

Sorption Properties of the Human Stratum Corneum

G.K. Johnsen^a L. Norlen^{c, d} Ø.G. Martinsen^{a, b} S. Grimnes^{a, b}

^aDepartment of Physics, University of Oslo, and ^bDepartment of Clinical and Biomedical Engineering, Rikshospitalet University Hospital, Oslo, Norway; ^cDepartment of Cell and Molecular Biology, Medical Nobel Institute, Karolinska Institutet, and ^dDermatology Clinic, Karolinska University Hospital, Stockholm, Sweden

Key Words

Skin · Stratum corneum · Hydration · Time constants · Hysteresis

Abstract

Water sorption is important for the overall structure and function of keratinized tissues such as the human epidermal stratum corneum (SC). In this study we report on a gravimetric method for studying sorption properties of human SC, both from heel and female breast skin. Changes in mass were measured as the relative humidity was altered in steps under controlled environmental conditions. The possibility of hysteresis is also discussed. Furthermore, we have found that the sorption time constants show triphasic behaviour during absorption, but not during desorption. This behaviour is connected to the 3 different types of water present in the SC. Water also enters the SC much more rapidly compared to its exit at relative humidities <50%. Finally, the amount of time between sample preparation and onset of measurement seems to have an effect on the absorption rate, but less on the total amount of water absorbed.

Copyright © 2011 S. Karger AG, Basel

Introduction

The foremost function of the skin is to constitute a barrier between the body and the environment [1]. The stratum corneum (SC), i.e. the most superficial layer of skin, is directly responsible for skin barrier function. It is composed of dead keratinized cells embedded in a continuous extracellular lipid structure. The major role of the keratinized cells is to constitute a both stiff and viscoelastic protective scaffold for the lipid structure. The protective mechanical properties of the SC cells are intimately linked to their water content [2–4] that also may influence the desquamation process [5]. The skin function is thereby largely dependent on the SC water balance. Also, the SC hydration properties are important for the understanding of skin diseases characterized by deficient barrier function, such as atopic dermatitis [6–8]. The SC water content will generally vary with both relative humidity (RH) and temperature, as well as with any applied occlusive effects [9], as the partial pressure in ambient air and SC tries to equal. Water contents of >10% are necessary in order for the skin to remain healthy and pliable [1].

In this study we report on a gravimetric method for studying the sorption properties of human SC, both from heel and from female breast skin obtained from plastic

surgery. Knowledge about SC sorption properties is central for our understanding of SC hydration. It will also aid the interpretation of electrical skin hydration measurements [10]. First, we investigated the absorption and desorption properties of SC in order to evaluate if hysteresis was detectable, meaning that the water content at a fixed RH is different during absorption and desorption. Water uptake as a function of RH was studied as well as time constants, which are the characteristic time needed in order to reach about 63% of the equilibrium value.

The study was performed with a dynamic vapour sorption (DVS) instrument from Surface Measurement Systems Ltd. (London, UK), where the RH is altered in steps under controlled environments. Kilpatrick-Liverman et al. [11] previously performed a similar study on porcine skin, but to our knowledge no such study has been performed on human skin. However, some results were presented in a preliminary study [13]. Also few, if any, considerations of the sorption time constant have earlier been carried out, although Martinsen et al. [10] commented on some of these aspects, and literature reports on concentration-dependent diffusion of water within the SC [13, 14].

Materials and Methods

The SC heel samples were taken from the heel of 10 volunteer test persons, 5 men and 5 women at the ages ranging from 24 to 29 years, all with no signs of unhealthy skin and with no history of dermatological diseases. The SC was removed by means of an Aesculap dermatome (Braun, Tuttlingen, Germany), and the sample pieces were about 4×5 mm in size after preparation. All samples were prepared with as equal size as possible in order to reduce any effect of geometry on the final results. Their thicknesses were 0.20 mm, which was the thinnest possible configuration provided by the dermatome.

Breast skin from 10 healthy females, without any sign of dry skin or other skin disease, aged between 32 and 68 years, was obtained from reconstructive surgery and kept in a freezer at -20°C for 3 months. After dissection of the subcutaneous fat, the samples were heated for 3 min at 60°C with the dermal side in contact with a metal plate. The superficial epidermis was then peeled off with a razor blade. Next, the samples were incubated in 0.5% trypsin with the epidermal side down for 2 h at ambient temperature to remove remnants of stratum granulosum adhering to the epidermal side of the SC. Finally, the sheets were washed 4 times in distilled water and dried and stored at ambient temperature under nitrogen for about 2 years.

One SC heel sample from one of the 5 male test subjects was also hydrated in HPLC water for 4 h and then contained in a freezer at -20°C for 72 h. This was done in order to investigate the effect of freezing on the sorption properties of the SC. Also, 3 heel samples from one of the 5 male test subjects were contained ex

vivo for several days prior to experiment onset in order to discover any effects on sorption properties with respect to time between sample preparation and measurement onset.

