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The effect of unsaturated fatty acids in benzyl alcohol on the percutaneous permeation of three model penetrants

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Abstract

The model penetrants butyl paraben (BP), methyl paraben (MP) and caffeine (CF), because of their different octanol/water partition coefficients and postulated routes of permeation through human skin, were selected to assess the enhancing activity of pre-treatment solutions consisting of monounsaturated (oleic (OA) and palmitoleic (PA)) and poly-unsaturated (linoleic (LA)) fatty acids in benzyl alcohol (BA) using Franz diffusion cells and HPLC detection. Prior to assessing the effect of penetrant lipophilicity, MP was chosen to investigate the concentration-dependent effect of fatty acids in pre-treatment solutions. At 5% (w/w) fatty acids in BA, only pre-treatment solutions containing palmitoleic acid (PA) increased the permeation of MP when compared to pre-treatment with BA alone, whereas at higher concentrations (10 and 20%, w/w), all pre-treatment solutions except 10% OA produced a significant increase in MP flux ($P < 0.05$). The general order of fatty acid effectiveness at any concentration was PA > LA > OA. At 20% (w/w) fatty acids in BA, all pre-treatment solutions significantly enhanced the permeation of all three penetrants ($P < 0.05$) and an inverse relationship between penetrant lipophilicity and enhancement effect was observed. The permeation of BP was enhanced to a similar extent by all three fatty acids, whereas PA caused a significantly greater enhancement in the flux of both MP and CF when compared to OA, LA and controls ($P < 0.05$). It was proposed that the synergetic enhancement mechanisms of fatty acids and BA in pre-treatment solutions were augmenting the polar route by way of interactions with both polar and non-polar regions of stratum corneum lipids. Furthermore, the combination of PA and BA appears to be a good candidate as a penetration enhancer for hydrophilic molecules.

Keywords: Palmitoleic acid; Fatty acids; Benzyl alcohol; Transdermal; Lipophilicity

1. Introduction

The dermal (topical) or transdermal administration of drugs for the treatment of local or systemic conditions overcomes several important limitations asso-

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ciated with more conventional forms of drug delivery (e.g. oral, injection) such as gastro-intestinal/hepatic first pass metabolism and inadvertent systemic drug absorption. Delivery of therapeutic agents topically however, is not without its complexities and involves overcoming the extensive barrier properties that are particular to the most superficial layer of the skin, the stratum corneum (SC) (Micali et al., 2001). The overall organisation of the SC is such that corneocytes (comprised of densely packed keratin networks) together with the multilaminar, lipid-rich matrix that surrounds them, obstruct the diffusion of many penetrating molecules, especially large or water-soluble ones. Nevertheless, alternating aqueous and hydrophobic phases within the intercellular lipid bilayers permit the diffusion of certain polar and non-polar compounds, provided they satisfy the stringent physicochemical characteristics (i.e. low molecular weight, adequate solubility and appropriate lipophilicity) necessary for passive diffusion across the strongly heterogeneous structure of the SC lipids (Bouwstra et al., 2002, 2003). The majority of drugs however, lack such an optimal balance and for that reason, numerous chemical and physical techniques aimed at improving inadequate permeation have been developed. Compounds that have the ability to promote the permeation of other molecules through the skin; i.e. chemical penetration enhancers, do so by either increasing the partitioning of penetrating molecules from their site of application into the SC, or interacting with skin components to compromise the barrier properties reversibly.

Fatty acids (FAs) have been shown, by way of interactions with intercellular lipid domains, to promote the skin permeation of drugs with a wide range of polarities (Cooper, 1984; Barry and Bennett, 1987; Green and Hadgraft, 1987). The efficacy of FAs is intrinsically linked to their structure, with differences evident between saturated and unsaturated forms and those of different hydrocarbon chain length (Tanojo et al., 1997; Kandimalla et al., 1999). Unsaturated FAs, particularly those of *cis* conformation and C18 chain lengths, have been shown to be more effective enhancers than their saturated counterparts, promoting the permeation of such penetrants as naloxone (Aungst et al., 1986) and flurbiprofen (Chi et al., 1995). When introduced into the predominantly saturated, straight-chained lipid environment of the SC, these kinked FAs are seen to intercalate and disrupt the ordered lipid array (Green et al., 1988) and form separate fluid states that disorder endogenous lipids (Ongpipattanakul et al., 1991; Naik et al., 1995). A prominent example is oleic acid (OA), which together with its methyl and ethyl esters, constitutes a large proportion of the considerable patent literature regarding FAs as transdermal enhancers (Santus and Baker, 1993). OA is both GRAS listed and included in the FDA Inactive Ingredients Guide (Kibbe, 2000) and its use in topical products such as Vivelle® for the transdermal delivery of estradiol is usually in conjunction with a polar solvent such as propylene glycol (PG) (FDA, 2000). OA, along with other FAs have been shown to be more potent penetration enhancers when combined with PG (Cooper, 1984; Mahjour et al., 1989; Larrucea et al., 2001), inciting drastic alterations in the membrane structure resulting in interfacial defects between solid and liquid domains that may serve to reduce either the diffusional path length or the resistance of the SC (Ongpipattanakul et al., 1991).

