Arch Pharm Res Vol 28, No 4, 458-462, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

Determination of Water Content in Skin by using a FT Near Infrared Spectrometer

Eun-Jung Suh, Young-Ah Woo, and Hyo-Jin Kim
College of Pharmacy, Dongduk Women's University, Seoul 136-714, Korea

(Received April 26, 2004)

The water content of skin was determined using a FT near infrared (NIR) spectrometer. NIR diffuse reflectance spectra were collected from hairless mouse, *in vitro*, and from human inner arm, *in vivo*. It was found that the variation of NIR absorbance band 1450 nm from OH vibration of water and 1940 nm from the combination involving OH stretching and OH deformation, depending on the absolute water content of separated hairless mouse skin, *in vitro*, using the FT NIR spectrometer. Partial least squares regression (PLSR) was applied to develop a calibration model. The PLS model showed good correlation. For practical use of the evaluation of human skin moisture, the PLS model for human skin moisture was developed *in vivo* on the basis of the relative water content of stratum corneum from the conventional capacitance method. The PLS model predicted human skin moisture with a standard errors of prediction (SEP) of 3.98 at 1130-1830 nm range. These studies showed the possibility of a rapid and nondestructive skin moisture measurement using FT NIR spectrometer.

Key words: FT near infrared (NIR), Skin moisture

INTRODUCTION

Skin covers the entire body and protects it from various types of external stimuli and damage as well as from moisture loss. The softness and pliability of skin are the main characteristic factors for protecting the body and assisting in motion (Obata and Tagami, 1990). These factors are dependent on the amount of moisture contained in the outermost layer of skin, the stratum corneum (SC). Changes in the water content of the SC have important consequences for the functional properties of human skin. Therefore, it is very important to maintain sufficient moisture in the SC for healthy skin.

Water levels in the superficial layers of the human skin are of utmost importance in determining many of its properties. A wide variety of techniques have been developed for measuring stratum corneum water content, including electric conductance (Courage, 1994; Martinsen *et al.*, 1995; Salter, 1998; Tagami, 1994), transepidermal water loss (TEWL) (Potts, 1986), and spectroscopic principles-attenuated total reflectance (ATR) fourier transform infrared or ATR-FTIR (Lucassen *et al.*, 1998; Prasch *et al.*, 2000; Wichrowski *et al.*, 1995) or confocal raman spectroscopy (Caspers *et al.*, 2001). Unfortunately all of these techniques have limitations. Electric conductance devices are easily influenced by the amount of electrolytes in the skin and by the contact area of the surface of the probe on the skin. TEWL is extremely environment-sensitive and requires several minutes of equilibration time for stable readings. ATR-FTIR measurements depend on the ambient conditions and are restricted to the uppermost stratum corneum, and the method is neither rapid nor portable.

In the near infrared, water molecules show two clear absorption bands at 1450 nm and 1940 nm. The water absorption bands that are most prominent in the NIR region are due to overtones and combinations of the fundamental vibrations active in the NIR range arising from hydrogen covalent bonds. The amplitudes are sufficiently high for them to be easily identifiable in the spectrum of the human skin.

NIR spectroscopy has the following advantages for biomedical applications. First, it is a noninvasive and nondestructive analytical technique. Second, one can use fiber optics for *in vivo* measurements. Third, it is possible to monitor not only the surface of biological tissues but also their insides because NIR light penetrate into the tissues (Egawa *et al.*, 2003).

The uses of near-infrared reflectance spectroscopy

Correspondence to: Young-Ah Woo, Spectrontech, Co., Ltd, Seongnam, 462-807, Korea
Tel: 82-2-940-4305, Fax: 82-2-943-9578
E-mail: wooy@spectrontech.com

related to skin moisture have been reported (Rigal *et al.*, 1993; Martin, 1993; Sowa *et al.*, 1999; Libnau *et al.*, 1994). *In vitro* or *in vivo*, they showed the good correlation between water content and NIR absorbance in addition to advantages of NIR analysis over other measurements (Walling and Dabney, 1989; Martin, 1998). These studies were performed with conventional NIR instruments with integrating sphere or moving grating, which are hard to move and handle for practical use. According to recent overview on NIR spectroscopy of skin (Heise, 2000; Kumar and Schmitt, 1997), diffuse reflectance spectra were recorded with the use of optical fiber probe and were valuably used for clinical diagnostics.

