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An Attempt to Clarify the Mechanism of the Penetration Enhancing Effects of Lipophilic Vehicles with Differential Scanning Calorimetry (DSC)*

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Abstract

In a previous in-vivo skin penetration study, it was observed that certain lipophilic liquid vehicles enhanced drug penetration, whilst others did not.

To clarify the mechanism of skin penetration enhancement, isolated sheets of human stratum corneum were measured by differential scanning calorimetry (DSC), either untreated or after pretreatment with various lipophilic liquids (highly purified light mineral oil, isopropyl myristate, caprylic/capric acid triglycerides containing 5% phospholipids, dibutyl adipate, dimethicone 100, cetearyl iso-octanoate, caprylic/capric acid triglycerides), commonly used in ointment bases.

All samples were analysed over a heating range of at least − 10–130°C. All DSC curves were evaluated with regard to the phase-transition enthalpies (peak areas) and peak maximum temperatures of the lipid-phase transitions at ca 75 and 85°C.

With the exception of dimethicone 100, cetearyl iso-octanoate and caprylic/capric acid triglycerides, all vehicles showed characteristic alterations of the phase-transition temperatures and enthalpies of the stratum corneum lipids. Mineral oil and isopropyl myristate caused a reduction of the enthalpy and a decrease of the phase-transition temperatures. These two vehicles are thought to fluidize the lamellar-gel phase of the stratum corneum lipids, and possibly partially dissolve the lipids. Dibutyl adipate and caprylic/capric acid triglycerides containing 5% phospholipids decreased the phase-transition enthalpy only, probably due to dissolution or extraction of the stratum corneum lipids.

These DSC results provide an explanation for the in-vivo penetration-enhancing effects observed previously.

According to our recently conducted human in-vivo penetration study with methyl nicotinate as a model drug, applied at the same relative thermodynamic activity in all vehicles (Leopold & Lippold 1993), the following lipophilic liquids were found to enhance drug penetration: mineral oil, isopropyl myristate, caprylic/capric acid triglycerides with 5% phospholipids, and dibutyl adipate. Dimethicone 100, cetearyl iso-octanoate, and caprylic/capric acid triglycerides were without effect on drug penetration. These observed enhancing effects might be explained by a specific alteration of the lamellar structure of the stratum corneum lipids, which are considered to be the main barrier of penetration. On the other hand, enhancing effects may be caused by a nonspecific increase in drug solubility in the barrier. A structural change of the α-keratin caused by the vehicles is not expected because of their lipophilicity. Besides infrared spectroscopy and X-ray diffraction, differential scanning calorimetry (DSC) is a widely used method for the examination of structural changes of the stratum corneum (Goodman & Barry 1985; Barry 1987, 1991; Bouwstra et al 1991). Calorimetric measurements allow us to investigate the effects of compounds applied to the skin on the structure of the stratum corneum. Isolated stratum corneum sheets show four characteristic endothermic transitions at about 40, 75, 85, and 105°C, respectively. The first three transitions are lipid-based since they disappear after extraction of the samples with organic solvents. The first transition, which until recently has been attributed to the melting of sebaceous lipids (Golden et al 1986), is thought to represent a transition from an orthorhombic (crystalline) to a hexagonal lipid (gel state) subcell arrangement within the lipid bilayers (White et al 1988; Bouwstra et al 1992). The two thermal transitions at 75 and 85°C have been reported to represent phase transitions of the lipid bilayers from the lamellar gel state to the liquid-crystalline state (Knutson et al 1985; Bouwstra et al 1989, 1991). The transition at 85°C is considered by several investigators to represent a phase transition of lipids which are associated with proteins (Knutson et al 1985; Golden et al 1987; Francoeur et al 1990; Barry 1991; Bouwstra et al 1991). The fourth transition at 105°C, which requires a water content of the sample of at least 15%, represents the denaturation of the protein portion of the stratum corneum, i.e. the α-keratin (Duzee 1975; Goodman & Barry 1985; Bouwstra et al 1989, 1991). Changes of the phase-transition enthalpies and temperatures of the intercellular lipids as well as alterations of the α-keratin denaturation peak indicate an interaction of the respective compounds with the stratum corneum (Knutson

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et al 1985; Goodman & Barry 1986; Bouwstra et al 1989, 1991; Francoeur et al 1990).