Furthermore, a DVS was used in order to reveal the SC sorption characteristics by altering the RH in steps from 0 until 90% and then back to 0%. The steps were all preprogrammed to be of a 10% increase in RH and the temperature was kept constant at 25°C throughout the entire experiment. The DVS apparatus provides a very sensitive microbalance weight with a resolution of 0.1 μg . The SC heel samples were prepared so that their weights were about 3–5 mg, whereas the breast samples, being much thinner, were about 0.4–0.6 mg, but still well within the resolution of the DVS instrument. The DVS was set to jump to the next stage, i.e. a new RH value, when the rate of sample mass, dM/dt , was <20 ppm/min over a period of at least 10 min. The measured sorption data were sampled with a rate of 1 min^{-1} .

The sorption time constants were found by fitting the data to an exponential time course, $y = a + \exp(-kt)$ by means of the 'curve-fitting tool' in MatlabTM, and standard deviations were found correspondingly. A course proportional to $\exp(-kt)$ would then yield time constants equal to $\tau = 1/k$.

As the breast samples were of unknown thickness, yet much thinner than the heel samples, and as the thickness will influence the numeric value of the time constants, giving lower values with reduced thickness [15], the results of each breast sample were normalized in order to find the curve form of the average curve of the time constants of the breast samples.

Results

Figure 1 shows the time course of one of the SC samples, taken from the heel, as the RH in the DVS chamber was altered in steps of 10%. The increment in mass corresponds to absorption of water caused by an increase in RH, whereas decreasing mass corresponds to desorption, i.e. the sample loses water to the environment. The increase (or decrease) in weight then equals the percentage of the weight of water in the skin sample relative to the dry weight of the sample. Initially, the SC sample was set to be in equilibrium with the environment at 0% RH, giving the dry weight of the sample. Part of this calibration is seen to the far left in the time course in figure 1, where the sample has a negative mass gain. The time evolutions of the 10 SC heel samples showed very similar time courses. We notice that the first steps in RH resulted in very small mass changes. These steps are better viewed in figure 2, where the steps corresponding to RH at 10, 20, 30 and 40% are enlarged. The corresponding desorption time courses at low RH followed similar patterns, only now with a negative mass rate.

The alterations in mass of the samples, initiated by the instantaneous increments in RH, followed courses that were close to exponential at high RH, indicating that the

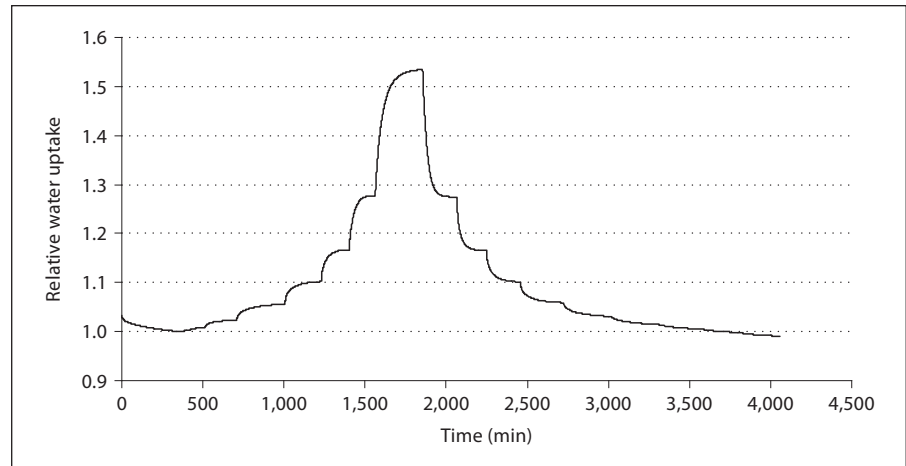


Fig. 1. Time course of relative water uptake for SC from the heel during absorption and desorption.

mass rate was proportional to the difference between the actual mass and the equilibrium mass for each step. This is in accordance with Fick's diffusion theory [14], and time courses of the mass for each step were therefore approximated as

$$M(t) \approx \exp(-k_i t)(M_1 - M_2) + M_2 \quad (1),$$

where M_1 and M_2 are the sample masses before and after a step in RH was initiated, respectively, $i = a, d$, so that k_a^{-1} is the time constant for the absorption process, whereas k_d^{-1} is time constant for desorption. The steps at the lower part of the RH interval deviated somewhat from a purely exponential behaviour, especially courses at 30 and 40% RH, which are shown in figure 2. However, all regressions of single measurements produced fits to the measured data with root mean square error <0.2%.

In figure 3, a corresponding course is shown for a full sorption cycle for one of the samples from SC isolated from breast skin. The 2 different types of SC sample, heel and breast, showed very similar full sorption courses, however, with more distinct steps for the latter sample type. This may be due to the fact that breast SC samples were much thinner than SC taken from the heel, which again led to a much larger surface to volume ratio, resulting in less time in water diffusing into the samples.

Figure 4 shows the average water uptake of the 2 different sample types of human SC at 25°C as a function of ambient RH. The red, dotted graphs correspond to absorption data, and the blue, continuous graphs show desorption data. The error bars represent the standard deviations of the measurements. We observed that the absorption and desorption graphs were very similar, but with the desorption data slightly elevated compared to

absorption, indicating that the samples contained nearly equal amounts of water during absorption and desorption at any given value of RH in the DVS closed chamber. The water sorption isotherms for heel SC showed small changes in water uptake for RH <50%, but a more rapid and exponential behaviour at the highest RH values. The isotherms for the SC isolated from breast skin were more elevated compared to heel data at RH <70%, indicating larger water uptakes. At the highest RH values, breast SC contained substantially less water compared to heel SC. Only at the midrange RH were there small signs of deviation between water content from absorption and desorption for heel SC, however mainly within the uncertainties in our measurements. For breast SC, the small signs of hysteresis were broader, spanning over a wider RH interval.

