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# Stratum corneum hydration : mode of action of moisturizers on a molecular level

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# Chapter 8

## Hydrophilic and lipophilic moisturizers have similar penetration profiles but different effects on SC water distribution in vivo

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### 1. Abstract

Dry skin is often treated with hydrophilic and/or lipophilic moisturizers. Hydrophilic moisturizers must penetrate the stratum corneum (SC) deeply to function properly, whereas lipophilic moisturizers should remain in the upper SC layers. In this study, both types of moisturizers were applied on volunteers for three hours, after which the relative amount of moisturizer and the water distribution in the SC were determined using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy in combination with tape-stripping.

The results show that while hydrophilic moisturizers penetrate much more readily than lipophilic moisturizers, the latter are abundantly present in the upper regions of the SC. It was also observed that a three-hour-treatment with lipophilic moisturizer did not result in increased water levels in the SC, whereas hydrophilic moisturizers retained water where they are located.

The results suggest that upon prolonged application, adequate amounts of moisturizer can be obtained in those regions where they may cause moisturization in the central part of the SC. However, a single application of 3 hours is probably too short to exert increased hydration as measured with ATR-FTIR.

## 2. Introduction

Dry skin is the most common symptom of dermatological diseases. Its characteristic symptoms itching, redness and scaliness are not only physically uncomfortable, but also affects patients psychologically (Gupta et al., 2005). The symptoms are all related to impaired enzymatic processes partly due to decreased stratum corneum (SC) water content. Two elements are important for the maintenance of SC humidity. Firstly, intercellular lipids form the main barrier against water. They consist of ceramides, cholesterol and free fatty acids and are organized in two types of lipid lamellae, the short and the long periodicity phase (SPP and LPP, respectively), the latter of which is thought to be essential for the barrier function of the SC (de Jager et al., 2006). Within these lamellae, the lipids are organized in dense lattices that are mostly orthorhombic in organization, although the hexagonal organization is also observed (Bouwstra et al., 1992). Of the two, the orthorhombic organization is very important for the barrier function of the lipids (Abrahamsson et al., 1978; Pilgram et al., 2001b).

The second element to play a key role in the regulation of SC water is the natural moisturising factor (NMF). This is a collection of small, hygroscopic substances that under normal circumstances retains water in the SC (Harding, 2000; Katagiri et al., 2003a; Rawlings, 2003; Scott, 1986). NMF is partly produced by the enzymatic breakdown of filaggrin, but only at optimal water levels in SC as the activity of these enzymes is strongly dependent on the water level in SC (Scott, 1986; Watkinson et al., 2001). This implies that SC of dry skin not only lacks water, but also contains reduced levels of NMF, which is required to bind water in SC. In this way a vicious circle has been created (Scott, 1986).

The dry skin cycle caused by the impaired NMF production can be disrupted by increasing water levels in the SC by the application of moisturizers, i.e. substances whose application results in increased water levels in the SC. In this study, the penetration of hydrophilic and lipophilic moisturizers into SC was examined. The hydrophilic moisturizer group consists of glycerol and glucose. It is assumed that their low molecular weight allows these substances to penetrate the SC, where they subsequently act as humectants (Sagiv and Marcus, 2003),

mimicking the role of NMF. Glycerol is one of the most commonly used moisturizers, and has been shown to increase skin moisturization (Fluhr et al., 2003) and to repair the barrier function of the skin (Fluhr et al., 1999). Recently, endogenous glycerol (derived from sebaceous lipids) has been speculated to play an important role in natural skin moisturization (11).

The lipophilic moisturizers in this study consist of three isostearic acid derivatives which differ in size and lipophilicity: isostearyl isostearate (ISIS, $C_{17}H_{35}COOC_{18}H_{37}$), isopropyl isostearate (IPIS, $C_{17}H_{35}COOC_3H_7$) and glycerol monoisostearate (GMIS, $C_{17}H_{35}COOC_3H_5(OH)_2$). Lipophilic moisturizers may have at least two working mechanisms. Firstly, they may remain on the skin surface and occlude it, thereby preventing evaporation of water from the SC. ISIS, IPIS and GMIS have been shown not to act in this way (Wiechers, 1999). Secondly, they may penetrate into the SC, thereby interacting with the SC lipids to provide an additional barrier to water loss. Previous studies have shown that the selected lipophilic moisturizers increase SC water levels *in vivo* and *in vitro* (Caussin et al., 2007b; Wiechers, 1999), whereas the action of hydrophilic moisturizers is dependent on the concentration at which they are applied (Caussin et al., 2007b). Interestingly, it has previously been shown using *in vitro* SC lipid mixtures that two of the lipophilic moisturizers, ISIS and IPIS, promote the formation of orthorhombic domains in these lipid mixtures (Caussin et al., 2007b) and that all three lipophilic moisturizers promote the formation of the long periodicity phase (LLP) in similar lipid mixtures (Caussin et al., 2007a). This promotion may explain the moisturizers' effect.

