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Glyceryl monocaprylate/caprate as a moderate skin penetration enhancer

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Abstract

Eleven widely used lipophilic formulation excipients have been screened for their skin penetration enhancing effects. The excipients tested were glyceryl tricaprylate/caprate, isopropyl myristate, glyceryl monocaprylate/caprate, decyl oleate, polyethylene glycol-6 glyceryl dicaprylate/caprate, isopropyl isostearate, isostearyl isostearate, glyceryl monoisostearate, polyglyceryl-3 diisostearate, vegetable squalane and isostearyl alcohol. Excipient effects were evaluated by measuring skin permeability coefficients towards a model hydrophilic drug, 5-fluorouracil (5-FU), before and after a 6-h treatment with neat excipient. The skin penetration enhancing mixture, 10% (w/w) Azone® in propylene glycol, was used as a positive control. Only one excipient, glyceryl monocaprylate/caprate, had enhancement effects significantly above the buffer control ($p < 0.05$). This excipient increased 5-FU penetration 10-fold. log $P_{\text{octanol/water}}$ and hydrophilic–lipophilic balance values were calculated for each of the excipients. It was concluded that, of the excipients screened, glyceryl monocaprylate/caprate is the only penetration enhancer because (1) it is the least lipophilic, (2) it has surfactant properties, and (3) it has the optimum alkyl chain length for surfactant-type skin penetration enhancers. Since glyceryl monocaprylate/caprate has only moderate enhancement effects, it should be useful as a mild, well-tolerated skin penetration enhancer.

Keywords: Azone®; Fatty acid ester; 5-Fluorouracil; Penetration enhancer; Structure–activity relationship

1. Introduction

The outermost layer of the skin, the stratum corneum, is the major rate-limiting barrier to the percutaneous absorption of most drugs (Blank, 1953, Monash and Blank, 1958, Scheuplein, 1965). The stratum corneum comprises terminally differentiated, flattened keratinocytes, rich in keratin, embedded in apolar, lamellar-phase lipids (Elias, 1991). This keratin–lipid–keratin–lipid multilayered structure makes it very difficult for

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molecules to permeate. Compounds that act as penetration enhancers work by disrupting lipid or protein structures in the stratum corneum, and/or by improving partitioning into the stratum corneum (Barry, 1991). Enhancers that disrupt lipid bilayer structures may do so by acting either at the polar head group region, or at the aqueous region between polar head groups, or at the lipophilic bilayer core (Barry, 1987). Recently, it has also been suggested that lipophilic enhancers might phase-separate from endogenous lipids and create new pore pathways at the phase interfaces (Ongpipattanakul et al., 1991, Cornwell et al., 1996).

A wide range of compounds has been shown to enhance skin penetration (Smith and Maibach, 1995). These include, for example, fatty alcohols and fatty acids (Aungst et al., 1986), fatty acid methyl esters (Chukwumerije et al., 1989) and terpenes (Williams and Barry, 1991, Cornwell and Barry, 1994). In the present study, the skin penetration enhancing effects of a series of 11 ingredients used in topical pharmaceutical and cosmetic formulations have been are tested. The excipients tested were predominantly fatty acid esters (i.e. glyceryl tricaprylate/caprate, isopropyl myristate, glyceryl monocaprylate/caprate, decyl oleate, polyethylene glycol-6 glyceryl dicaprylate/caprate, isopropyl isostearate, isostearyl isostearate, glyceryl monoisostearate, polyglyceryl-3 diisostearate). The other excipients tested were vegetable squalane and isostearyl alcohol. Some of these excipients are commonly used in topical formulations and personal care products. For example, 5–10% decyl oleate or 5–10% squalane are used to impart emolliency into formulations. Glyceryl monocaprylate/caprate is receiving increased interest from pharmaceutical formulators who use it as an emulsifier for lipophilic materials at concentrations of 5–10%, and as a bacteriostatic agent.

In this study in vitro permeation was used to test the effects of each excipient on overall skin permeability towards the model hydrophilic permeant, 5-fluorouracil (5-FU). In addition to the excipients, some skin samples were treated with 10% (w/w) Azone® in propylene glycol as a positive control. Azone® is a well-known and well-characterised skin permeation enhancer (Wiechers and De Zeeuw, 1990, Hadgraft and Williams, 1993). A mixture of 10% Azone® in propylene glycol has been shown to produce marked effects on skin structure (Bouwstra et al., 1989, 1992) and permeability (Hoogstraate et al., 1991).

2. Materials and methods

2.1. Chemicals

5-[6-^{3}H]fluorouracil (23.1 Ci/mmol, $>95\%$ radiochemical purity) and 5-FU ($\approx 99\%$) were purchased from Sigma (St Louis, MO).

The excipients, glyceryl tricaprylate/caprate (ESTASAN GT8-60 3575), isopropyl myristate (ESTOL IPM 1512), glyceryl monocaprylate/caprate (ESTOL GMCC 3601), n-decyl oleate (ESTOL DCO 3662), polyethylene glycol-6 glyceryl dicaprylate/caprate (ESTOL E03GCC 3684), isopropyl isostearate (PRISORINE IPIS 2021), isostearyl isostearate (PRISORINE ISIS 2039), glyceryl monoisostearate (PRISORINE GMIS 2040), isostearyl alcohol (PRISORINE ISOH 3515), polyglyceryl-3 diisostearate (PRISORINE PG3DI 3700) and vegetable squalane (PRIPURE SQV 3759) were obtained from Unichema International (Gouda, The Netherlands). Azone® was obtained from Nelson Research (Irvine, CA).