The aim of the present study was to assess the potential penetration enhancement activity of a series of unsaturated FAs when combined with the solvent, benzyl alcohol (BA). The FAs, OA (*cis*-9-octadecenoic acid), palmitoleic acid (PA) (*cis*-9-hexadecenoic acid) and linoleic acid (LA) (*all cis*-9, 12-octadecadienoic acid) were selected for their variability in chain length and degree of unsaturation. BA, a widely used, low-risk preservative and fragrance additive in pharmaceutical and cosmetic systems was chosen on the premise that it is able to solvate strongly hydrophobic compounds by forming micelles whilst, due to its moderate hydrophilicity, maintaining contact with aqueous solutions, thus making it ideal for penetration through the SC (Mikulak et al., 1998). The inclusion of BA in topical formulations has been shown to increase drug retention and accumulation within the skin significantly (Mikulak et al., 1998; Peng and Nimni, 1999), whilst lack of binding in the dermis and metabolic biotransformation reactions within the epidermis aid removal from the skin and so maintain sink conditions (Barry et al., 1985; Boehnlein et al., 1994).

The stability of FAs in penetration enhancement studies has received little attention despite the fact that in the presence of air and light, unsaturated FAs are subject to peroxidation, initiated by the reaction of lipid radicals with molecular oxygen, which leads to the formation of lipid hydroperoxides (Henderson et al., 1999). It was thought necessary therefore, to

establish whether the FAs used in this study remained stable under experimental conditions.

In order to monitor any effects exclusively, enhancers were applied as pre-treatment solutions to eliminate any interaction with the penetrants. First, the effect of varying the FA concentration in pre-treatment solutions was investigated with regard to methyl paraben (MP). Then, using a specific concentration (20% FA in BA), enhancement of three model compounds; i.e. MP, butyl paraben (BP) and caffeine (CF), which have been frequently used in skin permeation studies investigating the influence of penetrant lipophilicity (Kitagawa et al., 1997; Godwin and Michniak, 1999; Akomeah et al., 2004), was carried out.

2. Methods and materials

2.1. Materials

All FAs, model penetrants and triethylamine were procured from Sigma (Poole, UK). Orthophosphoric acid and potassium dihydrogen orthophosphate (KH_2PO_4) were purchased from BDH laboratories supplies (Loughborough, UK). Phosphate buffered saline (PBS, pH 7.4) tablets were supplied by Oxoid Ltd. (Basingstoke, UK). Deionised water was prepared using an Elgstat water purifier, Option 3A, Elga Ltd. (Elga, Buckinghamshire, UK). All solvents involved in this study were of HPLC grade and purchased from Rathburn Chemicals Ltd. (Wakeburn, Scotland).

2.2. Fatty acid stability studies

Normal phase thin layer chromatography (TLC) was selected as a semi-qualitative method to monitor FA stability during Franz cell experiments. The chromatographic conditions were adapted from a previously described method (Carr et al., 1996) and consisted of silica gel plates (10 cm × 20 cm aluminium backed 60 F_{254}) and a diethyl ether:petroleum ether (b.p. 60–80 °C):acetic acid (70:30:1, v/v/v) mobile phase. Visualisation was under long (365 nm) and short (254 nm) wave UV light followed by spraying with sulphuric acid:methanol (40:60, v/v) and heating over a hotplate (140 °C).

Aliquots (100 μl) of each FA were placed in parafilm-sealed vials containing air and immersed in a water bath at 32 °C (surface temperature of skin in Franz cell experiments). For comparison, FAs were stored under nitrogen or air at room temperature and under oxygen as part of a positive control. At predetermined time intervals over a period of 72 h, 10 μl samples were taken and dissolved in chloroform to a concentration of 200 μl ml^{-1}. Samples (1 μl) were then applied to TLC plates using 5 μl disposable micropipettes and allowed to dry before placing plates vertically in TLC tanks that had been pre-equilibrated with the aforementioned mobile phase for approximately 2 h. After solvent front migration, plates were removed and dried in air before viewing under a UV lamp and visualisation by spraying.

