Gravimetric Method for *in Vitro* Calibration of Skin Hydration Measurements

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Abstract—A novel method for *in vitro* calibration of skin hydration measurements is presented. The method combines gravimetric and electrical measurements and reveals an exponential dependency of measured electrical susceptance to absolute water content in the epidermal stratum corneum. The results also show that absorption of water into the stratum corneum exhibits three different phases with significant differences in absorption time constant. These phases probably correspond to bound, loosely bound, and bulk water.

Index Terms—Bioelectric phenomena, epidermis, stratum corneum, water.

I. INTRODUCTION

WATER content is the single most important parameter controlling the vital function of the epidermal stratum corneum (SC) [1], [2]. Electrical methods for measuring skin hydration have been studied for several decades and a low-frequency susceptance method has proved to be the most appropriate [3], [4]. Higher frequencies will in general focus the measurements on deeper skin layers, and the conductance of the SC is highly influenced by sweat duct filling and, hence, is a source of error in the measurements [5]–[7]. The relative contribution from these error sources has been discussed in an earlier paper [8]. So far, only relative hydration measurements have been reported with this method. The method is, however, immune to errors like sweat duct filling, viable skin layers, and temperature changes, and as it facilitates focused measurements on the SC, it is possible to calibrate against absolute water content.

In this paper, we report on a novel *in vitro* gravimetric method for finding the relationship between absolute water content in the SC and measured electrical susceptance. The basic idea of

TABLE I
SALTS USED FOR PRODUCING CONSTANT RH

Salt	% RH
Potassium carbonate	43.2
Sodium bromide	57.6
Sodium chloride	75.3
Potassium chloride	84.3

the method is to use closed chambers where the relative humidity (RH) and temperature can be controlled. Two different experiments were performed. One setup measures the weight changes in the skin samples as a function of RH and the other setup measures the electrical properties of the samples as a function of RH. The results from these experiments can then be combined to yield, for instance, the electrical susceptance of the samples as a function of the weight. Knowing the dry weight of the samples, which is the weight at 0% RH, enables calculation of absolute water content. Plantar stratum corneum is used in this particular study, but the principle will be the same also for studies on samples from other skin sites.

II. MATERIALS AND METHODS

An Aesculap Acculan dermatome was used to cut pieces of SC from the heels of five healthy, male volunteers within our research group (age range 27–67 years). The skin was mildly abraded with fine sand paper before the dermatome was used. The pieces were about 2 by 2 cm and with a thickness of 0.2 mm. One sample was taken from each heel of all persons.

The water sorption properties of one sample from each person were measured with a dynamic vapor sorption (DVS) instrument from Surface Measurement Systems Ltd., U.K. The samples were automatically weighed in a closed chamber as a function of ambient RH. Measurements were performed in the range 0–95% RH at 25 °C to obtain high resolution isotherms which were used to study the characteristics of absorption, desorption and diffusion of water molecules in skin.

The other SC sample from each person was used for electrical measurements. These measurements were done at 25 °C in a closed chamber where the RH was controlled by means of saturated salt solutions (see Table I).

Lower values of RH than those shown in Table I resulted in hard and brittle skin samples, from which it was impossible to achieve stable and reproducible measurements. Since this state represents a non-physiological condition for the skin, values below those shown in Table I were not used.

We used Ag/AgCl buttons from Quickels QN 600 ECG electrodes in a two-electrode setup. The Ag/AgCl electrodes
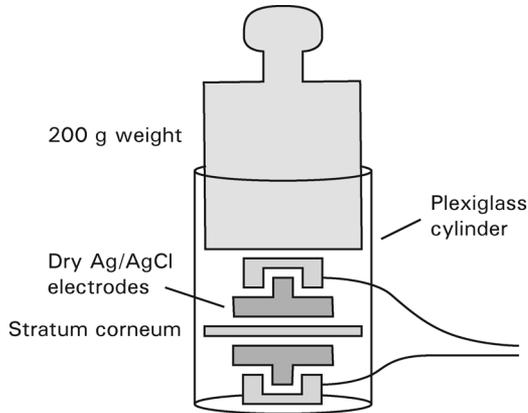
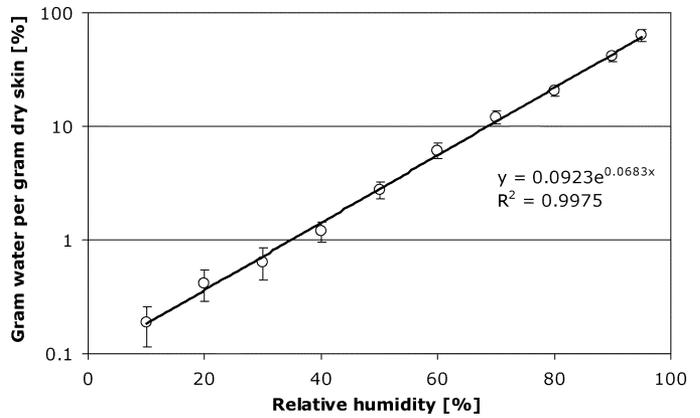Fig. 1. Electrode arrangement. Connectors for the two dry Ag/AgCl electrodes have insulating plastic backing.

