NIR for the study of human skin

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Introduction

Background

Near infrared (NIR) spectroscopy is still a developing analytical technique. The growth of its application is significant. In addition to industrial applications developed in the late 1980s, this technique has also been used in medical and cosmetic studies.[1,2] However, compared to other techniques the use of NIR applications in cosmetics is still quite limited, although the non-invasiveness, cost-effectiveness and simplicity of measurement of NIR make it a potentially attractive method for *in-vivo* skin studies.

An NIR project was initiated to investigate the possibility of visualising the skin moisturisation process and related influential factors for *in-vivo* skin measurement. In a previous publication,[3] we reported the penetration depth of NIR irradiation in human skin *in-vitro* to be 1.3 mm. The observed relationship of human emotions, gender, age and smoking habit with skin moisture levels has been reported as well.

In this paper, we continue the discussion on the feasibility of using NIR to monitor human skin water content variations *in-vivo* and explore the mechanisms of skin moisturisation. Finally, a skin moisturisation model is proposed.

Wavelength selection

On average, the total thickness of human skin is about 1–4 mm, comprising of four layers: the stratum corneum (10–20 μm), the viable epidermis (50–100 μm), the dermis (1–2 mm) and the hypodermis (1–2 mm).[4] It is very important to understand the presence of different types of water in the skin and its distribution at each layer. Near Infrared spectrum wavelengths around 1900 nm region was chosen for the determination of water as by using the second derivative spectrum, the free water and the protein-bound water band can be separated approx. 15–20 nm apart. Using the –OH overtone band at around 1400 nm will not serve the purpose of this study because at this wavelength the penetration depth of NIR incident light will be much more than the 1.3 mm we measured in the 1900 nm region during our previous studies.[3] The proportion of dermal water in the 1400 nm region signal will be even greater than that measured in the 1900 nm region.

On the other hand, the NIR light irradiation will be stronger and the noise level will be lower at the shorter wavelength region (~1400 nm) and as a consequence, the S/N ratio will be higher. However, by using this overtone region, we will lose important information on the amount of water in each layer, the types of water, for example, free water and protein-bound water, due to the penetration depth and resolution limit. The focus of this study is to visualise different types of water absorption bands and to quantify the band intensities in each layer of skin. As long as the S/N ratio is high enough to recognise the real signal, it satisfies the purpose. Moreover, only for a quantitative analysis a high S/N is critical. In this case, without a proper reference method that can give an accurate amount of free and protein-bound water in each layer of skin, the use of chemometrics to decompose the information of the data from the shorter wavelength region does not allow

differentiation of the various types of water and their relative amounts at different layers of the skin. Therefore, the combination band region of –OH absorption in NIR spectra around 1900 nm was preferred.

Skin spectra and band assignment

Our previous publication[3] showed an original NIR spectrum of human *in-vivo* forearm skin and the second derivative of the same spectrum in the water band absorption region around 1900 nm. The different types of water can be clearly seen. The patterns are the same as those measured by Martin,[2,5] despite the fact that the two studies used different NIR instruments, different software and different panellists. Martin[2] summarised NIR absorption band assignments in her early publications, based on IR, NMR and *in-vitro* pigskin studies. We have adopted the band assignments from Martin and other pioneers.

Experimental

Instruments and software

An NIRSystem 6500 module coupled with a regular bundle fibre (1 metre) optical trans-reflectance probe was used for this study. Both the Vision software version 2.21 supplied with the NIRSystem instrument and Chemometrics software Unscrambler Version 7.8 were used for data interpretation and data treatment (Norris second derivative with segment size 3).

Both a Corneometer (CM 820, Courage and Khazaka, Cologne, Germany) and a Tewameter (Courage and Khazaka) were used in this study to measure the capacitance of human skin (a measure for water content as this has a dielectric constant significantly higher than any other ingredient in human skin) as well as its barrier function, respectively. The Tewameter calculates the time taken for the amount of moisture at the top and bottom of a funnel-shaped probe to reach equilibrium, in this way giving an accurate assessment of the rate of evaporation of moisture from the epidermis.

Subjects

For the *in-vivo* repeatability and reproducibility tests of NIR skin measurement, all subjects were randomly chosen and without any skin treatment. Subjects did not undergo any specific acclimatisation typically done in clinical skin moisturisation studies. Measurements were taken on the left volar forearm.