Furthermore, the sorption time constants, which are the characteristic time of water entering or leaving the SC, of the heel samples are illustrated in figure 5. The rapidity of water entering or leaving the SC varied as a function of RH, and the absorption was seen to be triphasic (i.e. 3 separate phases) in this respect. We note the significant difference in the time constants between absorption and desorption at low RH. During absorption, SC rapidly reached its equilibrium state at very low RH and then decreased with an increase in RH until 50%. Above 50% the absorption time constant decreased with increasing RH until 80%, from which it again increased. The desorption time constant followed the pattern of absorption for RH >50%, but at lower humidities the desorption time constant was much higher and flattened out as RH approached zero.

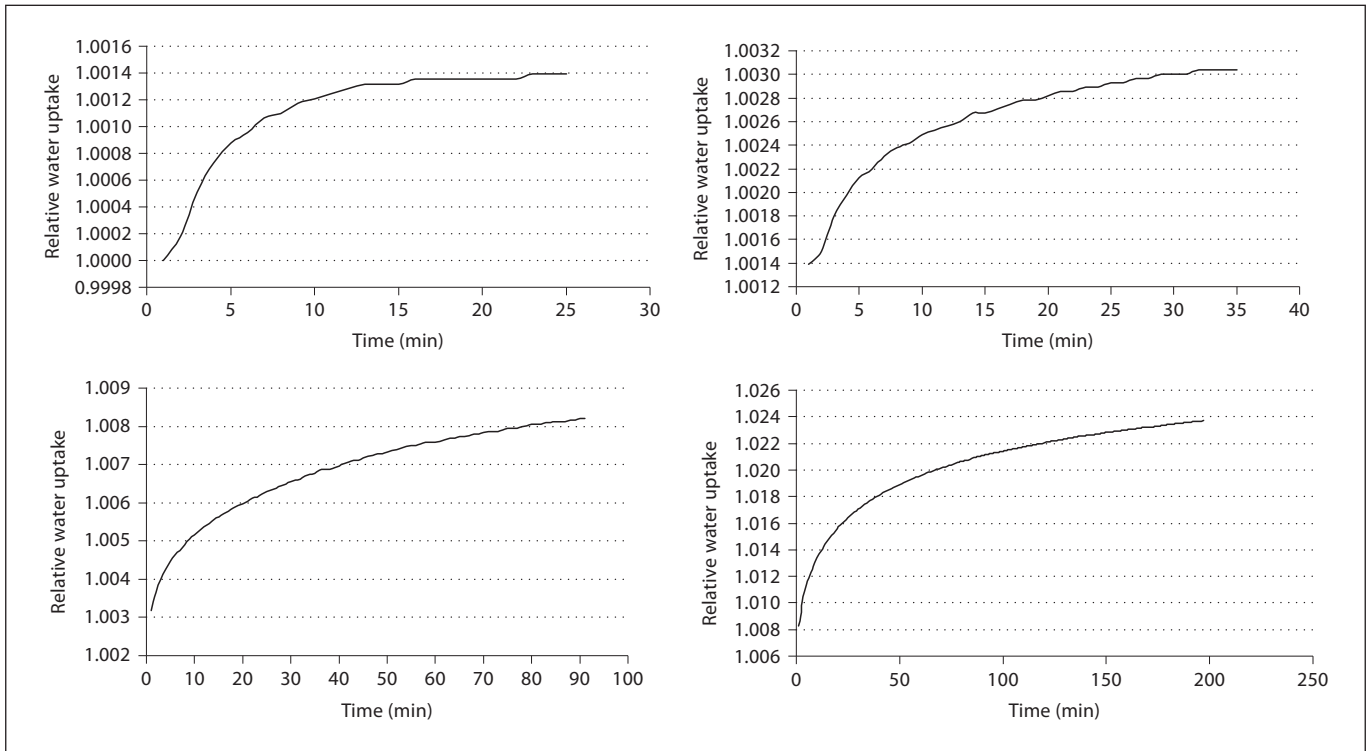


Fig. 2. Time courses of relative water uptake at RH corresponding to 10, 20, 30 and 40% for heel SC during desorption.