In this study, the rapid and nondestructive method for determination of the water content in SC was developed by using a FT NIR spectrometer with a fiber-optics probe.

MATERIALS AND METHODS

FT NIR spectrometer

We used a MPA™ FT NIR spectrometer (Bruker Optik GmbH, Germany). This system is equipped with a tungsten halogen lamp and a Ge diode detector. NIR reflectance spectra were collected over the 12000-4000 cm^{-1} spectral region at 4 cm^{-1} interval using a fiber-optics probe. The fiber-optics probe permits the direct measurement of solids and powders by diffuse reflectance. The probe uses a bifurcated (Y-shaped) fiber bundle to illuminate the sample and collect the diffusely scattered light. Each sample was obtained by averaging 16 scans. All of the hairless mouse skin spectra were recorded as log (1/R) with respect to a reference standard made of teflon. A standard ceramic was used as a reference for human skin spectra. NIR spectra were collected by using the OPUS software (Bruker Optik GmbH, Germany).

Hairless mouse skin

Dorsal skin from two hairless mice (8 weeks) was used. The epidermis parts were separated from the dermal tissues and were cut into 8 pieces. The pieces were labeled A to H. The labeled skin pieces were weighed and soaked for 1 h so that they would contain a maximum amount of water. After that, the skin samples were placed in the desiccators charged with silica gels. After 24 h, the skin samples were dried in an oven at 50°C during 22 h, 75°C during 7 h, and 105°C until they were of constant weight. NIR spectra of each piece of skin were acquired by using the FT NIR spectrometer with reflectance fiber-optic probe right after every weighing.

Through this procedure, the 157 NIR spectra of the hairless mouse samples with various ranges from 4.55% to 85.87% were acquired by using the FT NIR spectrometer.

Repeatability test

When acquiring NIR spectra of hairless mouse skin, repeatability tests of spectral measurement were implemented for the FT NIR spectrometer. Ten consecutive spectral measurements were acquired by using the fiber-optic probe. The coefficient of variation (%) was calculated in the 1150-1700 nm, and 1150-2250 nm wavelength range.

Human skin

To develop a PLS model for the determination of human skin moisture using the FT NIR spectrometer, 80 NIR spectra were collected from the arms of 2 persons. A capacitance method using Corneometer CM 825 (Courage-Khazaka, Köln, Germany) was used for reference values for the relative water content of human skin. The relative water content for all of the samples ranged from 24.3 to 67.3. NIR reflectance spectra and reference values using the Corneometer CM 825 were acquired at the same sites on the arms.

Evaluation of NIR model

The samples were divided into a calibration set for modeling and a prediction set for the evaluation of the developed model. The prediction set consisted of samples that were not used for the calibration set. Each developed NIR model was evaluated as the standard errors of calibration (SEC) for the calibration set and the standard errors of prediction (SEP) for the prediction set.

$$SEC = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n-p}}, \quad SEP = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}}$$

where $\hat{y}_i$ is the NIR predicted value, y_i is the reference value, n is the number of data spectra, and p is the number of used factors.

RESULTS AND DISCUSSION

Hairless mouse skin

Fig. 1 shows the water content changes of hairless mouse skin, depending on time. Since the hairless mouse skin was first soaked in water for 1 h in order to acquire a data set with a broad water content range, every second point had the highest water content. We acquired the hairless mouse skin samples with various water content ranges from 4.55% to 85.87%, and the near infrared reflectance spectra of the hairless mouse skin samples were collected.

