Development of a portable near infrared device for skin moisture by using a microspectrometer

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Synopsis

In recent years, a miniature spectrometer has been extensively developed due to the combination of fiber optics and semiconductor detector arrays. This type of miniature spectrometer has advantages of low price and robustness because of the capability of mass production and no moving parts are required such as lenses, mirrors and scanning monochromator. In this study, for skin diagnostics, a portable near infrared (NIR) system has been developed using a LIGA microspectrometer, which is photo-diode arrays-type. The measuring time is within 1 second and the spectral data have good repeatability. By using this developed system, skin moisture studies have been performed. We compared the portable device with a FT-NIR system to evaluate the performance of the new device for hairless mouse skin moisture. In order to develop a NIR model for skin calibration, rat skin was used with absolute water contents using loss on drying. The developed calibration model using rat skin could be successfully applied for human skin moisture calibration. In addition, we studied depth profiling in the near infrared region changing the distance from illuminating and receiving places of fiber optic probes. This study showed that the colleted light information could be controlled depending the distance of fiber optic probes. It was confirmed that the longer distance we used, the deeper site from the skin surface we could get information. Two kinds of probes with distances such as 0.03 mm and 0.5 mm were used.

Key words: near infrared (NIR), skin moisture, portable, microspectrometer, depth profiling

INTRODUCTION

Portable near infrared system

Recently, microspectrometers are getting much more attractive thanks to the versatility and low price especially in the field of medical diagnostics, environmental and process analysis. The small detector without moving parts is quite robust, particularly suitable for use in portable instruments. Moreover, with the use of fiber optics, various sample modules are available and the sample measurements become very simple and effective. Combined with suitable optical sensing heads, it can be employed both for reflection/remission measurements and absorption /transmission measurements. Almost microspectrometers has been resulted from the combination of fiber optics and semiconductor detectors such as CCDs (charge coupled devices), photodiode arrays (PDAs) and several hybrid devices[1]. In our study, using a LIGA technology, PDAs were developed for a detector and we integrated a near infrared portable system based on the development of this miniature spectrometer. For sample presentation, a fiber optics probe was used. The newly developed portable NIR system was applied to the calibration of skin moisture. The performance of the portable system was compared with that of a FT-NIR instrument.

Human skin moisture

Human skin consists of epidermis and dermis. The softness and pliability of skin are the main characteristic factors to protect the body and assist the motion[2]. These factors are dependent on the amount of moisture contained in the stratum corneum, which is the outermost layer of epidermis, and controlled by the barrier function that maintains adequate water content in the skin layer. The stratum corneum is about 10-40 micron thick, except on the palms and soles, and composed of partially flattened and keratinized layers[3]. If the health condition of stratum corneum is not maintained due to environmental changes, the efficiency of barrier function and moisture maintaining function of the skin will be dropped off. As a result, the skin becomes easily dried, roughed, and even more liable to infection. Therefore, it is very important to evaluate skin moisture.

Electric conductance[4-7], transepidermal water loss (TEWL)[8], infrared spectroscopy by attenuated total reflectance (ATR)[9-11] have been used as conventional skin moisture measuring devices. TEWL measures the rate of evaporation of water from the skin surface, which is extremely environment-sensitive and requires several minutes of equilibration time for stable readings. The infrared spectroscopy is a direct moisture measuring method at certain

wavelength range. Since this is not only very expensive but also difficult to operate, it is rarely used for commercial purpose. In addition, ATR measurements depend on the ambient conditions and are restricted to the uppermost stratum corneum. Electric conductance measuring devices including capacitance method has been widely used so far. When the alternating current with a constant frequency is applied to the skin, electric conductance is measured and the skin moisture is calculated from the electric conductivity depending on the water content of skin. However, these devices are influenced by external temperature and humidity, which requires to be kept at a constant temperature and humidity conditions. Also, the amount of electrolyte that skin contains can change the conductance value without regard to water content.

The uses of near-infrared reflectance spectroscopy related to skin moisture have been reported[12-15]. *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[16,17]. 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[18,19], diffuse reflectance spectra were recorded with the use of optical fiber probe and were valuably used for clinical diagnostics.

We reported the feasibility for calibration of human skin moisture by using a newly developed device and compared the performance with a scanning type NIR spectrometer[20]. In this study, the performance of NIR device using a microspectrometer was compared with that of a FT-NIR spectrometer for the calibration of hairless mouse skin moisture. In addition, an absolute moisture model using partial least squares (PLS) regression[21,22] which was developed using rat skin could be successfully applied to the calibration of human skin moisture. In addition, we investigated the skin depth profiling using rat skin for the measuring condition of fiber optic probes for skin moisture.

EXPERIMENTAL

LIGA microspectrometer

A planar grating spectrometer with self focusing reflection grating as shown in Figure 1 can be fabricated by moulding techniques, including fiber fixing grooves in one process step. A diode array for signal detection can be adapted easily using a 450 sidewall at the focus line. The obtained spectral resolution is in the area of 7 nm with grating constant between 2-5 um; the step height of the echeletter grating is between 0.2 and 0.6 um.

