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Effect of melting point of chiral terpenes on human stratum corneum uptake☆

Kirsty M.B. Mackay, Adrian C. Williams, Brian W. Barry *

Drug Delivery Group, Postgraduate Studies in Pharmaceutical Technology, School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK

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Abstract

The effect of melting point of chiral penetration enhancers on their stratum corneum uptake was investigated. The pure enantiomers of a chiral compound often possess different melting points, and therefore dissimilar solubilities, to the racemate because of variations in their crystal structure. Two terpenes, menthol and neomenthol, saturated in propylene glycol/water, were applied to stratum corneum. Racemic menthol melts at ≈33 °C, some 9 °C lower than the pure enantiomers, whereas racemic neomenthol melts at 26 °C higher than the study temperature, considered as the theoretical melting point of its enantiomers, which are both liquids. Terpene solubility increased with the propylene glycol content of the vehicle. The lower melting forms of both penetration enhancers possessed the highest solubility in every vehicle. Maximum stratum corneum uptake was obtained from formulations containing the lower melting forms of each enhancer in 60% w/w propylene glycol systems (highest concentration used). Compared with menthol, the larger melting point difference between optical forms of neomenthol produced bigger differences in their uptake. Thus melting point depression of menthol and neomenthol, by selection of the appropriate optical form, increased the amount of terpene delivered to the stratum corneum, in agreement with theoretical predictions.

Keywords: Chiral enantiomers; Melting point; Penetration enhancers; Stratum corneum uptake; Terpenes

1. Introduction

Transdermal delivery has become an important means of drug administration. This route offers several advantages compared with oral or parenteral drug delivery (Barry, 1983; Guy and Hadgraft, 1985; Chein, 1987). These include avoidance of first-pass metabolism, the production of relatively constant plasma levels of drugs, a concurrent decrease in side effects, relative ease of drug input termination, and improved patient compliance. However, before any topically applied drug can act either locally or systemically, it must penetrate the usual barrier layer of the skin, the stratum corneum, which limits the number of

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* Corresponding author. Tel.: +44-1274-234-760; fax: +44-1274-234-769.

E-mail address: bwbarry@bradford.ac.uk (B.W. Barry).

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drugs that can successfully penetrate into and through skin (Scheuplein and Blank, 1971; Barry, 1983).

To overcome the problems arising from skin's relative impermeability, various approaches to remove reversibly the barrier resistance have been investigated, including the addition of penetration enhancers to the formulation. These agents increase drug transport through the skin by altering the diffusion and/or partition coefficient of the permeant (Barry, 1991). Previous studies have reported the penetration enhancing effects of terpenes, naturally occurring components of plant oils (Williams and Barry, 1991; Cornwell and Barry, 1994; Yamane et al., 1995a,b). However, the magnitude of enhancer uptake by the stratum corneum is seldom considered in the literature, although a relationship between enhancer uptake and promoting effect has sometimes been reported. Thus, Schuckler and Lee (1992) observed a concentration-dependent enhancement of diazepam delivery at low stratum corneum loadings of Azone. The flux of piroxicam across human and hairless mouse skin was proportionate to oleic acid uptake (Francoeur et al., 1990) and Obata et al. (1993) correlated diclofenac permeation with membrane pre-treatment time for (−) menthol.

The effect of permeant melting point depression on transdermal drug delivery has been considered (Kaplun-Frischoff and Touitou, 1997; Stott et al., 1997). According to ideal solubility theory, the lower the melting point of a substance the greater its solubility in a given solvent, including skin lipids. The pure enantiomers of a chiral compound often possess different melting points to their racemic mixtures because of variations in their crystal structure (Fig. 1) (Jaques et al., 1981). This property has been used to enhance the transdermal permeation of various drugs (Lawter and Pawelchak, 1989; Touitou et al., 1994). However, a search revealed no such literature relating to penetration enhancers. Many terpenes possess optical activity. For example, racemic (±) menthol melts at a lower temperature than its pure (+) and (−) enantiomers. Conversely, racemic neomenthol melts at a higher temperature than its pure enantiomers, which are both oils at 25 °C.