2.3. In vitro skin permeation studies

2.3.1. Preparation of epidermal sheets

Excised skin from abdominoplastic surgery of a 58-year-old female (obtained following approval by the College Research Ethics Committee) was immediately stored at −20 °C. Frozen skin was partly thawed before removal of subcutaneous fat by dissection. In order to separate the epidermis from the dermis, fat-free skin was immersed in water (60 °C) for 45 s and then pinned, dermis side down, to allow gentle removal of the epidermis. This was then floated, stratum corneum side up, on water and immediately taken up onto a sheet of Whatman no.1 filter paper (Whatman International Ltd., Maidstone, England). The resultant epidermal sheet was left to dry thoroughly (∼2 h) and then stored flat in aluminium foil at −20 °C until needed.

2.3.2. Determination of drug binding to filter paper

The potential affinity of penetrants for the filter paper supporting epidermal sheets was investigated by incubating circular pieces of filter paper (∼0.6 cm^2) in 5 ml of MP, BP and CF standards in PBS (0.5, 5 and 20 μg ml^{-1}) at 37 °C for up to 8 h. At specified time intervals, 200 μl samples were removed and analysed by HPLC.

2.3.3. Franz diffusion cell studies

Unjacketed, pre-calibrated, vertical Franz diffusion cells, having areas of approximately 0.6 cm^2 available for diffusion and receptor compartment volumes of approximately 1.5 ml, were used for permeation experi-

ments. Donor and receptor chambers were separated by a circular section of epidermal sheet, orientated so that the SC faced uppermost. The interface of the two chambers was then sealed with parafilm and held together using a metal clamp and stand. Receptor compartments were carefully filled with PBS and stirred with small magnetic bars to ensure adequate mixing and maintenance of sink conditions (Akomeah et al., 2004). Skin samples were allowed to hydrate for 1 h prior to use by immersing receptor compartment sections in a 37 °C water bath. A minimum of three diffusion cells were used for each experiment.

Due to the limited amount of skin available from a single donor, preliminary experiments investigating the most effective concentration of FAs in pre-treatment solutions were carried out using MP alone, since this penetrant has a moderate lipophilicity compared to CF or BP.

2.3.3.1. Preparation and application of pre-treatment solutions. The FAs used in this study were mono-unsaturated FAs, oleic (C18:1) and palmitoleic (C16:1) acids and the poly-unsaturated FA, linoleic (C18:2) acid. Pre-treatment solutions with different concentrations of FAs in BA (5, 10 and 20%, w/w) were used to determine the most effective combination for further testing against model penetrants. Control pre-treatment solutions included PBS and BA.

Aliquots (300 μl) of each pre-treatment solution were applied directly on to the surface of epidermal sheets in donor chambers, which were then covered with parafilm and left for 14 h. Receiver chamber solutions were then replaced with fresh PBS and pre-treatment solutions were removed from the skin surface by emptying and quickly rinsing donor chambers three times with ethanol:deionised water (2:1, v/v) and then thoroughly with deionised water.

2.3.3.2. Preparation and application of donor suspensions. Suspensions containing undissolved/suspended solids of each penetrant were prepared by adding excess amounts of each to vials containing 20 ml PBS (vehicle). Containers were then sealed and the suspensions stirred for approximately 14 h at experimental temperatures to attain saturation. For permeation experiments, 1 ml aliquots were added to donor chambers following removal of pre-treatment solution and the cell was closed with parafilm. At specified time intervals over a period of 4 and 8 h for paraben and CF experiments, respectively, 200 μl samples were taken from the receiver compartments and immediately replaced by fresh receptor solution of equal volume and temperature. Samples were stored at −4 °C until analysed by HPLC.

2.3.3.3. HPLC analysis. A Perkin-Elmer series 200 LC Pump with Autosampler connected to an UV absorbance detector 785A was used for the analytical determination of all three penetrants. This system was connected, via a PE Nelson network chromatography Interface (NCI) 900 and PE Nelson 600 series LINK to a PC with Turbochrom Workstation software, which was used to set HPLC parameters and record analytical data.