The crucial information of the penetration depth of the moisturizers and changes in water levels is subject of the present study. Perdeuterated moisturizers are applied on volunteers *in vivo*. After the treatment period, the application sites are tape-stripped. The removal of each tape-strip is followed by an ATR-FTIR (attenuated total reflectance Fourier Transform Infrared) spectroscopy measurement (15, 16) to determine moisturizer and water distribution in the stratum corneum. Combination of these measurements with the quantification of the SC removed by tape-stripping (17, 18) results in distribution profiles of both water and the moisturizers throughout the upper two thirds of the SC.

## 3. Materials and Methods

### *Subjects*

25 healthy volunteers (14 females, 11 males) aged 19-28 (mean 23.2 2.3) participated in the study. The study was approved by the local ethics committee and all volunteers gave written informed consent. None of the volunteers had a history of dermatological diseases. All experiments were carried out on the skin of the ventral forearms. In order to simulate dry skin volunteers were asked to wash one or both arms with soap three times a day during the week before the experiments. In this way water retention by NMF was minimized. All volunteers were asked not to apply any skin products on their ventral forearms within 24 hours before the start of the experiments and to refrain from caffeine ingestion and physical strain on the day of the experiment.

### *Moisturizers*

Perdeuterated ISIS, IPIS, GMIS were kindly supplied by Uniqema (Gouda, The Netherlands). Glycerol-1,1,2,3,3-$d_8$ and D-glucose-1,2,3,4,5,6,6-$d_7$ were purchased from Sigma Aldrich (Zwijndrecht, The Netherlands).

### *Treatments*

Three separate studies were performed to check the effect of extensive washing on tape-stripping and the penetration of lipophilic and hydrophilic moisturizers In the first series of experiments the effect of washing was studied. Four volunteers were asked to apply the washing regimen on one arm only. On the day of the experiment, both arms were treated with 10 µl/cm$^2$ water for three hours. In the second series of experiments the effect of lipophilic moisturizers was studied. 7 µl/cm$^2$ of two of the perdeuterated moisturizers ISIS, IPIS or GMIS were applied neat for three hours on washing-pre-treated inner forearm of 14 volunteers under non-occlusive conditions. In the third series of experiments hydrophilic moisturizers were studied. 10 µl/cm$^2$ deuterated 35% glycerol and 50% glucose solutions in water were applied occlusively for three hours on the washing-pre-treated forearms of 8 volunteers. The concentrations of the hydrophilic moisturizers were chosen to ensure equal water activity (i.e. thermodynamic activity) between the moisturizer solutions and the SC. In all three studies, the locations of the application sites (left or right arm) were randomised using a rotation scheme. All application and tape-stripping sites measured 2 by 8 cm.

### *Pre-experimental Trans-Epidermal Water Loss (TEWL) (i.e. control) measurements.*

In order to estimate the number of tape-strips required to remove approx. two thirds of the SC, the number of tape-strips necessary to reach a TEWL value (Tewameter TM 210, Courage + Kazaka, Cologne, Germany) of 40 $gm^{-2}h^{-1}$ was determined. The areas used to determine this number was also used to determine the water distribution in control SC (see below). Tape-strips were applied and removed from a 2x8 cm moisturizer-untreated control site on one of the ventral forearms of each volunteer immediately prior to the moisturizer application experiment. The location of this site did not interfere with the sites used for the moisturizer application. In case of the study aiming to determine the effect of excessive washing on tape-stripping, a control site was tape-stripped on both arms. After each third tape-strip TEWL was measured. Tape-stripping was discontinued after reaching a TEWL value above 40 $gm^{-2}h^{-1}$. This number of tape-strips was removed from all application sites of this volunteer.

### *ATR-FTIR measurements*

ATR-FTIR spectra were collected using a BioRad Excalibur FTS 4000 XM spectrometer (BioRad Laboratories, Inc. Cambridge, USA), equipped with a SHA 10 FTIR air purifying system (Hitma BV, Uithoorn, The Netherlands) and a liquid nitrogen cooled mercury-cadmium-telluride detector. Measurements were taken in the ATR mode using an ATR accessory (Specac Ltd, Orpington, UK), which supports a flat zinc selenide crystal (45° face angle). All spectra were collected with a resolution of 1 $cm^{-1}$ in the frequency range of 4000-400 $cm^{-1}$ and were the average of 32 scans.