This study was performed to elucidate the mechanism of penetration enhancement by several lipophilic vehicles, using DSC on isolated sheets of human abdominal stratum corneum. Understanding of the underlying mechanism of action facilitates the design of ointment bases.

Materials and Methods

Vehicles

The following lipophilic liquids, which are components of many common ointment bases, were investigated in terms of their ability to alter the structure of the stratum corneum: dimethicone 100 (Baysilone M 100, Bayer AG, Leverkusen, Germany); highly purified light mineral oil (Parafluid Mineralölgesellschaft, Hamburg, Germany); cetearyl isooctanoate (PCL-liquid, Dragoco Gerberding & Co GmbH, Holzminden, Germany); isopropyl myristate (Henkel KGaA, Düsseldorf, Germany); caprylic/capric acid triglycerides (Hüls Troisdorf AG, Troisdorf, Germany); caprylic/capric acid triglycerides containing 5% phospholipids (phospholipids: Phospholipon 80, containing up to 80% phosphatidylcholine and up to 10% phosphatidylethanolamine, Nattermann Phospholipid GmbH, Köln, Germany); and dibutyl adipate (Henkel KGaA, Düsseldorf, Germany). The strong penetration enhancer oleic acid, 99% pure (Sigma Chemie, Deisenhofen, Germany), served as a reference.

Preparation of human stratum corneum samples

Sheets of human abdominal cadaver skin were obtained from autopsy. After cleaning the skin with 70% (v/v) ethanol and removing the hair with a razor blade, the dermis was excised using a scalpel (Cutfix 24, Braun Melsungen AG, Melsungen, Germany). The stratum corneum was separated from the epidermis with a pair of forceps after skin samples had remained for several hours in a Petri dish at 37°C, placed on a filter paper soaked with a phosphate buffer solution, pH 7·4, containing 0·5% (w/v) trypsin (Type II: Crude, Sigma Chemie, Deisenhofen, Germany) (Kligman & Christophers 1963). After rinsing the stratum corneum sheets with distilled water and placing them on pieces of wire gauze, they were allowed to dry and then stored in a desiccator with silica gel. Stratum corneum lipids were obtained by extraction of about 40 mg of dried sheets of stratum corneum with 50 mL of a mixture of chloroform/methanol (2:1, v/v) (Goodman & Barry 1985). The mixture was shaken for at least one day before removal of the extracted stratum corneum sheets. The solvent phase was allowed to evaporate to dryness and the residue was stored in a desiccator with silica gel until required.

DSC measurements

Dry sheets of stratum corneum were pretreated with the respective vehicles overnight at 32°C before DSC measurement. To investigate the effect of the duration of pretreatment on the extent of structural changes of the stratum corneum lipids, several samples were pretreated for 1–2 h. The samples were transferred into Petri dishes and moistened with a 0·5-mm thick layer of the respective vehicle using a Pasteur pipet. To remove the vehicles after pretreatment, the samples were blotted with laboratory wipes, leading to a sample weight equal to the initial weight before pretreatment $\pm$ 1 mg. The extracted lipids were pretreated directly in the DSC steel pans without removal of the vehicle before measurement. All samples were analysed at high sensitivity over a heating range of at least −10–130°C at 2 K min^{-1} (DSC 30 with TA3000 Processor, Mettler AG, Greifensee, Switzerland), approximately 10–20 mg being placed in each 120-μL steel pan. Untreated samples served as controls.