Portable near infrared system

The newly integrated system has been focused on the development of compact spectrometer equipped with optical fiber using a microspectrometer described above. A schematic diagram for portable NIR system is presented in Figure 2. This system includes a tungsten halogen lamp for light source, an InGaAs photodiode array for micro-spectrometer, an internal battery with portable size (DC 12V), and a software for interfacing and chemometrics. The RS-232c cable was used for the connection to a computer. For skin sample presentation, a fiber optic probe was used. The fiber optic probe has surrounding fiber optic bundles for illumination of light to sample and one centric bundle for receiving the light from sample. Each reflectance spectrum was derived from a mean of 50 scans collected between 1100 nm and 1750 nm at a 2 nm interval.

FT-NIR spectrometer

To test the performance of the developed portable system, FT-NIR spectrometer was compared. We used a VECTOR22/N FT-NIR spectrometer (Bruker Optics Inc.). 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 using a reflectance fiber-optics probe. Each sample was obtained by averaging 16 scans. All spectra were recorded as log(1/R) with respect to a reference standard made of teflon.

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 7 pieces. The pieces were labeled A to F. The

labeled skin pieces were weighed and soaked for 1 hour 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 hours, the skin samples were dried in an oven at 50℃ during 22 hours, 75℃ during 7 hours, and 105℃ until they were of constant weight. NIR spectra of each piece of skin were acquired by using the portable NIR system and FT-NIR spectrometer with reflectance fiber-optic probe right after every weighing.

Through this procedure, the 137 NIR spectra of the hairless mouse samples with various ranges from 4.55 to 85.87% were acquired by using the portable NIR system and the FT-NIR spectrometer.

Rat skin

Rat skin was used after hair removed with cream. Samples of rat skin were cut to 9 pieces. After that the rat skin was carried out by the same method as described for the hairless mouse skin. Rat skin was placed for in the desiccators charged with silica gel. After 24 hours, the skin samples were dried at 105℃ in an oven until they were of constant weight. NIR spectra of each piece of skin were acquired by using the portable NIR system. Through this procedure, the 143 NIR spectra of the rat skin with various ranges from 0.10 to 70.42% were acquired.

Human skin

To apply the developed PLS model using rat skin to human skin moisture directly, human skin NIR spectra were collected in the same condition. The spectra were collected from 12 sites, right and left forearm, right and left elbow, right and left palm, right and left hand, right and left finger, cheek and forehead. For repeatability test, the developed model were evaluated for 2 people during 4 days.

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.

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

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.

Skin depth profiling

The dorsal skin of rat skin with different thickness such as 1.0, 1.4, and 1.7 mm was used. The NIR spectra of rat skin were measured backing on the ceramic for reference and on the plastic for the investigation of lightening depth. We controlled the depth of skin information changing the distance from illumination and receiving of light. For the depth profiling, two kinds of fiber optic probes with different distances such as 0.03 mm and 0.5 mm were used.

RESULTS AND DISCUSSION

Hairless mouse skin – Portable NIR System.

Figure 3 shows the water content changes of hairless mouse skin, depending on time. Since the hairless mouse skin was first soaked in water for 1 hour 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.

Only five NIR spectra treated with basic offset were presented for the clear comparison as shown in Figure 4. The huge band at 1450 nm in the spectrum is the first overtone of OH band stretching of water. 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. 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, as listed in Table I. The optimum number of PLS factors was identified as the number of factors that gives a minimum SEP. Therefore, seven factors could be the optimum for modeling in this case. The PLS model predicted the water content for calibration and validation set with a SEC of 6.68% and a SEP of 7.25%, when first derivative were used. Figure 5a presents the scattering plot showing the correlation between the NIR predicted value and the water content using the best PLS model. The calibration and prediction data showed good correlation with water content of hairless mouse skin. Overall, the calibration of water content of hairless mouse skin was successfully performed by the newly integrated portable NIR system.

Hairless Mouse Skin – FT-NIR

To compare the performance of the developed system, FT-NIR was employed. FT-NIR spectra of hairless mouse skin were collected in the 833-2498 nm spectral range. PLS was used for the development of a calibration model for the water content of skin by using The Unscrambler® (Camo, Norway). We investigated the whole region, 1101-2250 nm, and 1101-1750 nm, which was the same region used in the portable system. When log(1/R) spectra with 1101-1750 nm

wavelength range were used, the better calibration result was acquired with a SEC of 6.64% and a SEP of 7.12%, as listed in TableⅡ. The performance of the calibration of FT-NIR spectrometer was approximately equivalent to that of the portable NIR system. Figure 5b shows the scattering plot showing the good correlation between NIR value and water content using the best PLS model.

Development of model for skin moisture using rat skin and its evaluation

Figure 6 shows the water content changes of rat skin. The water loss of separated rat skin was slow when the skin was placed for in the desiccators charged with silica gel. After 24 hours, the skin samples were dried at 105℃ in an oven until they were of constant weight, and the water content decreased rapidly. Calibration of water content of rat skin using portable NIR system was accomplished using the same method as implemented for the hairless mouse skin. The PLS models for rat skin using 1150 nm to 1700 nm were developed using log(1/R), first derivative, and second derivative spectra, as listed in TableⅢ. The calibration and prediction result was best with a SEC of 6.24% and a SEP of 6.64%, respectively, when log(1/R) were used. Figure 7 represents the scattering plot showing the correlation between NIR predicted value and water content with the best PLS model. Open and filled circles indicate calibration and prediction data, respectively. The calibration and prediction data showed good correlation with reference values from loss on drying.

In order to validate the developed calibration model, routine analyses were performed using newly prepared rat skin samples. Measurement of water content of new rat skin samples using the developed PLS model were listed in TableⅣ. The NIR prediction showed good results with determination of water content with a SEP of 7.11% compared with those of loss on drying.