To be able to discern the applied moisturizers from lipids, protein and other substances in the SC, perdeuterated varieties of the moisturizers were used (Dias et al., 2008). In these moisturizers all hydrogen atoms, with the exception of those in hydroxyl groups, have been substituted by deuterium atoms. Consequently, their atomic vibrations appear at a lower wavenumber, discernible from their hydrogenated counterparts. To determine the relative amount of moisturizer and the distribution of water in the SC, ATR-FTIR spectra of the treated regions were recorded before the first tape-strip and after the removal of each separate tape-strip. For ISIS, IPIS, GMIS the peak area (AUC) of the symmetric and asymmetric deuterated methylene ($CD_2$) stretching (2222 $cm^{-1}$ and 2110 $cm^{-1}$) were used. The vibration used for the determination of the relative amount of glycerol and glucose (971 $cm^{-1}$) is attributed to $CD_2$ rocking in a $CD_2OD$ group. Bulk water was determined using the peak area of around 3275 $cm^{-1}$

(hydroxyl bond). Because the ATR signal is dependent on the degree of contact between the applied arm and the crystal, all calculated peak areas were divided by the area between 1850 $cm^{-1}$ and 1800 $cm^{-1}$ to compensate for the difference in the degree of contact. In that region of the spectrum no peaks are detected and therefore the IR absorption in this region can serve as a baseline value.

To enable the comparison of the different moisturizers, it is necessary to determine the amount of moisturizer in the SC. This is problematic because the absorption of atom bond vibrations is influenced by the environment of a molecule, meaning that a calibration curve must be prepared in the same environment in which the moisturizer is measured. Therefore, it is impossible to determine absolute levels of moisturizers. However, relative amounts of moisturizer can be determined by correcting the FTIR contour for the difference in sensitivity of the FTIR signal of the different moisturizers. The relative moisturizer molarity was determined by preparing improvised calibration curves and applying them onto the ATR-crystal and determining the AUC as described at different molarities. The calibration curves of glycerol and glucose were prepared in water and those for ISIS, IPIS and GMIS by diluting perdeuterated moisturizer with protonated moisturizer. This made it possible to determine the relative moisturizer level differences within one moisturizer group.

### *Stratum corneum quantification*

The SC removed per tape-strip was quantified using Chao *et al*'s method (Chao and Nylander-French, 2004), with some modifications. Briefly, the tape-strips were extracted for 24 hours in 1N sodium hydroxide in a specially designed tray, after which the extracts were neutralized with 1N hydrochloric acid. To 50 l of the extract 200 l BCA reagent was added (Pierce BCA Protein Assay, Perbio Science BV, Etten-Leur, The Netherlands), followed by heating to 37°C for an hour and determination of the absorption at 595 nm. Very small pieces of isolated human SC dissolved in 1N sodium hydroxide and neutralized with 1N hydrochloric acid served as a stock solution for a calibration curve to determine the amount of SC removed by the tape-strip. Each well was diluted to contain approx. 1 to 10 µg of SC.

### *Data handling*

Firstly, the moisturizer distribution curves in the SC were fitted to an exponential equation:

equation 1: $ae^{bx}$

Herein coefficient *a* is a measure for the relative moisturizer molarity at the surface of the SC, while *b* determines the decrease of the relative molarity in depth. Secondly, the water distribution curves were fitted to quadratic (equation 2) and linear (equation 3) equations after hydrophilic and lipophilic moisturizer treatment, respectively:

equation 2: $ax^2 + bx + c$

equation 3: $bx + c$

In the waterdistibution curves in the SC after treated with hydrophilic moisturizers (equation 2) a negative coefficient *a* indicates that the (relative) amount of water increases in depth towards a maximum, before declining again, whereas a positive *a* indicates the opposite. This means that in case of a negative *a* indicates that the highest water levels are found in the central SC regions. The (relative) amount of water at the skin surface is equal to $x=0$, i.e. coefficient *c*. In the water profile in the SC after treatment with lipophilic moisturizers (equation 3), coefficient *c* is again a measure for the amount of water at the skin surface, whereas coefficient *b* shows the change in water level in SC depth. The equation's coefficients were compared using one-way ANOVA with Bonferroni post-tests using GraphPad 4.0 (GraphPad Software, Inc., La Jolla, CA). Differences with $p<0.05$ were deemed significant. In all calculations, the first measurement before removal of the first tape-strip was discarded, as this measurement is not representative of the relative molarity in the SC, but of the SC surface.

## 4. Results

Using tape-strip extraction, colorimetric measurements and a calibration curve prepared from SC, it is possible to determine the amount of SC removed per tape-strip in $\mu g.cm^{-2}$. The results of the SC quantification of the tape-strips reveal that tape-stripping removes SC relatively evenly per tape-strip, with only the first strips removing more than the average amount of SC per tape-strip. There are large fluctuations in the total amount of SC removed between subjects. However, combination with the inverse of the obtained TEWL data as performed previously by Kalia *et al* (1996), shows that in most cases, approx. 60% of the SC was removed (figure 1). It was also shown that the pre-experimental washing regimen, as well as 3-hour treatments with water or moisturizers had no effect on the amount of SC that was removed per tape-strip and in total (one-way ANOVA, not shown).

