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An attempt to clarify the influence of glycerol, propylene glycol, isopropyl myristate and a combination of propylene glycol and isopropyl myristate on human stratum corneum

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The present study is a comparison of the influences of glycerol, propylene glycol (PG), isopropyl myristate (IPM) and a combination of PG and IPM (1/1;w/w) on human stratum corneum (SC) by means of differential scanning calorimetry (DSC) and wide and small angle X-ray-diffraction (WAXD and SAXD). The effects of glycerol and PG on SC structure can be attributed to their functional groups. In DSC transition temperatures of lipid fractions are decreased whereas SAXD long distances of lamellar phases reveal an additional interference due to an integration into hydrophilic regions of hexagonally packed lipids (PG) or orthorhombically packed lipids (glycerol). The increased repeat distance is attributed to the polar character of both molecules. However, with IPM the long distance remains unaffected. IPM is integrated into the lipophilic regions of SC lipid matrix as concluded from an increase of WAXD reflections of orthorhombical lipids and a decrease of WAXD reflections of hexagonal lipids. The combination of PG/IPM affects SC microstructure in a specific manner. DSC shows a decrease in transition temperatures of the lipid fractions, although not as much as expected from the single substances. Additionally, the combination of IPM/PG affects the short distances of orthorhombically and hexagonally packed lipids in WAXD measurements similar as PG alone, whereas the long distance seems to remain unaffected as in the case of IPM pretreatment. Adjuvants with penetration enhancing potential reveal different effects on SC lipid microstructure, which have to be kept in mind in terms of formulating systems for transdermal administration.

1. Introduction

The stratum corneum (SC) is the outermost layer of the human skin and the main barrier in terms of drug permeation across human skin (Kranz et al. 1977). In order to elucidate the structure of this tissue many investigations have been performed. Elias (1988) presented the brick and mortar model of SC including several layers of corneocytes embedded in a lipid matrix. This intercellular lipid matrix is essential for the barrier properties of SC demonstrated in an increased drug permeation after extraction of these lipids (Potts and Francoer 1991). On the basis of the latter studies and from results of X-ray diffraction Bouwstra et al. (2000), developed an advanced model of a sublattice structure of SC lipid organisation. But the basic concept of hydrophilic and lipophilic lamellae is remaining unchanged.
On topical administration of a formulation, penetration of its components into SC microstructure must be taken into account. Depending on the site of incorporation the influence of each adjuvant has to be investigated individually. In the present study the functions of glycerol, propylene glycol (PG), isopropyl myristate (IPM) and a combination of PG and IPM on human SC lipids are investigated by differential scanning calorimetry (DSC), wide angle X-ray diffraction (WAXD) and small angle X-ray diffraction (SAXD). In topical formulations mainly PG, which causes a smooth and soft skin appeal, has widely been investigated in terms of its effects on human SC. According to Barry (1987) PG causes dissolution of alpha-keratin and adsorption to hydrogen-binding sites, but not an influence of lipid structure. On the other hand PG leads to an increased water retention in SC, although the mechanism of this effect is not fully understood. PG is commonly used as a vehicle for potential penetration enhancers (Barry and Bennet 1987; Bouwstra et al. 1989; Bouwstra et al. 1991a; Cornwell et al. 1996; Williams and Barry 1989), while PG also shows a modifying and in the considered combinations sometimes synergistical effect on SC lipid structure. Barry and Bennet (1987) already supposed an effect of PG on the non-polar route of drug permeation filling holes in the lipid structure, which are caused by other enhancing substances (Cornwell et al. 1996). It always has to be mentioned as a side effect that PG can change the saturation concentration of the drug in the formulation which results in a modified release rate of the drug from the vehicle. This can affect the permeation rate of the drug,