For the clinical tests, 20 volunteers of either sex, aged 18 to 60 years acclimatised for at least 30 minutes in a temperature-controlled room of 20 ± 1°C with the relative humidity set at either 45, 55, 65, 75 or 81%, prior to taking skin measurements. Cosmetic ingredients glycerine (Pripure 9091, Uniqema, Emmerich, Germany) and Vaseline Petroleum Jelly (INCI name: Petrolatum; Elida Fabergé, Bodegraven, The Netherlands) were applied at a rate of 2.24 μL cm^{-2}. At various times after application of these products (30 minutes, one, two, four and six hours) Corneometer, Tewameter and NIR measurements were taken in this sequence after removal of the dose.

Results and discussion

Reproducibility of NIR skin measurements and its signal to noise (S/N) ratio

The NIR wavelength region of interest for this study was from 1880–1910 nm (see Figure 1). The noise level of the instrument itself was determined by measuring an internal standard material,

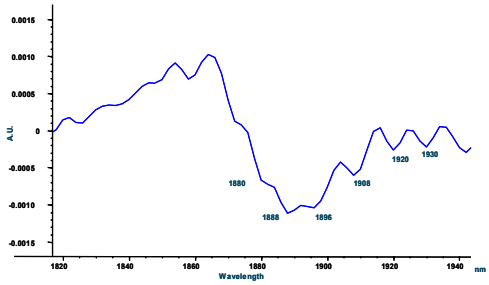

Figure 1. Derivative spectrum of skin.

polystyrene. The average of ten measurements resulted in an absorption at 1890 nm of 5.7E-06 abs, with a standard deviation of 9.6E-06 abs.

The "real" noise level, however, is a summation of the instrument noise and that introduced by the skin of unconditioned human subjects. In order to prove that the "real" noise level at the interesting wavelengths is not so high that the recognition of the water band variation is an issue, 247 spectra obtained from approx. 60 volunteers were used to calculate the average noise and signal. These data were obtained without control of subject gender, age, habit and health conditions, or the temperature and humidity of the measurement room. The peak height used as signal intensity and the units recorded are in an arbitrary scale ABS. The average absorptions at 1620 nm and at 1800 nm were used as reference "real" noise level, because there are no absorption peaks at these two wavelengths. They roughly represented the "real" noise of measurement including the error introduced by the skin and the instrument. Thereafter, these average values were used for the calculation of signal to noise ratio (S/N). They are summarised in Table 1.

Table 1. Average absorption intensity (ABS) of signal and noise bands, and S/N

Signal		Noise		S/N ratio			
1906 nm	1890 nm	1620 nm	1800 nm	1906/1620	1906/1800	1890/1620	1890/1800
0.00042	0.00097	0.000077	0.00005	5.45	8.4	12.6	19.2

Obviously, all average S/N ratios are above five. When we use the 1800 nm absorption as the noise level, the S/N ratio is as much as 19 for the free water absorption band at 1890 nm, the most important water absorption band in this study. The S/N ratio is 8.4 for the protein-bound water absorption band at 1906 nm. These ratios are sufficiently high for the qualitative purposes outlined in the introduction to give confidence to our interpretations.

Standard deviation is another statistical parameter to evaluate method reproducibility. For 247 spectra, the free water absorption band of 60 subjects at 1890 nm has an average signal value of 0.00097 ± 0.00028. Comparing this with the noise level at 1620 nm of 0.000077 ± 0.000037 STD, it can be seen that the magnitude difference is more than 10. We therefore conclude that the observed band intensity variations are due to the skin water content of the subjects rather than to measurement error.

Two different skin moisturisation mechanisms

A series of experiments was conducted to study the mechanism of skin moisturisation. This time, the study was performed under controlled conditions, i.e. after acclimatising for at least 30 minutes at a pre-set temperature and percentage relative humidity (RH%). Glycerine is a well-known standard moisturising cosmetic ingredient, giving excellent moisturisation to skin that is often used in commercial skin lotion formulations. Petrolatum is another moisturising personal care formulation. They were selected for the mechanism studies, because they represent typical examples of the humectancy and the occluding principle of yielding skin moisturisation, respectively.[6]

The room temperature was controlled at 20 ± 1°C, whilst the RH% was first controlled at 45, then 55, 65, 75 and finally 81%. Measurements only took place after a RH% had been established

for at least one hour. This study design allowed skin hydration measurements with three different techniques (capacitance via the Corneometer; transepidermal water loss (TEWL) via the Tewameter and water absorption bands via NIR) to take place at the same time on the same subjects, with and without the application of the cosmetic ingredients.