In order to measure human skin moisture by the developed model from rat skin, human skin spectra were acquired. There are some factors to consider so that the model will be applied to the measurement of human skin moisture. For example, there are some different scattering effects from different surface and tissues. Because it is very hard to measure the absolute water content directly, some model could not be developed for human skin moisture. In previous study, we used the reference value from electric conductance method for human skin moisture modelling. However, the values are not absolute one and must have some measuring errors. We could not get good prediction results. Therefore, we tried to apply the developed model to the calibration of human skin moisture and investigated feasibility from the repeatability test, for 2 people, during 4 days. The spectra were acquired from 12 sites such as right and left forearm, right and left elbow, right and left palm, right and left hand, right and left

finger, cheek, and forehead. Figure 8a-8d showed the evaluation results compared with corneometer values. It is noticeable that the NIR prediction results were similar to the corneometer results and the repeatability of NIR performance was better than that of corneometer.

Skin depth profiling

We supposed that the depth of skin information could be controlled changing the distance from illumination and receiving of light. Figure 9 showed the used fiber optic probes with different distance (a) and their expecting phenomena (b), where the scattering of light is not considered. For this study, plastic plate was backed under the rat skin. A plastic peak at 1612 nm was used for comparison as shown in Fig 10. In the case of sufficient thick skin, the plastic peak could be not seen because the light cannot go back to the receiving and therefore, skin information from NIR spectrum was not related to backing materials. However, for not sufficient thickness of skin, we could find the some peaks from backing materials. The same results could be found when making the distance of fiber optic probes longer.

In this study, two kinds of fiber optic probes were used. The distance between illumination and receiving place are 0.03 mm and 0.5mm, respectively. Rat skin with three different thicknesses such as 1.0 mm, 1.4 mm, and 1.7 mm were used. For 0.03 mm distance fiber optic probes, we had three figures for each piece of rat skin as shown Figure 11. There are three NIR spectra for each figure. One is only plastic NIR spectrum and the rest two are skin spectra with different backing material, plastic one and ceramic one. In spite of same probe, we had different results changing the thickness of sample skin. We selected the plastic peak at 1612 nm for comparison. For, 1.0 mm thickness-rat skin, we could find skin spectra including the clear plastic peak. However, for 1.4 mm and 1.7 mm, it could be difficult to find the plastic peak clearly. The small changes at 1612 nm were within the confidence interval acquired during 20 time measurements. From this result, some skin information below 1.4 mm from the skin surface could not be acquired when using 0.03 mm fiber optic probe.

For a 0.5 mm distance-fiber optic probe, we got the same result for 1.0 mm thick-skin that was acquired by using 0.1 mm probe. However, for 1.4 mm, we could find the clear increase of the peak and was thought to be from the plastic plate. When using 0.5 mm distance-fiber optic probe, the light from source passes through the skin sample down to the plastic plate and go back to the receiving part of probe. We also found some little peak at 1612 nm for 1.7 mm skin.

Overall, we could get some information up to 1.7 mm from the skin surface when using 0.5 mm distance-probe even though we could not get the skin information below 1.4mm by

using 0.03 mm-distance probe. This result showed the skin depth profiling would be possible changing the distance between illuminating and receiving place.

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Figure 1. Schematic diagram of spectrometer.

Figure 2. Schematic diagram of the portable NIR system[20].

Figure 3. Water content changes of hairless mouse skin, depending on the time. S means after soaking 1 h in water.

Figure 4. NIR spectra of hairless mouse skin by using portable NIR system.

Figure 5. (a) Scattering plot showing the correlation between the NIR value and the water content of hairless mouse skin using the 1100-1730 nm range (portable NIR system). (b) Scattering plot showing correlation between the NIR value and the water content of hairless mouse skin using the 1101-1750 nm range (FT-NIR).

Figure 6. Water content changes of rat skin. S^* means after soaking 1 h in water.

Figure 7. Scaterring plot showing the correlation between the NIR value and the water content of rat skin using the 1150-1700 nm range (portable NIR system).

Figure 8. Routine analysis results of the water content of human skin in the 1150-1700 nm wavelength range using NIR system. (a) NIR Predicted value of volunteer Ⅰ. (b) Corneometer value of volunteer Ⅰ. (c) NIR Predicted value of volunteer Ⅱ. (d) Corneometer value of volunteer Ⅱ.(a) Schematic diagram of two kinds of fiber optic probes, which has the different distance between illumination and receiving place. (b) Schematic diagram of skin showing possible pathways with lights according to distance between illumination and receiving place.

Figure 10. NIR spectra of skin and plastic

Figure 11. 2D spectra of plastic and skin with different backing material, plastic and ceramic, obtained by using 0.03 mm distance fiber optic probe. (a) skin thickness : 1.0 mm, (b) skin thickness : 1.4 mm, (c) skin thickness : 1.7 mm.

Figure 12. 2D spectra of plastic and skin with different backing material, plastic and ceramic, obtained by using 0.5 mm distance fiber optic probe. (a) skin thickness : 1.0 mm, (b) skin thickness : 1.4 mm, (c) skin thickness : 1.7 mm.