Fig. 1 shows the structural formulae of all the tested excipients and Azone®. Many of the excipients occur as mixtures of structural isomers. The structures shown in Fig. 1 are the structural isomers we have selected to calculate $\log P_{\text{octanol/water}}$ and hydrophilic–lipophilic balance (HLB) values. Isostearates contain a mixture of branched C_{18} alkyl chains. For the purposes of our calculations, we selected the C9-methyl form (e.g. isostearyl alcohol, glyceryl monoisostearate, etc.) in line with gas-chromatographic analysis. It was found that $\log P_{\text{octanol/water}}$ values changed as the methyl group moved from the α-position (C2) to the C3 position, but remained unchanged thereafter. The $\log P_{\text{octanol/water}}$ for the C9 branch is therefore representative of all methyl branched forms from C3 to C16 (NB: the $\log P_{\text{octanol/water}}$

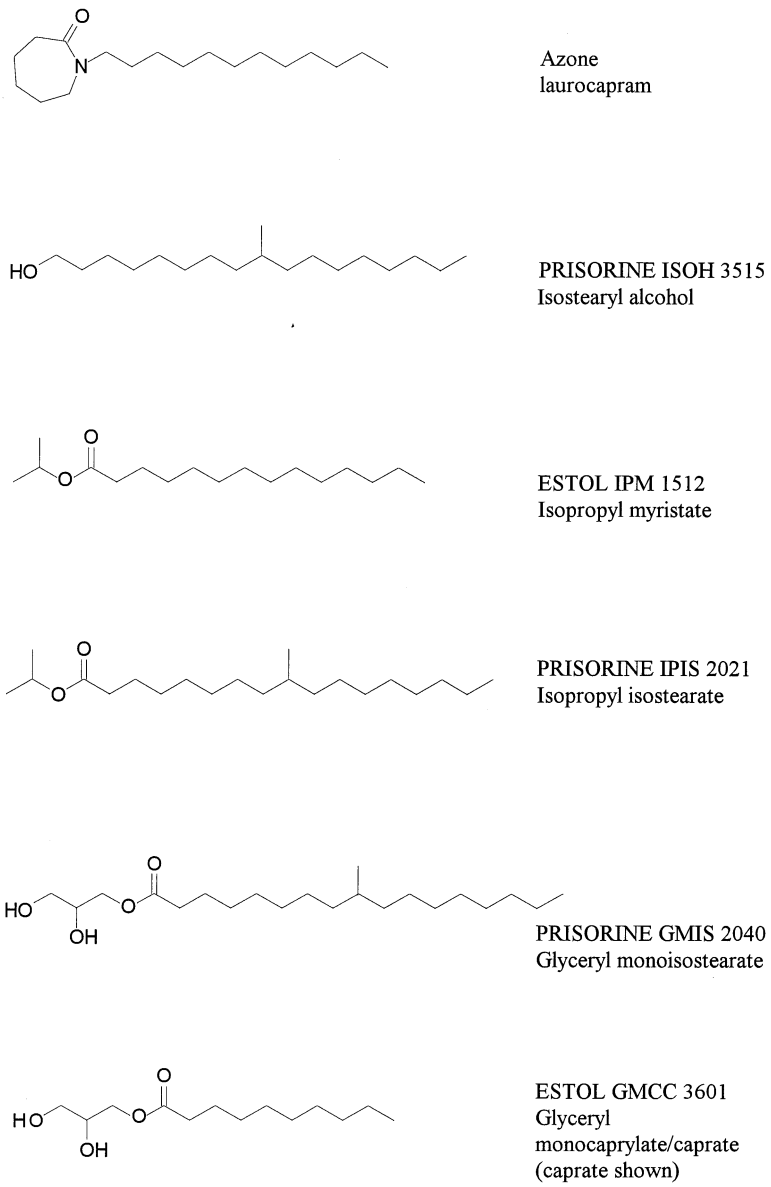
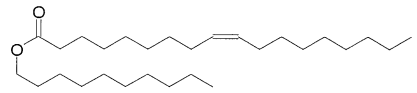
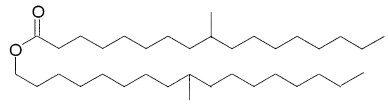


Fig. 1. Chemical structures of the tested excipients.

