drug was added to the vehicles at room temperature, heated to 60°C to dissolve the drug and then equilibrated at 32 ± 0·5°C for 72 h. The solubilities of 5-fluorouracil in propylene glycol/water saturated with terpenes were also determined, by adding the enhancers in excess quantities to the vehicles which were first saturated with the drug. The mixtures were shaken gently on a Vibrax VXR shaker (Janke and Kunkel GmbH, Germany) for 24 h at 32°C and then equilibrated for 48 h at 32 ± 0·5°C. Aliquots of the saturated vehicles were filtered, diluted with ethanol and analysed spectrophotometrically at 267 nm.

Permeation studies

In-vitro permeation experiments used an automated diffusion apparatus (Akhter et al 1984), and human epidermal membranes obtained from twelve donors (ten female) with a mean age of 75 ± 9 (s.d.) years.

Hydrated epidermal membrane samples were mounted into the diffusion cells (area 0·126 cm^2) equilibrated at 32°C. The drug preparations (150 μL) were applied as radiolabelled saturated aqueous solution or formulations which were propylene glycol/water systems at concentrations of 20, 50 or 80% w/w free or saturated with the study terpenes.

The receptor fluid was 0·002% sodium azide flowing at 2 mL h^{-1} to maintain sink conditions and collected every 2 h for 48 h. The donor formulations containing terpenes were replenished every 12 h to avoid enhancer depletion. The collected receptor solution was mixed with 5 mL Optiphase Hisafe III scintillation fluid (LKB) and analysed by liquid scintillation counting with a Tri Carb Liquid Analyzer, Model 1600 TR.

The steady-state flux of the drug (J, counts min^{-1} cm^{-2} h^{-1}) was estimated from the slope of the straight line portion of the cumulative amount absorbed (counts min^{-1} cm^{-2}) against time (h) profiles. The steady-state permeability coefficient (K_p, cm h^{-1}) was calculated from the flux and the donor concentration (C, counts min^{-1} cm^{-3}) from:

$$K_p = J/C \quad (1)$$

The values reported for each enhancer formulation are the mean of 5–6 replicates.

Partitioning studies

The partitioning of 5-fluorouracil into stratum corneum from water, from propylene glycol/water co-solvent systems and from the co-solvent systems saturated with the terpenes was determined in triplicate. Stratum corneum samples were obtained from four donors (three female) with a mean age of 76 ± 3 (s.d.) years.

Discs of dry stratum corneum were weighed (2–5 mg) and floated on 0·002% aqueous sodium azide for 72 h. The hydrated discs were blotted dry and immersed in radiolabelled subsaturated drug solutions for 48 h at 32°C. The drug solutions were either aqueous or propylene glycol/water co-solvent systems (free or saturated with terpenes) at concentrations of 10 mg mL^{-1} and with radiolabelled activity of about 0·01 mCi mL^{-1}. The equilibrated tissue samples were blotted dry and solubilized overnight in

1 mL Soluene-350 (Packard), which was then neutralized with 0·1 mL glacial acetic acid and mixed with 5 mL scintillation fluid; the concentrations of 5-fluorouracil in the membranes were evaluated by liquid scintillation counting. The concentrations of the drug in the bathing solutions were also determined in triplicate by liquid scintillation counting.

Differential scanning calorimetry

Samples of dry stratum corneum were weighed (8–12 mg) and hydrated over a saturated sodium sulphate solution (r.h. 97% at 25°C) to 20–40% hydration. Percent hydration was calculated using the formula:

$$\% \text{ hydration} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100 \quad (2)$$

Stratum corneum samples were immersed in one of the test formulations (20–90% w/w propylene glycol/water, free or saturated with terpenes) for 30 s, a short time which provides good discrimination of DSC effects, after which they were blotted clean and hermetically sealed in 75-μL stainless-steel capsules. The samples were equilibrated for one hour and then scanned on a DSC7 Differential Scanning Calorimeter (Perkin Elmer, USA) from 10 to 140°C at 10°C min^{-1}.

Due to poor baseline resolution typical of skin, transition midpoint temperatures were determined manually and transition enthalpies were measured from integration of partial areas. Enthalpies (ΔH, J g^{-1}) were calculated from:

$$\Delta H = \frac{\text{Peak area}}{\text{Sample dry weight}} \quad (3)$$

Transition temperatures and enthalpies of propylene glycol/water-treated samples were compared with untreated controls from the same cadaver. The results of the samples treated with terpene formulations were compared with samples from the same cadavers treated with the analogue propylene glycol/water solution. Stratum corneum samples used were obtained from 29 donors (13 female) with a mean age of 70 ± 13 (s.d.) years. A minimum of three replicate experiments were performed for each enhancer formulation using three different skin samples.

Results and Discussion

Radiolabelled 5-fluorouracil was used to determine its permeability coefficients across human epidermal membranes from aqueous vehicles and from propylene glycol/water system, free or saturated with terpenes. However, to calculate the flux in $\mu g\, cm^{-2}\, h^{-1}$ from the permeability coefficient, it was necessary to determine the solubility of the drug in the vehicles. Fig. 2 shows the data in propylene glycol/water co-solvent systems ranging from 0 to 100% w/w. The solubility in water was slightly higher than that in propylene glycol ($P < 0{\cdot}025$). However, as the percent propylene glycol increased, the solubility plot showed a broad peak with a maximum at 50% where the solubility was about 20% greater than in water. In spite of the gradual change in solubility, the solubility in 50% propylene glycol was significantly higher ($P < 0{\cdot}005$) than that in water or in pure propylene glycol. A previous study determined the solubility of 5-fluorouracil (log $P_{octanol/water} = -0{\cdot}92$) in 50% ethanol/water co-solvent system to be about 30% in excess of the drug solubility in water (Cornwell & Barry 1994). Since the drug is about half as soluble in ethanol as in water (Rudy & Senkowski 1973), a bell-shaped solubility curve would be expected.

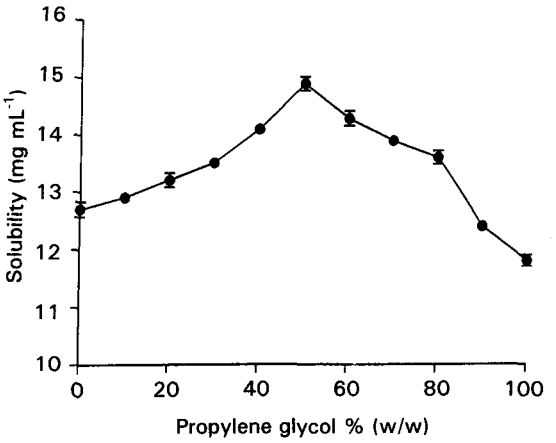

FIG. 2. Solubility of 5-fluorouracil in propylene glycol/water systems at 32°C. Data are mean ± s.e.m. (n = 6). For all figures, error bars within size of symbols are not shown.