Figure 2 is a PCA plot of NIR data of all the subjects without and with petrolatum and glycerine applied, measured at 0 and two hours. The results show that the skin moisture level has increased following the use of lotions in all subjects. In this Figure, the water level decreases from the left side to the right of the plot and a cluster of higher hydrated skin sites can be found on the left-hand side of the Figure.

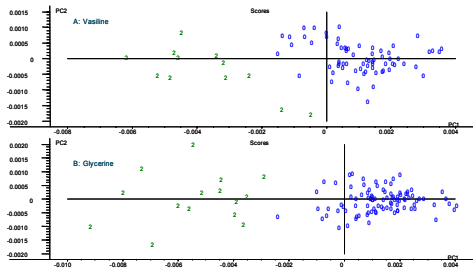

Figure 2. PCA of untreated skin and 2 hours after treatment with A: Petrolatum and B: Glycerine

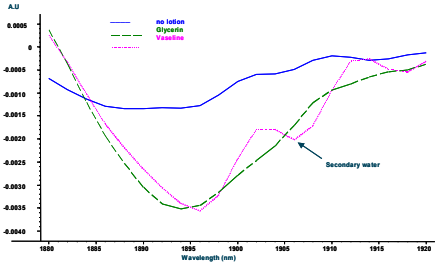

Figure 3. Spectra of untreated and petrolatum and glycerine treated skin.

Figure 3 shows overlapping NIR spectra of untreated skin and skin following the application of glycerine and petrolatum of one single subject. The spectrum obtained after the application of petrolatum shows that the water band is split into two separate peaks, one at 1890 nm and another around 1908 nm. The first band is the free water band whereas the second is the primary protein-bound water band. The water band in the spectrum following glycerine application, however, is not split. It remains as one overlapping peak.

We found this to be the situation with most subjects. Figure 4 is a principal component analysis (PCA) loading plot based on 60 subjects. Obviously, the loading line of petrolatum shows an extra shoulder peak in the 1906 nm region, which is the region where the additional band emerges, whereas the glycerine loading line is made of one smooth peak.

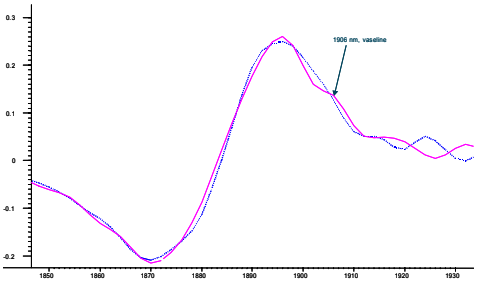

Figure 4. Loadings of PCA models with glycerine (…)and petrolatum (-).

If most subjects show the same tendency to two different lotions, this indicates that the skin moisturisation mechanisms of the two lotions are different as generally believed but never shown. The NIR data suggest that glycerine intends to collect free water and penetrates into the skin whereas petrolatum tends to increase the protein-bound primary water by blocking the water evaporation from the skin. Due to the occlusive nature of petrolatum, this increased bound water can only have migrated from the deep body, not from the environment. When this data is combined with that of the Corneometer and the Tewameter, a dynamic model of skin moisturisation can de derived.

A pseudo skin moisturisation model

Figure 5(a) illustrates that according to NIR measurements, the water level in human skin decreases as the RH increases until 75%, whereas according to the Corneometer measurements the water level increases with increasing humidity as shown in Figure 5(b). Furthermore, Figure 5(c) shows that the TEWL (Tewameter) is reducing with increasing RH. A possible explanation for this discrepancy can be that the various instruments measure water at different depths in skin: the Corneometer measures an integrated signal to levels as deep as 100 μm (almost exclusively the epidermis)[7] whereas the measuring depth of NIR light was determined to be about 1.3 mm which is well into the dermis, although the integrated signal will definitely contain information on water in the stratum corneum and the viable epidermis. Measurement of TEWL from the skin surface (Tewameter) is independent of depth.