The aim of the present study was to assess the effect of melting point variations on the stratum corneum uptake of the racemate and enantiomers of menthol and neomenthol. Propylene glycol is frequently used as a solvent in dermatological formulations and has a synergistic effect on the enhancement activity of terpenes (Barry and Williams, 1989; Cornwell, 1993). Most of the terpenes in our investigation were solid at the study temperature. Consequently they were formulated as saturated solutions in propylene glycol/water co-solvents. It was realised that this would inevitably lead to the complication that various amounts of the propylene glycol would be imbibed by the stratum corneum. This would then alter somewhat the uptake of the terpene. Therefore strict agreement with physicochemical theory, as applied to inert membranes unaffected by the solvent, was not expected. The inherent variations of human stratum corneum would also pose problems. However, the experimental approach adopted should be able to assess trends, even if quantitative agreement is not possible. The uptake of the (+) and (−) enantiomers of both terpenes was therefore measured to determine if the process was enantioselective.

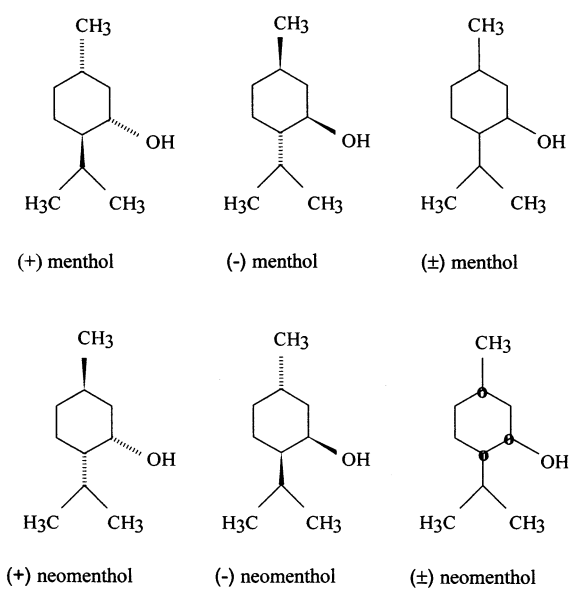

Fig. 1. Molecular structure of menthol and neomenthol. (○) Denotes chiral centres.

2. Materials and methods

2.1. Materials

(−) Menthol was purchased from Sigma Chemical Company, St Louis, MO, (+) and (±) menthol from Aldrich, Gillingham, UK, (−), (+) and (±) neomenthol from Fluka, Buchs, Switzerland. All terpenes were >99% pure. Propylene glycol (PG) was obtained from Sigma Chemical Company, St Louis, MO.

2.2. Differential scanning calorimetry

The melting points of the terpenes were determined on a DSC 7 Differential Scanning Calorimeter (Perkin Elmer, USA). Solid terpenes (7–8 mg) and liquid terpenes (10 μl) were hermetically sealed in stainless steel DSC pans. Samples were heated against a reference pan from −30 to 80 °C at 2 °C min^{-1}.

2.3. Preparation of stratum corneum

Caucasian mid-line abdominal skin was obtained post-mortem and stored in vacuum-sealed polythene bags at −20 °C (Harrison et al., 1984). Skin samples were from 17 donors, 12 female, with a mean age of 72.2 ± 9.4 years. Human epidermal membranes were prepared by a heat separation technique (Kligman and Christophers, 1963). Subcutaneous fat was removed from the skin, which was then immersed in water at 60 °C for 45 s. The epidermis was then teased away from the underlying dermis. Stratum corneum sheets were prepared by floating epidermal membranes (stratum corneum side up) on an aqueous solution of 0.001% w/v trypsin and 0.5% w/v sodium hydrogen carbonate for 12 h at 37 °C. The stratum corneum membranes were picked up on filter paper and the digested cells removed by wiping with cotton wool. The membranes were floated on water for 2 h to remove any remaining cells, lifted onto stainless steel wire mesh and left to dry at room temperature. Each sheet was rinsed for 10 s in ice-cold acetone to remove any surface contamination and stored over silica gel under vacuum.

2.4. Solubility studies

The solubilities of menthol and neomenthol in PG/water co-solvent systems, at concentrations ranging from 20 to 60% w/w, were determined at 25 °C. Excess terpene was added to the co-solvents and the mixtures heated to 40 °C for 30 min. The solutions were cooled and maintained at 25 ± 2 °C for 24 h with constant stirring, then equilibrated without stirring for a further 48 h. Excess terpene was removed by centrifugation at 13 000 rpm for 10 min and the samples were diluted with chloroform:methanol (2:1 v/v) prior to analysis (see below).