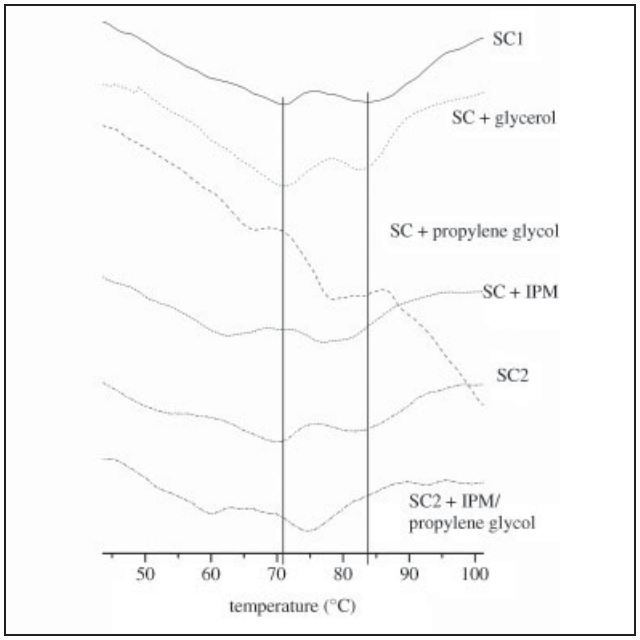

Fig. 1: DSC thermograms of human SC untreated and pretreated with glycerol, PG, IPM (measured with SC1) and the combination of IPM/PG (measured with SC2)

because the amount of dissolved drug molecules is altered. IPM is suggested to decrease the permeation flux of benzotropine mesylate (Gorukanti et al. 1999) and hydrocortisone (Brinkmann and Müller-Goymann 2003). But in combination with isopropyl alcohol a higher permeation rate of hydrocortisone is achieved than with isopropyl alcohol alone. From these surprising findings the question arises whether other alcohols exhibit a similar effect in combination with IPM.

The effect of IPM in combination with PG on human SC microstructure has previously not been reported and is therefore of particular interest in the present study.

Table 1: Transition shifts of SC pretreated with solvent mixtures with reference to the transition temperatures of untreated SC

	71.1 °C (SC1) 70.6 °C (SC2)	83.8 °C (SC1) 81.8 °C (SC2)
Glycerol	1.2 K	4.4 K
PG	4.1 K	5.5 K
IPM	8.4 K	6.2 K
IPM/PG	10.6 K	7.1 K

(donor SC1: female, abdomen, 46 years; donor SC2: female, breast, 47 years)

2. Investigations and results

2.1. Differential scanning calorimetry

DSC is an approved method to characterise the intercellular lipids of SC in detail. According to Van Duzee (1975) four endothermic transitions may be observed at 40 °C and 75 °C as thermal transitions of lipids, at 85 °C for denaturation of alpha-keratin and at 105 °C referring to the denaturation of a nonfibrous protein. The first three transitions were proved to be particularly reversible. The first transition at 40 °C is often missing in skin donations from various donors and is thus not easily detectable. The last one at 105 °C requires a certain water content to become apparent. Therefore in the present study focus was put on just two thermal transitions, giving sufficient information about interactions between vehicle and lipoidal structures of SC. Depending on the body site the skin was taken from, the first transition occurred between 70 °C and 72 °C and the second transition between 81 °C and 84 °C. Bouwstra et al. (1991a) described these transitions as a gel-liquid transition of lipid bilayers and a gel-liquid transition of protein-bonded lipids, respectively.

With pretreatment of each investigated compound lipid transition temperatures of SC were shifted to lower temperatures (Fig. 1, Table 1). The degree of this decrease