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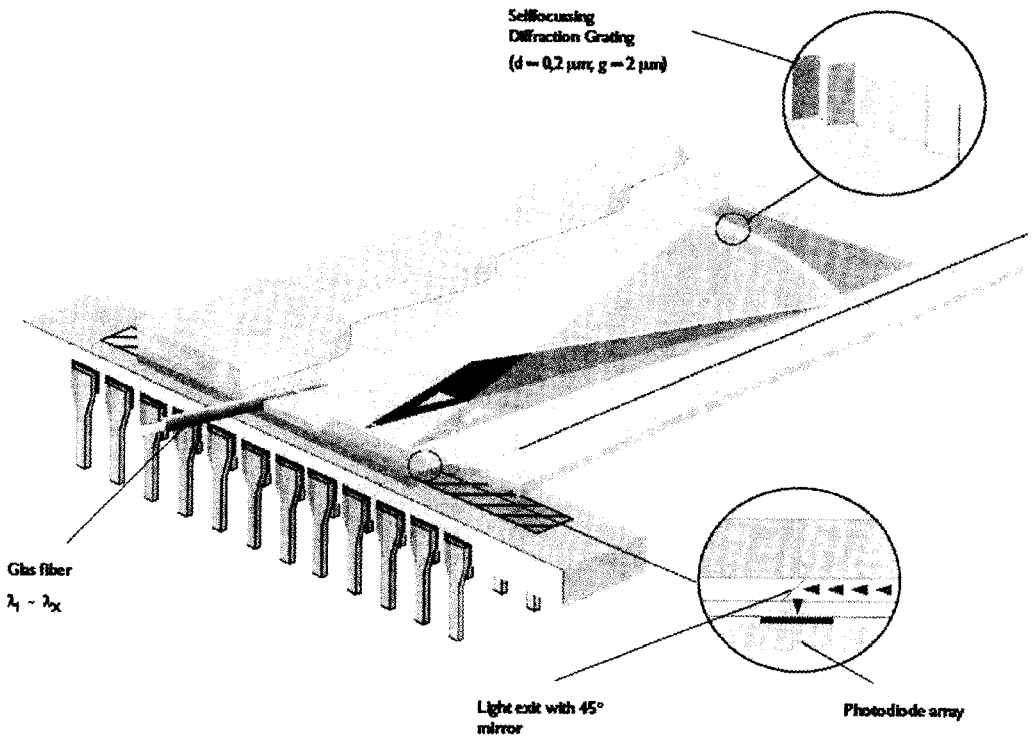
TableⅢ Calibration results of the water content of rat skin using the portable NIR system.

TableⅣ Routine analysis results of the water content of rat skin in the 1150-1700 nm wavelength range using NIR system.

Fig. 1


Selffocussing
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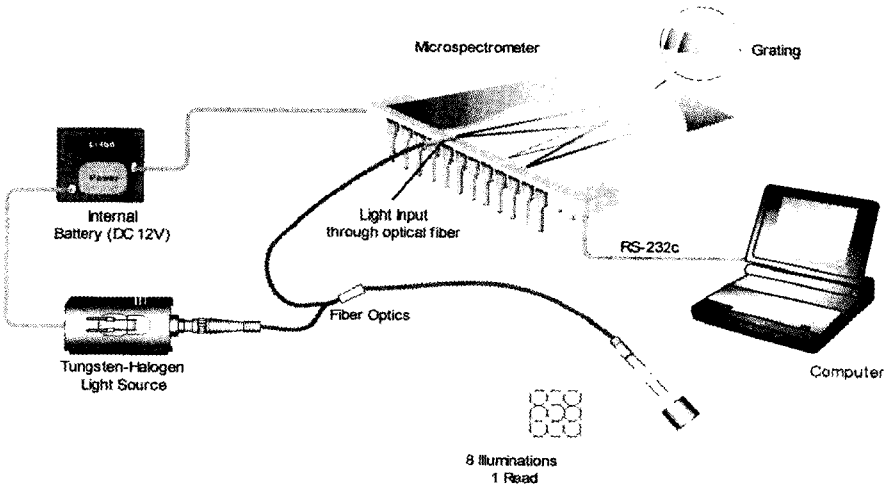
mirror
Photodiode array

Fig. 2


Microspectrometer
Grating
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Light Source
Computer
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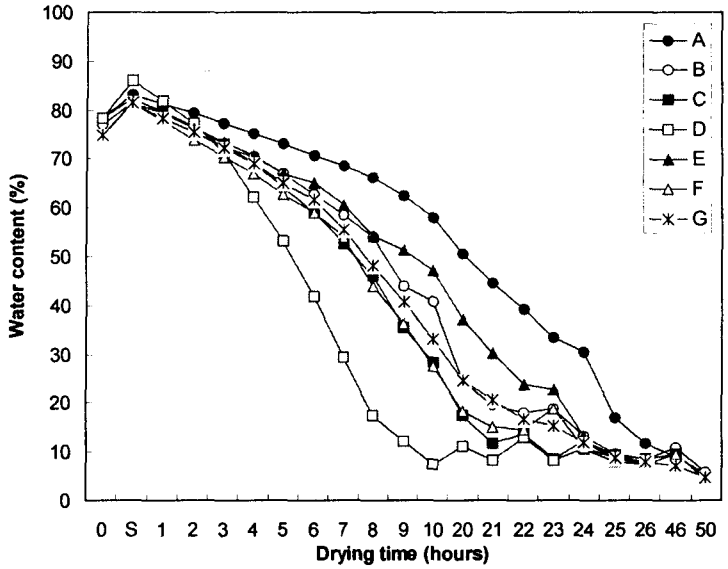
1 Read