Analysis of all three penetrants was adapted from the method described by Akomeah et al. (2004). The sample injection volume for MP and BP was 10 μl, whereas 50 μl was used for CF. A mobile phase flow rate of 1.0 ml min^{-1} was set for all penetrants. MP chromatographic conditions included a Hypersil™ C18 BDS column (150 mm × 4.6 mm, 5 μm particle size) together with the mobile phase, phosphate buffer (50 mM KH_2PO_4 containing 1% (v/v) triethylamine and adjusted to pH 3.5 using orthophosphoric acid):acetonitrile (65:35, v/v) with wavelength detection set to 254 nm. For BP, a Hichrom® C18 column (150 mm × 4.6 mm, 5 μm particle size) together with the mobile phase, phosphate buffer (20 mM KH_2PO_4 adjusted to pH 3.0 with orthophosphoric acid):acetonitrile (50:50, v/v) with detection at 256 nm was used. For CF, chromatographic conditions included a Phenomenex® Prodigy™ 5 μm ODS 2, C18 (150 mm × 4.6 mm, 5 μm) column with the mobile phase, phosphate buffer (20 mM KH_2PO_4 adjusted to pH 3.0 with orthophosphoric acid):acetonitrile (87.5:12.5, v/v). Wavelength detection was set to 215 nm.

2.3.3.4. Data analysis. Cumulative amounts of penetrant (μg) penetrating the unit diffusional surface area of the epidermal sheet (cm^2) were corrected for previous sample removal and plotted against time (h). The slope of the linear plot ($R^2 \geq 0.97$) was estimated as the pseudo-steady state flux (J_s) of in vitro penetrant permeation. Lag times were derived from the *x*-intercept of the slope at steady state and the permeability coeffi-

cient (k_P) calculated as:

$$k_P = \frac{J_s}{C_v} \quad (1)$$

where C_v is the concentration of the penetrant in the donor chamber. Enhancement ratios (ER) were derived from:

$$ER = \frac{J_s(E)}{J_s(C)} \quad (2)$$

where $J_s(E)$ and $J_s(C)$ are the flux values of the penetrant following pre-treatment with enhancers or controls (either BA or PBS), respectively. Results were expressed as the mean ± standard deviation (S.D.) of 3–5 determinations. Statistical comparisons were made using the Student's *t*-test (two-sample assuming equal variances) and analysis of variance (ANOVA, single factor) with the chosen level of significance at $P \leq 0.05$.

3. Results and discussion

3.1. Stability, solubility and binding studies

In the presence of oxygen, unsaturated lipids are susceptible to auto-oxidation, which leads to the formation of lipid hydroperoxides and hydroxides (Boyd et al., 1992). It was therefore necessary to verify that the compounds enhancing penetrant flux through the skin during Franz cell experiments were in fact those selected and not their oxidative derivatives. TLC showed that unmodified fractions of FAs subjected to diffusion cell experimental conditions migrated on silica plates as single, clearly defined bands. The poly-unsaturated FA, LA migrated with some minor bands representing lipid oxidative products, though after 14 h (length of pre-treatment in Franz cell studies) these were insignificant in comparison to pure fractions.

The binding of penetrants to filter paper on which epidermal sheets are mounted was shown to be minor. Recovery rates from standard solutions containing filter paper pieces were consistently ≥97% for a period of time equal to the duration of diffusion cell experiments.

3.2. In vitro permeation studies

Saturated suspensions; i.e. infinite doses of MP, BP and CF in PBS were used to maintain the thermodynamic activity of the penetrant in the vehicle for the duration of the experiment.

Due to the limited availability of human epidermis from the same donor, only one penetrant was used to evaluate whether FA concentration had any influence on the efficacy of pre-treatment solutions. MP was selected because although it is presumed to penetrate the skin mainly by the lipid route, a small fraction is also believed to partition down the polar pathway and therefore any pre-treatment solution that increased MP permeation is likely to increase the permeation of other compounds (Barry and Bennett, 1987).

Data for the in vitro permeation of MP through human skin from a saturated suspension following 14 h pre-treatment with FAs in BA (w/w) are summarised in Table 1. The transdermal permeation of MP was observed to increase as a function of FA content in pre-treatment solutions. At higher concentrations (10 and 20% FAs in BA, w/w), all pre-treatment solutions, except 10% OA, significantly enhanced the permeation of MP through the skin ($P \leq 0.05$). The general order of FA effectiveness at any concentration was PA > LA > OA, with 10% PA inducing an 11.31-fold enhancement in MP permeation, which was significantly greater ($P \leq 0.05$) than that achieved by either 20% LA or OA. Lag time values were reduced by skin pre-treatment with BA alone and FAs in BA in all cases. However, values for 5% OA, 20% LA and 10 and 20% PA were too small to determine.