These bell-shaped curves have been observed with other polar/semipolar solutes in binary systems of propylene glycol/water or ethanol/water. Yalkowski & Roseman (1981) reported this phenomenon with solutes having log octanol/water partition coefficients around zero. For example, parabolic solubility curves were obtained for the solubility of caffeine (log $P_{octanol/water} = 0{\cdot}57$) in ethanol/water and propylene glycol/water systems. Similarly, the plot of theophylline solubility (log $P_{octanol/water} = -0{\cdot}07$) against propylene glycol/water concentration has a maximum.

The solubilities of 5-fluorouracil in water and in 20, 50 and 80% propylene glycol/water co-solvent systems, and the fluxes and permeability coefficients across human epidermal membranes from these vehicles are given in Table 1. The mean permeability coefficient of 5-fluorouracil from its aqueous saturated solutions was $3{\cdot}12 \times 10^{-5}$ cm h^{-1} showing good agreement with previously reported values (for example, Williams & Barry (1991) – $2{\cdot}46 \times 10^{-5}$ cm h^{-1}; Cornwell & Barry (1994) – $2{\cdot}71 \times 10^{-5}$ cm h^{-1}; Yamane et al (1995) – $3{\cdot}06 \times 10^{-5}$ cm h^{-1}). The permeability coefficients and fluxes of the drug from the co-solvent systems show an apparent increase with increasing propylene glycol content in the vehicles. However, the values are not significantly different from each other (two-sided *t*-test at the level 5%).

This result agrees with theory, since the drug is at saturation in each vehicle (Higuchi 1960; Ostrenga et al 1971). The rate of drug transport increases with increasing applied concentration until saturation, at which the chemical potential of the drug in solution is equivalent to that of the solid and hence provides maximum flux. Therefore, if the drug is applied at saturation in different vehicles, constant fluxes should be achieved provided that the vehicle does not significantly change the properties of the membrane, i.e. the system behaves ideally. This is probably the case with the test propylene glycol/water co-solvent systems used in this study for which the maximum concentration of propylene glycol was 80%.

Table 1. Solubilities, fluxes and permeability coefficients (K_p) for saturated 5-fluorouracil using water and propylene glycol/water co-solvent systems as vehicles.

Vehicle Water : propylene glycol	Solubility ($mg\,mL^{-1}$)	Flux ($\mu g\,cm^{-2}\,h^{-1}$)	K_p ($cm\,h^{-1} \times 10^5$)
100 : 0	12·7 ± 0·13	0·39 ± 0·05	3·12 ± 0·43
80 : 20	12·2 ± 0·12	0·46 ± 0·11	3·50 ± 0·79
50 : 50	14·9 ± 0·12	0·53 ± 0·11	3·57 ± 0·71
20 : 80	13·6 ± 0·12	0·58 ± 0·12	4·24 ± 0·86

Solubility data are mean ± s.e.m. (n = 6). Flux and K_p data are mean ± s.e.m. (n = 10–12 except for water, n = 34).

This conclusion corresponds with previous research regarding the effects of propylene glycol acting as a penetration enhancer towards 5-fluorouracil (Goodman & Barry 1988; 1989a). Those studies illustrated that the enhancing effect of propylene glycol depends on the experimental conditions. Propylene glycol acted as an enhancer when partially hydrated epidermal membranes were treated with it for 12 h followed by the application of the drug as a solvent-deposited film (Goodman & Barry 1989a), while pretreating fully hydrated skin samples with propylene glycol did not increase drug permeation from its aqueous solution (Goodman & Barry 1988). Similarly, in the present study, fully hydrated skin samples were used and, in addition, propylene glycol was used in conjunction with water, and hence it is unlikely that propylene glycol will be effective as an enhancer under these maximized conditions. Moreover, recent studies in our laboratories measured the uptake of water and propylene glycol from their co-solvent systems into fully hydrated stratum corneum (Megrab 1994). The propylene glycol content in the stratum corneum increased with increasing percentage in the applied co-solvent. This was accompanied by a sharp decrease in water content in the stratum corneum when the propylene glycol percent in the vehicle was increased from 0 to 20%, followed by a gradual decrease in the water content when the propylene glycol percent in the vehicle was increased from 20 to 100%. For the model lipophilic drug, oestradiol, raising of the propylene glycol content in the stratum corneum led to an increase in the drug solubility in the membrane and subsequently to higher oestradiol flux from its saturated propylene glycol co-solvent systems. However, for the hydrophilic drug 5-fluorouracil, that change in the propylene glycol content in the stratum corneum is unlikely to affect significantly the solubility of the drug in the membrane since the difference in the solubility of 5-fluorouracil in water and in propylene glycol is comparatively insignificant (the solubility of 5-fluorouracil in water and propylene glycol is 12·7 and 11·8 $mg\,mL^{-1}$, respectively, while for oestradiol it is about 0·003 and 60·0 $mg\,mL^{-1}$, respectively).

The effects of the study terpenes (1,8-cineole, menthone, (+)-limonene and nerolidol) formulated in the propylene glycol co-solvent systems (20, 50 and 80% w/w propylene glycol/water) on the in-vitro permeation of 5-fluorouracil across human skin were examined (Table 2). Data in Tables 1 and 2 show that the enhancers slightly reduced the solubility of the drug in the co-solvent systems, probably because of its poor solubility in the terpenes (for example, the solubility of 5-fluorouracil in 1,8-cineole is about 0·249 $mg\,mL^{-1}$ and in (+)-limonene is about 0·0057 $mg\,mL^{-1}$). A similar reduction in the solubility in propylene glycol was observed in the presence of some fatty acids (Aungst et al 1990). Despite such slight reduction in the solubility, the fluxes and permeability coefficients increased in the presence of terpenes. For each enhancer the flux of 5-fluorouracil from 80% co-solvent system was higher than that from the 50% system which in turn was higher than the flux from 20% vehicle. As detailed above, the flux from pure vehicles insignificantly increased with increasing propylene glycol content. Nevertheless, to normalize the contribution of the direct effect of the vehicles to the observed increase in flux, enhancement ratios (flux ratios) were calculated by dividing the flux from a

Table 2. Solubilities, fluxes and permeability coefficients (K_p) for saturated 5-fluorouracil from propylene glycol/water systems saturated with terpenes.

Vehicle Water : propylene glycol	Enhancer	Solubility ($mg\,mL^{-1}$)	Flux ($\mu g\,cm^{-2}\,h^{-1}$)	K_p ($cm\,h^{-1} \times 10^5$)
80 : 20	(+)-Limonene	12·9 ± 0·09	0·74 ± 0·10	5·81 ± 0·79
	Nerolidol	12·1 ± 0·11	2·50 ± 0·39	20·4 ± 3·19
	Menthone	11·8 ± 0·12	3·36 ± 0·61	28·4 ± 5·14
	1,8-Cineole	12·6 ± 0·11	5·01 ± 0·87	39·6 ± 6·89
50 : 50	(+)-Limonene	14·0 ± 0·11	1·48 ± 0·25	10·4 ± 1·75
	Nerolidol	13·2 ± 0·12	4·93 ± 0·69	37·3 ± 5·28
	Menthone	12·4 ± 0·09	8·37 ± 1·66	67·8 ± 13·4
	1,8-Cineole	13·6 ± 0·10	9·59 ± 1·89	70·3 ± 13·9
20 : 80	(+)-Limonene	12·5 ± 0·08	2·44 ± 0·42	19·5 ± 3·34
	Nerolidol	11·4 ± 0·11	10·4 ± 1·71	91·4 ± 14·5
	Menthone	10·9 ± 0·09	11·9 ± 1·39	112 ± 12·8
	1,8-Cineole	11·9 ± 0·07	14·1 ± 2·20	118 ± 18·5

Solubility data are mean ± s.e.m. (n = 3). Flux and K_p data are mean ± s.e.m. (n = 5–6).