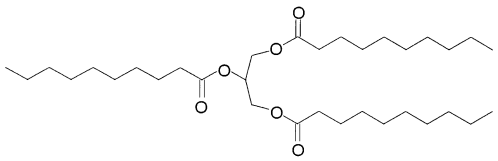
values for the α-methyl forms were also calculated; see Table 2). Some excipients contain mixtures of caprylate/caprate esters (C_8 and C_{10}, respectively). In these instances, the longest chain length was always selected. In the case of polyethylene glycol-6 glyceryl dicaprylate/caprate and polyglyceryl-3 diisostearate, there are many combinations in which the various side-chains could attach to the glycerol/polyglycerol backbones. The combination chosen for polyethylene glycol-6 glyceryl dicaprylate/caprate was selected arbitrarily. For polyglyceryl-3 diisostearate it was assumed that the hydroxyl groups at each end of the polyglycerol would be the most likely



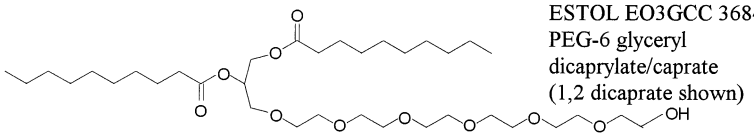
ESTOL DCO 3662
Decyl oleate



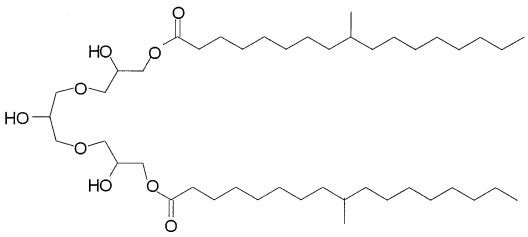
PRISORINE ISIS 2039
Isostearyl isostearate



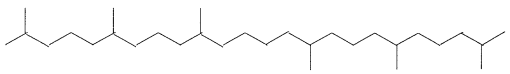
ESTASAN GT8-60 3575
Glyceryl
tricaprylate/caprate
(tricaprate shown)



ESTOL EO3GCC 3684
PEG-6 glyceryl
dicaprylate/caprate
(1,2 dicaprate shown)



PRISORINE PG3DI 3700
Polyglyceryl-3
Diisostearate



PRIPURE SQV 3759
Squalane

Fig. 1. (*Continued*)

sites for the positioning of the isostearate side-chains. It should be realised, however, that the composition of these oleochemical excipients might change from one supplier to the next, which could have an impact on the general applicability of the findings reported here.

2.2. Preparation of human skin

Upon receipt, freshly excised human abdominal skin samples were cut into 4 × 12 cm pieces with a scalpel blade and the subcutaneous fat was removed with a knife. The skin was then wrapped in aluminium foil and frozen at −70°C. Immediately prior to use, the skin was thawed and the upper surface dermatomed to a thickness of 300–500 μm (Deca Dermatome; DePuy Healthcare, Leeds, UK).

To minimise changes in hydration during the experiment, skin samples were floated on Dulbecco's modified phosphate buffered saline (pH 7.4, with 0.1% glucose; DMPBS) for 1–2 h prior to diffusion cell assembly. The skin was also allowed to hydrate further in the cells by filling both donor and receptor compartments with DMPBS. The total hydration time before the first 5-FU treatment was approximately 6 h.

2.3. Diffusion system set-up

Teflon® flow-through diffusion cells (Crown Glass Company, Somerville, NY) were used to mount skin samples (Bronaugh and Stewart, 1985). The cells had a receptor volume of 130 μl and an exposed surface area of 0.38 cm^2. They were maintained at a temperature of 37°C in aluminium block holders heated by a circulating water bath. The skin temperature was held at about 32°C in this way. The buffer was pumped through the cells at a rate of approximately 1 ml/h by means of a peristaltic pump (Cole-Parmar Instrument Company, Bishops Stortford, UK). This rate was sufficient to maintain sink conditions underneath the skin.

The integrity of the skin was checked by measuring water evaporation by a transepidermal water loss meter (Evaporimeter; ServoMed, Kinna, Sweden). Measurements of more than 6 g/m^2 per h indicated either that there was a hole in the skin or that the skin was not centred properly.

2.4. Permeation studies

For the first control 5-FU run, each cell was treated with 500 μl of saturated 5-FU solution and the donor compartments were sealed with Parafilm®. The saturated 5-FU contained approximately 40 μCi/ml radiolabel. The receptor fluid was collected at 2-h intervals for 40 h. Receptor solution samples and all other aqueous samples were mixed with Ready-Safe® scintillation cocktail (Beckman Instruments, Fullerton, CA) and then analysed using a LS60001C liquid scintillation counter (Beckman Instruments).

After the application time, each donor compartment was emptied and washed with nine rinses of 0.5 ml of buffer. Each donor compartment was then swabbed dry with cotton wool. Donor compartments were next filled with 0.5 ml of buffer to allow the 5-FU, which had partitioned into the skin, to wash out into both the donor and the receptor compartments for 24 h. After the wash-out period the buffer was removed and the skin blotted dry with cotton wool.

Each cell was treated with 250 μl of excipient/enhancer for 6 h, during which time the completion of the wash-out was monitored by collecting the receptor solution every 2 h. After treatment, the membranes were blotted free of excipient with cotton wool and the skin treated as before with 5-FU for 40 h. After the second treatment period the cell donor compartments were washed as before and the cells dismantled. Skin samples were solubilised overnight in an aqueous solution of 90% (v/v) Soluene-350® (Packard Instruments, Meriden). Solubilised skin samples were mixed with Hionic-Fluor® scintillation cocktail (Beckman Instruments) prior to liquid scintillation counting.