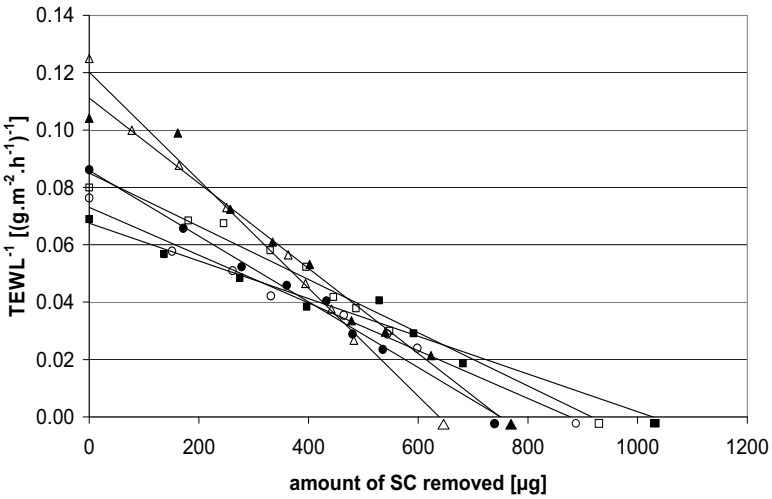


**FIGURE 1.** Typical examples of the inverse of obtained TEWL measurements versus the amount of SC removed of six typical volunteers. This results in an approx. linear relationship, which is shown by trend lines that can be identified by the symbols at their intercept with the y-axis. Extrapolation of the data indicates that the maximum mass of SC that can be removed is approx. 1000 g and that approx. 60% of the SC was removed, although occasionally more was removed (white triangle).

The average relative molarity of the hydrophilic moisturizers in depth is shown in figure 2a. The relative moisturizer molarity appears to decrease exponentially in depth. As expected, the highest relative molarity is seen close to the surface of the SC, indicating that the bulk of the moisturizer is present in approx. the upper 200 g of the SC. Deeper in the SC, only little moisturizer is detected. The decrease of moisturizer in depth is most clearly seen for glycerol, the curve of which shows a steep decrease in the upper regions, which levels off in the deeper SC. To determine a possible significant penetration difference between glycerol and glucose, the curves were fitted to an exponential equation (equation 1) and the coefficients *a* and *b* were compared using a paired t-test. The coefficient a, determining the relative amount of moisturizer close to the SC surface, showed that more glycerol than glucose is present after removal of one tape-strip ($p<0.001$, table 1a). The coefficient *b,* i.e. the gradual relative decrease in the moisturizer level in SC depth, however, was not found to be different (table 1b).

The average relative molarity of the lipophilic moisturizers in depth is shown in figure 2b. Although the relative moisturizer molarities are very different from those of the hydrophilic moisturizers, the shape of the curves defining their decrease in depth is very similar. The bulk of the moisturizer is again located at the surface of the SC and only very little is found in the deeper regions. The former is not surprising, given the short treatment period and the high molecular weight of the lipophilic moisturizers, which were expected not to penetrate the SC deeply. Fitting of the curves and comparison of the coefficients *a* and *b* (one-way ANOVA, table 1a and 1b) revealed that in this case neither *a* nor *b* are different between moisturizers, indicating that there are no differences in penetration profile between the three compounds after three hours.

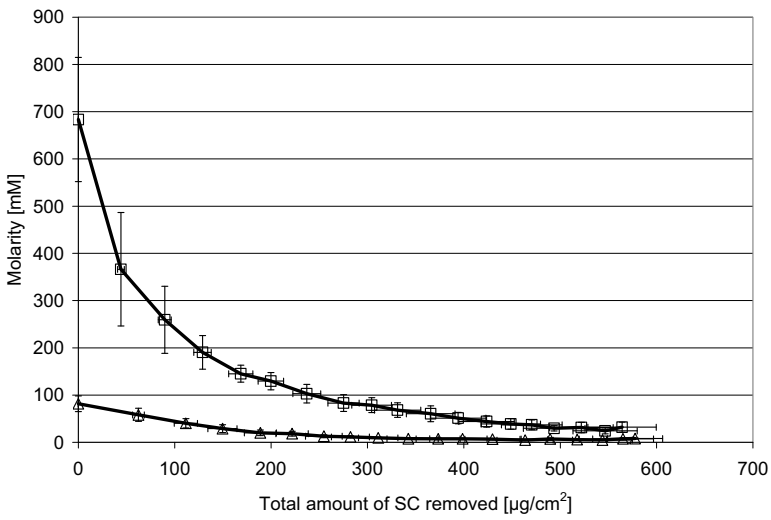


**FIGURE 2A.** The molarity of the moisturizers in approx. the top 1 µm of the treated site, after removal of a certain amount of SC. Glycerol penetrates the SC more readily than glucose, most likely due to their size difference. (-□- glycerol, -Δ- glucose)