2.5. Uptake studies

Discs of dry stratum corneum (13-mm diameter) were cut using a cork borer, accurately weighed and hydrated in 0.002% w/v aqueous sodium azide for 72 h at 25 ± 2 °C. The hydrated samples were then blotted dry and immersed in a saturated solution of terpene in PG/water co-solvent for times between 30 s and 12 h. For terpene extraction from the stratum corneum, the discs were blotted dry, ensuring all excess terpene solution had been removed, then placed in 1.5-ml glass vials with 1 ml of chloroform: methanol (2:1 v/v) (Bligh and Dyer, 1959) containing 0.048 mg ml^{-1} decane GC internal standard. The vials were crimped with PTFE rubber coated septa and shaken for 48 h on a Vibrax VXR shaker. Samples were analysed by GC.

2.6. Gas chromatography analysis

Samples were analysed using a 8320B Capillary Gas Chromatograph (Perkin–Elmer, USA with a 25 m BP-5 fused silica capillary column (SGE Inc., Australia). Ten μl of analyte was manually injected onto the column with an injection temperature of 250 °C, using helium as the carrier gas set at 280 °C and 15 p.s.i.. The oven was programmed to rise from 50 to 120 °C at 10 °C min^{-1} then held at 120 °C for 1 min. The eluted samples were analysed by a hydrogen flame–ionisation detector heated at 280 °C. Both menthol and neomenthol eluted from the column with a

Table 1
Peak melting points of terpenes

Terpene	Melting point (°C) ± S.D. ($n = 3$)
(−) Menthol	42.2 (0.1)
(+) Menthol	42.8 (0.2)
(±) Menthol	33.4 (0.2)
(±) Neomenthol	50.9 (0.1)
(−) Neomenthol	Liquid-taken as 25 °C
(+) Neomenthol	Liquid-taken as 25 °C

retention time of 6.9 min. Validation of the extraction procedure gave recoveries of 90.8 (2.86), 92.5 (2.94) and 84.8 (5.59)% (standard error of the mean) for (−), (+) and (±) menthol respectively. (−), (+) and (±) neomenthol yielded recoveries of 86.4 (4.73), 87.9 (3.98) and 74.6 (1.83)% respectively.

3. Results and discussion

3.1. Differential scanning calorimetry

Initially, the melting point differences between the enantiomers and racemate of menthol and neomenthol were determined to assess if they were large enough to produce marked differences in terpene solubility and thus stratum corneum uptake. (±) menthol melted 9–10 °C lower than the (−) and (+) enantiomers (Table 1). (±) neomenthol had a melting point of 50.9 °C while both enantiomers showed no evidence of solid formation throughout the temperature range of the DSC analysis. If the solute to be studied is a liquid, its melting point may be taken as the study temperature (25 °C) (Yalkowsky and Roseman, 1981). This represents a 26 °C difference between the melting points of racemic neomenthol and its enantiomers. Lawter and Pawelchak (1989) suggested that a compound whose racemate and enantiomers melt at a temperature difference of 10 °C or more holds promise as a means of enhancing transdermal delivery. Therefore, for both menthol and neomenthol, the theoretical potential exists for significant differences in their stratum corneum uptake.

3.2. Solubility studies

The effect of melting point on the solubility of menthol and neomenthol in the PG/water co-solvent systems was investigated. The solubility for each individual terpene increased markedly with increasing percentage of PG. The lower melting (±) menthol possessed slightly higher solubility than the pure enantiomers in all PG/water co-solvents (Table 2). Increasing the PG content of the solutions had no marked effect on the racemic/enantiomer solubility ratios. The larger melting point difference between the racemate and enantiomers of neomenthol was reflected in a larger solubility ratio; the lower melting enantiomers were approximately two-fold more soluble in the PG/water mixtures, again a trend that was independent of PG concentration.

Table 2
Solubilities of terpenes in propylene glycol/water co-solvent systems at 25 °C

Propylene glycol (%w/w)	Solubility (mg ml^{-1}) ± S.D. ($n = 3$)		
	(−) Enantiomer	(+) Enantiomer	(±) Racemate
Menthol			
20	0.373 (0.030)	0.371 (0.055)	0.432 (0.035)
40	1.36 (0.178)	1.29 (0.197)	1.42 (0.193)
60	9.49 (0.866)	9.27 (0.681)	10.0 (1.49)
Neomenthol			
20	0.452 (0.041)	0.496 (0.029)	0.248 (0.006)
40	1.09 (0.263)	1.14 (0.126)	0.546 (0.064)
60	9.22 (0.527)	9.01 (0.234)	4.46 (0.192)

Enantiomer solubilities for either menthol or neomenthol were not significantly different at any PG concentration. This is as expected as the enantiomer melting points are essentially the same; additionally, the results provide further confidence in the accuracy of the analytical method.