All DSC curves were evaluated with regard to the phase-transition enthalpies (peak areas) and peak maximum temperatures of the lipid-phase transitions at about 75 and 85°C, respectively. Significance limits had to be determined in order to be able to evaluate the extent of the peak shifts and thus the extent of structural changes of the lipid bilayers caused by the vehicles. They were obtained by measurement of 4–5 untreated stratum corneum samples from each donor body and subsequent calculation of the respective enthalpy and peak temperature means and ranges. The significance limits correspond to the maximum range of the enthalpies and the peak temperatures, respectively. In the case of the phase-transition enthalpy, an effect was considered to be statistically significant when there was more than 17% of an enthalpy change in comparison with the respective mean of the untreated samples, using the sum of both peak areas divided by the sample weight as a measure for the enthalpy. Typical enthalpy values for untreated stratum corneum samples are 2–4 J g^{-1} (Leopold 1992). Analogous to the evaluation of the enthalpy, a statistically significant change of the phase-transition temperatures (peak maxima) was assumed in the case of peak shifts of more than 3°C. With at least 75% of the samples ($n = 8–11$) showing a significant change in either the phase transition temperatures or the enthalpy or in both, an overall significant effect of the respective vehicle was considered to be proven.

To ensure the absence of any interfering peaks, all vehicles were also measured separately.

Results and Discussion

Typical DSC thermograms of untreated and vehicle-pretreated stratum corneum samples are shown in Fig. 1. Except for the samples pretreated with dimethicone 100, all curves were evaluated only up to a temperature of 120°C because of an endothermic peak appearing in all thermograms at about 170–180°C, caused by the oxidative degradation of the vehicles themselves. In the case of oleic acid this degradation occurred even at lower temperatures (140°C) and allowed an interpretation of the curves only up to 100°C.

It has to be mentioned that the first lipid-phase transition at about 40°C, which is not easily detectable (Winfield & Taylor 1990; Potts et al 1991), could not be found. The α-keratin denaturation peak, which requires a certain water content of the sample in order to become apparent, could not be detected in every thermogram because of the low water content of the samples. Storage of the samples at

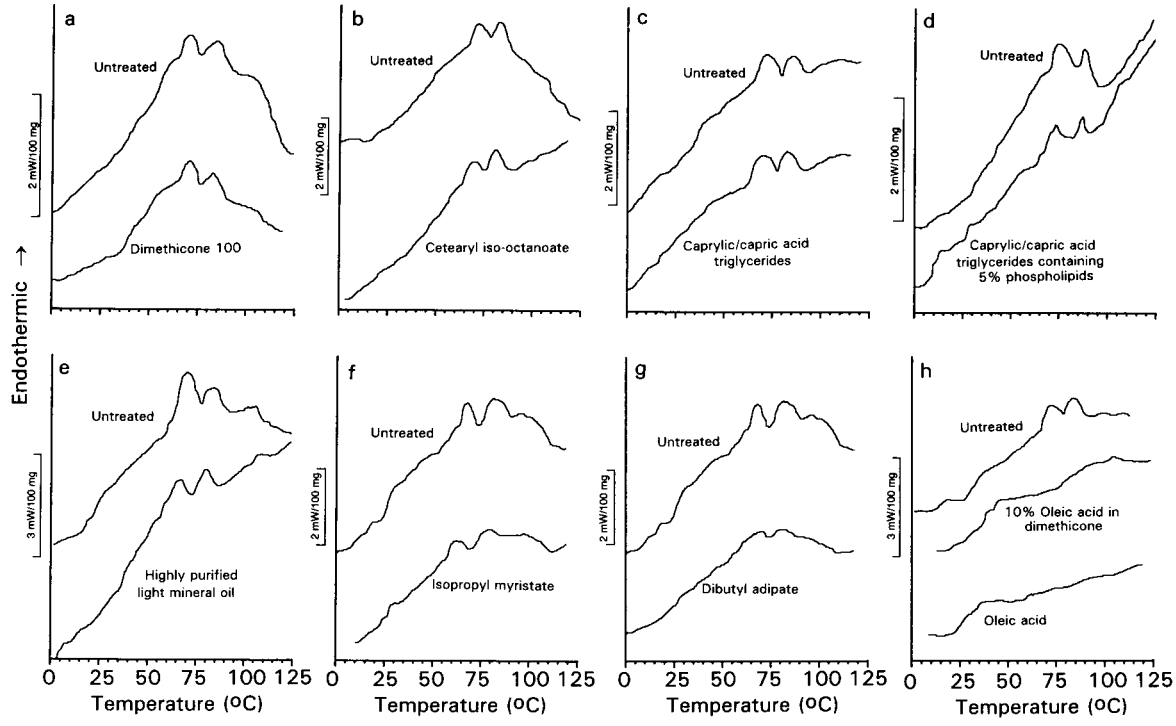

FIG. 1. Typical DSC thermograms for human stratum corneum after pretreatment with different vehicles.