FT NIR spectra of hairless mouse skin were collected in the 12000-4000 cm^{-1} spectral range. For finding OH band, we transformed from wavenumber (cm^{-1}) to wavelength (nm) by using the OPUS software (Bruker Optik GmbH, Germany), Only five NIR spectra were presented for the

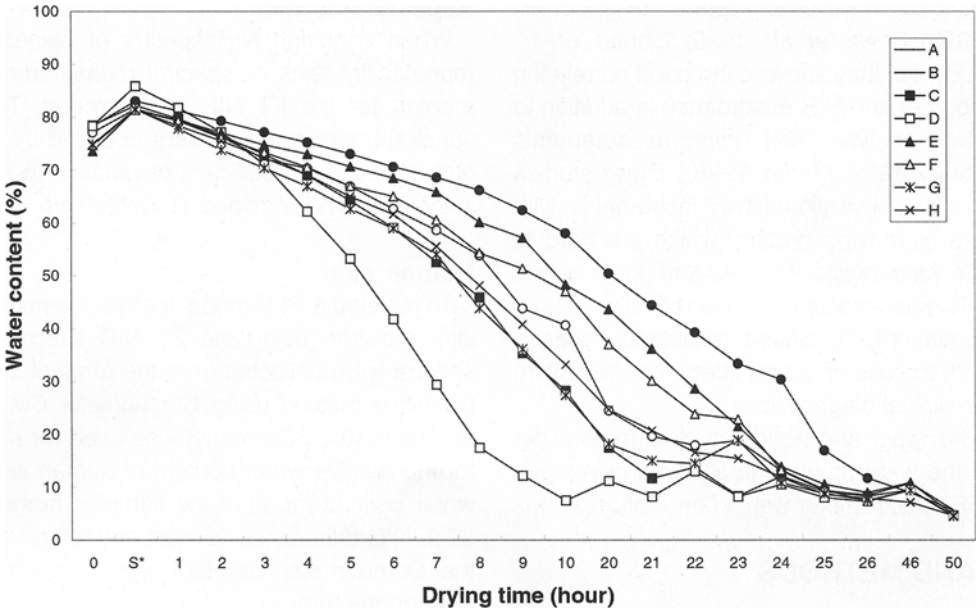

Fig. 1. Water content changes of hairless mouse skin, depending on time. S* means after soaking 1 h in water.

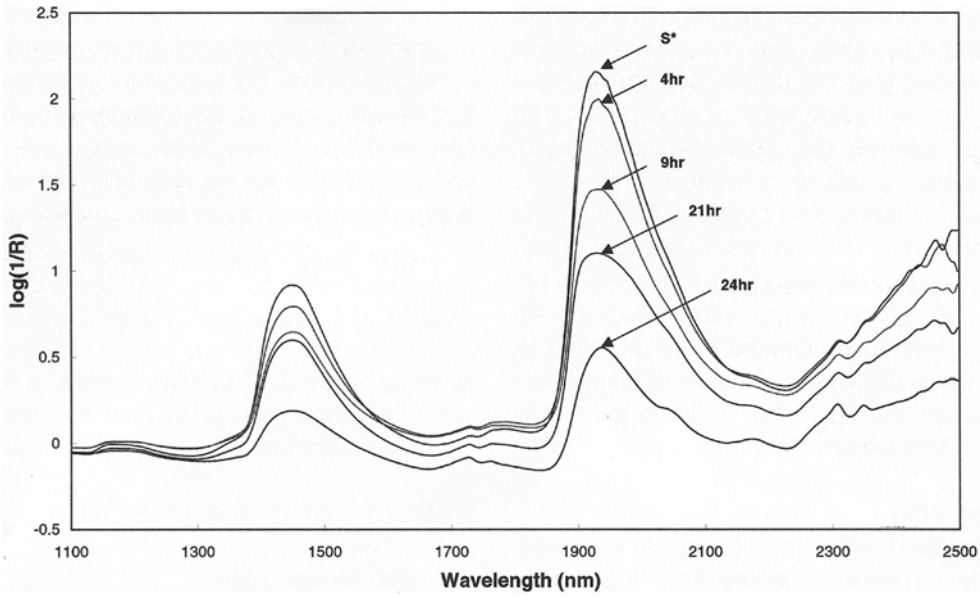

Fig. 2. NIR spectra of hairless mouse skin by using the FT NIR spectrometer

clear comparison as shown in Fig. 2. The huge band at 1450 nm and 1940 nm in the spectrum is the first overtone of OH band stretching of water and the combination band involving OH stretch and OH bend. Although the water band changes depending on the water content were clearly observed, it is difficult to use a classical univariate calibration method for water content of skin because of dominant scattering effect on NIR spectra from skin surface.