2.5. Calculation of skin penetration data

In vitro permeation studies used saturated solutions of 5-FU. Since donor depletion was not significant, the 5-FU may be described as an 'infinite dose'. Zero-order kinetics can, therefore, reasonably be applied to the data. Under zero-order conditions and at steady-state the cumulative amount of 5-FU permeating through an ideal membrane, Q_t (dpm/cm^2), can be described by:

$$Q_t = [(P_{m/w} \times D \times C_o)/h] \times [t - (h^2/6D)]$$

where $P_{m/w}$ is the membrane/water partition coefficient, D is the diffusivity (cm^2/h), h is the membrane thickness (cm), C_o is the concentration in the donor solution (dpm/ml) and t is the time (h). When Q_t is plotted against time, the slope of the curve at steady-state is given by the equation:

$$J = [(P_{m/w} \times D)/h] \times C_o = K_p \times C_o$$

where J is the steady state flux (dpm/cm^2 per h) and K_p is the permeability coefficient (cm/h). For the purposes of this study we will assume that our dermatomed skin preparation acts, under the conditions of this study, as an ideal membrane towards 5-FU. This is clearly an over-simplification.

Since the slope at pseudo-steady-state could be easily estimated (with linear regression) from our experimental data and the concentration in the donor compartment determined, the apparent K_p could readily be calculated using the equation above.

Enhancer activities were expressed as enhancement ratios (ERs):

$$ER = (K_p \text{ after treatment})/(K_p \text{ before treatment})$$

Each ER was calculated with data from the same cell to reduce intra- and inter-subject variability.

There is considerable literature evidence to suggest that K_p and ER values for 5-FU in human skin in vitro follow log–normal distributions (Williams et al., 1992, Cornwell and Barry, 1995). This was also confirmed by our own data. Data are thus summarised using geometric means and geometric standard errors.

2.6. Calculation of log $P_{octanol/water}$ values

In order to estimate these values for the used excipients, chemical structures were constructed using Sybyl 6.1 software (Tripos Associates, Bracknell, UK). After energy minimisation, log $P_{octanol/water}$ values were calculated using the CHEMICALC system (Suzuki and Kudo, 1990) linked to Sybyl.

2.7. Calculation of hydrophilic–lipophilic balance (HLB) values

The method for calculating HLB values is described in detail by Davies and Rideal (1963). HLBs represent an empirical numerical correlation of the emulsifying and solubilising properties of different surfactants. The HLB values reported in this study were calculated using the following formula:

$$HLB = (\text{hydrophilic group numbers}) - m(\text{group number per } -CH_2- \text{ group}) + 7$$

where m represents the number of $-CH_2-$ groups. Group numbers were as described in Davies and Rideal (1963).

2.8. Histological monitoring of the skin with time

Qualitative assessments of tissue integrity over time were made by taking samples at different time points during the experiment. For safety reasons, samples were treated with DMPBS instead of 5-FU solution but the other treatments, as stated above for the determination of the K_p, were the same. Samples were fixed in 10% formaldehyde solution, dehydrated with alcohol, embedded in paraffin, sectioned to a thickness of approximately 4 μm and stained with haematoxylin and eosin.

3. Results

Typical permeation profiles obtained before and after 6 h of treatment with selected excipients are shown in Fig. 2. All treatments, including the buffer control, apparently increased the rate of penetration of 5-FU. The most dramatic increases, however, were observed following treatment with the positive control, 10% Azone® in propylene glycol, and glyceryl monocaprylate/caprate.

Table 1 summarises the results of all the skin penetration studies. Penetration data from various treatments are grouped against their respective DMPBS treatment controls. Within each group of treatments an analysis of variance (ANOVA) was