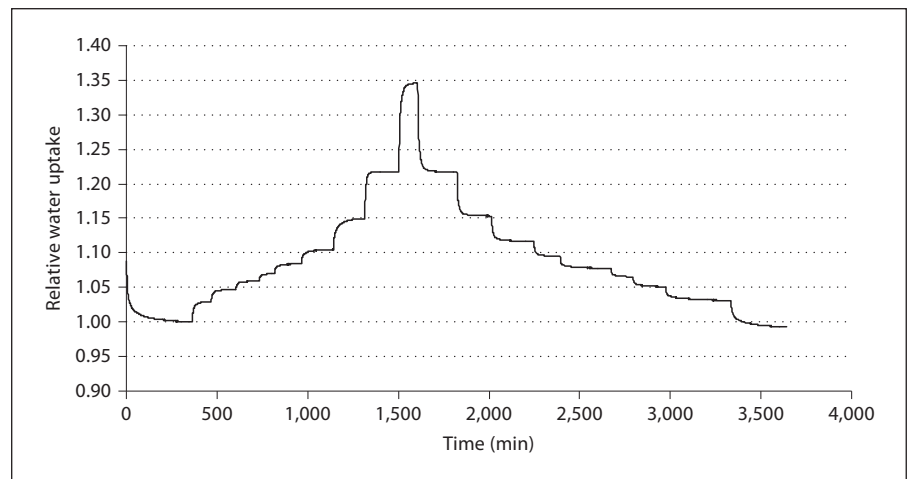
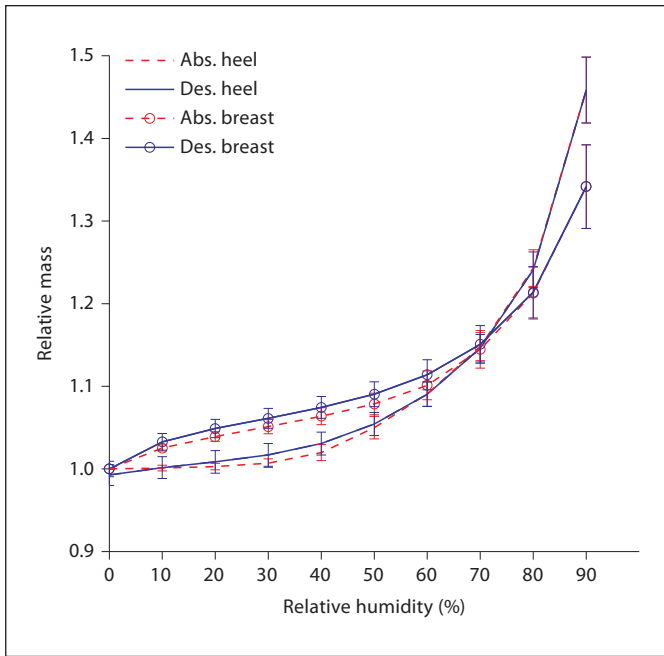


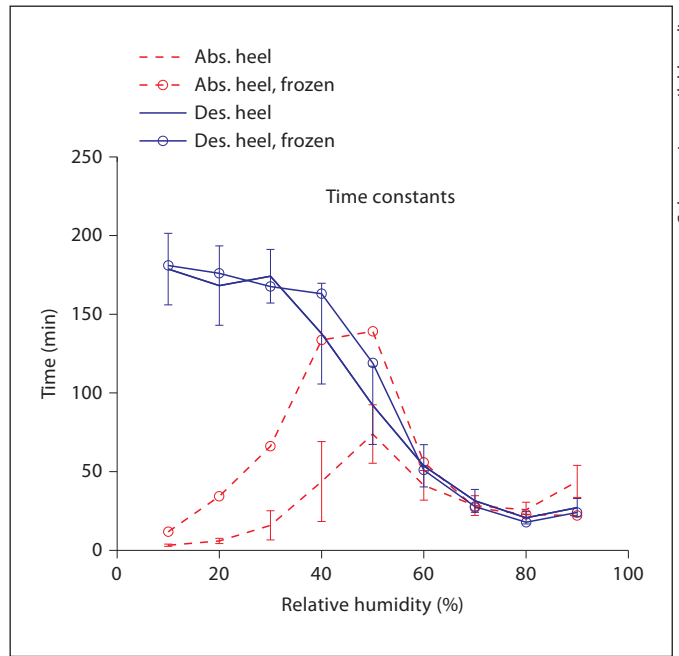
Fig. 3. Time course of relative water uptake for SC isolated from breast epidermis during absorption and desorption.

The corresponding sorption time constants, now for the breast samples, are shown in figure 6. Here, the time constants are normalized with respect to the value of the time constant of the first absorption step. This was done in order to be able to compare at least curve forms with the heel data.

We noted that there were similar trends in curve form for the time constants of heel and breast SC, although there were also substantial differences. The time constants of both sets of samples experienced a peak followed by a rapid drop during absorption, but with different rates compared to the corresponding desorption trends.