Partial least squares (PLS) regression was used for the development of a calibration model for the water content of skin, which is a powerful multivariate calibration method used elucidate the correlation between NIR absorbance and concentration of interest, even in the complex system. The Unscrambler® (Camo, Norway) was used for PLS modeling. We investigated the 1150-2250 nm, which contain OH band at 1450 nm and 1940 nm, and 1150-1700 nm, which contain only OH band at 1450 nm. Derivative techniques were used to remove or suppress constant background signals and to enhance the visual resolution before PLS modeling. To develop a robust model, several conditions, such as first and second derivatization, were considered. When first derivative spectra with 1150-2250 nm wavelength range were used, the better calibration result was acquired with a SEC of 6.65% and a SEP of

Table I. Calibration results of the water content of hairless mouse skin using the FT NIR spectrometer.

Spectral range (nm)	Spectral treatment	No. factors	SEC (%)	SEP (%)
1150-2250	none	3	6.98	7.04
	1D	3	6.65	6.74
	2D	3	6.55	7.50
1150-1700	none	3	6.92	7.18
	1D	3	6.13	6.91
	2D	2	6.53	7.23

6.74%, as listed in Table I. Fig. 3 shows the scattering plot showing the good correlation between NIR value and water content using the best PLS model. Overall, the calibration of water content of hairless mouse skin was successfully performed by the FT NIR spectrometer.

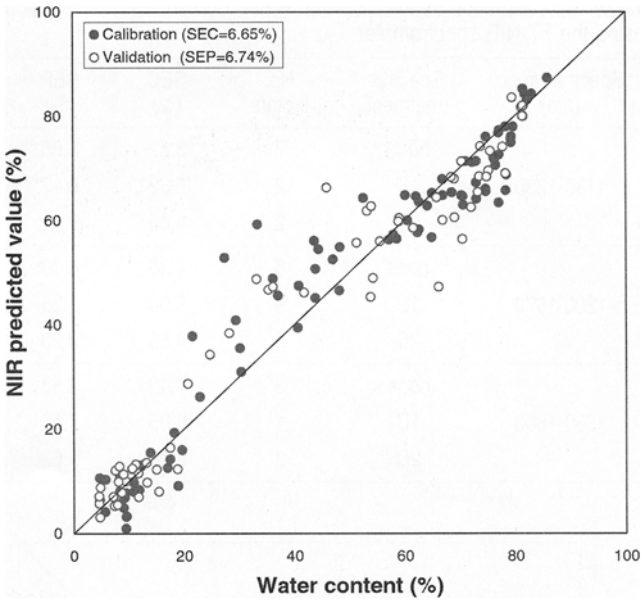

Fig. 3. Scattering plot showing correlation between the NIR value and the water content of hairless mouse skin using the 1150-2250 nm range

Repetability test

The coefficient of variation (%) was calculated by obtaining the NIR spectra of hairless mouse skin 10 times. The average of CV (%) in the region from 1150 to 1700 nm and in the region from 1150 to 2250 nm was 2.12% and 1.39%, respectively. The CV (%) was low and spectral treatment such as derivatives could be applied for complementary measures.

Human skin

The FT NIR spectrometer was applied for the determination of the water content, which is an important factor for healthy skin, of the stratum corneum layer of human skin. We used the conventional capacitance method for the reference value and investigated the correlation between the NIR value and the reference value. 80 NIR reflectance spectra were acquired from the inside of the arm of 2 persons. Fig. 4 shows the human skin spectra. All of spectra were separated in to two sets for the development of PLS modeling and the evaluation of the PLS models. PLS regression was performed in the same method as used for the hairless mouse skin moisture calibration. Three regions, 1130-1830 nm, 1200-1670 nm, and 1380-1600 nm, were used to find a robust model. Also, to enhance the spectral features, derivative tech-