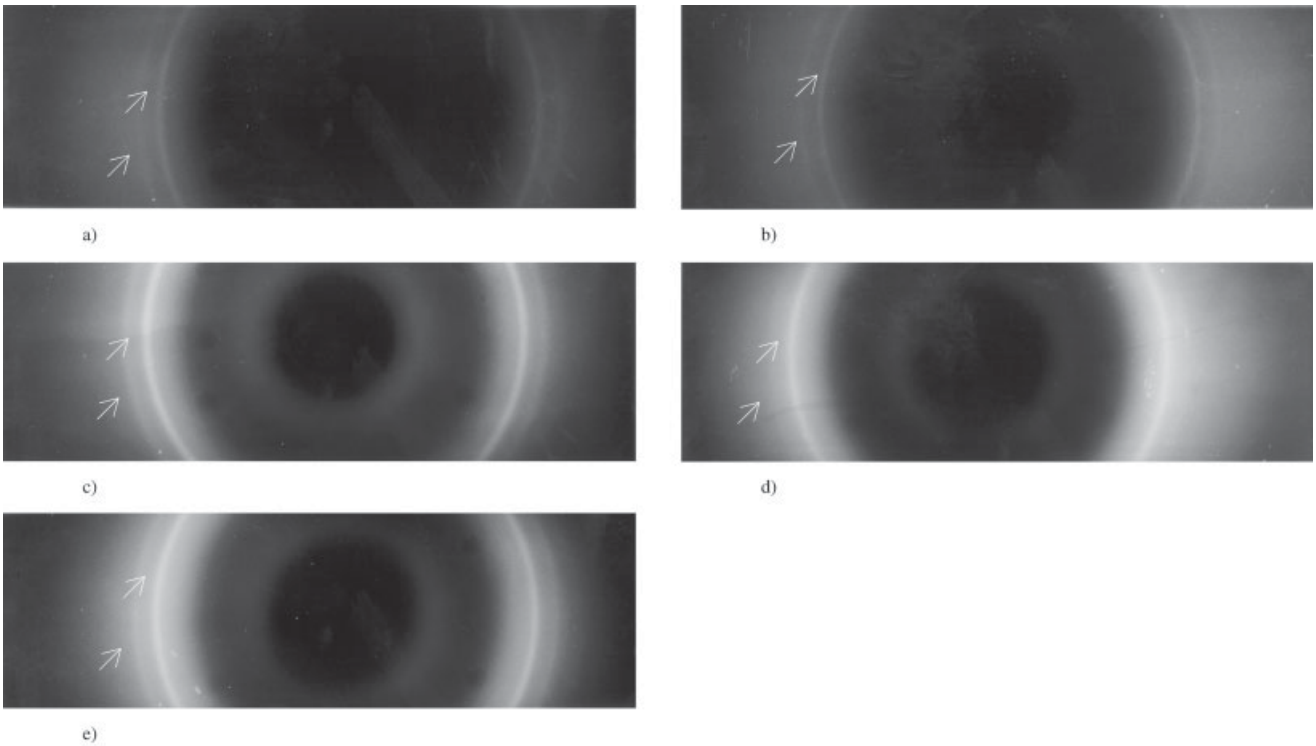

Fig. 2: Debeye-Scherrer films/WAXD: a) untreated SC; b) SC pretreated with glycerol; c) with PG; d) with IPM; e) with IPM/PG

was in ascending order: glycerol < PG < IPM < combination. This agrees with Cornwell et al. (1996) who also observed a shift of the transition temperatures and even a disappearing of the transition at 105 °C after pretreatment with PG. Particularly the pretreatment of the combination leads to a strong decrease in lipid transition temperatures although not as much as expected from summing up the temperature shifts of each single substance. In the thermogram of SC2 a further transition at 55 °C is detected. In the literature this transition is ascribed to covalently bound lipids at the outside of the corneocyte envelope (Cornwell et al. 1996). As we did not detect this transition in the thermograms of SC1 and in that of pretreated SC1 either interindividual variations between SC from different donors become evident. Therefore we will not consider this transition further.