The solvent, BA was shown to significantly ($P \leq 0.05$) enhance the rate of MP permeation through the skin when compared to pre-treatment with PBS and was more effective in doing so alone than when supplemented with 5% of any FA, excluding PA. Saitoh et al. (1995) showed that BA caused significant reductions in the skin's water holding capacity and hygroscopicity, with the effect on the former being considerable. This, together with differential scanning calorimetry (DSC) and X-ray diffraction spectrum data, signified that changes were occurring within the lipid fraction of the SC and that BA effectively loosens the lamellar structure whilst maintaining the overall lipid organisation. The reduction in its ability to do so associated with the incorporation of small amounts of FAs in this study may be the result of interactions between the two compounds. As an amphipathic organic solvent, BA is capable of solvating strongly hydrophobic compounds such as FAs (Mikulak et al., 1998). This may

Table 1
Skin permeation parameters for MP following 14 h pre-treatment with BA, PBS and different concentrations of OA, LA and PA in BA

Pre-treatment (FA in BA, w/w)	Flux ($\mu g\,cm^{-2}\,h^{-1}$)	Lag time ($\times 10^{-1}$ h)	k_P ($\times 10^{-3}$ cm h^{-1})	ER
PBS	76.51 ± 21.95	6.82 ± 2.87	30.04 ± 8.62	1
5% LA	113.99 ± 9.91	3.85 ± 1.68	44.76 ± 3.89	1.49
10% OA	119.35 ± 25.65	1.63 ± 1.31	46.86 ± 10.07	1.56
5% OA	123.14 ± 40.72	–	48.35 ± 15.99	1.61
100% BA	147.41 ± 45.44	4.04 ± 0.99	57.88 ± 17.84	1.93
20% OA	182.83 ± 34.76	1.84 ± 0.41	71.78 ± 13.65	2.39
10% LA	215.17 ± 49.98	3.26 ± 1.69	84.48 ± 19.62	2.81
5% PA	235.04 ± 81.28	2.50 ± 1.68	92.28 ± 31.91	3.07
20% LA	296.41 ± 43.76	–	116.37 ± 17.18	3.87
10% PA	851.22 ± 112.73	–	334.20 ± 44.26	11.13
20% PA	1027.44 ± 90.15	–	403.39 ± 35.4	13.43

Values are the mean ± S.D. (n = 3–5). Missing lag time values too small to determine.

however, reduce the thermodynamic activity of BA and its resulting disruption of the skin. The small amounts of FAs present may have been insufficient to compensate for this reduction, which consequently diminished the overall efficacy of the pre-treatment solution. The inclusion of 5% PA however, offset this diminution in pre-treatment solution effectiveness, resulting in a 3.07-fold enhancement in the permeation of MP, which was similar to that achieved by 20% OA and 10% LA. These observations are somewhat surprising since it has been generally observed that the mechanisms by which all FAs perturb the SC barrier depend greatly on their unsaturation. Along with forming separate fluid domains and causing interfacial defects, *cis*-unsaturated FAs like those employed in this study are thought to intercalate with SC lipids where they incite further disruptions as a result of structural kinks produced by their double bonds. In the absence of such kinks (e.g. lauric acid) enhancement is reduced (Green et al., 1988), whereas increasing the number of double bonds to two results in an increase (Tanojo et al., 1997). In this study, LA appeared to be the better enhancer of MP permeation at concentrations of both 10 and 20% when compared to OA. Tanojo et al. (1997) compared OA with LA in the penetration enhancement of *para*-amino benzoic acid across human SC and found that LA gave the highest enhancement factor. It was concluded that just as the single 'kink' in OA is thought to be responsible for SC lipid disruptions, the additional double bond present in LA may exacerbate these effects.

OA and PA on the other hand, are considered to have comparable enhancing properties and the extent to which PA enhanced the permeation of MP in this study contradicts the work of other authors. For example, whilst comparing phospholipid penetration enhancers to known unsaturated fatty acids, Yokomizo and Sagitani (1996) demonstrated that PA was second to OA at enhancing the flux of indomethacin through dorsal guinea pig skin in vitro. Taguchi et al. (1999), using Fourier transform/attenuated total reflection (FT-IR/ATR) analysis, found that the maximum PG flux into the dermis and increased dermal steady state levels were conveyed by OA and PA. This effect however, did not differ significantly and it was suggested that these FAs disrupted SC structures in similar ways. Cooper (1984) also showed that PA enhancement of the lipophilic molecule, salicylic acid from a PG vehicle through human epidermal sheet was only slightly greater than that achieved with OA. Loftsson et al. (1995), during studies assessing FAs from cod-liver oil as skin penetration enhancers, are the only group to report a significantly greater enhancement obtained with PA when compared to OA and other unsaturated FAs. They found that with the addition of PA, the flux of hydrocortisone (a model hydrophobic penetrant) through hairless mouse skin from a PG vehicle increased 640-fold.