3.3. Uptake studies

Previous studies in our laboratories used a membrane pre-treatment time of 12 h with enhancers to obtain a maximum enhancement effect when undertaking permeation studies (Goodman and Barry, 1988; Williams, 1990; Cornwell and Barry, 1994). For the present investigation, the amount of menthol taken up by the stratum corneum was measured at times between 30 s and 12 h and expressed as weight terpene/weight dry stratum corneum.

After 30 s exposure to saturated solutions in 20 and 40% w/w PG/water co-solvents, menthol uptake by the stratum corneum was below the limit of detection of the gas chromatographic method used for sample analysis (Fig. 2a and b). In general, the validity of data from uptake studies may be questionable as failure to remove fully the excess formulation from the membrane surface could falsely elevate the amount of analyte detected. However, the fact that negligible terpene was detected after 30 s exposure to test solution validates the cleaning protocol used during this study, indicating that any menthol detected has been taken up by the stratum corneum and is not simply surface contamination. The 30-s uptake of all three forms of menthol from 60% w/w PG/water was detectable (Fig. 2c).

With the exception of the 4 h uptake from the 60% PG vehicle, there was no significant ($P > 0.05$) difference in the uptake of (−) and (+) menthol from all the PG/water co-solvents, suggesting the process is not stereospecific. Reports of enantioselective transdermal permeation are contradictory. The stratum corneum contains keratin and ceramides, which could potentially provide a chiral environment. Differential binding of enantiomers to keratin or interactions with ceramides may alter the permeation profiles of the

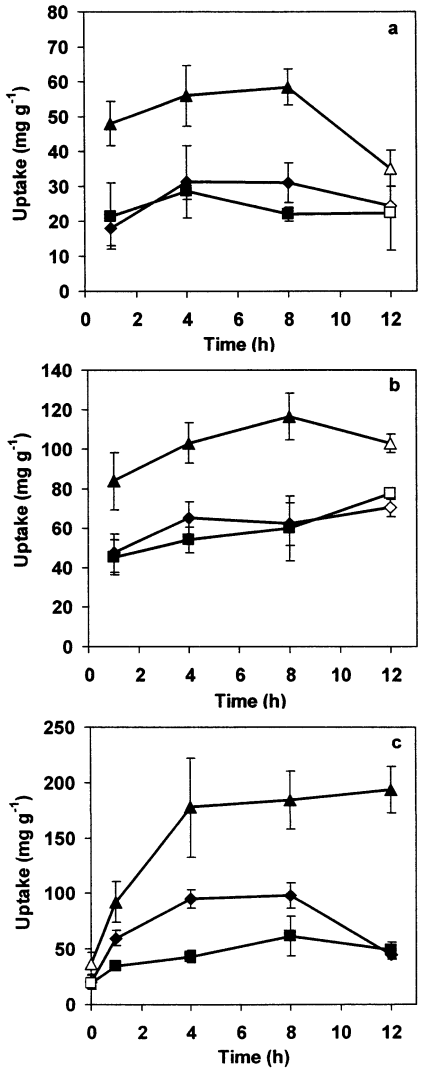

Fig. 2. Uptake into human stratum corneum of (−) menthol (◆), (+) menthol (■) and (±) menthol (▲) from (a) 20, (b) 40 and (c) 60% w/w propylene glycol/water. Data are mean ± S.E.M. ($n = 3$). Open symbols denote uptake from skin donors different to those used for corresponding closed symbols.

enantiomers (Heard and Brain, 1995). Heard et al. (1993) were unable to detect enantioselective transport of propranolol across human and rat skin, as previously reported by Miyazaki et al. (1992).

The 9 °C difference in melting point between (±) menthol and its enantiomers was reflected in

a clear trend of 1–3-fold increases in terpene uptake of the racemate over the (+) and (−) forms. The uptake of the racemate was significantly higher ($P < 0.05$) than that of either enantiomer in all the PG/water vehicles at all time points, with the exceptions of (−) menthol at 4 h and (+) menthol at 12 h in the 20% PG/water system. Increasing the treatment time and PG content of the vehicle had no marked effect on the uptake ratio of (±) menthol relative to its pure enantiomers. The racemate/enantiomer uptake ratios were consistently higher than their corresponding solubility ratios, in all vehicles. This suggests that the PG content of the vehicle has a greater influence on the membrane partitioning of the more soluble (lower melting) (±) menthol than its enantiomers.