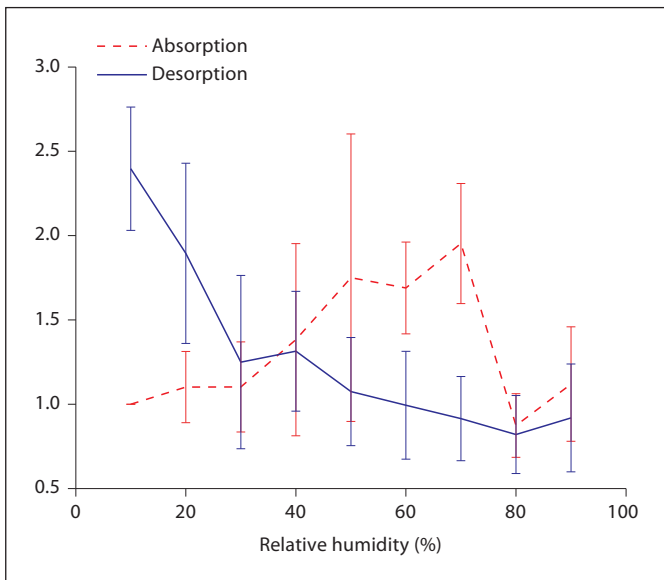
Color version available online



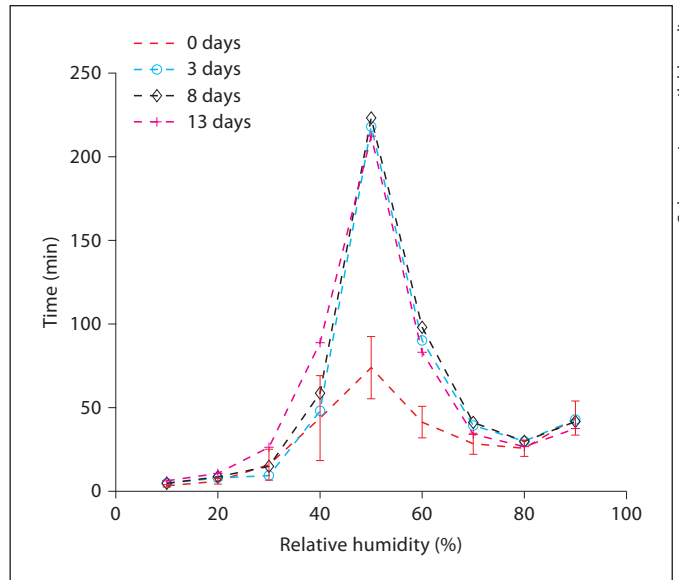
Color version available online

Fig. 4. Relative water uptake for heel and breast SC during absorption and desorption.

Fig. 5. Time constants of heel SC during absorption and desorption.



Color version available online



Color version available online

Fig. 6. Normalized time constants of SC isolated from breast epidermis.

Fig. 7. The effect of time between heel sample preparation and onset of measurement on the absorption time constant.

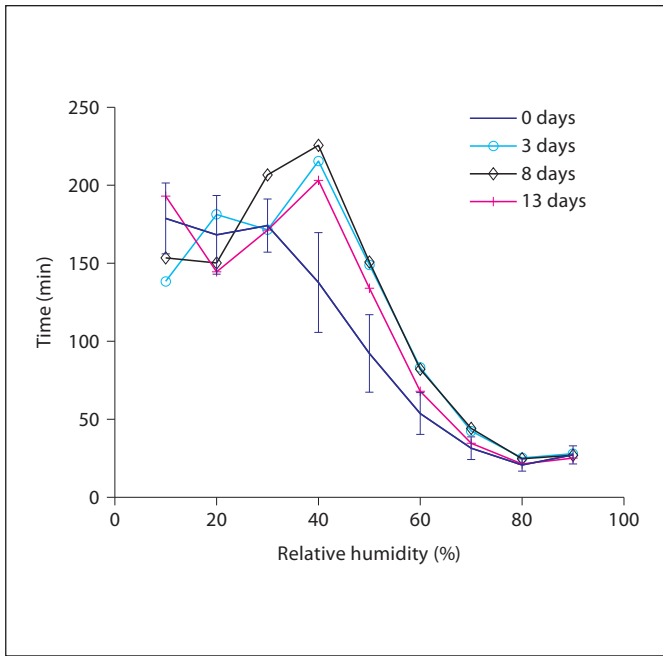


Fig. 8. The effect of time between heel sample preparation and onset of measurement on the desorption time constant.

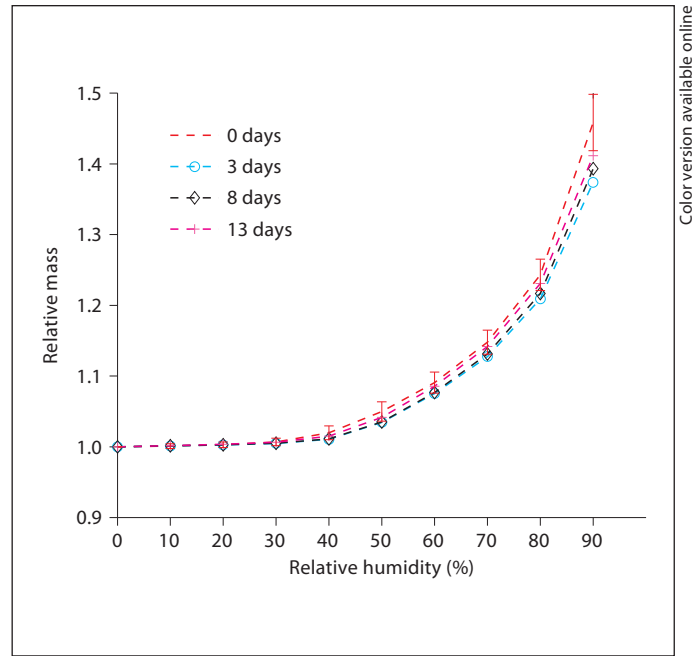


Fig. 9. The effect of time between heel sample preparation and onset of measurement on the relative water uptake during absorption.

