Direct measurement of moisture in skin by NIR spectroscopy

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Synopsis

A method to directly determine water content in skin as well as the nature of water binding using near-infrared reflectance spectroscopy is introduced. The method is able to quantitate water on a relative basis and to distinguish free, bulk, and bound water. In addition, scattering of near-infrared radiation off the skin surface can be used as a measure of skin smoothness. As examples of the method's potential, effects of both humidity and moisturizer application on moisture content, water type, and scattering effects have been studied on dry legs.

INTRODUCTION

According to Kligman (1), skin characterized as dry is not necessarily lacking in moisture but is more often considered to have a rough, uneven surface that scatters light efficiently, leading to a dry, matte appearance. Normal dry skin can be caused by climate, cleansing, age, or heredity. The function of a moisturizing product is to retard water loss by creating a barrier to surface evaporation, to create a smoother, softer feel to the skin, and to improve the appearance of the skin.

The outermost layer of skin is the stratum corneum (SC), about 10–20 microns thick and composed of partially dehydrated cells in a lipid matrix. It is this layer that is considered to be responsible for the barrier function of skin. Below the SC is the epidermis, about 100–200 microns thick, and below that the dermis, about 2–4 mm thick. Skin becomes increasingly more hydrated at the deeper layers.

Obata and Tagami indicate that the main role of water in the stratum corneum is to control softness and pliability (2). Several studies on the effect of humidity on both the strength and number of water-binding sites in the SC have been performed and are summarized by Potts (3). Generally, increasing relative humidity causes an increase in tissue hydration, the rate of hydration being greater at higher relative humidity. The nature of water in the SC was concluded to be different at different relative humidities. Using NMR and IR, Hansen and Yellin (4) determined that at a water content of below 10% the water present was tightly bound, presumably to the polar sites of the proteins. At water contents between 10% and 40%, they found less tightly bound water, which

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Purchased for the exclusive use of nofirst nolast (unknown) From: SCC Media Library & Resource Center (library.scconline.org) they suggested was hydrogen-bonded to the protein-bound water. Above 50% water content, the water resembled the bulk liquid.

The efficacy of skin moisturizers *in vivo* is usually determined by indirect measurements of hydration. Tests include high frequency electrical conductivity, TEWL (transepidermal water loss), biomechanical measurements, and subjective clinical evaluations. All suffer from low precision and no well-understood relationship to water content.

Spectroscopic measurements, on the other hand, are related to water content in a straightforward manner through a classical Beer's Law type relationship via absorption of the hydroxyl moieties. The absorbance of a sample is proportional to both concentration and the path length that light travels. In the mid-infrared, ATR (attenuated total reflectance) has been used to measure water content of skin *in vivo*. The ratio of the amide I to the amide II band of skin protein has been used as a measure of water content by exploiting the overlap of the water-bending mode with the amide I band (5). This method assumes that neither the amide I nor amide II band is affected by water content, an unproven assumption. Potts *et al.* (6) have used the 2100 cm⁻¹ combination band of water, which has the advantage of being far removed from bands due to skin or products. ATR measurements, however, require occlusion of the skin, which can affect water content. In addition, the degree of contact between the skin and the internal reflectance element is not constant, particularly after skin is treated with a lotion, and the refractive index of skin may change upon hydration, thus affecting the depth of penetration.

In this paper, near-infrared reflectance (NIR) spectroscopy is used to determine skin water content *in vivo*. NIR has several advantages over mid-infrared spectroscopy. The near-infrared region is more sensitive to hydrogen-bonding differences and thus can distinguish different types of water; scattering effects can be used to determine changes in the character of the skin surface, and non-occlusive measurements can be made.

The possibility of using NIR for this purpose was previously demonstrated in our laboratory by Walling and Dabney (7). In those experiments, a 10-foot fiber optic cable was coupled to the spectrometer to collect spectra on the skin of legs treated with moisturizer. In situ experiments on pigskin distinguished free and bound water, although *in vivo* experiments did not. This paper presents *in vivo* results determined on skin without a fiber optic cable. The improved resolution allows several types of water, including free, bulk, and protein-bound, to be distinguished.