Fig. 3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70
60
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30
20
10
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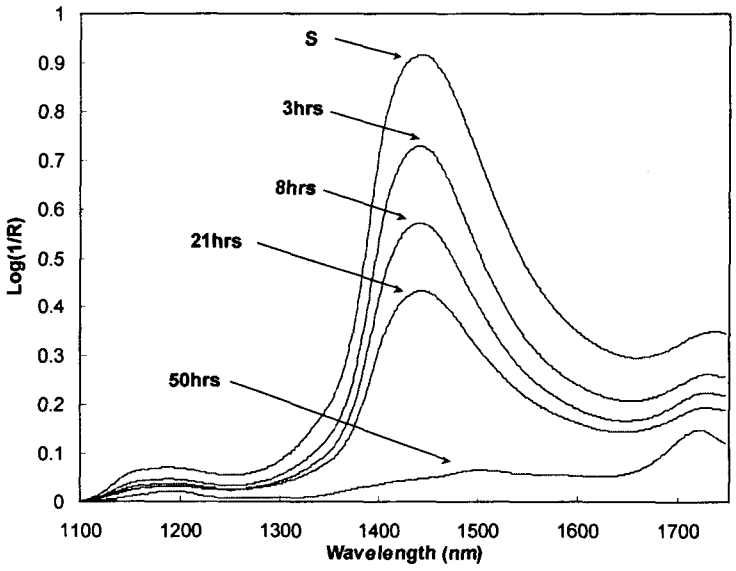
Drying time (hours)
A
B
C
D
E
F
G

Fig. 4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0.4
0.3
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Wavelength (nm)
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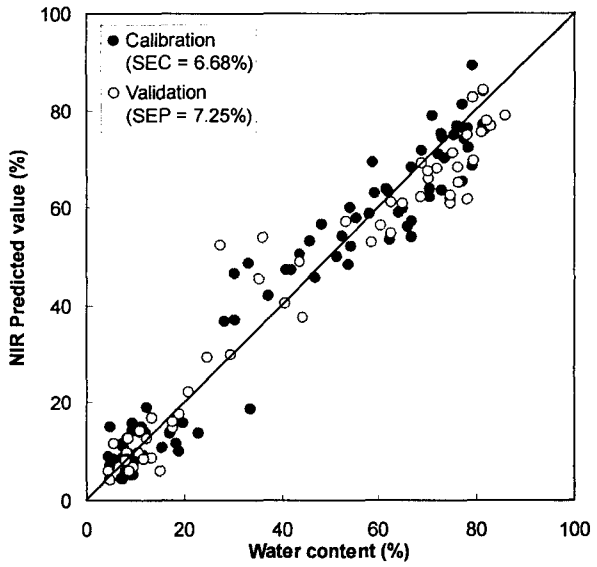
50hrs

Fig. 5

(a)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Validation
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Water content (%)
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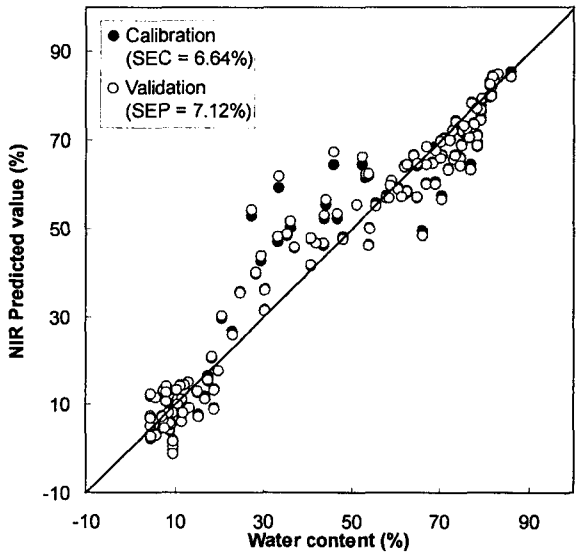
100

Fig. 5

(b)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Water content (%)
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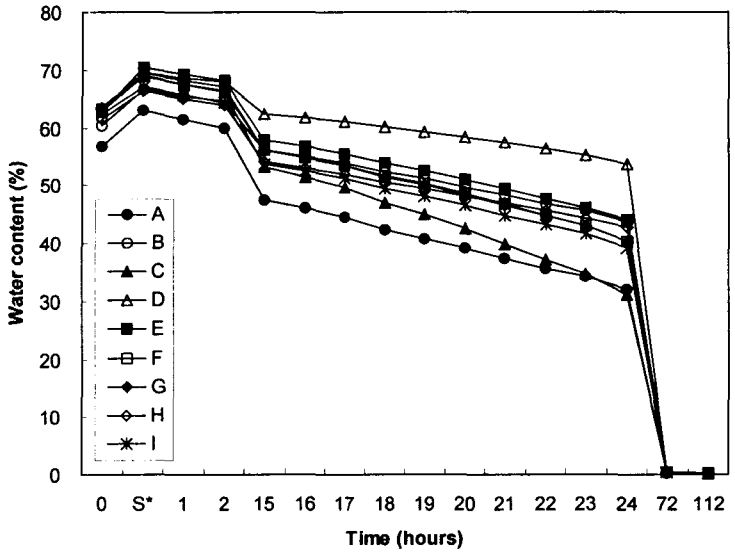
70
90