elevated relative humidity (75·2%) was found in preliminary experiments not to result in alterations of the DSC thermograms, except for a more pronounced keratin denaturation peak. However, the water content of these samples was only about 15 ± 1%, a concentration probably too low for an effect on the lipid-phase transitions to become obvious. In comparison, the samples stored in a desiccator showed a water content of 4 ± 1%, both concentrations determined by Karl Fischer titration.

The results of the DSC measurements show that several of the investigated vehicles are indeed able to interact with the stratum corneum lipids. According to the DSC thermograms of samples pretreated with dimethicone 100, cetearyl iso-octanoate and caprylic/capric acid triglycerides, respectively (Fig. 1a–c, Table 1), none of the latter induce significant changes of the lipid-phase transitions. Dimethicone 100 was expected not to interact with the stratum corneum lipids because of its high molecular weight of about 6700 Da. The inert behaviour of cetearyl iso-octanoate, which is similar to the oil gland secretion of waterfowl with regard to its chemical structure, and of caprylic/capric acid triglycerides cannot be explained by their physicochemical properties.

All the other investigated vehicles (Fig. 1d–h, Table 1) showed characteristic alterations of the phase-transition temperatures and enthalpies of the stratum corneum lipids: the vehicles dibutyl adipate and caprylic/capric acid triglycerides with phospholipid reduced only the peak areas, i.e. the phase-transition enthalpies, which indicates a dissolution or extraction of stratum corneum lipids. These two processes cannot easily be distinguished from each other by DSC measurements. The thermograms are similar to those

Table 1. Summarized DSC results with average peak shifts and transition-enthalpy changes caused by the investigated vehicles.

Vehicle	Total number of samples n	Average peak shifts* (°C)	Number of samples with significant peak shifts	Average ΣΔH-decrease* (%)	Number of samples with significant enthalpy changes
Oleic acid	8	−25	8	90	8
Dibutyl adipate	8	0	2	50	8
Caprylic/capric acid triglycerides containing 5% phospholipids	11	0	3	50	9
Highly purified light mineral oil	11	−5	10	30	8
Isopropyl myristate	7	−8–10	7	70	6
Caprylic/capric acid triglycerides	7	0	1	0	1
Cetearyl iso-octanoate	8	0	2	0	1
Dimethicone 100	7	0	0	0	0

* For details see Leopold (1992).

obtained after partial extraction of stratum corneum sheets with the chloroform/methanol mixture. Extraction of stratum corneum lipids has mainly been observed with organic solvents as vehicles (Catz & Friend 1990; Kai et al 1990; Kurihara-Bergstrom et al 1990; Bommannan et al 1991; Franz et al 1991), but it has also been shown with penetration enhancers such as oleic acid in propylene glycol, dimethylsulphoxide and decylmethylsulphoxide (Yamada et al 1987; Touitou 1988; Winfield & Taylor 1990). The observed lipid extraction with dibutyl adipate was not unexpected since it shows a solubility parameter which is similar to that of the stratum corneum lipids (20·5 $MPa^{1/2}$) (Liron & Cohen 1984) and may, therefore, act as a solvent. In the case of caprylic/capric acid triglycerides with phospholipids, the incorporated phospholipids are considered to be responsible for the observed decrease of the phase-transition enthalpies. It is likely that stratum corneum lipids become solubilized by inverse micelles formed by the phospholipids. This formation of inverse micelles has already been observed with isopropyl myristate as solvent (Hamann 1990). Both vehicles led to an enthalpy decrease of up to 70%, in two cases even up to 100% which results in thermograms similar to those from lipid-extracted samples. However, the small amount of vehicle applied to the dried stratum corneum sheets during the pretreatment period is not likely to lead to a complete extraction of the lipids. The fact that there are lipid-phase transitions present in the thermograms after a second heating run even after complete disappearance of the peaks during the first run, shows that a considerable amount of lipid remains within the stratum corneum.