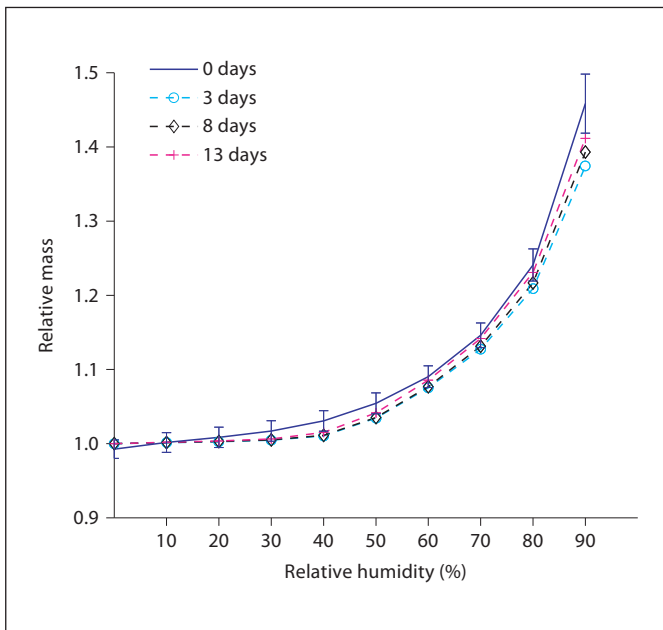


Fig. 10. The effect of time between heel sample preparation and onset of measurement on the relative water uptake during desorption.

In figure 5, the time constant of a heel SC sample that had been hydrated and then frozen for 72 h is shown in order to see if freezing, which had been applied to all breast samples, would affect the time constants. We noted that the absorption data were elevated compared to the 10 other heel samples when RH was <60%, but that the desorption curve followed more or less the same trend as for the 10 unfrozen heel samples.

Figure 7 shows that the absorption time constant increased substantially at the RH interval of 40–70% when the samples were contained in the lab for several days prior to onset of experiment. The same effect was found for desorption data (fig. 8), however with smaller deviations from the average value of the time constants. The number of days >3 did not seem to influence the data much.

In figures 9 and 10, the corresponding time effect is viewed through the relative water uptake of one heel SC sample during absorption and desorption. We noted only small differences in water uptake in the RH regions where the time constants altered, but there may be a weak tendency that the samples took up less water after having been stored for several days prior to the onset of measurement.

Discussion

In this paper, we have studied sorption properties of human SC and found that the sorption time constants varied greatly with RH of ambient air with triphasic behaviour during absorption. The sorption time courses were best described by single exponential time courses at high RH, whereas at the lowest RH both absorption and desorption showed a behaviour with slightly more pronounced deviations from exponential courses. A single exponential dependence with time may indicate that there is one underlying sorption mechanism that is strongly dominating, and a deviation from such a course could therefore be a combination of 2 or more mechanisms, both sorbing water, in the SC.

Studies on the diffusion properties of polymers such as keratins have shown that these types of material show a behaviour governed by Fickian diffusion above a certain and material-dependent temperature, the glass transition temperature, but being more complex below T_g . This has usually been ascribed to the differences in mobility, above and below T_g , of keratin filaments. Diffusion of water in between the keratin filaments is therefore possible since the filaments are free to move above T_g [19]. A possibility for the behaviour at low RH (i.e. at low sample water uptakes) may therefore be due to the effect of vapour-induced glass transition, below which water content diffusion can be expected to deviate from purely exponential. A topic for further studies should therefore be to reveal more of the underlying mechanisms of SC water binding and to see if SC experiences a vapour-induced RH glass transition.

The isotherm shapes illustrated in figure 4 correspond well with others found in earlier sorption studies [2, 17, 18]. From the figure we noted that our measurements showed very small, if any at all, amounts of hysteresis, being of a size comparable to the uncertainties between the different samples, and may therefore be due to the relatively large inter-individual spread with respect to skin sorption properties. Hysteresis is reported, however, much more substantial, in porcine skin [11], but not found in human SC from the hip [18]. Some hysteresis has been reported to be found in horn keratin [19], and Hey et al. [20] mentioned a low-pressure SC hysteresis. Hysteresis associated with sorption isotherms may indicate that there are mechanisms keeping more of the water within the samples during desorption compared to absorption. Significant hysteresis will in general require structural changes in the SC, which again affects the water-holding capacity. Such changes are, according to literature, expected to take

place at temperatures below the glass transition limit. For SC this limit is reported to be $<20^\circ\text{C}$ [21], meaning that large amounts of hysteresis cannot, in our study, be expected to occur due to glass transition structural changes.

One cannot rule out the possibility that some of the deviations in absorption and desorption water uptakes are due to the necessarily present gap between absorption and desorption equilibrium masses due to the fact that one has to specify a cut-off in mass rate (we used 20 ppm/min) as a criterion for RH to step to the next level as well as the fact that SC varies much between individuals. The numeric value of the smallest allowed mass fluctuation in the DVS setup will influence the result, especially if it is chosen too large. A large equilibrium rate will result in short time intervals before RH is programmed to initiate a step of 10%. This would allow little time for the SC samples to fully reach the equilibrium state, causing deviations between absorption and desorption, that is hysteresis. Still, as the mass rate criterion was chosen very small, and much smaller than in the similar study with SC from porcine skin [11], this effect can be assumed to be small, and is not likely to produce large gaps between absorption and desorption water uptake in a sorption cycle.