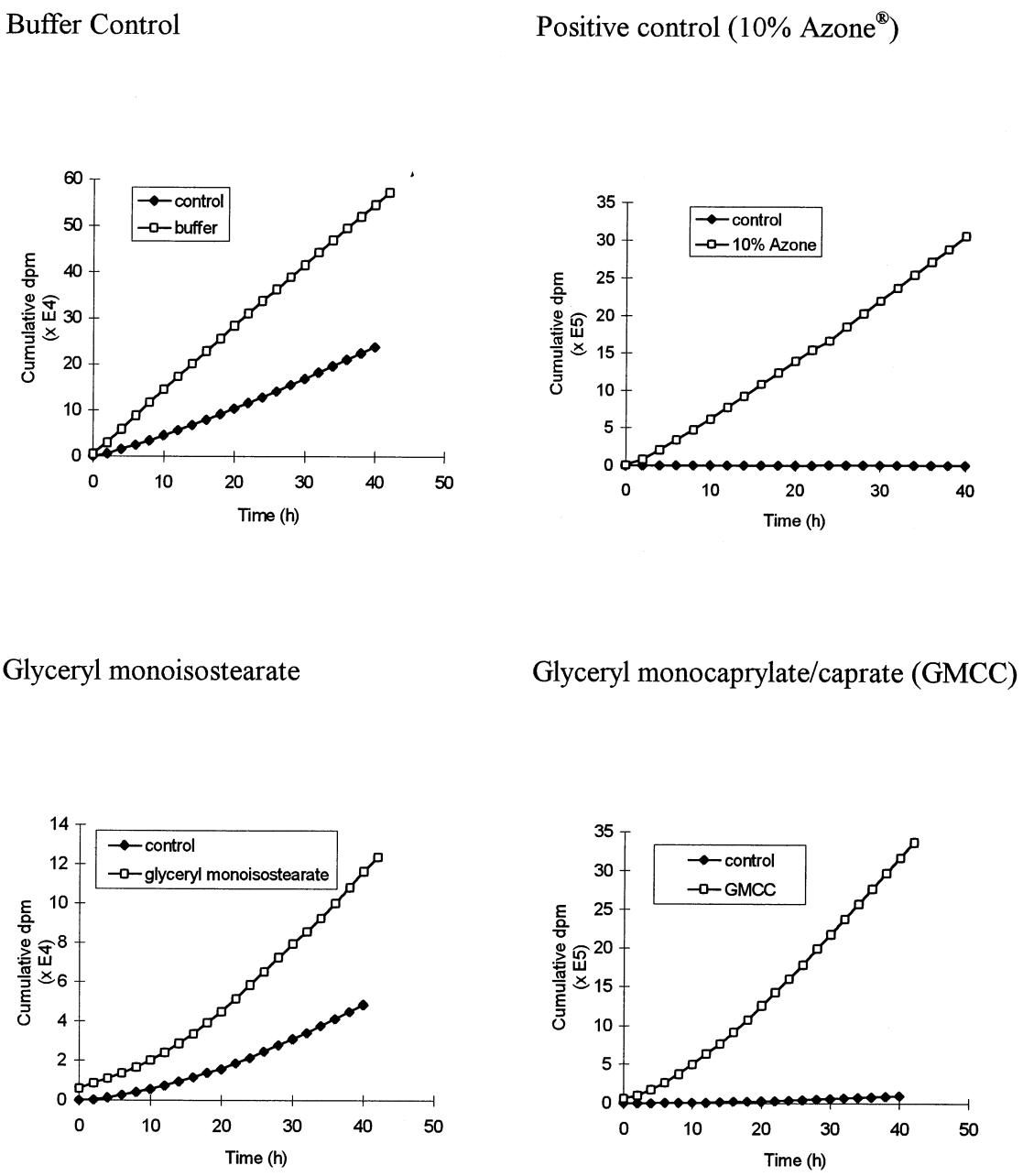


Fig. 2. Typical permeation profiles of 5-FU through human abdoinal skin before and after 6 h of treatment with a series of Unichema excipients.

performed to test whether there was any difference between the control K_ps for the different treatments. No evidence was found in any of the groups that initial K_ps were significantly different ($p < 0.05$) between treatments.

For each treatment, the Student's t-test was used to investigate whether ER values were significantly different from unity (i.e. no effect). Using this criterion, apparent enhancement effects were observed for 10% Azone® in propylene gly-

Table 1
Summary of permeability coefficients (K_p) before and after treatment and the respective enhancement ratios (ERs)

Treatment	n	K_p ($\times 10^5$, +/− 2S.E.M.)		ER (+/− 2S.E.M.)
		Before	After	
Buffer control	7	2.69 (+2.35/−0.71)	5.89 (+7.05/−1.69)	2.40 (+0.37/−0.26)
10% Azone®	7	2.51 (+1.92/−0.64)	589 (+299/−128)	324 (+292/−86.0) [a]
Isostearyl isostearate	7	1.20 (+0.32/−0.19)	2.34 (+0.62/−0.36)	2.00 (+0.34/−0.23)
Squalane	7	1.74 (+0.60/−0.31)	2.29 (+0.51/−0.32)	1.32 (+0.27/−0.18)
Buffer control	11	5.13 (+6.39/−2.84)	13.51 (+24.3/−8.68)	2.77 (+1.10/−0.79)
Glyceryl tricaprylate/caprate	11	2.90 (+3.07/−1.49)	7.41 (+7.63/−3.76)	3.15 (+2.65/−1.44)
Isopropyl isostearate	16	2.57 (+2.45/−1.25)	4.95 (+2.98/−1.86)	1.94 (+1.59/−0.87)
Isostearyl alcohol	14	1.82 (+1.32/−0.76)	4.59 (+2.91/−1.78)	2.52 (+1.26/−0.84)
Buffer control	5	5.99 (+12.6/−4.06)	10.0 (+28.6/−7.42)	1.67 (+0.69/−0.49)
Polyglyceryl-3 diisostearate	8	14.1 (+17.5/−7.81)	13.6 (+17.5/−7.66)	0.96 (+0.19/−0.16)
Polyethylene glycol-6 glyceryl dicaprylate/caprate	5	7.38 (+11.4/−4.48)	24.5 (+38.6/−15.0)	3.36 (+1.73/−1.14)
Glyceryl monoisostearate	8	11.5 (+17.9/−7.01)	14.0 (+17.9/−7.84)	1.21 (+0.72/−0.45)
Isopropyl myristate	8	5.42 (+3.71/−2.17)	14.1 (+8.66/−5.37)	2.69 (+1.13/−0.79)
n-Decyl oleate	7	8.60 (+9.56/−4.53)	7.87 (+4.15/−2.72)	0.92 (+0.59/−0.36)
Glyceryl monocaprylate/caprate	8	9.18 (+9.85/−4.75)	91.9 (+141/−55.7)	10.0 (+6.17/−3.82) [a]

Data are expressed as geometric means and geometric standard errors, and are grouped together against their respective buffer controls.
[a] Significantly different from buffer control ($p < 0.05$).

col, each of the buffer controls and most of the test excipients ($p < 0.05$). Only squalane did not significantly affect skin permeability. However, when treatment ERs were compared with their respective buffer controls, only 10% Azone® in propylene glycol and glyceryl monocaprylate/caprate were found to have enhancement effects significantly above the buffer control (Dunnett's test, $p < 0.05$). We can deduce from these data, therefore, that, under the conditions of this study, glyceryl monocaprylate/caprate was the only excipient better than the buffer control. It was therefore the only excipient in this study that acted as a skin penetration enhancer apart from Azone®.