Light mineral oil, isopropyl myristate and especially the reference oleic acid exhibited a reduction of the peak areas and a decrease of the phase-transition temperatures (Fig. 1h, Table 1). This phenomenon may be interpreted as an increase in fluidity of the lipid bilayers; apparently, interactions between the penetration-enhancing compounds and the intercellular lipids lead to a less ordered state of the latter. The formation of eutectic mixtures of the lipids and high amounts of the respective vehicle does not seem to be likely, since this would lead to a drastic decrease of the phase-transition temperatures due to the low melting points of the investigated vehicles. Regarding the vehicles as eutectic impurities on the other hand does not provide a satisfactory explanation for the observed effects, because of their high concentration during the pretreatment period. More likely, interpretations would be the development of arrangements with periodic undulations which have already been observed with liposomes (Rolland et al 1991) or the formation of solid solutions (lamellar gel states consisting of a homogeneous mixture of the lipids and the vehicle molecules). These effects are referred to as fluidizing actions throughout this paper. The observed enthalpy decrease caused by dissolution or extraction of the intercellular lipids cannot be distinguished from enthalpy changes caused by fluidization of the lipid bilayers. For this reason, fluidizing vehicles also must be regarded as potential solvents for intercellular lipids. Such a simultaneous action might be assumed in the case of oleic acid, which leads to very pronounced enthalpy decreases even as a mixture with 90% dimethicone 100 (Fig. 1h). Strong fluidizers such as oleic acid lead to a broadening of the phase-transition peaks (Beastall et al 1988; Winfield & Taylor 1990; Rolland et al 1991) and may even cause a fusion of the peaks (Fig. 1h).

To investigate whether the observed effects are indeed caused by a structural change of the lipid bilayers, extracted stratum corneum lipids were measured after pretreatment with isopropyl myristate (Fig. 2). Isopropyl myristate was chosen because of its known lipid-fluidizing effect (Sato et al 1988). Untreated lipids show only one phase transition at about 60°C with an enthalpy of about 17 $J g^{-1}$ which decreases slightly when the sample is reheated after a cool-