The differences between SC from heel and breast in curve form, as seen in figures 4 and 5, may possibly be due to different preparative methods and further discussions are therefore of little value. Cryo-electron microscopy of vitreous skin sections is likely the method to obtain such structural changes upon freezing in the future.

We noticed that the absorption time constant, given in figures 5 and 6, showed triphasic behaviour. Our results are similar to those found by Martinsen et al. [10], but in this study the SC samples were allowed sufficient time to reach the equilibrium states after the initial calibration at 0% RH, and a full sorption study was performed, including also desorption data. The observed triphasic behaviour may be related to the 3 different types of water present in the SC (strongly bound, bound and bulk) [18, 22–24] and to their corresponding sorption mechanisms. Silva et al. [25] reported on 3 regimes for isolated corneocytes where the water contents were $<5\%$ wt, 5–17% wt and $>17\%$ wt, respectively. This corresponds to our findings of the time constants for absorption in figures 5 and 6 combined with the mass gains in figure 4. The strongly bound water, potentially bound to polar functional groups of lipid bilayers until monolayer coverage was reached [17, 23], entered the SC very rapidly at low RH, and the increase in time constants with RH could be induced by less available sites to attach. Thereafter, as a new type of water was bound in the SC, the time constant de-

creased, which is an expected behaviour since the diffusion coefficient of water in SC, inversely related to the absorption time constant, usually increases with concentration [15, 16]. The final and slight increase in the time constant above RH values of 80% may be due to bulk water entering the SC combined with the onset of a swelling process that is time-consuming, i.e. giving higher time constants, although bulk water in itself is expected to yield a more rapid diffusion. This part of the RH domain is within the swelling area which has been reported to start at 22–33% wt [17, 18, 24, 26].

The initial increase in absorption time constant, meaning that it took more time for water to enter the SC as RH was increased, is the opposite of the effect caused by an increasing diffusion coefficient with concentration. Rouse [27] reported on a decreasing diffusion constant (i.e. increasing time constant) with increasing concentration for polymers that were not strongly hydrophilic. This effect was proposed to be due to an increasing proportion of immobilized water as the water content increases, leading to water entering the polymer more slowly. It is clear that keratin filaments are not very polar as they are quite insoluble in nondenaturing buffers [28]. This relatively low polarity of keratin is further underlined by its close association with lipids *in vivo* [4]. It has also been reported on a rapid increase in water uptake at low and high RH, but with a slower uptake in the intermediate range [24].

The absorption and desorption time constants from heel SC differed substantially at RH <50% as seen in figure 5. This regime is likely to be the strongly bound water, and thus it seems reasonably clear that this type of water entered the SC much more rapidly compared to its corresponding exit. The underlying mechanism for this peculiar difference is not clear other than that bound water prefers to remain bound. However, the increasing time constant with decreasing RH was as expected since the diffusion coefficient usually decreases with decreasing water content [15, 16, 18]. Thus, free and more mobile water will evaporate first and eventually bound water, being more strongly attracted, will desorb.

The peculiar effect of time between preparation of the SC samples and the onset of the DVS measurements, present mainly for RH between 40 and 70%, may be due to structural changes in the SC when being stored *ex vivo* over time. With time one can expect the extracellular lipid matrix as well as the keratin filament matrix of the corneocytes to suffer from irreversible distortions, such as sorting of lipids in the lipid matrix with crystalline domain building [29], or irreversible glass transition of the keratin filament matrix as consequences. This effect did

not seem to have much influence on the total water uptake, comparing the time constants in figures 7 and 8 with the relative water gains during absorption and desorption in figures 9 and 10, although the water holding capacities seemed to decrease slightly with time. The statistical material in this manner is very limited with only one test sample investigated, and more effort is needed to reveal a more complete picture.

An improved understanding of the characteristics and location of the 3 different water phases in the SC will be of importance regarding clinical treatments and knowledge of the human skin, and it is therefore to be a topic for future studies.

Conclusion

In this study we did not find signs of significant hysteresis for human SC, although there were small spreads in mass from absorption and desorption data. Any hysteresis of SC is important and must be accounted for in calibrations of susceptance-based hydration measurements, which are robust methods for determining the hydration state of human SC.

We found the time constants to be triphasic during absorption, possibly reflecting the 3 different types of water in the SC, and increasing with decreasing RH during desorption. The latter may be interpreted as a protective property, providing the skin with sufficient moisture in order to retain all its protective functions, whereas the former is connected to the weak polarity of SC keratin filaments. Knowledge of these types of human SC water content and their sorption characteristics is vital for a structure-function-based knowledge of SC properties.