EXPERIMENTAL

Dry leg studies were conducted on 16–22 female subjects in successive clinical trials. Four to five sites on the outer lower legs, each about 2 inches square, were used. An untreated control site was included and used to study the effects of ambient humidity on skin water content. The effects of three formulations used in a single trial were compared by NIR. These formulations were 1) a plasticizer (the exact nature of which is proprietary) in an aqueous gel base containing hydroxyethyl cellulose and preservatives, 2) propylene glycol (a penetration enhancer) in the same gel base, and 3) a non-ionic oil-in-water emulsion containing 22% oil phase and a water phase that includes the plasticizer, dipropylene glycol, glycerol, and emulsifiers. Product applications were made twice a day for two to four weeks, followed by a regression period of no treatment. NIR reflectance measurements were made prior to treatment and at least once a week for the duration of the study. Ambient temperature and relative humidity were not closely controlled but were monitored over the measurement period.

Reflectance measurements were made with a Quantum 1200 analyzer (LT Industries) in the region of 1200–2400 nm, using 30 scans per spectrum. The subjects' legs were placed directly against the detector port, with a foam cushion around the rim of the port to block stray light. The spectrometer was placed on a low stool, and subjects could easily reach the detector port from a sitting position. Spectra were collected at least eight hours following the most recent application to ensure that no residual product was present.

DATA TREATMENT

The spectra were converted to apparent absorbance (log 1/R, where R is reflectance) and averaged over all the subjects for each product at each time point. Averaging the data in this manner minimizes the effect of individual variation by limiting the influence of outliers. The averaged spectra within a study were subjected to a multiplicative scatter correction (MSC) to correct for the changes in apparent absorption due to scattering. MSC was first proposed by Geladi *et al.* for determinations of meat composition (8) and has since been used in a variety of NIR applications (9). MSC corrects each spectrum to an "ideal" spectrum, in this case an average spectrum, and determines both additive and multiplicative scattering effects. MSC assumes a constant scattering coefficient over the wavelength interval used and only minor changes in concentration. Over the wavelength interval used here, 1400–2200 nm, the scattering coefficient of skin has been shown to be fairly constant (10,11), and the variation in spectral intensity is small. From the MSC-corrected data, concentration changes can be determined, and from the difference in the original and MSC-corrected spectra, changes in scattering can be determined.

Relative concentrations were determined from second derivative intensities. Differentiation is a common means of resolution enhancement, and because it is a linear operation, the resulting spectral intensities follow Beer's Law if it held for the original spectrum (12).

RESULTS

BAND ASSIGNMENTS

The log 1/R spectra of skin and water are compared in Figure 1. The major features in both are the bands near 1450 and 1920 nm, corresponding to the first overtone of the OH stretch in water and the combination mode of the OH stretch and HOH bend in water, respectively. Skin also shows weak features near 1730 and 1750 nm due to lipids. The features in the water combination region are enhanced using second derivatives, as shown for both water and skin in Figure 2.

Water shows a major band at 1892 nm and weaker bands at 1906 and 1924 nm. Longer wavelengths generally indicate greater hydrogen bonding. According to Luck, these wavelengths correspond to unbonded (free) water and water with one and two hydrogen bonds, respectively (13). The same three bands appear in skin, with different relative



Figure 1. Apparent absorbance spectra of skin and water.

intensities, plus a stronger band at 1879 nm. These wavelengths suggest slightly different assignments from those of Luck. The band at 1879 nm is due to weakly bonded water (free water), and may be the water giving rise to the evaporative flux across the SC. The 1890-nm band of skin corresponds to the strongest band of liquid water and will be referred to as bulk water, while the longer wavelength bands at 1909 and 1927 nm are due to more strongly bound water, which may be that associated with protein. The free water and protein-bound water are expected to be found within the stratum corneum, while bulk water, and possibly some protein-bound water, are expected to be found in the epidermal layer just below the SC. These assignments are summarized in Table I.

EFFECT OF HUMIDITY ON SKIN WATER CONTENT

Figure 3 compares the second derivative spectra of untreated skin at two humidities. At an absolute humidity of 3.7 g/m^3 (corresponding to 19% relative humidity at 22°C), the water bands near 1879, 1909, and 1927 nm shift to longer wavelength (stronger hydrogen bonding) compared to the spectrum at 8.2 g/m³ (42% RH at 22°C) and become less intense, while the band at 1890 nm (bulk water) does not shift but becomes more intense. At lower humidity, therefore, the free and protein-bound water molecules are somewhat more strongly hydrogen-bonded.