In the present study, however, it has been found that the effect of PA intensifies with increasing penetrant hydrophilicity (Fig. 1), causing a respective 1.85, 6.97 and 51.47-fold increase in the skin permeation of BP, MP and CF when compared to BA controls (Table 2). The same can be said for LA, although, whereas PA caused a significantly greater enhancement in the permeation of both CF and MP when compared to other

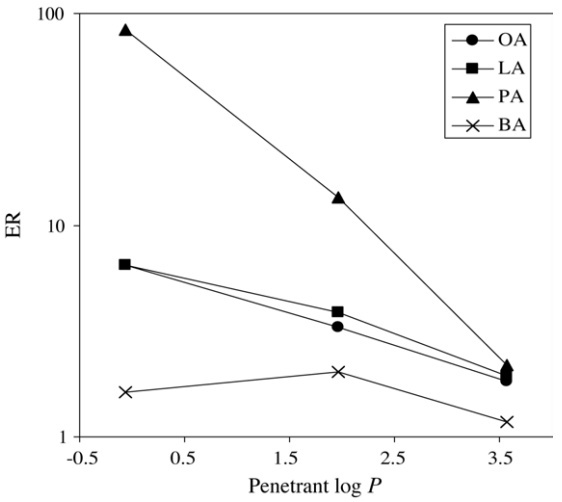

Fig. 1. The relationship between *n*-octanol water partition coefficients of model penetrants (log *P*, o/w) and enhancement ratios (ER) using benzyl alcohol (BA) and 20% oleic (OA), linoleic (LA) and palmitoleic (PA) acids in BA (w/w) as penetration enhancers. PBS control used for calculating ER.

FAs ($P \leq 0.05$), LA only caused a significantly greater enhancement in the permeation of MP when compared to OA (Fig. 3), which itself enhanced penetrants in the order CF > BP > MP. Although the permeation of BP was significantly increased by skin pre-treatment, the degree of enhancement did not vary significantly between FAs (Fig. 2). With the exception of BP permeation, lag time values were also reduced in all instances by skin pre-treatment, with certain values that corresponded to high levels of enhancement being too small to determine (Table 2).

Penetrating molecules are considered to traverse the skin by one of three pathways: the polar (aqueous), non-polar (lipoidal) or polar/non-polar route depending on their physicochemical properties. One such property is the *n*-octanol/water partition coefficient, or log *P* (o/w), which is a measure of how well a substance partitions between a lipid (oil) and water. In conjunction with the diffusion law, which predicts an inverse relationship between membrane diffusivity and penetrant size (Lieb and Stein, 1971), the log *P* value of a drug molecule can be used to predict its distribution within a biological system (Potts and Guy, 1992).

Since the three penetrants selected for this study have similar molecular weights, their rates of permeation through skin should reflect their log *P* values, which are 3.57, 1.96 and −0.07 for BP, MP and CF, respectively (Akomeah et al., 2004). Due to its optimal log *P* value (Yano et al., 1986; Potts et al., 1991), MP is readily absorbed through human skin (Soni et al., 2002), and despite its moderate hydrophilicity, is presumed to do so principally via the non-polar SC lipid route (Kitagawa et al., 1997). BP, a highly lipophilic molecule, is also expected to penetrate the skin by this route, whereas CF, which is hydrophilic, should utilise the polar pathways.

Table 2
Skin permeation parameters for BP, MP and CF following 14 h pre-treatment with BA, PBS and 20% PA, OA, LA in BA (w/w)

Penetrant	Pre-treatment	Flux ($\mu g\,cm^{-2}\,h^{-1}$)	Lag time ($\times 10^{-1}$ h)	k_P ($\times 10^{-3}\,cm\,h^{-1}$)	ER
BP	PBS	24.30 ± 4.75	0.91 ± 0.32	93.82 ± 18.32	0.85
	BA	28.74 ± 3.03	1.78 ± 0.54	110.97 ± 11.71	1
	OA	44.83 ± 4.05	1.41 ± 0.41	173.10 ± 15.64	1.56
	LA	46.96 ± 2.61	0.63 ± 0.25	181.30 ± 10.07	1.63
	PA	53.13 ± 13.1	3.07 ± 1.44	205.14 ± 50.58	1.85
MP	PBS	76.51 ± 21.95	6.82 ± 2.87	30.04 ± 8.62	0.52
	BA	147.41 ± 45.44	4.04 ± 0.99	57.88 ± 17.84	1
	OA	182.83 ± 34.76	1.84 ± 0.41	71.78 ± 13.65	1.24
	LA	296.41 ± 43.76	–	116.37 ± 17.18	2.01
	PA	1027.44 ± 90.15	–	403.39 ± 35.4	6.97
CF	PBS	4.49 ± 0.76	4.49 ± 0.09	0.17 ± 0.03	0.61
	BA	7.34 ± 0.89	2.42 ± 1.60	0.27 ± 0.03	1
	OA	28.63 ± 12.08	–	1.07 ± 0.45	3.9
	LA	29.25 ± 7.77	–	1.09 ± 0.29	3.99
	PA	377.80 ± 48.32	–	14.14 ± 1.81	51.47

Values are the mean ± S.D. ($n = 3$–5). Missing lag time values too small to determine.