Table 3. The effect of the skin penetration enhancers 1,8-cineole, menthone, nerolidol and (+)-limonene in propylene glycol-water co-solvent systems on the flux of 5-fluorouracil across human epidermal membranes expressed as enhancement ratios (flux from formulation containing enhancers: flux from pure vehicle).

Vehicle Water : propylene glycol	Enhancement ratio			
	1,8-Cineole	Menthone	Nerolidol	(+)-Limonene
80 : 20	10·9 ± 2·0	7·3 ± 1·3	5·4 ± 0·8	1·6 ± 0·2
50 : 50	16·5 ± 3·5	15·8 ± 3·2	9·3 ± 1·3	2·8 ± 0·5
20 : 80	24·3 ± 3·8	20·5 ± 2·5	17·9 ± 3·0	4·3 ± 0·8

Data are mean ± s.e.m. (n = 6).

formulation containing enhancer (given in Table 2) by that from the analogue pure vehicle (given in Table 1), and data are given in Table 3. The values show that the enhancers increased the flux of the drug and that 1,8-cineole is the most effective followed by menthone and nerolidol; the mildest effect was obtained with (+)-limonene.

The terpenes produced the same rank order previously when they were applied as unformulated oils using the pretreatment protocol (Yamane et al 1995). The maximum enhancement ratios obtained following 12-h pretreatment with 1,8-cineole, menthone and nerolidol were 95, 42 and 25, respectively. These ratios are higher than the maximum values obtained in this study for the enhancers formulated in 80% propylene glycol co-solvent systems which are 24, 21 and 18, respectively. The poor enhancer (+)-limonene behaved ideally and provided nearly the same enhancement in the pure and in the formulated form (enhancement ratios were 3·6 and 4·3, respectively).

The terpenes are applied at saturation in the co-solvent systems and theoretically they should provide the same enhancement effect as the pure oils since they are at their maximum thermodynamic activity. The reason why formulated 1,8-cineole, menthone and nerolidol were less effective than the neat terpenes is not clear. Previous studies (Cornwell 1993) measured the uptake of 1,8-cineole, (+)-limonene and nerolidol into stratum corneum following 12-h pretreatment with terpenes to be 26·2, 8·9 and 39·6% w/w, respectively. However, the uptake of such large quantities may suggest that the uptake of the terpene from the pure oil depends only on the solubility of the terpene in the stratum corneum and on the capacity of the stratum corneum, while the uptake of the terpene from the formulations is a conventional partitioning phenomenon and hence will depend on the relative affinity of the terpene towards the two partitioning phases. Indeed, the uptake of terpenes saturated in propylene glycol has been shown to be less than that from the pure enhancer (Cornwell 1993).

Table 3 also shows that the terpenes' enhancement potential is greater in the vehicles with high propylene glycol content ($P < 0·025$). Since the enhancers are applied at saturation in each vehicle, this result suggests that the vehicles affected the enhancer action. Hence, further study was directed to investigate the mechanisms of action of the different formulations.

An increase in the steady-state flux of a drug from its enhancer-containing, saturated solutions is probably due to the effects of the enhancer on the membrane. Assuming that the thickness of the stratum corneum does not change appreciably, the enhancer effect could be a result of an increase in the diffusivity of the drug through the stratum corneum or an increase in partitioning between the stratum corneum and the vehicle, or both.

The partitioning of 5-fluorouracil into stratum corneum was investigated from aqueous, and from propylene glycol/water co-solvent systems, free or saturated with terpenes. The stratum corneum/water partition coefficient of 5-fluorouracil was determined to be 1·04 ± 0·21 (mean ± s.d. n = 4) showing good agreement with previously reported values (Cornwell & Barry (1994), about 0·94; Yamane et al (1995), about 1·05). Table 4 shows the partition coefficient of the drug into stratum corneum from propylene glycol/water co-solvent systems in the presence and in the absence of terpenes. The partitioning of the drug from enhancer-free co-solvent systems was not significantly different from the stratum corneum/water partition coefficient (using two-sided t-test at the level 5%), in agreement with the permeation results where the drug permeability coefficient was not affected by the propylene glycol co-solvent systems.

The effect of terpenes on drug partitioning is clearly

Table 4. Stratum corneum/vehicle partition coefficients of 5-fluorouracil from propylene glycol/water co-solvent systems, free or saturated with terpenes.

Vehicle Water : propylene glycol	Stratum corneum/vehicle partition coefficients in the presence of the enhancer				
	None	1,8-Cineole	Menthone	Nerolidol	(+)-Limonene
20 : 80	0·94 ± 0·06	0·91 ± 0·06	0·92 ± 0·04	0·86 ± 0·05	0·86 ± 0·07
50 : 50	1·12 ± 0·20	1·62 ± 0·33	2·81 ± 0·54	3·25 ± 0·17	1·42 ± 0·23
80 : 20	0·91 ± 0·16	1·12 ± 0·03	2·01 ± 0·31	2·59 ± 0·04	1·01 ± 0·03

Data are mean ± s.e.m. (n = 3).

Table 5. Effect of the skin penetration enhancers 1,8-cineole, menthone, nerolidol and (+)-limonene in propylene glycol/water co-solvent systems on the partitioning of 5-fluorouracil into human stratum corneum expressed as partitioning ratios (partition coefficient from formulation containing enhancer: partition coefficient from pure vehicle).

Vehicle Water : propylene glycol	Partioning ratio			
	1,8-Cineole	Menthone	Nerolidol	(+)-Limonene
80:20	1·0 ± 0·1	1·0 ± 0·1	0·91 ± 0·2	0·91 ± 0·1
50:50	1·5 ± 0·3	2·5 ± 0·7	2·9 ± 0·5	1·3 ± 0·1
20:80	1·2 ± 0·3	2·2 ± 0·8	2·8 ± 0·6	1·1 ± 0·2

Data are mean s.e.m. (n = 3).

presented in Table 5 in terms of partitioning ratio, which is the ratio of the drug partition coefficient from the co-solvent system containing terpene to that from the pure analogue vehicle. The table shows that the terpenes saturated in 20% propylene glycol vehicles did not improve drug partitioning into stratum corneum, while the presence of the terpenes in the higher propylene glycol content vehicles (50, 80%) significantly increased drug partitioning (except (+)-limonene, $P > 0{\cdot}025$). The increase in partitioning was about 1·5-, 2·5- and 3-fold with 1,8-cineole, menthone and nerolidol, respectively. Comparing the effect of each enhancer on the drug partitioning from 20 and 50% vehicles indicates that the effect of terpenes on drug partitioning is modified by the propylene glycol content of the vehicle. However, the enhancers increased drug partitioning from 50 and 80% vehicles to about the same level, which may suggest that a certain quantity of the co-solvent is essential to induce the improvement in partitioning.