Using the structures described in Fig. 1 and the techniques outlined above, $\log P_{\text{octanol/water}}$ and HLB values were calculated for each of the excipients. The predicted values are summarised in Table 2.

Histological examination of skin samples was performed as a function of time to evaluate changes in skin structure with time. Fig. 3a shows a section of skin fixed directly after dermatoming. The skin shows reasonably good integrity with the intercellular spaces in the viable epidermis clearly visible. Under higher magnification desmosomal junctions were visible (not shown). The loose 'basket-weave' appearance of the stratum corneum is a well-known artefact of the fixation and embedding technique that extracts stratum corneum lipids and is not indicative of barrier disruption. Some perinuclear vacuoles are visible in the viable layers of the epidermis indicating slight tissue damage. Presence of damage at the beginning of the experiment was not remarkable since the skin had been stored frozen and ice formation would be expected to disrupt viable cells. Fig. 3b shows a sample fixed after the 40-h pretreatment run. Epidermal cells are not as well stained as in the first sample and cell boundaries appear much less distinct. Many perinuclear vacuoles and pycnotic nuclei are visible. Fig. 3c shows a sample fixed at the end of the experiment. Here the epidermis is severely autolysed and the epidermis separated

Table 2
Predicted physicochemical properties of the tested excipients

Excipient	Predicted log $P_{octanol/water}$	Predicted log $P_{octanol/water}$ (α-methyl form)	Predicted HLB
Isostearyl isostearate	17.399	16.835	−7.2
Squalane	16.086		−6.8
Polyglyceryl-3 diisostearate	14.166	13.268	−0.3
Decyl oleate	12.797		−3.4
Isopropyl isostearate	9.244	8.795	−0.1
Isostearyl alcohol	8.309		0.4
Glyceryl tricaprylate/caprate	8.129		1.4
Isopropyl myristate	7.175		1.8
Glyceryl monoisostearate	7.063	6.614	3.7
Polyethyleneglycol-6 glyceryl dicaprylate/caprate	6.144		5.8
Glyceryl monocaprylate/caprate	2.850		7.5

The structural formulae used to calculate log $P_{octanol/water}$ and HLB values are show in Fig. 1.

from the dermis. Remarkably, the stratum corneum appeared reasonably intact. Overall, therefore, histological data show severe deterioration in the structure of the viable skin layers over the 5-day experiment. In contrast to the viable tissue, no major changes in the stratum corneum cell layers or overall stratum corneum integrity were noted.

4. Discussion

The skin penetration enhancing activities of 11 excipients were tested in this study. Of all the excipients tested, only one, glyceryl monocaprylate/caprate, has been shown to have skin penetration enhancement effects whereas the others, for example isopropyl myristate, showed no significant enhancement effects.

The well-known permeation enhancer Azone® was used in this study as a positive control to validate the experimental methodology. The strong enhancement effect observed was in good agreement with the literature. Previous studies using the same model permeant have noted a 20-fold increase in human skin permeability following 12 h of treatment with 2% (v/v) Azone® in propylene glycol (Barry, 1987), and a 100-fold increase in permeability after 12 h of treatment with 3% (v/v) Azone® in propylene glycol (Goodman and Barry, 1988). In the present study, 6 h of treatment with 10% (w/w) Azone® in propylene glycol produced a 324-fold increase in 5-FU permeation. The higher ER observed here was probably the result of using a much higher concentration of Azone®: 10% (w/w) as opposed to 2 or 3% (w/w).

In addition to the positive control, a negative control was performed using DMPBS as the treatment. A small, yet significant, increase in permeability was detected (i.e. the ER was significantly different from 1). The reasons for this are unclear. It is possible that this may have been related to the gradual hydration of the stratum corneum over the 5-day experiment. This would be in agreement with Bond and Barry (1988) who report that human skin permeability towards water (also a model polar compound) doubled over a period of 10 days of hydration. It may also be related to the gradual loss of integrity of the viable skin layers observed in the present study. Although the viable skin layers do not form a major resistive barrier to 5-FU penetration, the major losses in integrity observed might have caused the slight increase in overall permeability.