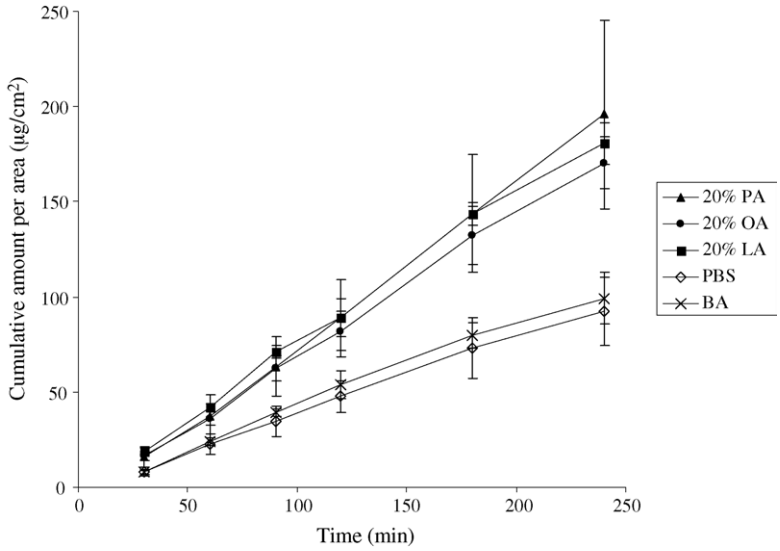

Fig. 2. Permeation profile of BP following 14 h pre-treatment with PBS, benzyl alcohol (BA) and 20% oleic (OA), linoleic (LA) and palmitoleic (PA) acids in BA (w/w). Each point represents the mean ± S.D. of 3–5 determinations.

In a similar study, Barry and Bennett (1987) selected three model drugs representing a spectrum of penetrant lipophilicity. They speculated that lipophilic molecules that move with relative ease through the SC do not have the same opportunity to act as indicators of enhancement. Highly polar molecules, however, owing to their low partition coefficient and high hydrogen-bonding potential, would show a dramatic increase in permeation with suitable enhancers. It is also possible that the experimental system may not provide optimal sink conditions for lipophilic compounds (Pozzo et al., 1991). The intrinsic permeabilities of both hydrophilic and lipophilic penetrants, however, are governed by the composition of the skin, with the former limited by their partitioning into the lipophilic SC and the latter, by partitioning from the SC into the less lipophilic

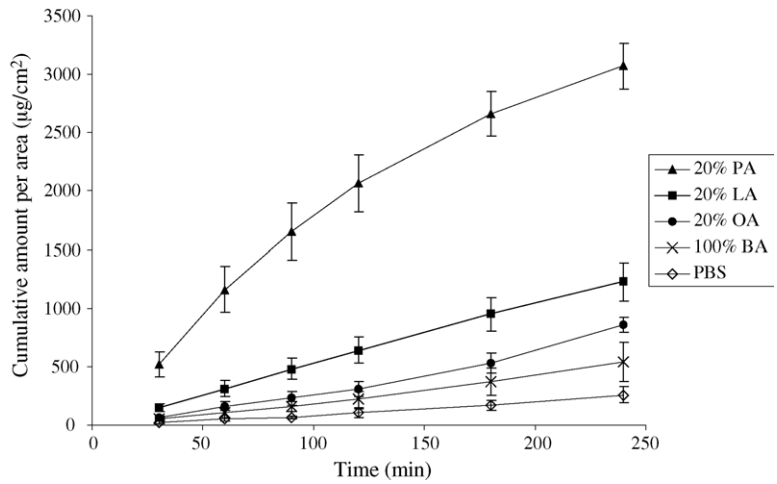

Fig. 3. Permeation profile of MP following 14 h pre-treatment with PBS, benzyl alcohol (BA) and 20% oleic (OA), linoleic (LA) and palmitoleic (PA) acids in BA (w/w). Each point represents the mean ± S.D. of 3–5 determinations.