Fig. 6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10
0
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B
C
D
E
F
G
H
I
0
S*
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17
18
19
20
21
22
23
24
72
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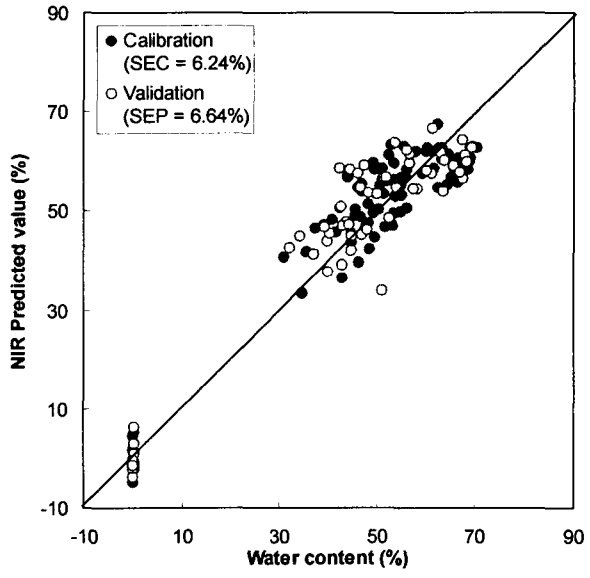
Time (hours)

Fig. 7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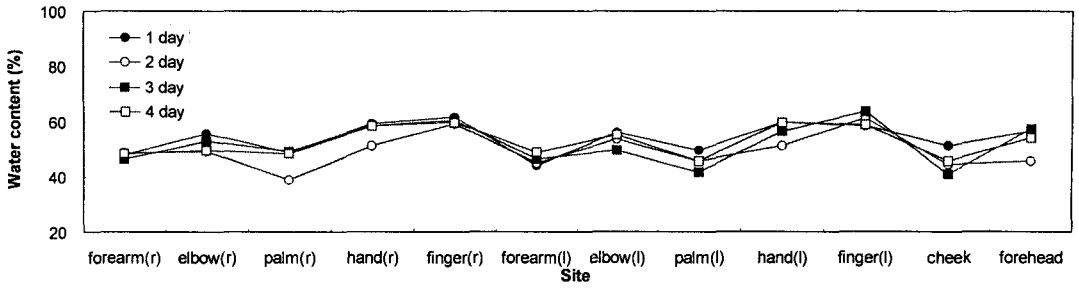
Water content (%)

Fig. 8

(a)



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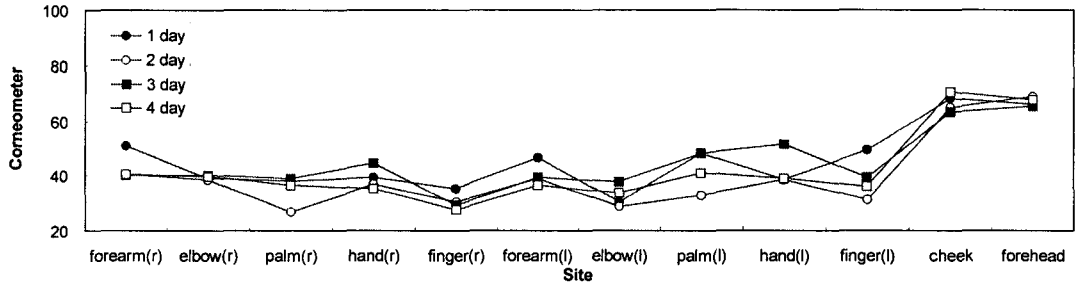
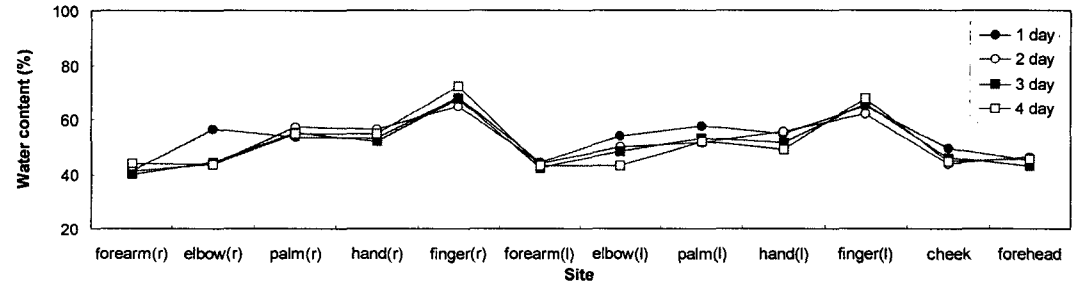


Fig. 8

(c)



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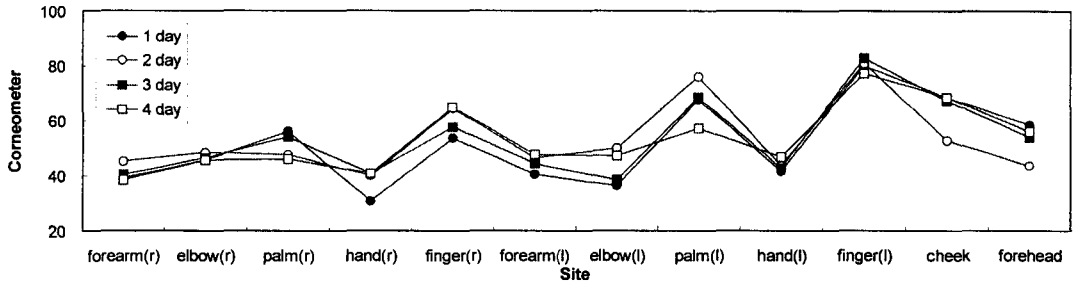