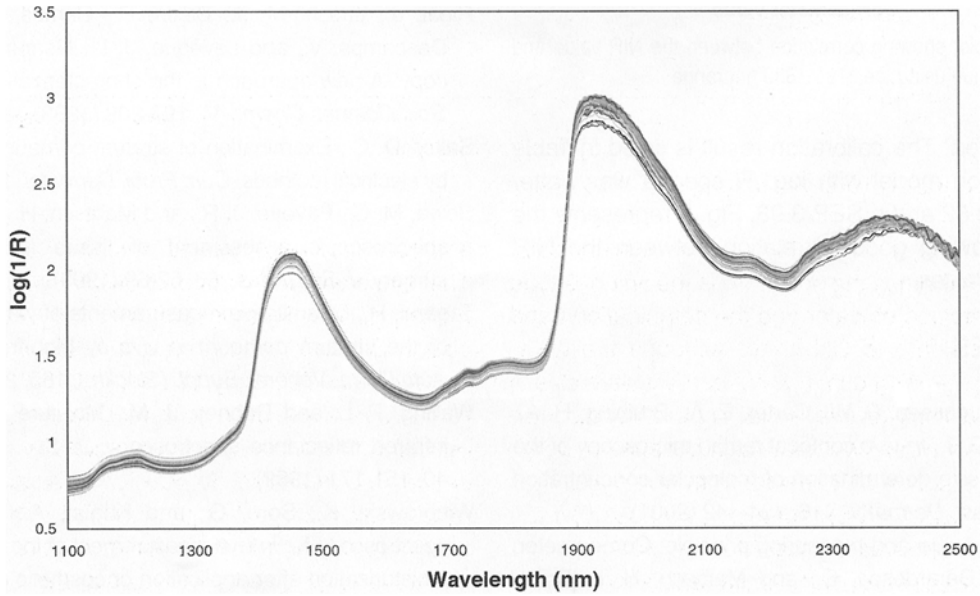

Fig. 4. NIR spectra of human skin by using the FT NIR spectrometer

Table II. Calibration results of the water content of human skin using the FT NIR spectrometer

Spectral range (nm)	Spectral treatment	No. factors	SEC (%)	SEP (%)
1130-1830	none	7	3.82	3.98
	1D	2	5.52	6.17
	2D	2	4.65	7.21
1200-1670	none	6	4.35	4.90
	1D	3	5.06	7.35
	2D	3	4.56	9.20
1380-1600	none	2	7.43	6.53
	1D	1	6.95	7.50
	2D	3	4.90	9.63

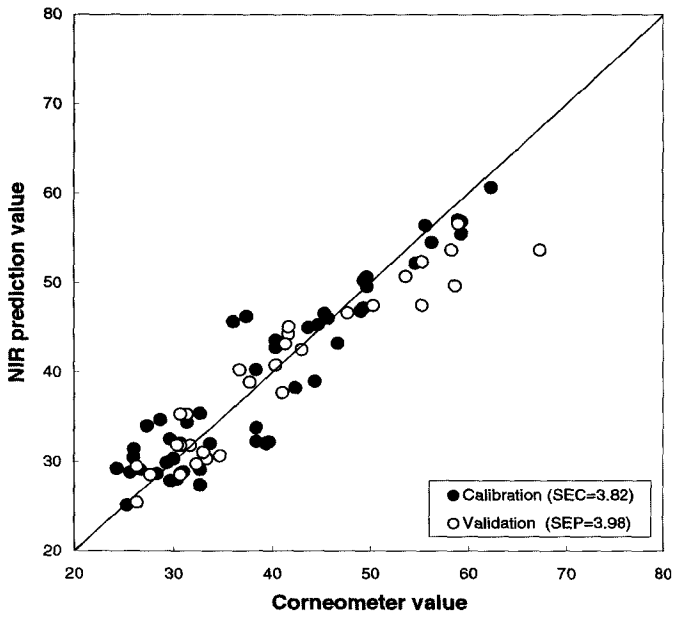

Fig. 5. Scattering plot showing correlation between the NIR value and the corneometer value using the 1130-1830 nm range

niques were used. The calibration result is listed in Table II. The calibration model with log1/R spectra was better with a SEC of 3.82 and a SEP 3.98. Fig. 5 represents the scatter plot showing good correlation between the NIR and reference values.