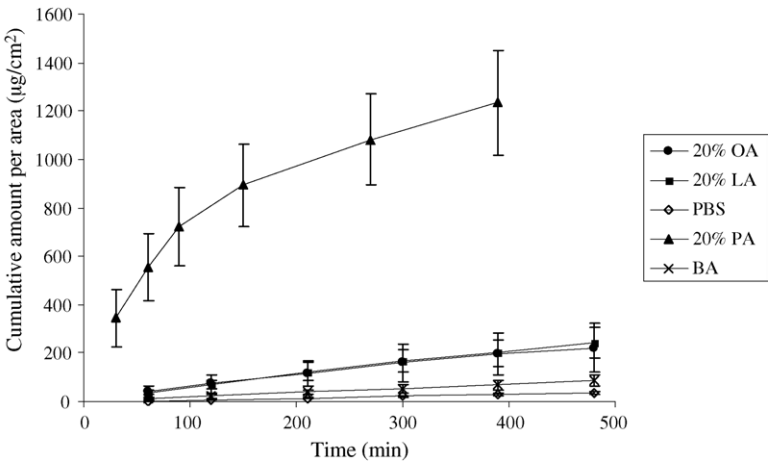

Fig. 4. Permeation profile of CF following 14 h pre-treatment with PBS, benzyl alcohol (BA) and 20% oleic (OA), linoleic (LA) and palmitoleic (PA) acids in BA (w/w). Each point represents the mean ± S.D. of 3–5 determinations.

epidermis (Lee et al., 1994). It is understandable therefore, that disruption of the lipid barrier of the SC could lead to a reduction in the membrane controlling permeation, depending on penetrant lipophilicity (Tsai et al., 2001). This is reflected in the present study by the respective high (93.82×10^{-3} cm h^{-1}) and low (0.17×10^{-3} cm h^{-1}) intrinsic k_P values of BP and CF and the degree to which these increased following skin pre-treatment. Whereas the k_P of BP was only marginally increased (205.14×10^{-3} cm h^{-1}), a substantial increase in the k_P of CF (14.14×10^{-3} cm h^{-1}) was observed after skin pre-treatment with PA/BA (Table 2 and Fig. 4).

The greater disruption of SC barrier properties obtained with PA/BA could be attributed to more favourable interactions between the two compounds. Whilst assessing the enhancing properties of a binary ethanol/water vehicle containing 4% lauric acid, Lee et al. (1994) also observed an inverse correlation between enhancement ratios and log P, which they attributed to the co-operative enhancing mechanisms of the lauric acid and the ethanol/water vehicle. Similarly, the findings of the present investigation are believed to reflect a synergistic enhancement resulting from FA interactions with BA. Wang et al. (2004) used spectroscopic studies to investigate the influence solvents have on the interactions between FA-based formulations and SC lipids. They found that while short-chained FAs were only able to disrupt lipids when applied in a lipophilic mineral oil-based formulation, perturbation with long-chained FAs, including OA and LA was limited to applications with hydrophilic, polar solvents such as PG. The use therefore, of a moderately hydrophilic solvent such as BA in conjunction with a more rigid and less lipophilic, medium sized FA like PA may have been favourable in terms of permeation through the SC and subsequent FA partitioning into SC lipids. With respect to the ways in which FAs are generally observed to perturb the SC barrier, it is possible that PA amplifies polar channels within the lipid bilayers by way of interactions with both polar and non-polar regions of SC lipids. This might result in the disruption of areas of the skin associated with the permeation of hydrophilic penetrants such as MP and in particular, CF. Other explanations such as FA interactions with the penetrant, FA interaction with BA and FA interactions with the different pathways suggested for each penetrant are also feasible and thus warrant further investigation.

4. Conclusion

Pre-treatment solutions containing 20% OA, LA or PA in BA (w/w) significantly enhanced the skin permeation of three model penetrants with wide ranging lipophilicities. Whereas all pre-treatment solutions enhanced the flux of the lipophilic penetrant BP to

a similar extent, the greatest difference in the effect of skin pre-treatment was observed with the more hydrophilic penetrants, MP and CF. MP permeation through the skin changed as a function of FA content in BA pre-treatment solutions, and maximum flux was attained for 20% FAs in BA. At this concentration, PA/BA produced a significantly greater enhancement of MP and CF when compared to LA/BA or OA/BA. The observed inverse relationship between enhancement and penetrant lipophilicity, thought to result from FA interactions with BA, lends support to the important role that solvents play in the interactions between FAs and SC lipids, influencing both their enhancement properties and ultimate effect on the transdermal delivery of drug molecules.

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