Fig. 2. Steps in chamber RH (left curve) and resulting change in skin sample mass (right curve).

Fig. 3. Semi-log plot of water content of skin samples as a function of RH. Mean and standard deviation.

Fig. 4. Semi-log plot of 88 Hz electrical susceptance of skin samples as a function of RH. Mean and standard deviation.

were used to avoid influence from electrode polarization [9]. The electrode area was 1 cm^2, and connections were made by placing the skin samples vertically between the two electrodes and placing a 200 g weight on top. This electrode setup, shown in Fig. 1, was placed inside a sealed glass chamber with the saturated salt solution to ensure constant RH. We then used a Solartron 1260/1294 system to measure the 88 Hz electrical susceptance. At each level of RH, the skin samples were allowed to stabilize for 24 hours inside the glass chamber before electrical measurements first were carried out and the RH then was increased again.

III. RESULTS

Fig. 2 shows the results from the DVS absorption measurements on one of the skin samples. This particular run was performed with RH steps of 20% for the sake of clarity in the figure, while all other measurements were done with RH steps of 10%. The left curve shows the steps in the RH inside the chamber, and the right curve shows the resulting increase in weight of the skin sample (which will be equal to the percentage of the weight of the water in the skin sample relative to the weight of the dry skin).

It is obvious from Fig. 2 that the absorption process was not fully completed at each step in RH before the next step was introduced. Due to lack of re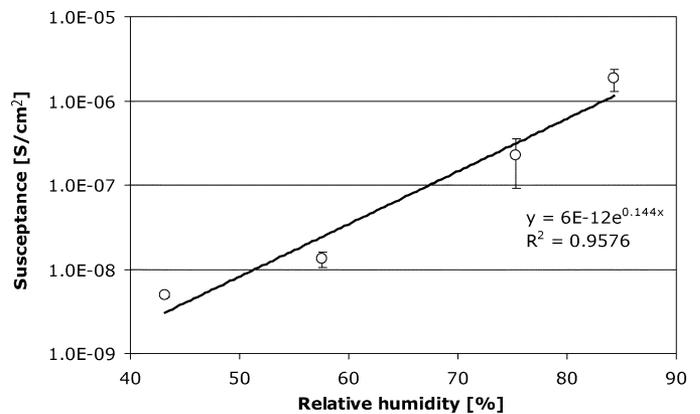sources, we were unable to rerun the samples with higher time delays at each RH, and therefore chose to perform numerical extrapolation to the data instead. The change in skin sample weight following a step in RH turned out to closely follow an exponential time course, which is also in accordance with the solution of Fick's second law of diffusion [10], [11]. The time course was hence modeled for all the skin samples according to the equation

$$y \approx a - b \cdot \exp\left(-c \cdot x\right) \quad (1)$$

where y is the change in mass, x is time, and a, b, and c are constants. The constant a will then be the calculated stable value for the water content at each absorption process and the time constant for the process will be c^{-1}. All regressions produced precise fits to the measured data, yielding results with standard error (SE) of less than 0.5% of the calculated value for the water content a.

Fig. 3 shows the mean and standard deviation (SD) of the calculated water content for all five skin samples and at each level of RH. The results show an almost perfect exponential dependency of water content to RH, with an R^2 of 0.9975.

The results from the electrical measurements are shown in Fig. 4 as mean and SD of the 88 Hz electrical susceptance of the five skin samples as a function of RH. The relationship is exponential with an R^2 of 0.9576.
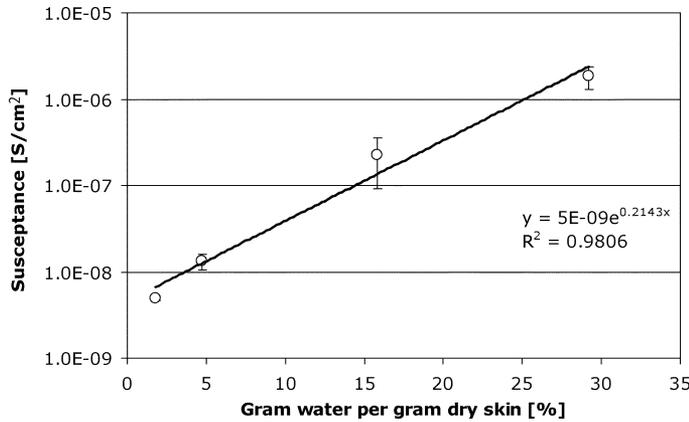
Fig. 5. Semi-log plot of 88 Hz electrical susceptance of skin samples as a function of absolute water content. Mean and standard deviation.
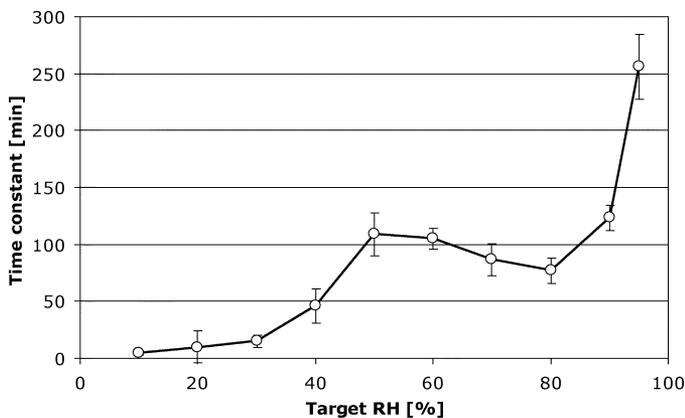