2.2. Wide Angle X-ray Diffraction

According to literature data on crystallized lipid fractions (Bouwstra et al. 1992a; Elias et al. 1983), untreated SC shows three diffraction interferences: i) the interference at 0.371 nm belongs to orthorhombically packed lipids, ii) the interference at 0.413 nm is due to a fraction consisting of both orthorhombically and hexagonally ordered lipids reflecting the lateral packing of the hydrocarbon lipid chains (Bouwstra et al. 1992a; White et al. 1988) and iii) the usually very weak and sometimes absent interference at 0.467 nm is assigned to corneocyte-bonded lipids (Bouwstra et al. 1992a). Pilgram et al. (1999) concluded that an increase in temperature from 32 °C up to 45 °C induces a transition from orthorhombical to a hexagonal sublattice, because the interference at 0.41 nm remained and that at 0.371 nm had disappeared. As even after cooling from 90 °C to 27 °C no recrystallisation from the hexagonal into the orthorhombical lipid packing occurred, an irreversible transition could be assumed (Wilkes et al. 1973). Since in the present study after SC equilibration at 37 °C for 30 min both interferences could be determined, both the hexagonal packing and the orthorhombical packing are present side by side. Although considering Pilgrim's assignment of the interference at 0.41 nm to both hexagonal and orthorhombical packing we refer to the interference at 0.413 nm as hexagonal and to that at 0.371 nm as orthorhombical in the following text, just for the sake of simplification. Comparing these interferences with those obtained from pretreated SC the influence of

Table 2: Wide angle X-ray diffraction data (calculated from the centre of the reflection ring width +/− ring half-width) of SC untreated and pretreated with glycerol, PG, IPM and a combination of IPM/PG (1/1; w/w)

SC	0.413+/−0.003 nm	0.373+/−0.004 nm
SC + Glycerol	0.413+/−0.004 nm	0.378+/−0.004 nm
SC + PG	0.418+/−0.004 nm	0.373+/−0.004 nm
SC + IPM	0.409+/−0.004 nm	0.377+/−0.001 nm
SC + IPM/PG	0.420+/−0.004 nm	0.373+/−0.005 nm

(donor: female, abdomen, 46 years)