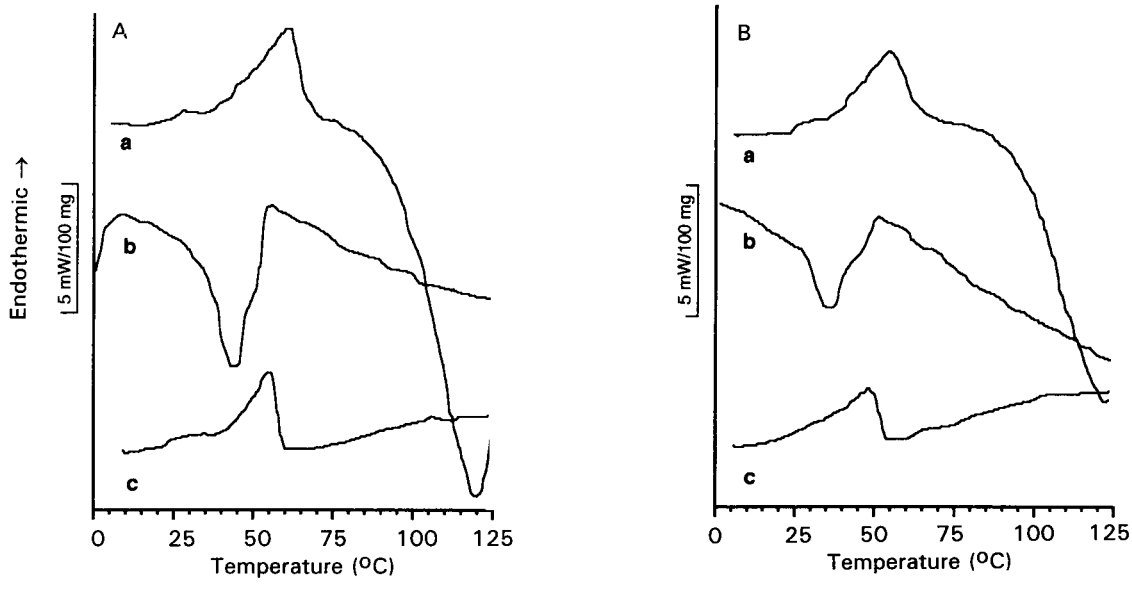

FIG. 2. DSC thermograms for human stratum corneum lipids. A. Untreated lipids: a, first heating; b, cooling; c, second heating. B. Lipids after 2-h pretreatment with isopropyl myristate (3:2, w/w): a, first heating; b, cooling; c, second heating.

ing run. The enthalpy values correspond well with those determined by measurement of whole stratum corneum sheets, assuming a lipid content of the stratum corneum of about 15%. A similar change of the lipid-phase transitions can be observed after reheating whole stratum corneum samples. This phenomenon may be explained by the formation of a eutectic mixture consisting of the two lipid domains originally appearing in the thermograms at 70 and 85°C, respectively (Winfield & Taylor 1990). Another explanation could be the disappearance of the third peak originally at 85°C, because this peak is considered by several investigators to represent a phase transition of lipids which are associated with proteins (Knutson et al 1985; Golden et al 1987; Francoeur et al 1990; Barry 1991; Bouwstra et al 1991). It is important to mention that at least part of the isolated lipids are obviously in the lamellar-gel state (Fig. 2). Therefore, vehicles with fluidizing properties should be able to alter the structure of the lipid bilayers of isolated stratum corneum lipids in the same way as they do if the latter are located within the intercellular space of the whole stratum corneum. Isopropyl myristate pretreatment led to a peak shift of -6°C and an enthalpy decrease of 35% which confirms its action as a fluidizer of the intercellular lipids. Interestingly, an enthalpy increase was found by Walker & Hadgraft (1991) after pretreatment of stratum corneum lipids with oleic acid. They concluded that oleic acid may introduce fluid-like channels within the stratum corneum which leads to a disruption of the intercellular lipid bilayers.

The reason for the fluidizing effect of mineral oil and isopropyl myristate might be their branched structure. In the case of oleic acid the *cis* double bond leads to a kink of the molecule which may cause a decrease of the ordered structure of the lipid lamellae. Moreover, isopropyl myristate and especially oleic acid are able to dissolve considerable amounts of cholesterol (about 70 and 175 mg mL^{-1}, respectively) (Leopold 1992) which may act as a membrane stabilizer (Imokawa et al 1989). The fact that mineral oil fluidizes lipid bilayers to some extent is a surprising result since mineral oil is considered to be an inert vehicle. Isopropyl myristate (Sato et al 1988) and oleic acid (Goodman & Barry 1986; Barry 1987; Golden et al 1987) are already known as fluidizing agents.

The observed effects are hardly dependent on the duration of pretreatment. The maximum effect can already be seen after a pretreatment period of 1–2 h as shown with isopropyl myristate (Leopold 1992) and oleic acid (Hsu et al 1991; Leopold 1992).

From the DSC results presented in this study it may be concluded that the observed decrease of only the phase-transition enthalpy by the vehicles dibutyl adipate and caprylic/capric acid triglycerides with phospholipids is due to dissolution or extraction of the stratum corneum lipids. Vehicles which cause both a reduction of the enthalpy and a decrease of the phase-transition temperatures (mineral oil, isopropyl myristate and oleic acid), are thought to fluidize the lamellar-gel phase of the stratum corneum lipids, and possibly also partially dissolve the lipids. Thus, the results of the DSC measurements provide an explanation for the in-vivo observed vehicle effects.

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