The dependence of terpene enhancer action on the propylene glycol content might be related to the previously reported synergistic effect between terpenes and propylene glycol (Barry & Williams 1989). Using the pretreatment protocol, propylene glycol has been shown to increase synergistically the enhancing ability of the terpenes towards 5-fluorouracil permeation by about 4-fold. Differential scanning calorimetry and small angle X-ray diffraction results suggested that the synergy might be, at least in part, due to increased lipid disruption. Increased uptake of the terpene into stratum corneum did not contribute to the observed synergy; in fact, the uptake of some terpenes significantly decreased (Cornwell 1993). This present study has shown that terpenes in the presence of high quantities of propylene glycol are able to improve drug partitioning. A similar effect may contribute to the mechanisms underlying propylene glycol synergy with terpenes.

Differential scanning calorimetry (DSC) measured the effects of propylene glycol/water vehicles and terpenes in propylene glycol/water formulations on stratum corneum constituents. Untreated human stratum corneum undergoes four major endothermic phase transitions (Fig. 3, upper thermogram). The first transition T1 appears at around 38°C. It has the lowest enthalpy and it was completely removed by lipid-extracting solvents. It has been attributed to sebaceous secretions (Golden et al 1986) or surface contaminations and to minor structural rearrangements within the bilayers (White et al 1988; Bouwstra et al 1992). However, a more recent study, based on IR results, argued that this transition is not related to lattice packing variations and attributed it to solid-to-liquid phase changes for a discrete subset of stratum corneum lipids (Gay et al 1994). The second and the third endotherms T2 and T3 appear at about 71 and 83°C, respectively. They were lost following treatment of stratum corneum samples with lipid-extracting solvents. It is generally accepted that T2 derives from the melting of the bilayer lipids (Van Duzee 1975; Golden et al 1986). The origin of T3 has been the subject of much discussion (see Williams & Barry 1992). However, recent evidence (White et al 1988; Rehfeld et al 1990) indicates that the lipids in the stratum corneum are heterogeneously arranged and hence supports the argument that T3 arises from a lipid component. The fourth endotherm appears at around 98°C. It was not affected by the lipid-extraction solvents and was heat irreversible. It has been attributed to intracellular keratin denaturation (Goodman & Barry 1989b).

Stratum corneum samples (20–40% hydrated) were treated with propylene glycol/water systems ranging from 20 to 90% w/w. The resultant thermograms were compared with thermograms of untreated samples from the same cadavers. Transition temperatures of the controls were subtracted from those of the treated samples and presented as temperature shifts. Transition enthalpy ratios were calculated by dividing transition enthalpies of treated samples by those of the controls. As shown by the thermograms in Fig. 3 and the data in Tables 6 and 7 the co-solvent systems had a very mild effect on the transition temperatures and enthalpies. The systems with the highest concentration of propylene glycol (80 and 90%) were the most effective on the lipid-

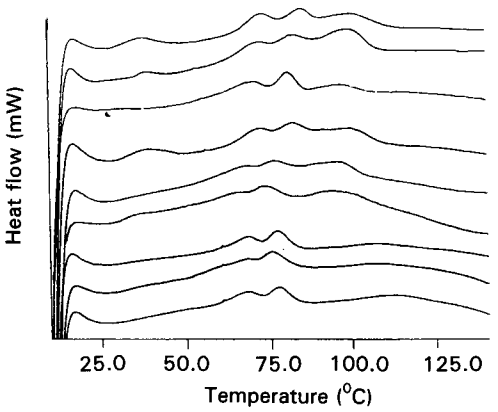

FIG. 3. Differential scanning calorimetry thermograms showing, from top to bottom, 20–40% hydrated human stratum corneum (control) and stratum corneum treated with propylene glycol co-solvent systems at concentrations 20–90% (at 10% increments).

Table 6. Effects of propylene glycol co-solvent systems on the transition temperatures and mean shift from control values (°C) of human stratum corneum.

Sample treatment (% propylene glycol)	T1		T2		T3		T4	
	Transition temperature	Mean shift	Transition temperature	Mean shift	Transition temperature	Mean shift	Transition temperature	Mean shift
Control	38·1 ± 0·3	0·0	70·9 ± 0·4	0·0	83·2 ± 0·5	0·0	98·1 ± 0·5	0·0
20	38·4 ± 0·3	−0·4	71·9 ± 0·3	−1·3	82·6 ± 0·5	−3·0	95·8 ± 2·5	−3·7
30	37·1*	−0·5	69·6 ± 0·5	−1·1	79·5 ± 1·1	−1·6	95·3 ± 0·8	−3·0
40	39·2 ± 0·2	−1·0	72·2 ± 0·3	−0·5	81·6 ± 0·8	−1·9	96·9 ± 2·0	−3·4
50	37·5 ± 0·8	−0·3	69·4 ± 0·5	−1·2	77·5 ± 0·6	−2·9	96·5 ± 1·1	−2·3
60	37·6*	0·9	67·5 ± 1·2	−2·3	77·4 ± 1·0	−2·6	96·2 ± 2·8	−1·6
70	38·4 ± 1·7	1·1	69·5 ± 1·5	−1·2	79·6 ± 2·3	−2·8	96·3 ± 2·2	−0·6
80	39·1 ± 0·5	1·5	67·2 ± 1·6	−3·9	77·4 ± 0·9	−3·4	102·3 ± 2·2	2·9
90	39·5 ± 0·6	2·4	67·7 ± 0·9	−4·3	75·7 ± 1·4	−4·2	102·7 ± 2·3	3·6

*T1 appeared in one sample only. Transition temperature data are mean ± s.e.m. (n = 3–18).

Table 7. Effects of propylene glycol co-solvent systems on the transition enthalpies (J g^{-1}) and mean ratios compared with control values of human stratum corneum.

Sample treatment (% propylene glycol)	T1		T2		T3		T4	
	Transition enthalpy	Mean ratio	Transition enthalpy	Mean ratio	Transition enthalpy	Mean ratio	Transition enthalpy	Mean ratio
Control	1·93 ± 0·22	1·0	6·65 ± 0·45	1·0	5·88 ± 0·48	1·0	7·09 ± 0·43	1·0
20	1·25 ± 0·15	1·0	4·59 ± 0·20	0·9	4·42 ± 0·08	0·9	7·77 ± 1·06	1·2
30	1·08*	1·0	5·48 ± 0·56	0·9	4·99 ± 0·38	0·9	6·56 ± 0·20	0·9
40	2·11 ± 0·32	1·2	4·76 ± 0·19	0·9	5·49 ± 0·39	1·1	5·58 ± 0·46	1·1
50	1·47 ± 0·40	1·2	6·13 ± 0·64	0·9	6·31 ± 0·64	1·1	6·81 ± 0·43	1·0
60	2·35*	1·1	6·04 ± 0·35	1·1	6·61 ± 0·52	1·0	6·52 ± 0·55	1·0
70	1·93 ± 0·25	1·2	5·78 ± 0·39	0·9	5·51 ± 0·78	1·0	6·95 ± 0·61	1·1
80	1·89 ± 0·21	1·1	5·70 ± 0·24	1·1	6·62 ± 0·46	1·2	7·18 ± 0·46	1·2
90	2·05 ± 0·23	1·2	5·96 ± 0·53	0·9	5·37 ± 0·45	1·0	8·95 ± 0·96	1·2

*T1 appeared in one sample only. Transition enthalpy data are mean ± s.e.m. (n = 3–18).

related transitions T2 and T3, reducing their temperatures by about 4°C. The transition T4 was also affected by high propylene glycol concentrations. It was broadened and shifted to higher temperature because of tissue dehydration.