With the exceptions of (+) and (±) menthol saturated in 60% w/w PG/water, the terpene uptake from all formulations approached a maximum within 1 h of application to the stratum corneum. Menthol is a small lipophilic compound, therefore rapid partitioning into the stratum corneum is expected. However, increasing the PG content of the vehicle increased the terpene solubilities in the co-solvent systems (Table 2). Correspondingly, the stratum corneum/vehicle partition coefficient for menthol from the PG/water vehicle should reduce. The enhancement effect of (−) menthol, in combination with ethanol, on the permeation of diclofenac across hairless rat skin was found to reach a maximum after 1 h pre-treatment of the membrane (Obata et al., 1993). The same study found that increasing the amount of menthol applied to the membrane increased drug permeation. These results correlate with the time taken (1 h) to achieve maximum terpene uptake from PG/water in the current study, suggesting a relationship between the amount of menthol in the stratum corneum and its enhancement potential. Yamane et al. (1995a) assessed the effects of enhancer pre-treatment time on the permeation of 5-fluorouracil (5-FU) across human epidermal membrane. Some enhancers (D-limonene and oleic acid) had a saturable enhancement effect while others (1,8-cineole, menthone and nerolidol) showed an increased enhancement with time. The non-saturable effect of the latter group was linked to increased 5-FU partitioning into the membrane.

A study in our laboratory by Cornwell et al. (1996) found the uptake of pure 1,8-cineole, (+) limonene and nerolidol into human stratum corneum, following 12 h pre-treatment with neat terpenes, to be 262, 89.0 and 396 mg g^{-1} respectively. In comparison, the current study obtained lower uptake results for menthol after a 12-h treatment time. Because menthol was applied at saturation in the co-solvent systems it will exhibit maximum thermodynamic activity within the vehicle. Therefore, theoretically, it will possess the same driving force to permeate the stratum corneum as the solid, i.e. pure terpene. However, the uptake of a pure terpene depends only on its solubility in the membrane. If, as in this case, the terpene is applied as part of a formulation, its partitioning behaviour between the skin and the vehicle will influence its uptake. For example, the human stratum corneum uptake of 1,8-cineole and nerolidol saturated in PG was found to be less than that from pure enhancer (Cornwell et al., 1996).

Increasing the PG content of the co-solvent system increased terpene solubility and stratum corneum uptake for each individual form of menthol. Differential uptake of menthol, at saturation, from different co-solvents suggests that the vehicles affected enhancer uptake. Megrab et al. (1995) measured the uptake of PG into fully hydrated stratum corneum from a range of PG/water co-solvent systems. Increasing the PG content of the vehicle produced an almost linear increase in the PG content of the stratum corneum. This increase raised the membrane solubility of another lipophilic compound, oestradiol.

Conversely to menthol, the neomenthol enantiomers melt 26 °C lower than (±) neomenthol. This larger difference in melting point resulted in enantiomeric/racemic uptake ratios of between 2.5 and 8. Indeed, for all PG/water systems at all time points, the uptake of the higher melting racemate was always significantly lower than that of either enantiomer ($P < 0.05$). Other than the 4 h data in 60% PG/water, there was no significant difference in the uptake of (−) and (+) neomenthol in any PG/water co-solvent system, strongly suggesting that neomenthol uptake was not stereospecific.

(±) Neomenthol reached approximately maximum uptake within 1 h of application to the membrane in all vehicles. The (−) and (+) enantiomers reached maximum uptake from 20% w/w PG/water (Fig. 3a) at the 1 h time point but their time to maximum uptake increased with increasing PG content of the co-solvent (Fig. 3b and c).