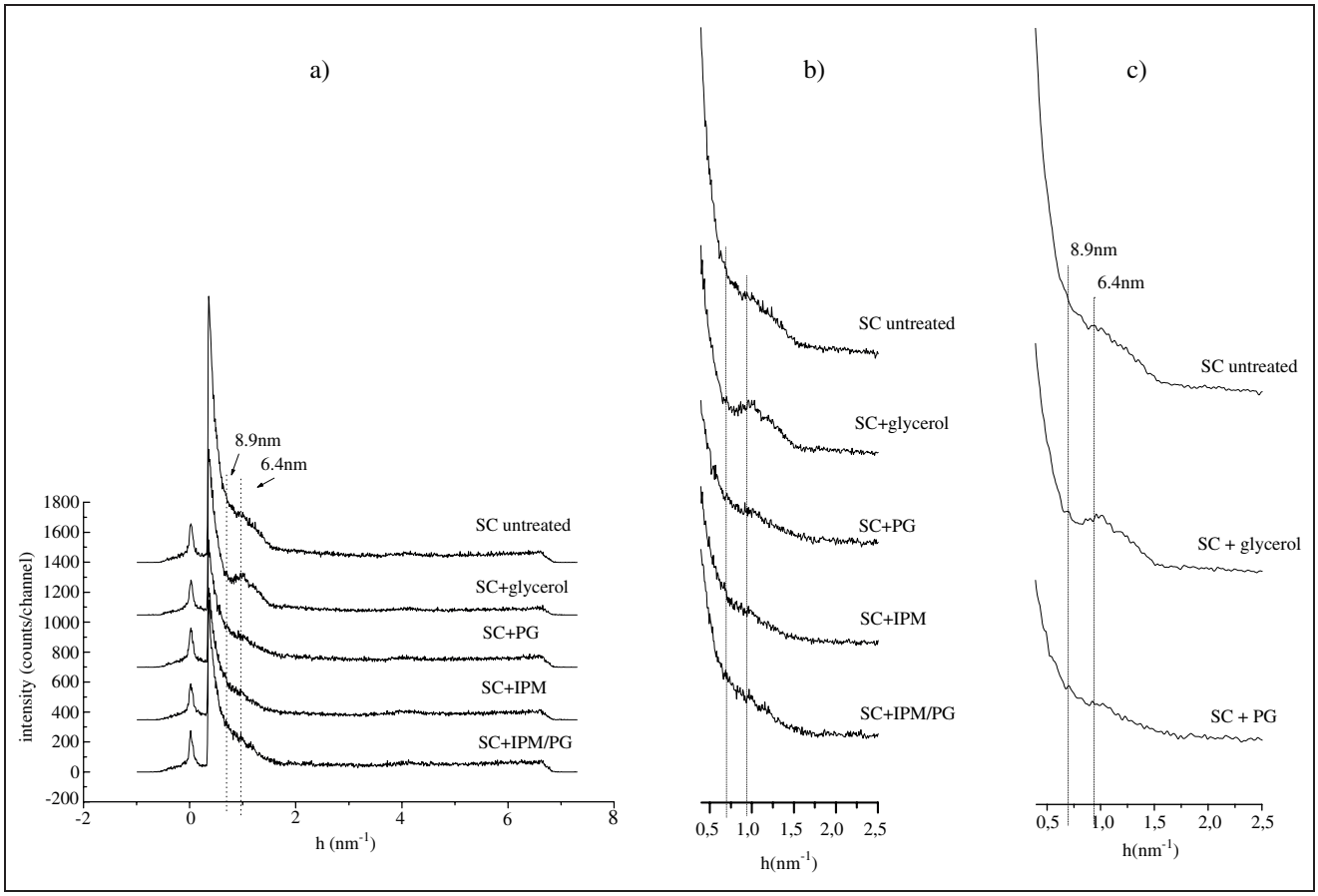

Fig. 3: SAXD data of untreated SC and SC pretreated with glycerol, PG, IPM and IPM/PG (donor: female, abdomen, 46 years) (h: vector of scattering in nm^{-1}): a) original data with the primary beam at 0 nm^{-1} (h ranges from –2.0 to 8.0 nm^{-1}); b) magnification of the original data (h ranges from 0.4 to 2.5 nm^{-1}); c) data of untreated SC and SC pretreated with glycerol and PG after Fast Fourier Transformation (h ranges from 0.4 to 2.5 nm^{-1})

the additives on chain packing in SC microstructure can be recognised directly (Table 2).

After pretreatment with glycerol the short distance of orthorhombically oriented lipids with an interference at 0.371 nm in the untreated status is increased indicating an integration of glycerol into the SC chain packing of lipids whereas the fraction of hexagonally packed lipids with the interference at 0.413 nm remains unaffected. The pretreatment of SC with PG leads to an increase of the short distance of hexagonally packed lipids, in contrast to the pretreatment with pure IPM, which interferes with both lipid chain packings. In accordance with a previous study, the short distance of hexagonally packed lipids is decreased whereas the orthorhombical chain packing is increased after pretreatment with pure IPM (Brinkmann and Müller-Goymann 2003). Furthermore the latter diffraction ring becomes sharper. The pretreatment with the combination of IPM/PG acts like that of PG alone on short distance chain packing. In this case only the interference of hexagonally packed lipids is shifted to higher distances while that of orthorhombically packed lipids remains unaffected.

The third interference at 0.467 nm cannot be detected in this study and is thus excluded from further considerations.

2.3. Small Angle X-ray Diffraction

SAXD-measurements of SC reveal an interference assigned to a long range order of approximately 6.4 nm in accordance with results from other authors (Bouwstra et al. 1991b). In agreement with the conclusion of Van Duzee (1974) that water itself is not necessary for lipid crystallisation but contributes to the ordering effects on protein and lipid microstructures, the measurements of SC pretreated with the investigated substances were carried out with hydrated SC.