The effects of terpenes saturated in propylene glycol co-solvent systems were also studied. Thermograms of samples treated with terpene formulations were compared with those obtained from samples treated with enhancer-free vehicles (as control), and hence any observed change could be assumed to be due to the terpenes. The enhancers did not significantly change the temperature and enthalpy of the transition T4. Transition T1 was absent in some samples, and in addition, recent studies have shown that the barrier function towards 5-fluorouracil permeation remains essentially unchanged at temperatures above T1 and below T2 (Cornwell & Barry 1993). Thus, only the effects of the formulations on the major lipid transitions T2 and T3 will be presented. The shift in the transition temperatures and the enthalpy ratios of the lipid-related endotherms T2 and T3 were calculated as above. Table 8 shows that the terpenes shifted the transitions T2 and T3 to lower temperatures. However, the effect of each enhancer at the different propylene glycol concentrations was very similar (no significant difference, Tukey's HSD test, $\alpha = 0.05$), except for nerolidol where its effect when applied in 80 and 90% vehicles was slightly higher than its effect in the other co-solvents.

Table 9 shows the effects of terpenes on the transition enthalpies. While (+)-limonene has no major effect on transition enthalpies, 1,8-cineole, menthone and nerolidol reduced the enthalpy of the transition T2 and their effect was

Table 8. Effects of the terpenes saturated in propylene glycol co-solvent systems on the transition temperature and mean shifts from control values (°C) of the lipid-related endotherms in human stratum corneum.

Vehicle (% propylene glycol)	1,8-Cineole				Menthone				Nerolidol				(+)-Limonene			
	T2	Shift	T3	Shift	T2	Shift	T3	Shift	T2	Shift	T3	Shift	T2	Shift	T3	Shift
20	48·6 ± 2·0	−23·7	65·7 ± 0·9	−17·6	56·5 ± 1·8	−15·3	68·0 ± 0·8	−14·5	68·5 ± 0·9	−3·2	79·5 ± 0·6	−3·6	49·4 ± 2·3	−22·4	69·5 ± 2·5	−13·2
30	48·4 ± 1·8	−22·8	65·3 ± 1·9	−16·7	65·0 ± 1·2	−14·9	68·2 ± 1·9	−13·1	67·1 ± 0·6	−3·8	77·2 ± 0·7	−4·0	46·9 ± 2·1	−24·2	66·4 ± 1·8	−14·4
40	47·0 ± 1·7	−24·7	66·0 ± 1·7	−17·8	54·3 ± 1·1	−17·6	67·8 ± 1·2	−14·2	68·8 ± 0·8	−3·6	78·3 ± 0·4	−3·8	48·5 ± 1·2	−23·3	68·4 ± 2·6	−13·8
50	45·7 ± 2·3	−24·2	61·3 ± 0·9	−16·7	55·9 ± 2·0	−14·6	63·1 ± 0·9	−14·7	65·6 ± 1·2	−4·1	73·5 ± 0·8	−4·3	47·0 ± 2·3	−22·8	63·1 ± 1·1	−15·2
60	44·0 ± 1·5	−26·3	62·1 ± 1·3	−17·2	53·3 ± 1·7	−15·9	65·3 ± 0·7	−13·1	65·0 ± 1·1	−3·8	73·1 ± 1·0	−4·8	45·7 ± 1·3	−23·6	63·5 ± 0·9	−14·6
70	43·2 ± 0·8	−26·7	63·2 ± 0·4	−18·2	51·4 ± 1·9	−18·2	65·1 ± 1·4	−15·6	64·9 ± 0·8	−4·8	75·4 ± 1·4	−5·5	46·4 ± 2·5	−23·8	67·3 ± 1·9	−13·9
80	42·3 ± 1·4	−28·3	62·0 ± 0·8	−16·7	53·1 ± 2·5	−15·8	64·5 ± 0·6	−15·2	62·7 ± 1·3	−5·9	70·9 ± 0·5	−8·3	45·3 ± 2·4	−24·1	63·5 ± 0·6	−15·2
90	42·0 ± 0·6	−27·5	58·1 ± 0·2	−18·9	52·8 ± 2·5	−14·9	62·3 ± 1·9	−14·7	60·8 ± 0·8	−6·5	68·9 ± 0·9	−7·6	45·1 ± 0·9	−23·4	61·8 ± 1·1	−15·4

Data are mean ± s.e.m. (n = 3–5).

Table 9. Effects of the terpenes saturated in propylene glycol co-solvent systems on the transition enthalpies ($J\ g^{-1}$) and mean ratios compared with control values of the lipid related endotherms in human stratum corneum.

Vehicle (% propylene glycol)	1,8-Cineole				Menthone				Nerolidol				(+)-Limonene			
	T2	Ratio	T3	Ratio	T2	Ratio	T3	Ratio	T2	Ratio	T3	Ratio	T2	Ratio	T3	Ratio
20	3·81 ± 0·15	0·82	4·78 ± 0·19	1·10	4·32 ± 0·09	0·92	4·42 ± 0·10	0·98	4·35 ± 0·13	0·94	4·14 ± 0·20	0·96	5·12 ± 0·13	1·10	4·89 ± 0·18	1·13
30	3·84 ± 0·19	0·70	5·53 ± 0·15	1·12	5·19 ± 0·18	0·94	5·48 ± 0·22	1·13	5·10 ± 0·19	0·90	5·00 ± 0·19	0·98	5·23 ± 0·22	0·96	5·61 ± 0·14	1·10
40	4·19 ± 0·08	0·75	5·25 ± 0·20	0·98	5·49 ± 0·07	0·89	4·53 ± 0·19	1·08	5·33 ± 0·10	0·89	4·95 ± 0·06	1·10	5·30 ± 0·08	0·98	4·92 ± 0·13	1·04
50	4·13 ± 0·25	0·63	5·89 ± 0·14	0·94	5·16 ± 0·21	0·83	7·23 ± 0·25	1·16	5·44 ± 0·22	0·85	6·45 ± 0·16	1·04	6·14 ± 0·15	0·95	6·21 ± 0·23	0·98
60	3·51 ± 0·10	0·55	6·23 ± 0·20	0·94	4·53 ± 0·14	0·74	6·98 ± 0·14	1·07	5·32 ± 0·09	0·87	5·98 ± 0·18	0·92	6·65 ± 0·18	1·12	6·89 ± 0·12	1·06
70	3·25 ± 0·24	0·45	4·10 ± 0·18	0·71	3·93 ± 0·12	0·69	5·52 ± 0·21	0·94	4·49 ± 0·06	0·76	6·03 ± 0·06	0·98	4·57 ± 0·12	1·08	5·85 ± 0·24	0·97
80	2·83 ± 0·18	0·41	4·63 ± 0·23	0·68	3·28 ± 0·08	0·58	6·28 ± 0·23	0·97	3·95 ± 0·20	0·69	7·20 ± 0·21	1·07	5·61 ± 0·09	0·98	7·10 ± 0·19	1·10
90	2·54 ± 0·31	0·34	3·42 ± 0·12	0·58	3·10 ± 0·25	0·46	5·09 ± 0·13	1·09	3·81 ± 0·13	0·67	5·27 ± 0·13	0·95	5·42 ± 0·06	0·89	4·39 ± 0·21	1·08