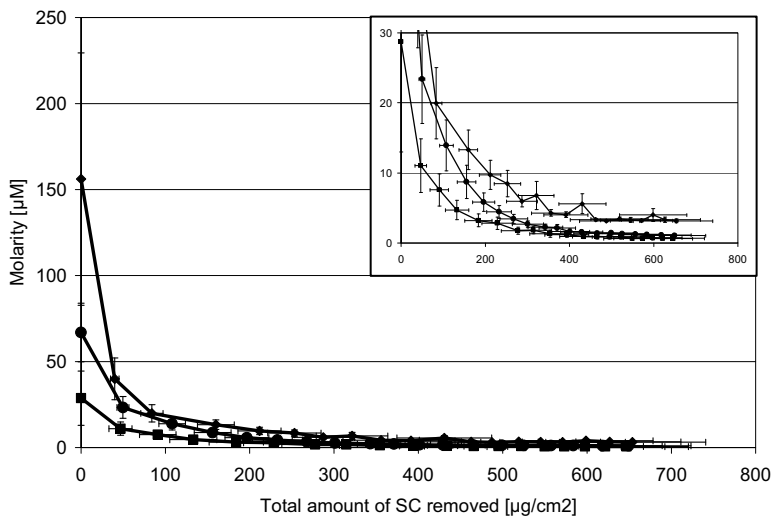


**FIGURE 2B.** The molarity of the moisturizers in approx. the top 1 µm of the treated site, after removal of a certain amount of SC. There is no significant difference between the penetration of ISIS, IPIS and GMIS. However, an enlargement of the graph (inset) clearly shows a trend for the least lipophilic moisturizers GMIS to penetrate deepest, whereas the largest moisturizer ISIS penetrates the least. ( -■- ISIS, -●- IPIS, -♦- GMIS)

Using the contour of the FTIR spectrum caused by the vibrations of the hydroxyl group at 3275 $cm^{-1}$, the distribution of water in SC depth could also be determined. First, it was determined whether the washing pre-treatment had any effect on the water distribution in SC before and after application of water. No difference was found (not shown).

*Table 1a: Mean differences, 95% confidence intervals and p-values of the coefficient* a *of equation 1 after moisturizer treatment.*

| Treatments | Mean difference | 95% Confidence Interval | $p_{adj}$-value |
|---|---|---|---|
| glycerol vs glucose | 279.1 | 204.3 to 353.8 | $p < 0.001$ |
| ISIS vs IPIS | 0.004413 | -69.42 to 69.43 | $p > 0.05$ |
| ISIS vs GMIS | 0.002026 | -77.32 to 77.33 | $p > 0.05$ |
| IPIS vs GMIS | -0.00239 | -72.24 to 72.23 | $p > 0.05$ |

*Table 1b: Mean differences, 95% confidence intervals and p-values of the coefficient* b *of equation 1 after moisturizer treatment.*

| Treatments | Mean difference | 95% Confidence Interval | $p_{adj}$-value |
|---|---|---|---|
| glycerol vs glucose | -0.00024 | -74.70 to 74.70 | p > 0.05 |
| ISIS vs IPIS | -0.00317 | -69.43 to 69.42 | p > 0.05 |
| ISIS vs GMIS | -0.00416 | -77.33 to 77.32 | p > 0.05 |
| IPIS vs GMIS | -0.001 | -72.24 to 72.24 | p > 0.05 |

The water distribution in control skin (no treatment) and after treatment with water or the hydrophilic moisturizers is shown in figure 3a. In the control SC, there is little water at the surface of the SC and levels increase slowly in the deeper regions. After glycerol and glucose treatment, large amounts of water are present in the superficial region of the SC. Glycerol treatment appears to increase the water content more than glucose does, although in the deeper SC regions, the water levels in SC treated with glycerol or glucose arevery similar. The amount of water decreases moving towards the central region of the SC. Treatment with water results in an entirely different water profile than treatment with hydrophilic moisturizers. In this case, water levels are low close to the SC surface and increase in the upper 200 µg of the SC to approx. twice the amount as after treatment with hydrophilic moisturizers. After this maximum, the amount of water slowly decreases towards the central region of the SC. Halfway through the SC, at approx. 500 µg depth, the levels are similar to those after treatment with hydrophilic moisturizers.

To determine whether there is a difference in the water distribution between control, water, glycerol or glucose treatment, all curves were fitted to a quadratic (equation 2), or, in case of the control, a linear equation (equation 3). The coefficients *a*, *b* and *c* of the curves were determined.

As expected from the different shapes of the curves after treatment with the glycerol and glucose and that after water alone, the coefficients *a* were mostly positive for the former equations, and negative for the latter. However, a significant difference was found only between the glycerol and the water treatment. No difference was found for glycerol vs glucose or water vs glucose (one-way ANOVA, table 2a).