In contrast to Bouwstra et al. (1992b), who did not determine any effect of PG on small angle X-ray reflections, we measured an additional weak interference at 8.9 nm in SC after pretreatment with glycerol or PG besides the interference at 6.4 nm in untreated SC. In terms of the long distance reflections the pretreatment with both IPM and the combination IPM/PG results in the same data as in the case of untreated SC (Fig. 3a). The original data of these measurements are given in Fig. 3a and b whereby the latter illustrates a magnification of Fig. 3a. A clearer identification of the interference at 8.9 nm is possible in Fig. 3c, which presents the diffractograms of untreated SC and those of SC pretreated with glycerol

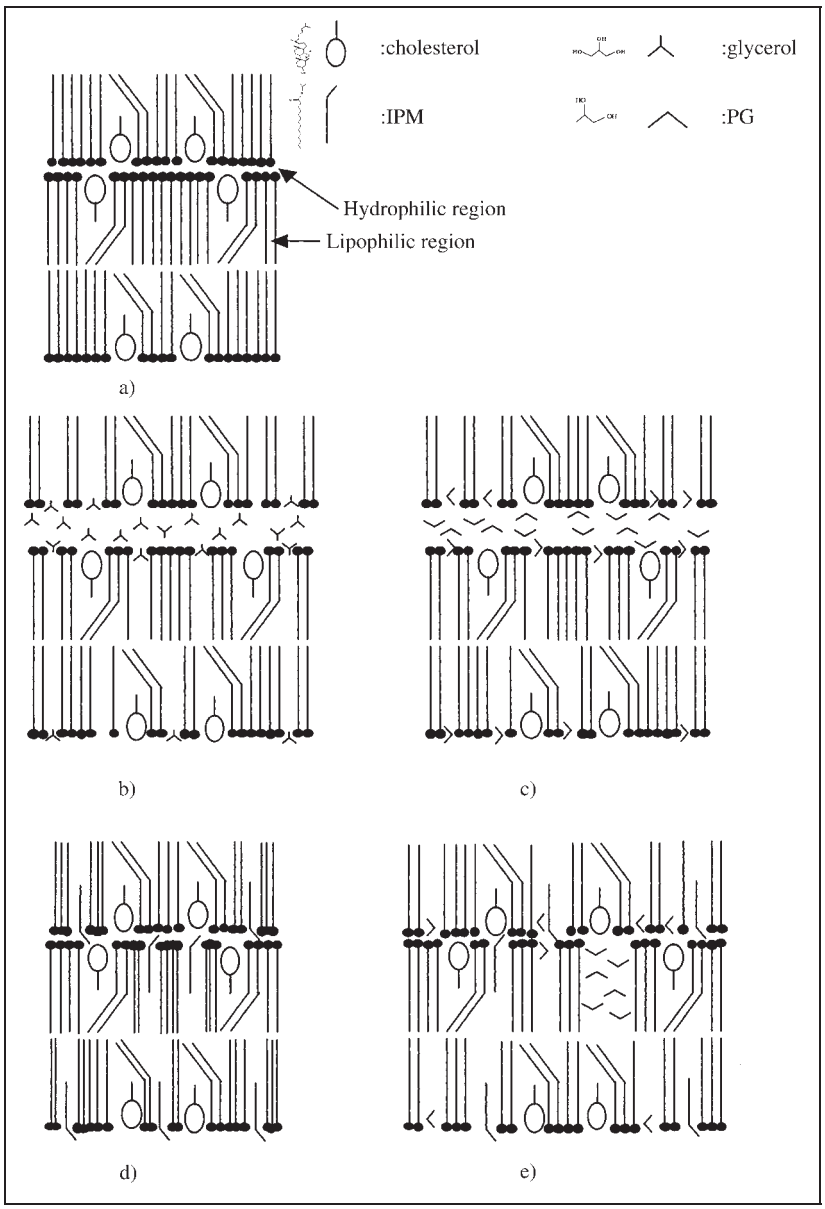

Fig. 4:
Schematical model as a conjecture of adjuvant insertion into lipid chain packing of human SC (one repeat distance): a) untreated SC; b) pretreated SC with glycerol; c) with PG; d) with IPM; e) with IPM/PG

and PG, respectively. These data have been converted with a Fast Fourier transformation for the purpose of baseline smoothing.