Data are mean ± s.e.m. (n = 3–5).

more pronounced as the propylene glycol percent in the vehicle increased. The enthalpy of the transition T3 was influenced (reduced) only by 1,8-cineole saturated in the high propylene glycol content vehicles (70–90%).

To relate the changes in the lipid transition temperatures and enthalpies, which are produced by the formulations, to events occurring in skin at physiological temperature and hence to permeation results, we will interpret the phase transitions of the skin lipids by applying classical thermodynamics as used to describe first-order melting behaviour of pure compounds. Such an approach has been used to analyse differential thermal analysis results obtained with stratum corneum even though skin lipids consist of a complex mixture of components (Bouwstra et al 1989).

At the transition temperature, Tm, solid and liquid phases are in equilibrium and $\Delta G = 0$. Therefore:

$$\Delta G = 0 = \Delta H - Tm\Delta S \quad (4)$$

thus:

$$\Delta H = Tm\Delta S \quad (5)$$

for which:

$$\Delta S = S_{final} - S_{initial} \quad (6)$$

where $S_{initial}$ is the entropy of the lipids before the transition and S_{final} is the entropy of the lipids after the transition. Hence, we will deal with two cases.

Case 1

A decrease in Tm on enhancer treatment ($Tm_{treated} < Tm_{control}$) should lead to an increase in ΔS of the treated sample in comparison with the control ($\Delta S_{treated} > \Delta S_{control}$), providing that ΔH remained essentially constant. Such an increase in ΔS is most likely to be due to an increase in S_{final} of the treated sample ($S_{final\ treated} > S_{final\ control}$), which means that above the phase transition temperature, the entropy of the lipids in the presence of the enhancer (in the treated sample) is higher than the entropy of the lipids alone (in the control sample). This phenomenon is a freezing point-depression effect, indicating little enhancer/lipid interaction below the transition temperature. A decrease in $S_{initial}$ of the treated sample ($S_{initial\ treated} < S_{initial\ control}$) is less likely, since it would mean an improvement in lipid organization in enhancer-treated samples below the transition temperature.

Case 2

A decrease in ΔH on enhancer treatment ($\Delta H_{treated} < \Delta H_{control}$) could decrease ΔS of the treated sample ($\Delta S_{treated} < \Delta S_{control}$) depending on the magnitude of Tm shift. A decrease in $\Delta S_{treated}$ is most likely to be due to an increase in $S_{initial}$ of the treated sample ($S_{initial\ treated} > S_{initial\ control}$), which would mean an increase in the entropy of the lipids in the presence of the enhancer at temperatures below the transition. We assume that these lower temperatures include physiological temperature and this decrease in entropy change indicates a fluidization or reduction in the lipid order at normal skin temperature.

Using the mean data of transition temperatures and enthalpies presented in Tables 6 and 7, the combined

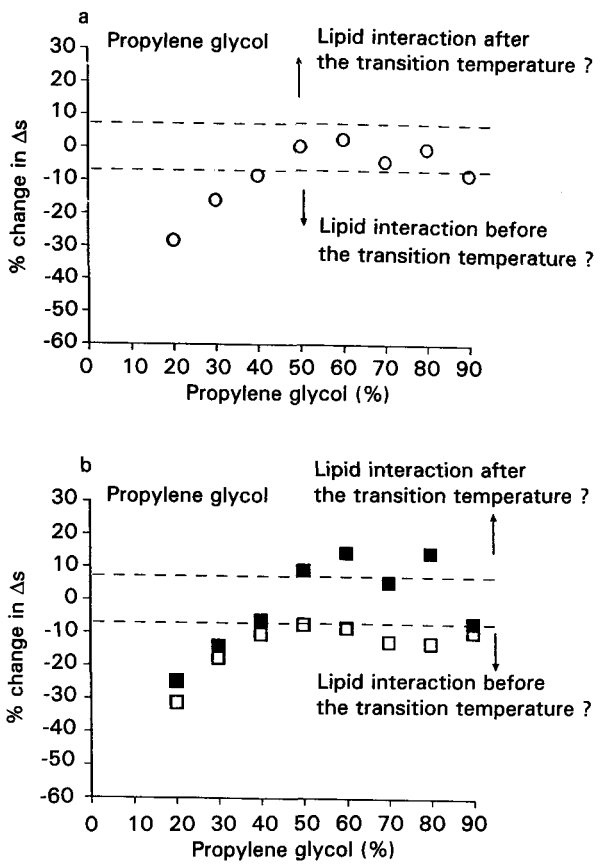

FIG. 4. a. The effects of propylene glycol/water co-solvent systems on the combined entropy changes (ΔS, $J\ K^{-1}\ g^{-1}$) associated with the major lipid transitions (T2 + T3) in 20–40% hydrated human stratum corneum. b. The effects of propylene glycol/water co-solvent systems on the entropy changes (ΔS, $J\ K^{-1}\ g^{-1}$) associated with T2 (□) and T3 (■) in 20–40% hydrated human stratum corneum. Dotted lines illustrate ± 7% level of experimental uncertainty.