Figure 4 shows the correlation between absolute humidity and the second derivative intensities of free water (1879 nm) from the untreated control sites using data pooled from five separate studies. The absolute humidity in these studies is limited to the range corresponding to 3%-50% RH at 22°C. The increase in intensity at 1879 nm with



Figure 2. Second derivative spectra of skin and water in the water combination region.

increasing humidity indicates a greater concentration of free water and supports the assignment that this is water that is readily transported across the skin barrier and thus gives rise to the flux across the SC. In his review on measurements of water in skin, Potts noted that the SC thickens with hydration, providing a more substantial barrier to water loss (3). The maximum flux found in those studies, as measured by TEWL, was found at 30%-40% relative humidity (6.9-9.2 g/m³) at 25°C. Figure 4 seems to correlate with this observation.

The increases in more tightly bound water (1909 and 1927 nm) are shown in Figures 5 and 6. These parallel the increase observed for free water, suggesting that there may be free exchange between the two types of water.

Bulk water (1890 nm), on the other hand, decreases with increasing humidity, as shown in Figure 7. Although the data for water at 1879, 1909, and 1927 nm were pooled from five studies without discontinuity, differences between the five clinical studies are apparent in the data at 1890 nm. The reason for the discontinuity may be related to minor changes in the thickness of the cushion between the detector port and the

Assignments in Water Combination Region of Skin	
nm	Assignment
1879	Free water
1890	Bulk water
1909	Protein-bound water
1927	Protein-bound water

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Figure 3. Effect of humidity on the second derivative spectra of skin.

subjects' legs, leading to differences in the depth of penetration of near-infrared radiation. Within each study, however, the negative correlation with humidity holds. Bulk water should be that which is located below the stratum corneum and is expected to be constant. The apparent decrease can only be attributable to another phenomenon such as decreased penetration of radiation below the SC. Recall that Potts has observed a thickening of the SC with humidity (3). The longer resulting path length for NIR radiation through the SC could leave a shorter path length below the SC. Thus, the SC appears to plump out as the humidity increases. The total path length of radiation, however, does not appear to change significantly.

EFFECT OF PRODUCT APPLICATION ON SKIN WATER CONTENT

After treatment with all three product types, skin shows a noticeable increase in apparent absorbance throughout the wavelength range of 1400–2200 nm, accompanied by an increase in the baseline offset (Figure 8). The effect is most likely due to decreased scattering of NIR radiation, possibly accompanied by an increase in water content. Subjective evaluations under visible light indicate a glossier appearance for treated skin, suggesting that visible light, at least, is less scattered. The MSC correction to the spectra was used to eliminate the apparent increase in absorbance due to scattering and to leave only effects due to changes in water content.

Because of the strong effects of humidity on water band intensities, relative water concentration changes produced by different products were compared at single time points (constant humidity). For example, Figure 9 shows relative concentration changes for the three formulations at the four water wavelengths after two weeks of product



Figure 4. Correlation of free water (1879 nm) and absolute humidity.

application. The data were collected at 35% RH at 23°C and were corrected by subtracting the control and baseline measurements. The results are thus relative to the baseline measurements that were collected at 30% RH.

The plasticizer ingredient in the simple base (A) and in the more complex formulation (C) induce similar effects; the levels of free (1879 nm) and protein-bound water (1909 and 1927 nm) decline while that of bulk water (1890 nm) increases. Differences in the net water band intensities between these two formulations are not significant. Propylene glycol (B), which is not expected to act as a moisturizer, shows decreases in free water and protein-bound water comparable to those of the other two formulations, but also results in a decrease in bulk water. The decrease in free water (1879 nm) with all three products occurs in spite of the small increase in humidity relative to the baseline measurements. It may arise from increased flux across the skin surface, which would result in less free water trapped within the SC, or from an overall decrease in SC thickness. The protein-bound water follows the behavior of the free water, as it does when the humidity changes. The increase in bulk water (1890 nm) with the plasticizer may signal an increase in the depth of penetration of reflected radiation into the epidermis as the SC becomes thinner. Product application, therefore, appears to have the opposite effect on stratum corneum thickness than does increasing humidity.

EFFECT OF PRODUCT APPLICATION ON SCATTERING

All three formulations result in decreased scattering after product application, observed as an increase in apparent absorbance and calculated as the difference between the



Figure 5. Correlation of bound water (1909 nm) and absolute humidity.

original and MSC-corrected spectra (Figure 10). The relative change in scattering among the three formulations is independent of the wavelength used, and C > A > B, where B, the penetration enhancer, shows the least decrease in scattering compared to the untreated site. The two formulations containing the plasticizer, A and C, cause a greater decrease in scattering and are comparable to each other.