3. Discussion

3.1. Comparison between glycerol and PG

From WAXD and DSC data can be concluded that PG is integrated into hexagonally ordered lipids whereas glycerol is incorporated into the orthorhombical chain packing. PG and glycerol differ in terms of just one additional hydroxyl group, and thus a different hydrophilicity and stereochemistry which obviously result in the preferential integration of either molecule. Both, glycerol and PG insertion coincide with an increase of the long distance of the lamellar phase, which can be interpreted in terms of an insertion of these hydrophilic molecules between the hydrophilic head groups of the bilayers in perpendicular direction to the bilayer. Taking together the results from WAXD and SAXD, an orientation of either PG or glycerol within the lamellar phase in both lateral and perpendicular direction to the lamellar plane can be suggested. The present interpretation is not in complete agreement with that from the literature in which due to an unchanged interference in SAXD PG positioning is suggested to be inserted laterally between the lipid head groups of the SC lipid bilayers (Bouwstra et al. 1991a). However, we assume an enrichment of either glycerol or PG in the hydrophilic areas of SC bilayer structure as well as an integration in lateral direction between the lipid head groups as shown in Fig. 4.

3.2. IPM

The influence of IPM on SC microstructure becomes evident in terms of a slight increase of the short distance of orthorhombically arranged lipids while that of hexagonally packed lipids decreases. From these findings an integration into lipid chain packing has been derived. Additionally the long distance of the lamellar structure is unaffected. In accordance with a previous study IPM insertion causes a more densely packed lipid order (Brinkmann and Müller-Goymann 2003). However DSC data suggest a fluidisation and disruption of the lipid chain order (Leopold and Lippold 1995), although a shift of both transition temperatures to lower temperatures is just an evidence of an altered structure in this tissue. In contrast to Leopold and Lippold (1995) we suggest a lateral insertion of IPM into lipophilic areas of SC microstructure with an anchoring of the isopropyl group in the polar region of the layer as presented in Fig. 4.

3.3. Combination of IPM and PG

The combination of IPM and PG affects SC lipids in a specific manner compared to the effects of the single substances. In terms of DSC transition temperatures the most distinct shifts were determined for the combination although not as much as expected from simply summing up the shifts of the single substances. WAXD data reveal a change in hexagonally packed lipids whereas orthorhombically packed lipids remain unaffected. The same has been observed with pure PG. However, the SAXD long distance seems not to be changed as in the case of pretreatment with PG alone. Taking these results together hints at a lateral integration of PG into lipid chain packing only, especially because of the unchanged long distance (Fig. 4). It is suggested that IPM enables PG's lateral insertion between lipid head groups so that the long distance remains unaffected. However, the mechanism and the conformation of this simultaneous incorporation is not fully understood.

In conclusion, glycerol, PG, IPM and a combination of PG and IPM affect SC lipid structure in a different manner. According to their hydrophilic characteristics glycerol and PG increase the long distance of the lamellar structure due to an incorporation into hydrophilic regions of the lamellas. Simultaneously the short distance of either the hexagonally packed lipid modification (PG) or the orthorhombically packed lipid modification (glycerol) is affected, respectively. From the effects on short and long distances both a lateral and a perpendicular integration of the hydrophilic molecules into the lipid bilayers can be concluded. IPM, however, reduces the short distance of the hexagonally packed lipid modification. Hence just a lateral insertion between the lipid molecules of the bilayers is suggested. The combination of PG and IPM affects the short distance the same way as PG alone whereas the long distance seems to remain unaffected as in the case of IPM. Therefore lateral integration of IPM molecules between lipid molecules together with an insertion of hydrophilic PG between polar headgroups of the lipid molecules in lateral direction between the bilayers may be concluded.

The results of the present study reveal the influences of pretreatment with different compounds on SC lipid microstructure. Although the modifications described do not directly hint at effects on drug solubility within the formulations as well as within SC and on percutaneous penetration which have to be studied separately, administration of topical formulations enriched with the respective compounds should be handled with care keeping in mind a potential penetration enhancement.

4. Experimental

4.1. Materials

Glycerol (Henry Lamotte GmbH, D-Bremen), isopropyl myristate (Unichema Chemiegesellschaft mbH, D-Emmerich), propylene glycol (BASF AG, D-Ludwigshafen am Rhein).