Fig. 9

(a)

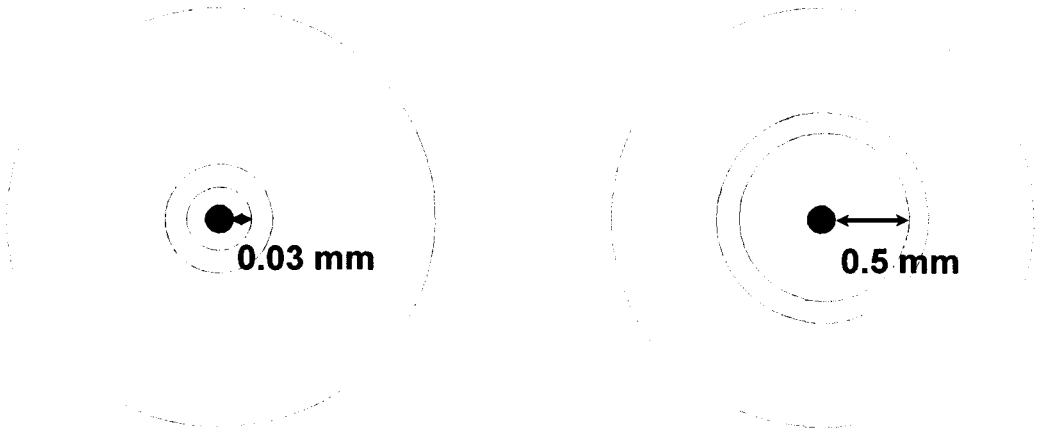


Fig. 9

(b)

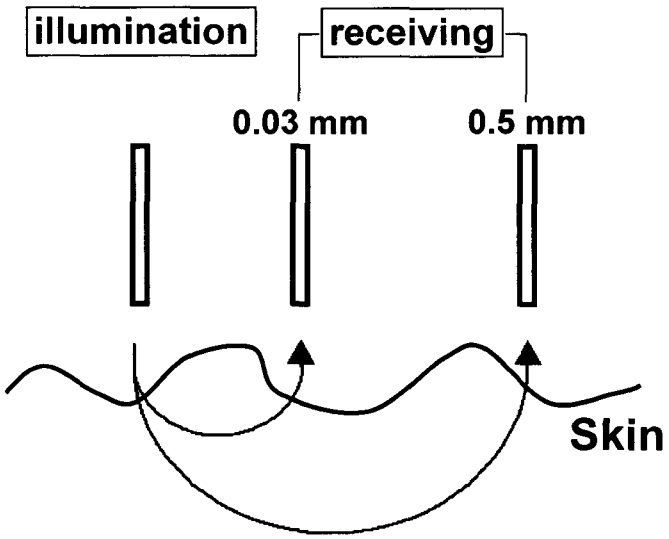


Fig. 10

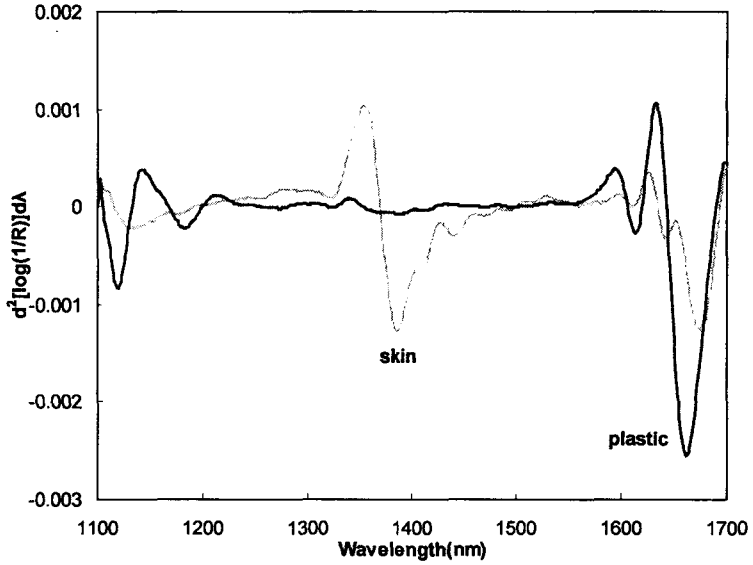

0.002
0.001
0
-0.001
-0.002
-0.003
d²[log(1/R)]dλ
skin
plastic
1100
1200
1300
1400
1500
1600
1700
Wavelength(nm)