These seemingly conflicting results suggest that different phenomena are taking place at different depths in skin. Based on the TEWL values depicted in Figure 5(c), it can be concluded that human skin loses water to the environment under all RH tested, the extent of which is controlled by the RH of this environment. As with all Fickian diffusion, also the magnitude of TEWL is determined by the water concentration gradient over the skin. The amount of water in the human body is estimated to be approx. 70% w/w and may be assumed to be constant. At low RH's, there will be a larger concentration gradient over skin and therefore a greater TEWL. As the RH increases, the gradient decreases and so does the TEWL [see Figure 5(c)].

With respect to the Corneometer values, we see that they increase with increasing RH's. First of all, the stratum corneum is in equilibrium with the external environment and will contain more water if the RH increases. But the absolute quantities of water in air and stratum corneum are not the same; they are higher in skin than in the external environment. To allow such a thermodynamically unfavourable state to be achieved and maintained, the stratum corneum contains the so-called Natural Moisturiser Factor (NMF), a hygroscopic mixture of pyrrolidone carboxylic acid, lactic acid and amino acids.8 As a consequence, the majority of water in the stratum corneum must be bound, the so-called primary water whilst a smaller fraction must be free to allow the TEWL, which is made up of free water. Now combine this with the results obtained for the cosmetic ingredients, glycerine only increasing free water and petrolatum increasing both bound and free water. Glycerine penetrates rapidly into the skin, increasing water levels in both the stratum corneum (as measured with the Corneometer; data not shown here but details can be found elsewhere[9]) and the dermis. It rapidly penetrates the skin to its full depth, including the dermis where it acts as a humectant, absorbing water of which the majority is free water in a water jacket surrounding the glycerine. Based on water-sorption profiles of glycerine, it can be concluded that each glycerine molecule is associated with three water molecules. In contrast to glycerine, petrolatum does not penetrate the skin and only prevents the evaporation of out-going free water. If the water level of the stratum corneum is still below 40% of its dry weight, this water will be bound whereas above 40-50%, it will be available as free water. The subjects that participated in this study will have had low water levels as we see both an increase in bound and free water.

We already know that the penetration depth of NIR irradiation into human skin is wavelength dependent and that it was found to be approx. 1 mm at wavelength region of 1890–1910 nm.[3] With such a penetration depth, the majority of the integrated signal will originate from the dermis. Whereas it has always been assumed that the deeper water levels were constant, we now have some preliminary indications that this may not be the case. The body is trying to compensate for the low water levels at the skin surface (at low RH's) by increasing the deeper water levels in the dermis, as this will lift the water concentration in the stratum corneum somewhat. It should be noted, however, that the variability in dermal hydration levels as assessed by NIR is high and that more experiments.

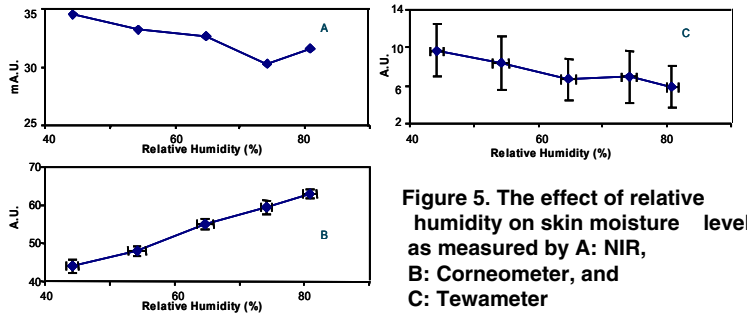

Figure 5. The effect of relative humidity on skin moisture levels as measured by A: NIR, B: Corneometer, and C: Tewameter

are required to assess whether the above is truly happening or whether dermal water levels are indeed constant The interpretation of the three different measurements all point in the same direction, namely to keep our skin reasonable hydrated under all circumstances. Skin moisturisation maintenance is a dynamic equilibrium process; the skin constantly adjusts water migration from its deeper layers to the surface of the skin or from the surface of the skin to its deeper layers, according to environmental conditions. This model can be expressed as follows.

$$Equilibrium \quad K = Absorption \underset{R2}{\overset{R1}{\Leftrightarrow}} Evaporation \qquad (1)$$

The amount of water in the stratum corneum is determined by the flux of water through this membrane but it needs to be bound to prevent it from being lost immediately. All such observations were only possible due to the use of NIR that can distinguish between bound and free water under dynamic environmental conditions.

Conclusions

The technique of NIR spectroscopy is able to differentiate the presence of different types of water in skin and to detect such amounts at a semi-quantitative level. The obtained data suggest that a dynamic equilibrium model can describe skin moisturisation.

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