DISCUSSION

The validity of using NIR reflectance data for assessing skin condition depends upon both the depth from which the measured radiation is coming and the degree of change in this depth upon product application. Ideally, in evaluating moisturizing products, the depth of penetration of near-infrared radiation should be limited to the stratum corneum. Increased depth of penetration could result in a higher apparent concentration of water, especially that which is termed here as bulk, due to the greater degree of hydration of deeper tissue.

The depth of penetration of radiation into the skin depends on several factors. When NIR radiation impinges on skin, it undergoes regular (specular or mirror-like) reflectance, absorption, internal scattering, and diffuse reflectance. The degree to which each occurs depends on the structure, the refractive index, the absorptivity, and the scattering coefficient.

Figure 11 illustrates some possible pathways for light reflected off skin. Anderson *et al.* (11) suggest that at the 1950 nm combination band of water, specular reflectance from



Figure 7. Correlation of bulk water (1890 nm) and absolute humidity.

the epidermis dominates, with only 5%-7% diffuse reflectance. Light that does not penetrate into the SC can undergo single scattering or multiple internal scattering. The stratified nature of the stratum corneum, with its layers of flattened cells, provides several opportunities for internal scattering. Some light may also be completely absorbed



nm

Figure 8. Apparent absorbance spectra before and after product application.



Figure 9. Net concentration changes after product application.

into the deeper tissues. According to Scheuplein (14), IR radiation at the water absorption bands that is not absorbed or scattered by the SC will be absorbed by the first few viable cell layers of the epidermis. Radiation that penetrates deeper into the skin is less likely to be returned to the surface and thus will not be measured.







Figure 11. Possible pathways for NIR radiation in the skin: a) specular reflectance; b) diffuse reflectance; c) absorption; d) multiple internal scattering.

Changes in the depth of penetration of radiation depend on changes in the refractive index or in skin surface morphology. The refractive index of the stratum corneum is about 1.55 at the sodium D line (14), but has been found to decrease upon hydration

Purchased for the exclusive use of nofirst nolast (unknown) From: SCC Media Library & Resource Center (library.scconline.org) (15,16). A lowered refractive index should result in decreased scatter and, consequently, deeper penetration of radiation and higher apparent absorbance. Humidity-induced hydration, however, does not appear to cause a significant increase in the path length of near-infrared radiation into the skin. Under high humidity, the increase in free water content apparently gives rise to swelling of the SC, while the decrease in bulk water suggests that less radiation consequently penetrates below the SC. Thus, there is no major change apparent in the net depth of penetration of radiation or significant change in the refractive index in this region with hydration.

Therefore, the humidity results provide evidence that NIR reflectance is a superficial measurement at 1879 nm since free water is presumed to be restricted to the SC. On the other hand, the presence of bulk water suggests that some portion of the reflected radiation has penetrated into the epidermis. The depth into the epidermis is likely to be small; otherwise the bulk water absorbance would overwhelm the spectrum.

In contrast to the humidity effects, certain products can affect scattering properties by smoothing the skin surface. Normal skin has been shown to have fewer and shallower crevices and an absence of flaky, uplifted scales compared to dry skin (17,18). The formation of a smoother surface should result in less scattering of radiation, as noted for the three formulations in Figure 10. Because all three show decreased scatter without increased free water, the mechanism by which skin is made smoother may not necessarily be hydration.

In fact, it appears that no hydration *per se* occurs with product application in the cases examined here. The decrease in free water combined with the increase in bulk water suggests that the SC is becoming thinner, opposite to the plumping effect observed with increasing humidity. The thinning may be due to compacting of the tissue or to removal of the driest, outer portion of the SC. Thus, maintenance of at least moderate humidities and application of moisturizers may both be beneficial to skin, but through different mechanisms. Increased humidity hydrates the skin, while moisturizers of the type applied in this study appear to smooth skin.

NIR reflectance thus appears to show potential as a method for evaluating both the smoothness and water content of skin. Further work to validate the treatment data with regard to separating the effects of scattering and concentration needs to be done. NIR may also offer a means for measuring SC thickness on free water/bulk water intensities.

SUMMARY

We have introduced NIR reflectance as a method to directly determine changes in free, bulk, and protein-bound water and to assess scattering effects in skin in the evaluation of skin care products. In addition, the effect of humidity on different types of water in skin can be examined. From our initial studies, it appears that increasing ambient humidity (up to 50% RH) is associated with increased levels of free water in the skin, while the products we used act primarily to smooth the skin surface, observed as a decrease in the scattering of radiation. Our results further indicate that although the water we see is primarily in the stratum corneum, some of the reflected radiation comes from the epidermis.

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