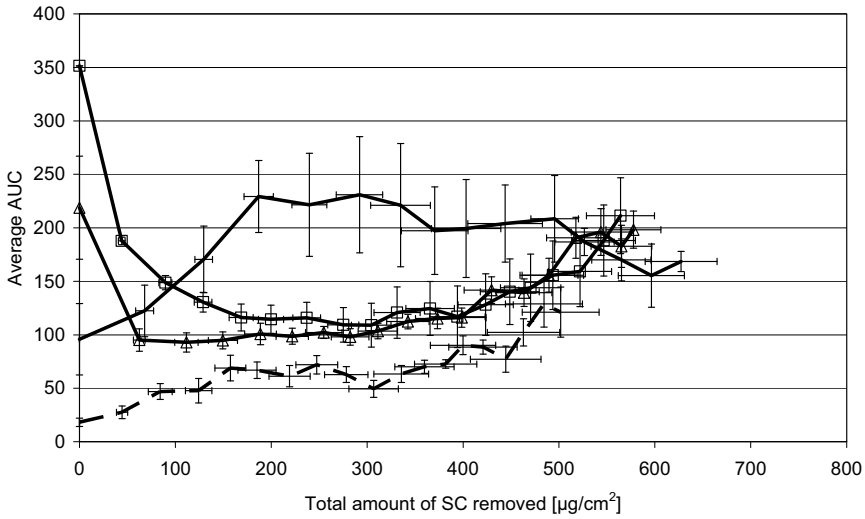


**FIGURE 3A.** Water distribution in the SC, expressed as the AUC of the water peak at 3275 $cm^{-1}$ after removal of a certain amount of SC. The water level in the upper region of the SC is relatively lower than that of the glycerol or glucose treated SC, but increases until a very high level is reached in the central SC. In contrast, the water levels in SC treated with glycerol or glucose are very high in the uppermost SC, but decrease sharply. The difference in water profile clearly demonstrates the humectant properties of glycerol and glucose: the applied water evaporates quickly from the top layers of the SC due to the lack of NMF in washing-pre-treated skin; addition of glycerol and glucose retains water in the uppermost regions of the treated SC. ( - - - dry, --- water, - □ – glycerol, - Δ – glucose)

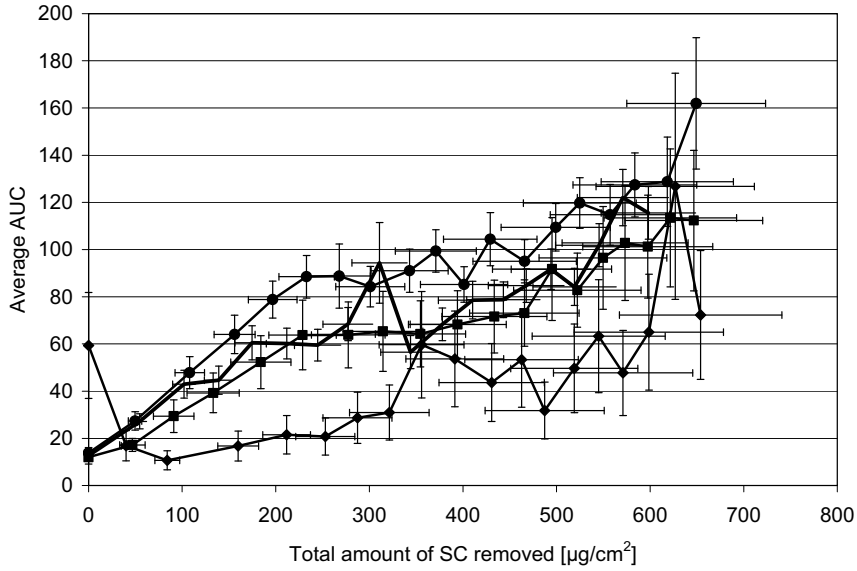


**FIGURE 3B.** Water distribution in the SC, expressed as the AUC of the water peak at 3275 $cm^{-1}$ after removal of a certain amount of SC. Water levels in SC treated with lipophilic moisturizers show no differences to each other or to control (no treatment). The higher AUC of GMIS on the surface of the SC is most likely caused by the hydroxyl groups of this moisturizer. ( - - - dry, --■--ISIS, -●- IPIS, -♦- GMIS)

*Table 2a: Mean differences, 95% confidence intervals and p-values of the coefficient* a *of equation 2 after moisturizer treatment.*

| Treatments | Mean difference | 95% Confidence Interval | P-value |
|---|---|---|---|
| glycerol vs glucose | $7.625.10^{-4}$ | $-4.386.10^{-4}$ to $1.964.10^{-3}$ | P > 0.05 |
| glycerol vs water | $1.858.10^{-3}$ | $6.564.10^{-4}$ to $3.059.10^{-3}$ | P < 0.01 |
| glucose vs water | $1.097.10^{-3}$ | $1.061.10^{-4}$-to $2.296.10^{-3}$ | P > 0.05 |