Fig. 11

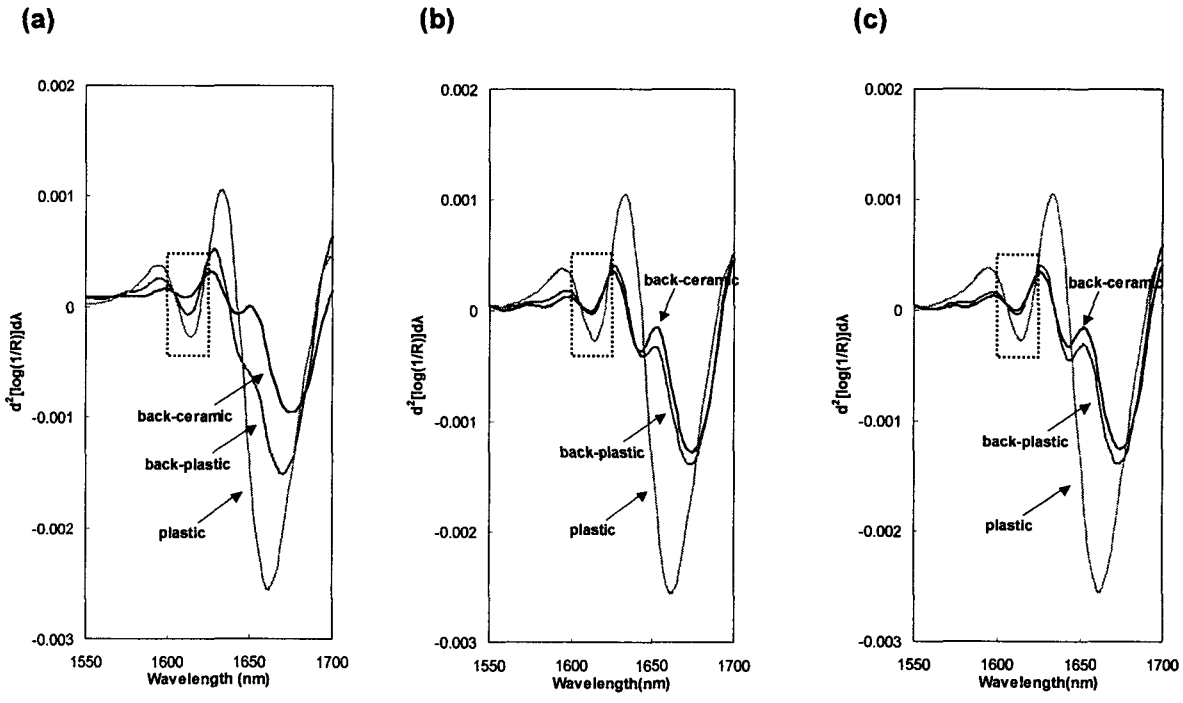
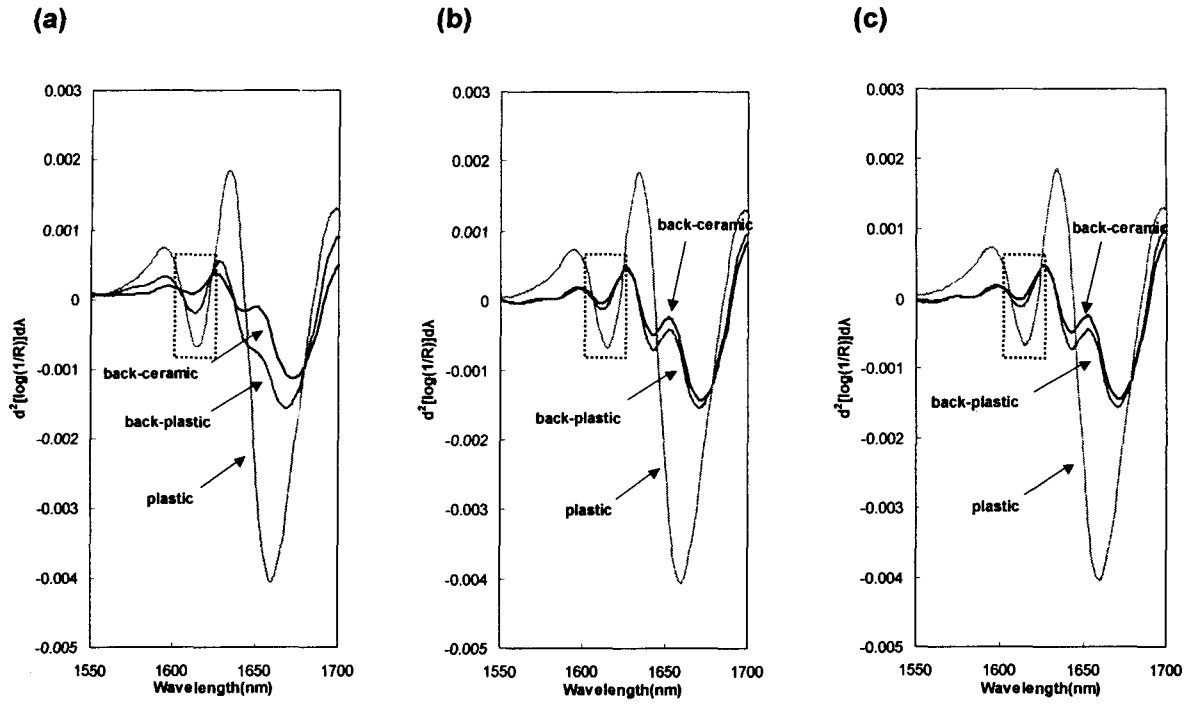

(a)
(b)
(c)
0.002
0.001
0
-0.001
-0.002
-0.003
d²[log(1/R)]dλ
back-ceramic
back-plastic
plastic
1550
1600
1650
1700
Wavelength (nm)
Wavelength(nm)

Fig. 12


(a)
(b)
(c)
0.003
0.002
0.001
0
-0.001
-0.002
-0.003
-0.004
-0.005
1550
1600
1650
1700
Wavelength(nm)
back-ceramic
back-plastic
plastic