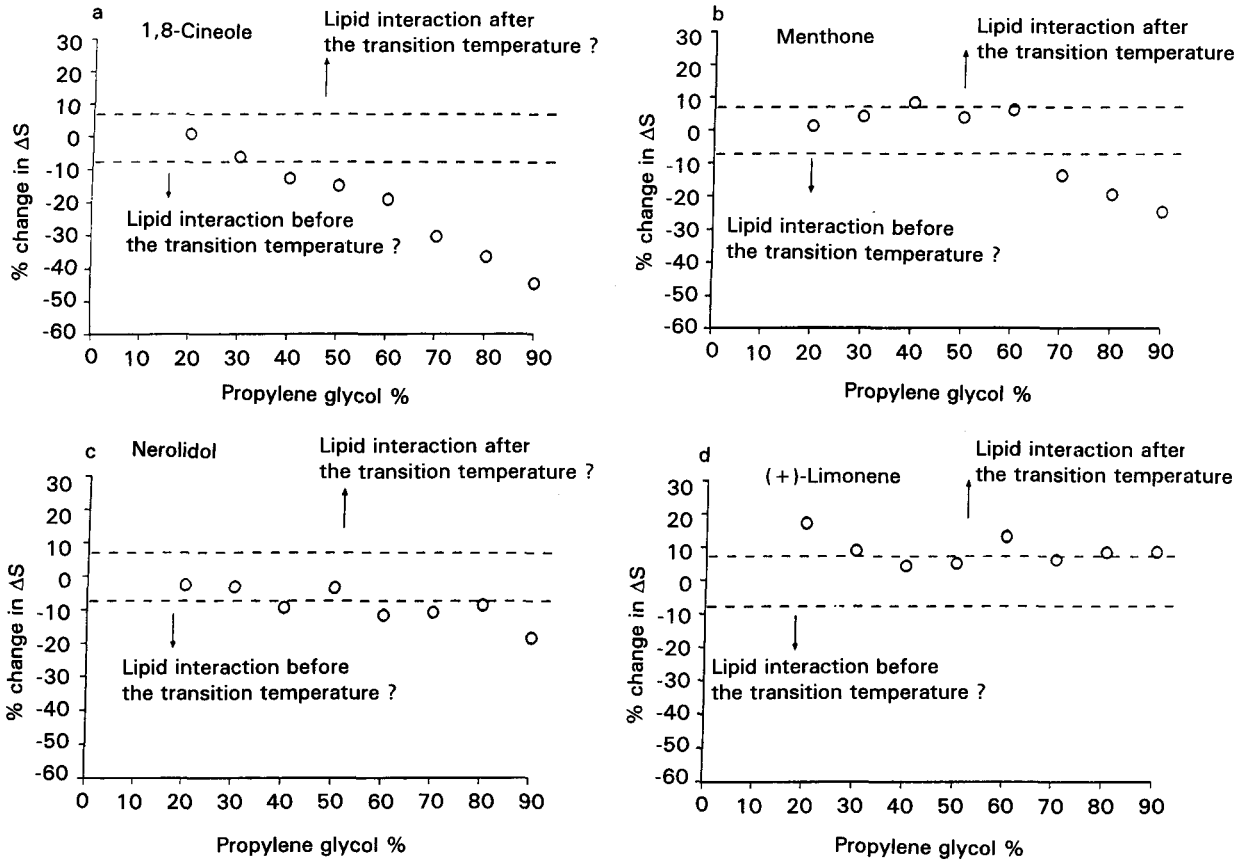

FIG. 5. The effects of (a) 1,8-cineole, (b) menthone, (c) nerolidol and (d) (+)-limonene saturated in propylene glycol/water co-solvent systems on the entropy changes (ΔS, $J K^{-1} g^{-1}$) associated with the major lipid transitions (T2 + T3) in 20–40% hydrated human stratum corneum. Dotted lines illustrate ± 7% level of experimental uncertainty.

entropy changes associated with the major lipid transitions (T2 + T3) and the entropy changes associated with each transition (T2 and T3 separately) were calculated. The combined entropy changes associated with the transitions on treatment with the co-solvent systems were compared with the combined entropy changes associated with the transitions in the control samples. The entropy change associated with each transition on treatment with the co-solvent system was compared with the entropy change associated with that transition in the control sample. The difference between the entropy changes associated with the control and the treated samples was calculated as percentages of the control value. In the light of the aforementioned, unavoidable baseline problems typical of human stratum corneum thermograms, the reported enthalpy values are approximate. Hence, up to 7% increase or decrease in the entropy of the treated samples compared with the controls is considered to be within experimental error.

Data in Fig. 4a show that the combined entropy changes reduced markedly on treatment with 20 and 30% co-solvent systems, and reduced slightly on treatment with 40 and 90% systems, while the effects of the systems 50–80% were within experimental error limits. The effects of the co-solvents could be better understood by interpreting the entropy changes associated with each transition separately. Fig. 4b shows that the co-solvent systems decreased the entropy changes associated with T2. The reduction was less as the propylene glycol percent increased from 20 to 50%, after which the effect was almost the same. Similarly, the entropy changes associated with T3 were reduced with the high water-content systems (20 and 30% propylene glycol) while remaining unchanged or showing a slight increase on treatment with 40–90% propylene glycol systems. Since we have used 20–40% hydrated stratum corneum as the control membrane, the reduction in the entropy changes on treatment with the high water-content systems (20 and 30% propylene glycol) is probably due to the uptake of water into stratum corneum which caused some fluidization or disorder in the lipids. As the percent propylene glycol in the vehicles was further increased its uptake into stratum corneum would increase and the uptake of water would decrease and hence a lesser effect was observed. These results suggest that propylene glycol is less lipid disruptive than water and that T2-related lipids are more susceptible to disruption than T3-related lipids.

The permeation experiments used fully hydrated epidermal membranes and hence the stratum corneum lipids were maximally conditioned in water and also kept in contact with an aqueous receptor. Consequently, the change in the quantity of water or propylene glycol taken up into the stratum corneum in contact with aqueous receptors from the different vehicles is unlikely to affect significantly the state of lipids. This explains the essentially similar flux of 5-fluorouracil from the different propylene glycol/water systems.