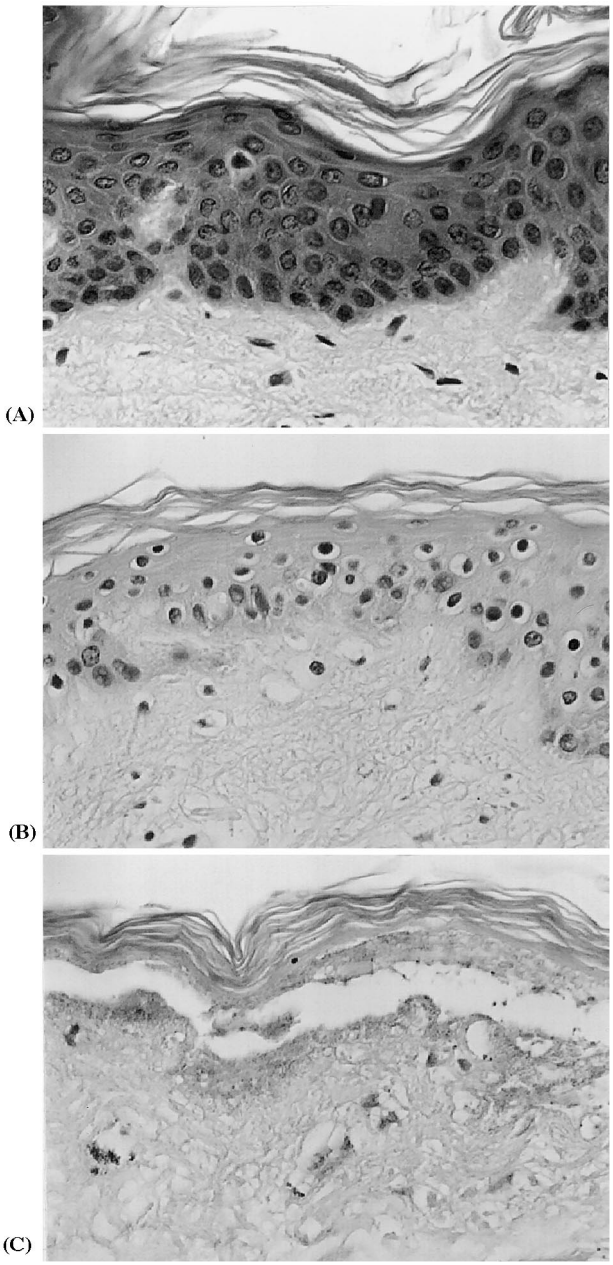


Fig. 3. Transverse sections through human abdominal skin directly after dermatoming (A), at 40 h (B) and at 5 days (C). Magnification × 600.

The lack of enhancement achieved with isopropyl myristate in our studies is surprising since evidence in the literature suggests that it is a good enhancer. For example, Ozawa et al. (1988) have shown that isopropyl myristate can enhance the penetration of hydrocortisone 17-butyrate 21-propionate (HBP; $\log P_{\text{octanol/water}} = 3.3$) across rat skin; 0.02% HBP was applied in an ethanolic gel with and without 3% enhancer. The reasons why isopropyl myristate increased drug penetration under these conditions and not in the present study may be that: (1) rat skin is more susceptible to isopropyl myristate actions than human skin; or (2) isopropyl myristate changed the thermodynamic activity of the HBP in the gel; or (3) isopropyl myristate works best when co-applied with ethanol (i.e. ethanol may enhance isopropyl myristate penetration into the stratum corneum); or (4) isopropyl myristate works better for lipophilic compounds than for a hydrophilic compound such as 5-FU.

The possibility that ethanol may enhance isopropyl myristate's penetration enhancing effects seems to be confirmed by Nakamura et al. (1996). Using hairless rat skin, the authors describe significantly higher penetration of ketotifen fumarate (a model lipophilic drug) from an ethanol–isopropyl myristate mixture than from isopropyl myristate alone. Leopold and Lippold (1995) have investigated the mechanisms of action of isopropyl myristate with differential scanning calorimetry. Studies on isolated sheets of stratum corneum revealed that treatment with undiluted isopropyl myristate reduces the transition temperatures and enthalpies of the stratum corneum lipids, suggesting that the enhancer acts through fluidising the lamellar-gel phase of the lipids.

The enhancement effect of glyceryl monocaprylate/caprate observed in our study is in good agreement with the literature. Okumura et al. (1989) investigated the penetration enhancing effects of a series of medium chained fatty acid esters on the penetration of a series of catecholamine analogues (levodopa, dopamine hydrochloride and isoproterenol hydrochloride) across excised rat skin. All drugs were highly water soluble, and were applied saturated in buffer, with or without enhancer. It was shown that glyceryl monocaprylate/caprate was an effective enhancer for all three drugs. Peak enhancement effects were observed at 3% enhancer. Under the conditions of their study glyceryl

monocaprylate was more effective than Azone® (probably because of the lack of a co-solvent such as ethanol or propylene glycol). It is interesting to note that Okumura et al. also tested glyceryl tricaprylate. As in our study they concluded that this compound has no penetration enhancing activity.

Calculation of log $P_{\text{octanol/water}}$ values for all the tested excipients revealed that the skin penetration enhancer, glyceryl monocaprylate/caprate, was the most hydrophilic of the group (log $P_{\text{octanol/water}} = 2.85$) (Table 2). All of the remaining excipients were very lipophilic (log $P_{\text{octanol/water}} > 6$). In good agreement with this finding, a previous study, using the same in vitro methodology and model penetrant (5-FU), has shown that, for a series of sesquiterpene penetration enhancers, enhancement effects decrease with increasing lipophilicity (Cornwell and Barry, 1994). Lipophilicity will affect skin penetration enhancer action for two major reasons. Firstly, it will affect uptake of the enhancer into the stratum corneum. It is likely that an optimal log $P_{\text{octanol/water}}$ exists for enhancer delivery, as there does for the transdermal delivery of a series of related compounds. Secondly, the log $P_{\text{octanol/water}}$ will affect the distribution of the enhancer within the stratum corneum, and hence the mechanism of action. It is possible that highly lipophilic enhancers might distribute into the cores of the intercellular lipid bilayers and not affect the packing of the lipid head-groups. A less lipophilic, surface-active compound, such as glyceryl monocaprylate/caprate, might pack alongside the lipids and fluidise the head-group region.