Fig. 6. Time constant for the absorption process as a function of target RH. Mean and standard deviation.

The results shown in Figs. 3 and 4 are then combined to give Fig. 5, where the 88 Hz electrical susceptance is plotted as a function of absolute water content. The relationship is exponential with an R^2 of 0.9806.

It is furthermore interesting to note that the time constant for the absorption process varies as a function of hydration level. This relationship is plotted in Fig. 6. The time constant increases with increasing target RH up to about 50%, then decreases up to about 80% and finally increases again above 80%.

IV. DISCUSSION

Absorption of water was found to be log-linear over the RH range studied, which is in agreement with earlier reports [12]. When the water is absorbed in the SC, it is usually considered to occur in three different phases [12], [13]. The first phase typically covers RH values up to about 30%, or 6% absorbed water [13]. In this phase, the water molecules are bound to the polar headgroups of the lipid bilayers until monolayer coverage is reached. The second water phase involves more loosely bound water molecules and goes up to an RH value of about 60% or an additional 6% adsorbed water [13]. Water adsorbed above this level is generally considered to exhibit properties close to those of bulk water and the SC can retain a large amount of water in this phase. Hence, Anderson *et al.* found that occlusion-treated skin samples were able to absorb more water than

non-treated skin [14]. The limits given above are not precise and may vary between skin types and also between porcine and human skin. Kasting and Barai [12] report, for instance, that water adsorbed up to a relative humidity of 90% may be considered bound water. This corresponds to a hydration level of about 30%–50%.

The electrical susceptance fits reasonably well to an exponential function of both RH and absolute water content, which is in agreement with earlier findings [4], [15]. However, there are only measurements at four different values of RH and the RH range spans two of the above-mentioned ranges for water phases. Hence, a more extensive study should be carried out in the future to investigate this dependency in more detail. This study should also focus more on the free-water hydration range since this is the most physiologically relevant range for the SC [1].

The time constant presented in Fig. 6 shows triphasic behavior. This is consistent with the theory of three different water phases, and hence sorption mechanisms, in the SC. It is also in agreement with Lévêque [16] who reports that the increase in water content is very rapid at very low and high values of RH and with slower uptake at mid-range values. As shown in Fig. 6, the absorption rate increases with increased target RH up to about 50% RH, then decreases again when going up to 80% RH and then finally increases again. The time constant ranges from 5 to 256 minutes. This means, for instance, that one has to wait for 17 hours to reach 98% of the final weight of the SC when going from 90% to 95% RH.

The method described in this paper can be used for calibration of skin hydration measurements, but a larger study should be carried out to provide the statistical data necessary for the calibration. Some of the issues that should also be addressed in a future study are the following.

- Kilpatrick–Liverman and Polefka [17] report on hysteresis between absorption and desorption in SC. How does that influence the calibration?
- SC samples *in vitro* will be uniformly hydrated, whereas SC *in vivo* will have a hydration gradient. What will be the correspondence between *in vivo* and *in vitro* measurements?
- The measurements were performed on 0.2-mm-thick plantar SC. Earlier studies indicate that the results on non-palmar or non-plantar SC will be qualitatively, but not necessarily quantitatively, similar. There is also evidence for departure from the simple log-linear absorption behavior for non-palmar/plantar skin at high values of RH [12].
- Temperature dependency is an issue, although the literature is contradictory on this point [12].

V. CONCLUSION

A novel method for calibration of skin electrical measurements for the assessment of hydration level has been presented, and the results show that calibration is feasible with this technique. The method may also be used in other studies involving water sorption dynamics and physical properties of skin or other materials.

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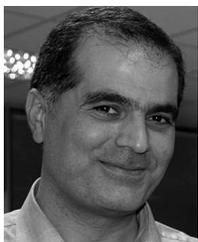
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