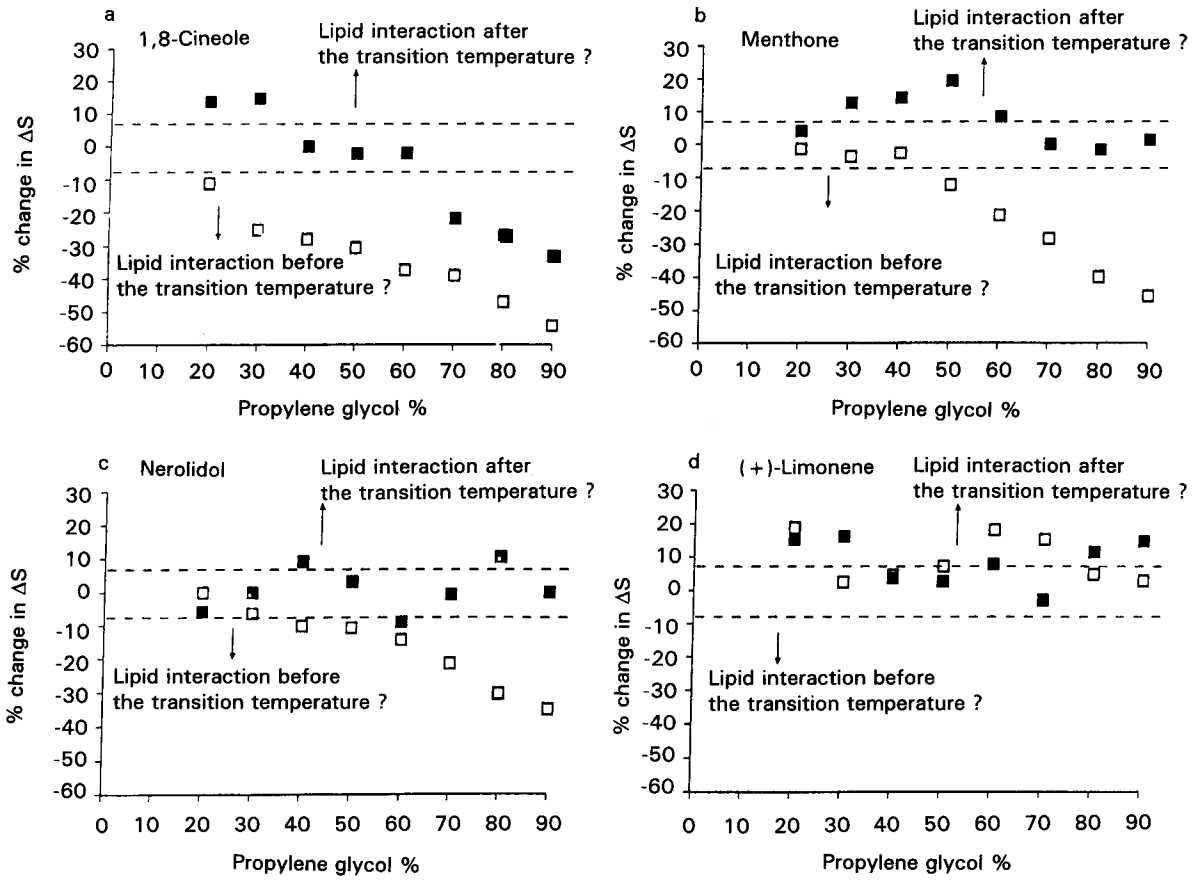

FIG. 6. The effects of (a) 1,8-cineole, (b) menthone, (c) nerolidol and (d) (+)-limonene saturated in propylene glycol/water co-solvent systems on the entropy changes (ΔS, $J K^{-1} g^{-1}$) associated with T2 (□) and T3 (■) in 20–40% hydrated stratum corneum. Dotted lines illustrate ± 7% level of experimental uncertainty.

Using the mean data of transition temperatures and enthalpies presented in Tables 8 and 9, the combined entropy changes associated with the major lipid transitions (T2 and T3) and the entropy changes associated with each transition (T2 and T3 separately) on treatment with terpene-containing co-solvent systems were calculated. To normalize the direct effect of the co-solvent systems as discussed above, the combined entropy changes associated with the transitions on treatment with terpene formulations were compared with the combined entropy changes associated with the transitions on treatment with the analogue, terpene-free co-solvent system. Results are presented in Fig. 5. Entropy changes associated with each transition (T2 and T3 separately) on treatment with the terpene formulations were compared with those associated with the analogue, terpene-free co-solvent systems (Fig. 6).

Fig. 5 shows the effects of the terpenes on the combined entropy changes. The effect of 1,8-cineole (Fig. 5a) in 20 and 30% co-solvent systems was small. However, 1,8-cineole decreased the combined entropy changes markedly when applied in 40–90% co-solvent systems, and its effect increased with increasing propylene glycol content in the vehicles in agreement with the permeation results. The combined entropy changes were also reduced by menthone (Fig. 5b) when applied in the systems with high propylene glycol content (70–90% vehicles), and by nerolidol (Fig. 5c) when applied in 40 and 60–90% systems, suggesting lipid disruption by the enhancers at skin temperature. However, (+)-limonene (Fig. 5d) increased the combined entropy changes for some vehicles, which suggests only a freezing point-depression effect.

More information about the interaction of the enhancers with stratum corneum lipids was again gained by interpreting the entropy changes associated with each transition alone (T2 and T3 separately). Data in Fig. 6a show that 1,8-cineole decreased the entropy changes associated with T2. Its effect was higher as the propylene glycol in the vehicle was increased from 20 to 90%. Menthone (Fig. 6b) decreased markedly the entropy changes associated with T2 when it was applied in a 50% propylene glycol system, and like 1,8-cineole, its effect increased sharply with increasing propylene glycol content. Nerolidol (Fig. 6c) decreased the entropy changes associated with T2 gradually and continuously. As discussed above, these reductions suggest a disruption or fluidization in the T2-related lipids at skin temperature and in agreement with the permeation results they suggest that the disruptive effect of the enhancers is affected by the propylene glycol content of the vehicle.

The entropy changes associated with T3 (Figs 6a–c) suggest that T3-related lipids are less affected by the terpenes (1,8-cineole, menthone and nerolidol) than T2-related lipids. The effects of these enhancers on the entropy changes

associated with this transition were either within the range of experimental error or they caused a slight increase, both effects providing no proof of lipid disruption or fluidization at low temperature. 1,8-Cineole at the high propylene glycol content systems (70–90%) was the exception, since it reduced the enthalpy changes associated with T3 significantly which may suggest a further disurption in the lipids. Fig. 6d shows that (+)-limonene, which provided the mildest enhancement effect, did not significantly reduce the entropy changes associated with either T2 or T3; in fact, it increased them for some vehicles and hence entropy changes provide no evidence of lipid disruption by (+)-limonene at physiological temperatures.

In agreement with a previous study (Yamane et al 1995) the effect of terpenes on the lipid transition enthalpies (and in this study the associated entropy changes) correlates well with the permeation results. Formulations containing (+)-limonene which slightly increase 5-fluorouracil flux, shifted the transition temperatures of the lipid endotherms without affecting the enthalpies and consequently without decreasing the entropy changes. This suggests a freezing point-depression effect and provides no proof of lipid disruption at physiological temperatures. Formulations containing 1,8-cineole, menthone and nerolidol, which significantly increased 5-fluorouracil flux, produced (in addition to their effect on the transitions temperature) a reduction in T2 enthalpies and the associated entropy changes in a manner dependent on the propylene glycol content in agreement with the permeation results. These entropy changes suggest lipid disruption at skin temperature.

In conclusion, these studies have shown that formulations of terpenes in propylene glycol/water provide good enhancement effects on the permeation of 5-fluorouracil across human epidermis. DSC results indicated that the action of 1,8-cineole, menthone and nerolidol formulations involved lipid disruption at skin temperature as manifested by shifts in the lipid transition temperatures and reductions in the lipid transition enthalpies and entropy changes which were related to increases in the propylene glycol content in the vehicles. Partitioning studies suggest the involvement of increased drug partitioning by enhancer formulations containing large quantities of propylene glycol. (+)-Limonene showed no evidence of lipid disruption or increased drug partitioning. Its mild enhancement effect might be due to heterogeneous distribution of the oil in the stratum corneum lipids.

The application of thermodynamic principles to interpret DSC results proved to be a useful approach. By calculating the entropy changes associated with the lipid transitions it was possible to relate DSC data to events occurring at physiological skin temperature and consequently to permeation results. Moreover, the percentage change in the entropies associated with the lipid transitions appeared to be a predictive and reliable measure of enhancer activity and it provided valuable information about the interactions between the enhancers and the lipids of the stratum corneum. T2-related lipids appeared to be more susceptible to interaction with the enhancers than T3-related lipids. Presumably, this is because the higher melting lipids have longer alkyl chains, pack more efficiently and are thus more resistant to penetration by the enhancers. It is thus worthwhile in general considering the effect of enhancers on the two main subclasses of lipids separately.

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