In addition to predicting log $P_{\text{octanol/water}}$ values, HLB values were also calculated. Again, the penetration enhancer, glyceryl monocaprylate/caprate, stood out from the rest of the group having the highest value (HLB = 7; see Table 2). According to the classification system, this would make glyceryl monocaprylate/caprate a wetting agent. How wetting agent properties impact on penetration enhancement is unclear. Of the remaining excipients, only two, glyceryl monoisostearate and polyethylene glycol-6 glyceryl dicaprylate/caprate (HLB = 3.7 and 5.8, respectively), were classified as emulsifiers. The HLB of glyceryl monoisostearate has been measured experimentally to be 3.8 (Davies and Rideal, 1963). Both glyceryl monoisostearate and polyethylene glycol-6 glyceryl dicaprylate/caprate would be expected to be water-in-oil surfactants. Again, how this property would impact on enhancer activity is not understood. All the remaining excipients were too lipophilic (HLB < 3.5) to be expected to have significant surfactant properties.

It was interesting to note that HLB values for the excipients correlated very well with the predicted log $P_{\text{octanol/water}}$ values ($r^2 = 0.8926$). This relationship was in good agreement with the literature. Davies and Rideal (1963) argue on theoretical grounds that the following relationship should hold:

$$HLB = 7 + 0.829 \log P_{water/octanol}$$

Changing the partition coefficient to log $P_{\text{octanol/water}}$ we get:

$$HLB = 7 - 0.829 \log P_{octanol/water}$$

which is reasonably close to the equation of the regression line obtained in this study:

$$HLB = 9.9057 - 0.9707 \log P_{octanol/water}$$

It is perhaps not unexpected that a good correlation exists since both values reflect the balance of hydrophilic and lipophilic properties within a molecule. The reason why the correlation works so well here might be that the molecules concerned are relatively uncomplicated: i.e. there are no ring systems, no ionised functional groups, no unusual elements, etc.

In addition to considering the physicochemical properties of the tested excipients, it may also be important to consider their chemical structures. It was interesting to note that the only compound found to act as an enhancer, glyceryl monocaprylate/caprate, had a simple surfactant type structure with a hydrophilic head group and a single lipophilic, alkyl chain tail group. This type of structure compares very well with that of the established penetration enhancer Azone®, which also has a head group and a single alkyl chain tail. It is possible that excipients with two or more alkyl chains (e.g. isostearyl isostearate), in addition to being too lipophilic, do not have the

required structure to be effective penetration enhancers. In the present study, the penetration enhancing activities of both glyceryl monoisostearate and glyceryl monocaprylate/caprate were measured. Since the C_8/C_{10} caprylate/caprate ester was successful as an enhancer and the C_{18} isostearate not, our data suggest that enhancer effects may be affected, amongst other things, by the alkyl chain length. This would be in good agreement with many studies performed on homologous series of fatty acids, alcohols, amines, etc. For example, in vitro permeation studies on fatty alcohol and fatty acid enhancers have shown a parabolic relationship between enhancer chain length and naloxone delivery through human skin (Aungst et al., 1986). Interestingly, C_{10} or C_{12} enhancers produced peak enhancement effects. Chukwumerije et al. (1989) have measured the in vitro enhancement effects of a series of methyl esters of n-alkyl fatty acids in hamster ventral ear skin. Peak fluxes of minoxidil were observed following treatment with C_9–C_{11} compounds. Hori et al. (1992) have measured the in vitro penetration enhancement effects of a series of aliphatic alcohols towards both propranolol hydrochloride and diazepam in rat skin. Again, a parabolic relationship was observed and the best enhancers were the C_9 and C_{10} compounds. Finally, Cornwell and Barry (1992) have investigated the penetration enhancing abilities of a series of terpene alcohols. As in the studies mentioned above, a parabolic relationship was noted with the best enhancer, nerolidol, having a chain length of ten carbons.

5. Conclusions

This study has shown that glyceryl monocaprylate/caprate with its composition as present in ESTOL GMCC 3601 is a skin penetration enhancer for the hydrophilic permeant, 5-FU. None of the other ten excipients tested had enhancer effects. Analysis of the chemical structures and physicochemical properties of all the excipients tested suggests that glyceryl monocaprylate/caprate is successful as a penetration enhancer because: (1) it is not too lipophilic; (2) it has surfactant properties (it is a wetting agent); and (3) it has the optimum alkyl chain length (C_8–C_{10}) for surfactant type skin penetration enhancers.

Glyceryl monocaprylate/caprate is under investigation as an excipient in topical pharmaceutical preparations as an emulsifier for lipophilic materials at concentrations of 5–10% and as a bacteriostatic agent. Its history of safe use, and the data presented here, suggest that glyceryl monocaprylate/caprate has the potential to be a moderately effective, well-tolerated skin penetration enhancer.

Further studies are in progress to determine the effect of other formulation ingredients on the penetration enhancing effects of the excipients tested here. It is likely that co-solvents such as ethanol and propylene glycol will have dramatic effects on the efficacy of, for example, glyceryl monocaprylate/caprate.

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