REFERENCES

Caspers, P. J., Lucassen, G. W., Carter, E. A., Bruining, H. A., and Puppels, G. J., *In-vivo* confocal raman microscopy of the skin: Non-invasive determination of molecular concentration profiles. *J. Invest. Dermatol.*, 116, 434-442 (2001).

Courage W., Hardware and measuring principle: Corneometer, In Elsner, P., Berardesca, E., and Maibach, H. I. (Eds.). Bioengineering of the skin: Water and the Stratum Corneum. CRC press, Boca Raton, Chapter 14, (1994).

Egawa, M., Fukuhara, T., Takahashi, M., and Ozaki, Y., Determining Water Content in Human Nails with a Portable Near-Infrared Spectrometer. *Appl. Spectrosc.*, 57, 473-478 (2003).

Heise, H. M., Clinical applications of near- and mid- infrared spectroscopy, In Gremlich, H. U., and Yan, B. (Eds.). Infrared and raman spectroscopy of biological materials. Marcel Dekker, New York, (2000).

Kumar, G. and Shmitt, J. M., Optimal probe geometry for near-infrared spectroscopy of biological tissue. *Appl. Optics,* 36, 2286-2293 (1997).

Libnau, F. O., Kvalheim, O. M., Christy, A. A., and Toft, J., Infrared spectra of water in the NIR and MIR region. *Vib. Spectrosc.*, 7, 243-254 (1994).

Lucassen, G. W., Veen, G. N. A., and Jansen, J. A. J., Band analysis of hydrated human skin stratum corneum attenuated total reflectance fourier transform intrared spectra *in vivo. J. Biomedical Optics*, 3, 267-280 (1998).

Martin, K. A., Direct measurement of moisture in skin by NIR spectroscopy. *J. Soc. Cosmet. Chem.*, 44, 249-261 (1993).

Martin, K., *In vivo* measurements of water in skin by near-infrared reflectance. *Appl. Spectrosc.*, 52, 1001-1007 (1998).

Martinsen, O. G., Grimnes, S., and Karlsen, J., Electrical methods for skin moisture assessment. *Skin Pharmacol.*, 8, 237-245 (1995).

Obata, M. and Tagami, H., A rapid *in vitro* test to assess skin moisturizers. *J. Soc. Cosmet. Chem.*, 41, 235-241 (1990).

Potts, R. O., Stratum corneum hydration : Experimental techniques and interpretations of results. *J. Soc. Cosmet. Chem.*, 37, 9-33 (1986).

Prasch, T. H., Knübel, G., Schmidt-Fonk, K., Ortanderl, S., Nieveler, S., and Förster, T. H., Infrared spectroscopy of the skin: influencing the stratum corneum with cosmetic products. *Int. J. Cos. Sci.*, 22, 371-383 (2000).

Rigal, J., Losch, M. J., Bazin, R., Camus, C., Sturelle, C., Descamps, V., and Lévêque, J. L., Near-infrared spectroscopy: A new approach to the characterization of dry skin. *J. Soc. Cosmet. Chem.*, 44, 197-209 (1993).

Salter, D. C., Examination of stratum corneum hydration state by electrical methods. *Curr. Probl. Dermatol.*, 26, 38-47 (1998).

Sowa, M. G., Payette, J. R., and Mantsch, H. H., Near-infrared spectroscopic assessment of tissue hydration following surgery. *J. Surg. Res.*, 86, 62-69 (1999).

Tagami, H., Quantitative measurements of water concentration of the stratum *corneum in vivo* by high-frequency current. *Acta Derm. Venerol. Suppl. (Stockh.)*, 185, 29-33 (1994).

Walling, P. L. and Dabney, J. M., Moisture in skin by near-infrared reflectance spectroscopy. *J. Soc. Cosmet. Chem.*, 40, 151-171 (1989).

Wichrowski, K., Sore, G., and Khaïat, A., Use of infrared spectroscopy for *in vivo* measurement of the stratum corneum moisturization after application of cosmetic preparations. *Int. J. Cos. Sci.*, 17, 1-11 (1995).