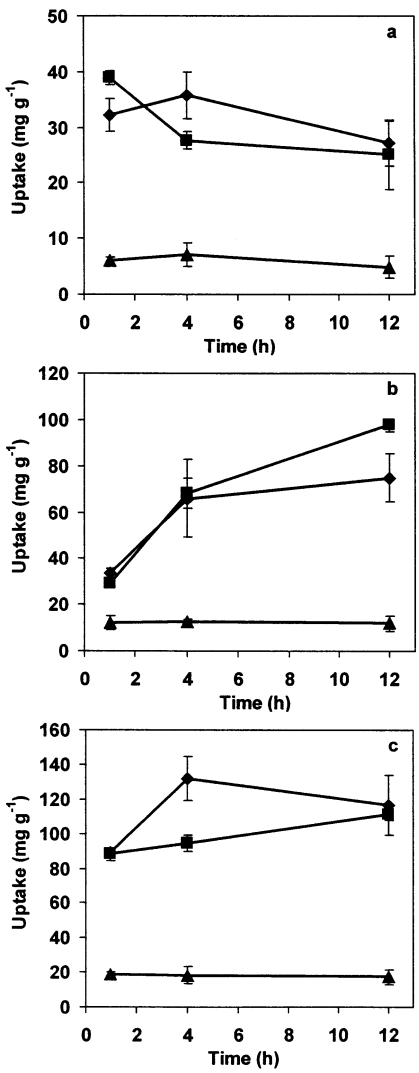

Fig. 3. Uptake of (−) neomenthol (◆), (+) neomenthol (■) and (±) neomenthol (▲) from (a) 20, (b) 40 and (c) 60% w/w propylene glycol/water by human stratum corneum. Data are mean ± S.E.M. ($n = 3$).

Comparing the uptake of menthol and neomenthol from each vehicle, it can be seen that similar amounts of corresponding enantiomers were taken up by the stratum corneum, despite (−) and (+) neomenthol having 8 °C lower melting points than (−) and (+) menthol. However, no correlation between their melting points and uptake is expected. As diastereomers, menthol and neomenthol have the same chemical structure but differ in their stereotypical and physicochemical properties. Therefore they will exhibit differences in their solubility and partitioning behaviour.

The significance of the results of the menthol and neomenthol uptake studies on their potential for permeation enhancement is two-fold. Firstly, the amount of terpene delivered to the stratum corneum depends on enhancer melting point and on the co-solvent composition. Yamane (1994) examined the enhancing effects of a range of terpenes applied to human epidermal membranes in PG/water formulations. It was suggested that the quantity of terpene delivered to the stratum corneum was an important determinant of the enhancer effects; 1,8-cineole, menthone and nerolidol, applied saturated in the co-solvents, showed reduced enhancement towards 5-FU (Yamane et al., 1995b) compared with the action of neat terpenes (Yamane et al., 1995a). This correlated with a decreased stratum corneum uptake of the terpenes when formulated in PG (Cornwell et al., 1996)

Terpenes, such as menthol and (±) neomenthol, which are not liquids at physiological temperature, have to be applied formulated in a suitable vehicle. In the current study, increasing the PG content of the co-solvent systems increased the amount of terpene taken up by the stratum corneum. Terpene activity has also been shown to depend on the PG content of the vehicle (Yamane et al., 1995b), and therefore on the amount of PG taken up by the stratum corneum, suggesting a correlation between the amount of enhancer delivered to the skin and its enhancing effect.

Additionally, the implications of menthol and neomenthol stereochemistry on their enhancement potential may be considered. Equal amounts of (−) and (+) enantiomers of both terpenes were

delivered to the stratum corneum from each PG/water co-solvent system, indicating their uptake was independent of their stereochemistry. However once in the skin they may still have the potential to cause enantioselective enhancement of a drug's permeation. Literature reports differ regarding this possibility. Kommuru et al. (1998) examined the effect of terpene chirality on the transport of (−) and (+) ketoprofen across hairless mouse skin. Addition of (−) menthol to the donor solution produced no evidence of enantioselective ketoprofen permeation. However, stereospecific transfer of S metoprolol across the same membrane by (−) menthol has been reported (Kommuru et al., 1999). Another study (Zahir et al., 1998) found preferential permeation enhancement of the (−) enantiomer of propranolol across guinea pig skin in the presence of (−) menthol. However, in the absence of terpene, (−) propranolol showed greater permeation than the racemate, despite being formulated at equal sub-saturation concentrations. This contradicts the findings of Touitou et al. (1994) with respect to the same permeants passing across human skin. It should be noted that these literature studies were performed on mammalian skin other than human, and may thus yield different results. For example, rat skin is intrinsically different from human skin in composition, thickness and permeability (Kommuru et al., 1998).

The examples cited above tested the effect of one optical form of terpene on the permeation of chiral drugs. Comparing the enhancement effects of the (−), (+) and (±) forms of a terpene would yield more relevant information about the relationship between terpene stereochemistry and their potential for increasing transdermal permeation.

3.4. General conclusion

These results demonstrate how knowledge of the melting behaviour of chiral terpenes can be used to rationalise the amount of penetration enhancer taken up by the stratum corneum. Compared with menthol, the larger melting point difference between optical forms of neomenthol produced bigger differences in their uptake.

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