4.2. Human stratum corneum

The skin was obtained from plastic surgeries of abdominal and breast regions of female donors (#1: female, abdomen, 46 years; #2: female, breast, 47 years). After mechanical removing of fat tissue the skin was frozen in liquid nitrogen and stored in a refrigerator (−2 °C−4 °C). For isolation of SC the skin was thawed at room temperature and after that trypsinated (Kligman and Christophers 1963). In order to avoid SC degradation, SC was stored at room temperature in a desiccator over silica gel not longer than 4 months.

Prior to the experiments SC was hydrated by storing it for 48 h in a desiccator over a saturated sodium chloride solution (rel. humidity: 75% at room temperature) to yield a water content of 20% as similar starting condition for each SC sample.

All comparative studies were performed with skin from the same donor.

4.3. Differential Scanning Calorimetry

In order to investigate the influence of the formulations on lipid matrix of SC, differential scanning calorimetry (DSC 220C with a disc station 5200H Seiko, J-Tokyo) was used in the temperature range from −20 °C to +140 °C with a heating rate of 5 °C/min.

For sample preparation hydrated human SC was inserted into the additives such as glycerol, PG, IPM and a combination of PG/IPM (1/1; w/w) for 30 min at 37 °C, respectively. After that the formulation was dabbed off carefully with filter paper and the SC was folded in an aluminium crucible. An empty pan served as reference.

Prior to DSC measurements of pretreated human SC, all substances were run to ensure the absence of any interfering transitions. Every measurement was performed twice.

4.4. Wide Angle X-ray Diffraction

For sample preparation hydrated human SC was inserted into either glycerol, PG, IPM or the combination of IPM/PG for 30 min at 37 °C, respectively. After careful cleaning with filter paper the pretreated SC was inserted into a X-ray amorphous glass capillary (d = 0.5 mm) (Glas, D-Berlin). The sample was measured for 24 h using a Debeye-Scherrer camera (circumference 360 mm) with following measurement settings:
X-ray generator: PW 1830 (Philips, D-Kassel)
X-ray tube: PW 2253/11 (Philips, D-Kassel)
Accelerating voltage: 40 kV; anode current 40 mA
Radiation: CuKα, (λ = 0.154 nm)
The X-ray beam was focused with the help of a fluorescence screen in the middle of the lead window. The diffraction rings were visualised on X-ray Structurix D 7 FW film material (Agfa, D-Köln). After developing, the diffraction rings were measured with a graduated ruler (accuracy 0.01 mm) and the interlayer spacings were calculated using Bragg's equation. Fig. 2 shows Debeye-Scherrer films with sharp reflections (bright white diffraction rings with superimposing halos). In Table 2 the interlayer spacings calculated from the centre of the ring width are summarized. For an exact sizing of the rings the negative films were candled on a strong source of light in order to become more visible.

4.5. Small Angle X-ray Diffraction

Hydrated SC was inserted into either glycerol, PG, IPM or a combination of IPM and PG for 30 min at 37 °C and afterwards carefully cleaned with filter paper. The sample was folded in a cube-like sample holder between capton foils (Krempel, D-Vaihingen). Exposition time was 400s in a Kiessig camera equipped with a position sensitive detector (PSD) from MBraun, D-Garching. The measurement settings were as follows:
X-ray generator: PW 1710 (Philips, D-Kassel), X-ray tube: PW 2213/20 (Philips, D-Kassel), Accelerating voltage: 45kV; anode current 30mA, Radiation: CuKα, (λ = 0.154 nm).
The resulting interferences were detected by PSD-50M and analysed by PC-APD Version 3.6 (Philips, D-Kassel). For calibration of the Kiessig OED cholesterol was used because of its well-known interlayer spacing of 0.335 nm and a corresponding angle of diffraction at 1.319°. The distance between sample holder and detector was 264 mm. The interlayer spacings were calculated using Bragg's equation. Fig. 3 shows the diffraction curves.
Prior to measurements of pretreated human SC, all substances were run to ensure the absence of any interfering signals. All X-ray measurements were performed once because of the limited access of donor skin.

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