*Table 2b: Mean differences, 95% confidence intervals and p-values of the coefficient* b *of equation 2 after moisturizer treatment.*

| Treatments | Mean difference | 95% Confidence Interval | P-value |
|---|---|---|---|
| no treatment vs water | 0,2273 | -0.3254 to 0.7800 | P > 0.05 |
| no treatment vs glycerol | 1,026 | 0.4736 to 1.579 | P < 0.001 |
| no treatment vs glucose | 0,2368 | -0.3159 to 0.7895 | P > 0.05 |
| water vs glycerol | 0,799 | 0.2463 to 1.352 | P < 0.01 |
| water vs glucose | 0,009475 | -0.5432 to 0.5622 | P > 0.05 |
| no treatment vs ISIS | -0,06696 | -0.5764 to 0.4425 | P > 0.05 |
| no treatment vs IPIS | -0,0845 | -0.5489 to 0.3799 | P > 0.05 |
| no treatment vs GMIS | -0,08017 | -0.6117 to 0.4513 | P > 0.05 |
| ISIS vs IPIS | -0,01754 | -0.5443 to 0.5092 | P > 0.05 |
| ISIS vs GMIS | -0,01321 | -0.6000 to 0.5735 | P > 0.05 |
| IPIS vs GMIS | 0,004327 | -0.5438 to 0.5525 | P > 0.05 |

*Table 2c: Mean differences, 95% confidence intervals and p-values of the coefficient* c *of equation 2 after moisturizer treatment.*

| Treatments | Mean difference | 95% Confidence Interval | P-value |
|---|---|---|---|
| no treatment vs water | -17,25 | -108.7 to 74.25 | P > 0.05 |
| no treatment vs glycerol | -187,3 | -278.7 to -95.75 | P < 0.001 |
| no treatment vs glucose | -86,9 | -178.4 to 4.596 | P > 0.05 |
| water vs glycerol | -170 | -261.5 to -78.50 | P < 0.001 |
| water vs glucose | -69,65 | -161.1 to 21.85 | P > 0.05 |
| no treatment vs ISIS | 2,471 | -81.86 to 86.80 | P > 0.05 |
| no treatment vs IPIS | -12,73 | -89.62 to 64.15 | P > 0.05 |
| no treatment vs GMIS | -22,56 | -110.5 to 65.42 | P > 0.05 |
| ISIS vs IPIS | -15,2 | -102.4 to 72.00 | P > 0.05 |
| ISIS vs GMIS | -25,04 | -122.2 to 72.10 | P > 0.05 |
| IPIS vs GMIS | -9,831 | -100.6 to 80.91 | P > 0.05 |
| no treatment vs water | -17,25 | -108.7 to 74.25 | P > 0.05 |

When comparing the coefficients *b* and *c*, the control (no treatment) was also included. Two differences for coefficient *b* were found. Coefficient *b* was smaller in the curves describing the water distribution after glycerol treatment than in those describing it for the control (no treatment) and after water treatment (one-way ANOVA, table 2b).

Also coefficient *c* was found to be different for the pairs glycerol treatment vs. control and glycerol treatment vs. water treatment. In this case *c* was larger for glycerol, indicating a higher water level in the upper SC regions after glycerol treatment than after water treatment or in the control (one-way ANOVA, table 2c).

Figure 3b shows the distribution of water in SC after treatment with the lipophilic moisturizers ISIS, IPIS and GMIS and after control (no treatment). Water levels are very low in the upper SC and slowly increase in depth. After GMIS treatment, a small decrease from the first to the second tape-strip is seen, most likely caused by the free hydroxyl group that this moisturizer possesses.

To compare the water distribution after ISIS, IPIS, GMIS or control (no treatment), the obtained curves were fitted to a linear equation (equation 3) and their coefficients *b* and *c* were compared. This comparison (one-way ANOVA, table 2a and 2b) did not reveal any differences, neither between the control and the treatments, nor between the lipophilic moisturizers themselves. This increase in relative water level in SC depth is therefore quite similar for the control and the three moisturizers.

## 5. Discussion

Tape-stripping has long been used to determine the penetration of substances into SC. By applying and removing successive pieces of adhesive tape, layers of SC are removed. The amount of applied substance on the tape-strips can then be quantified (Chao and Nylander-French, 2004; Kalia et al., 1996). However, using ATR-FTIR an applied substance can also be detected *in the superficial layers of the SC* at the application site itself, rather than on the tape-strip itself (Gorzelanny et al., 2006; Honeywell-Nguyen et al., 2004). The tape-strip can then be used for SC quantification.

Quantification of the SC on the removed tape-strips revealed that SC was evenly removed, with only the first to third tape-strips removing more SC than the average amount per tape-strip. Comparison of the amounts of SC removed when tape-stripping control skin from washed or unwashed skin, as well as after water, hydrophilic or lipophilic moisturizer

treatment showed that in this study neither of these treatments affected the amount of SC removed. This suggests that one week of excessive washing, as well as a three hour treatment with water or moisturizers does not affect the degradation of corneodesmosomes leading to increased or decreased adhesion of corneocytes. In general, tape-stripping to a depth resulting in a TEWL of 40 $g.h^{-1}.m^{-2}$ or higher resulted in approx. 60% of the entire SC to be removed (figure 1).