References

- 1 Kessner D, Ruettinger A, Kiselev MA, Wartewig S, Neubert RHH: Properties of ceramides and their impact on the stratum corneum structure: a review. *Skin Pharmacol Physiol* 2007;21:58–74.
- 2 Blank IH: Factors which influence the water content of the stratum corneum. *J Invest Dermatol* 1952;18:433–440.
- 3 Blank IH: Further observations on factors which influence the water content of the stratum corneum. *J Invest Dermatol* 1953;21: 259–271.
- 4 Norlen L, Al-Amoudi A: Stratum corneum keratin structure, function, and formation: the cubic rod-packing and membrane templating model. *J Invest Dermatol* 2004;123: 715–732.

- 5 Houben E, Hachem JP, Paepe K De, Rogiers V: Epidermal ceramidase activity regulates epidermal desquamation via stratum corneum acidification. *Skin Pharmacol Physiol* 2008;21:111–118.
- 6 Blichmann CW, Serup J: Hydration studies on scaly hand eczema. *Contact Dermatitis* 1987;16:155–159.
- 7 Fartasch M: Atopic dermatitis and other skin diseases; in Fluhr J, Elsner P, Berardesca E, Maibach HI (eds): *Bioengineering of the skin: water and the stratum corneum*. London, CRC Press, 2005, pp 159–170.
- 8 Martinsen ØG, Grimnes S, Kirkhus T, Mørkrid L, Aas K: Increased moisture content in children's atopic skin. *Eur J Pediatr Dermatol* 2007;17:17–20.
- 9 The influence of a non-occlusive bi-layer composite membrane on skin barrier properties. *Skin Pharmacol Physiol* 2008;21:50–55.
- 10 Martinsen ØG, Grimnes S, Nilsen JK, Tronstad C, Yang W, Kim H, Shin K, Naderi M, Thielmann F: Gravimetric method for in vitro calibration of skin hydration measurements. *IEEE Trans Biomed Eng* 2007;55:728–732.
- 11 Kilpatrick-Liverman L, Polefka TG: Use of dynamic vapour sorption meter to measure skin hydration properties in vitro. *Skin Res Technol* 2006;12:36–42.
- 12 Johnsen G, Martinsen ØG, Grimnes S: A preliminary investigation of the sorption properties of the stratum corneum in vivo. *Proc IFMBE* 2009;25:597–599.
- 13 Kasting GB, Barai ND, Wang TF, Nitsche JM: Mobility of water in human stratum corneum. *J Pharm Sci* 2003;92:2326–2340.
- 14 Liron Z, Clewell HJ, McDougal JN: Kinetics of water vapor sorption in porcine stratum corneum. *J Pharm Sci* 1994;83:692–698.
- 15 Crank J, Park GS: *Methods of measurement; in Crank J, Park GS (eds): Diffusion in Polymers*. London, Academic Press, 1968, pp 1–39.
- 16 Fujita H: Organic vapors above the glass transition temperature; in Crank J, Park GS (eds): *Diffusion in Polymers*. London, Academic Press, 1968, pp 75–105.
- 17 Kasting GB, Barai ND: Equilibrium water sorption in human stratum corneum. *J Pharm Sci* 2003;92:1624–1631.
- 18 Anderson RL, Cassidy JM, Hansen JR, Yellin W: Hydration of stratum corneum. *Biopolymers* 1973;12:2789–2802.
- 19 King G: Permeability of keratin membranes to water vapor. *Trans Faraday Soc* 1945;41:479–487.
- 20 Hey MJ, Taylor DJ, Derbyshire W: Water sorption by human callus. *Biochim Biophys Acta* 1978;540:518–533.
- 21 El-Shimi AF, Princen HM: Diffusion characteristics of water vapor in some keratins. *Coll Polym Sci* 1978;256:209–217.
- 22 Potts RO: Stratum corneum hydration: experimental techniques and interpretations of results. *J Cosmet Chem* 1986;37:9–33.
- 23 Pieper J, Charalambopoulou G, Steriotis T, Vasenkov S, Desmedt A, Lechner RE: Water diffusion in fully hydrated porcine stratum corneum. *Chem Phys* 2003;292:465–476.
- 24 Leveque JL: Water-keratin interactions; in Fluhr J, Elsner P, Berardesca E, Maibach HI (eds): *Bioengineering of the Skin: Water and the Stratum Corneum*. London, CRC Press, 2005, pp 15–26.
- 25 Silva CL, Topgaard D, Kocherbitov V, Sousa JJS, Pais AACC, Sparr E: Stratum corneum hydration: phase transformations and mobility in stratum corneum, extracted lipids and isolated corneocytes. *Biochim Biophys Acta* 2007;1768:2647–2659.
- 26 Inoue T, Tsujii K, Okamoto K, Toda K: Differential scanning calorimetric studies on the melting behavior of water in the stratum corneum. *J Invest Dermatol* 1986;86:689–693.
- 27 Rouse PE: Diffusion of vapors in films. *J Am Chem Soc* 1947;69:1068–1073.
- 28 Martys JL, Ho CL, Liem RKH, Gundersen GG: Intermediate filaments in motion: observation of intermediate filaments in cells using green fluorescent protein-vitamin. *Mol Biol Cell* 1999;10:1289–1295.
- 29 Norlen L: Skin barrier structure and function: the single gel phase model. *J Invest Dermatol* 2001;117:830–836.