The hydrophilic moisturizers penetrate the SC readily. After three hours of treatment, they are present in large amounts in the upper 200 g of the SC, albeit that the relative molarity of glycerol is up to 10 times as high as that of glucose. This difference may partially be caused by a difference in molecular weight. Furthermore, at such a short time interval and non-occlusive application, the moisturizer distributions have not yet reached steady state at which the difference between the moisturizers may be different.

The lipophilic moisturizers ISIS, IPIS and GMIS penetrate the SC at lower levels. However, in some cases they were also found in the deeper SC regions, despite their large molecular weight. Curve fitting to an exponential equation combined with the comparison of the equation's coefficients did not show any differences between the three moisturizers. This may be due to the fact that also the lipophilic moisturizer diffusion into the SC had not yet reached steady state. However, their localization in the upper regions of the SC suggests that they could have interactions with the intercellular lipids there, possibly rendering them less penetrable to water. It must also be noted that the relative molarity in the deeper regions of the SC are so low that they are not likely to have a clinical effect here.

Although the penetration of the moisturizer is very important, their effect, i.e. changes in SC water levels can only be measured by the amount of water present in the SC at different depths. This was achieved by measuring the AUC of the peak at 3275 $cm^{-1}$.

The pre-treatment of the skin by washing with soap did not result in a detectable difference in water levels before or after application of water. This could be the result of already low NMF levels due to the season in which the studies were performed (winter and spring) or a less aggressive soap used in our studies in inducing dry skin than used in some other studies, such as sodium lauryl sulfate (Fluhr et al., 2005; Paye et al., 2007).

The greatest effect on water levels was obtained by applying water or hydrophilic moisturizers dissolved in water. Indeed, the coefficients *b* and *c* were found to be significantly different for the curves describing the water distribution in the control and after glycerol

treatment. More curious, however is the difference between the curve of the water application and that of the moisturizer application (fig 3a). While the water level after moisturizer application is very high and immediately decreases in depth, the water level in the superficial regions of SC treated with only water appears to be lower. Interestingly, after this application, the water levels appear to increase a little deeper in the SC. This is most likely caused by evaporation of water from the superficial SC immediately after the 3 hours of application due to a lack of NMF. In SC treated with hydrophilic moisturizers, the superficial water levels are higher, as the applied moisturizer retains water. The difference in curve shape after water treatment and glycerol treatment is underlined by significant differences of the coefficients *a, b* and *c*.

The lipophilic moisturizers appeared not to increase the water levels in the SC, even though IPIS, and to a lesser extent GMIS, were shown to increase skin moisture using corneometry (Wiechers, 1999) and the same was visualized for ISIS using cryo-SEM (Caussin et al., 2007b). Recent studies (Caussin et al., 2007a) have shown that ISIS and IPIS have the ability to stabilize the orthorhombic lateral packing of SC lipids mixtures and to promote the formation of the LPP. Both features are thought to be very important for the barrier function of the SC (Abrahamsson et al., 1978; de Jager et al., 2006). These observations indicate that interactions between the moisturizers and SC lipids may be responsible for the moisturizing effect of ISIS, IPIS and GMIS. It is very likely, however, that a 3 hour treatment period is too short for these effects to occur in the environmental conditions used in the present study. Most likely, they can only be shown with this method after prolonged and/or repeated treatments. As already mentioned, a repeated application does lead to a higher water level in stratum corneum (Wiechers, 1999).

This study determined the localization of lipophilic and hydrophilic moisturizers and water in SC after a three hour moisturizer application. To understand the consequences of the distribution of lipophilic moisturizers on their effect, it is essential to identify their working mechanism. Lipophilic moisturizers are hypothesized to increase SC water levels either by occlusion on the surface of the SC, or by changing the packing and/or lamellar organisation of the intercellular lipid matrix in the upper SC layers as described, making them less permeable to water. For either mechanism, it is vital that lipophilic moisturizers do not penetrate the SC too deeply, because this would result in decreased hydration in the SC layers above their penetration depth. The moisturizers used in this study were measured to be non-occlusive

(Wiechers, 1999) and are therefore suggested to function by penetration into and interaction with intercellular SC lipids (Caussin et al., 2007a; Caussin et al., 2008). This study shows that the bulk of the penetrated lipophilic moisturizer remains in the superficial layers of the SC after 3 hours of application. This is the location in which they could interact with SC lipids. In this way, they could affect an internal occlusion of the SC, rather than forming a layer on the surface of the SC, which is perceived as uncomfortable by many patients using emollients.

The hydrophilic moisturizers are also mostly present in the upper region of the SC. For a proper effect, they have to penetrate the central part of the SC to be effective. However, judging from the amount penetrated after only 3 hours, this appears realistic, especially after prolonged and/or repeated application.

Concluding, this study has shown that hydrophilic and lipophilic moisturizers penetrate the SC, the former more readily than the latter. Hydrophilic moisturizers appeared to result in higher water levels in the region that they are localized in. No increase in water levels was found after treatment with lipophilic moisturizers, most likely due to the short application time.

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## 7. References

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