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Moisture Measurement in the Stratum Corneum Through a Coating Formulation Using Terahertz Attenuated Total Reflection Spectroscopy

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Water in the stratum corneum (SC) is essential for maintaining healthy skin, making accurate measurement of the SC moisture content vital for developing effective skincare formulations. However, accurately evaluating the moisture content of the SC when coated with various formulations has been challenging. To address this, we previously introduced a terahertz attenuated total reflectance (THz-ATR) system that could determine the water content of the SC, even when a formulation was applied. Nevertheless, the consistency of the THz-ATR system with conventional skin moisture measurements has not been clarified, and the system cannot overcome the thick formulation interposition because it decreases the interacting field of the electromagnetic wave with the SC. In this study, we compare the THz-ATR measurements with skin moisture meters that use conductance or capacitance as indicators and investigate methods to mitigate the influence of thick formulations. This study demonstrates the consistency of our system with conventional skin moisture meters and mitigates the influence of formulation thickness by using the ratio of ATR signals at two different frequencies as a moisture index. Thus, the corrected ATR signal is useful for evaluating the moisture content of SCs coated with cosmetics. Therefore, it could potentially improve the accuracy of skincare product evaluation.

Key words: terahertz attenuated total reflection spectroscopy, attenuated total reflectance signal, conductance, capacitance, stratum corneum, moisture, obstacle, coating, formulation, film

1. Introduction

Water in the stratum corneum (SC) plays an important role in maintaining a healthy SC by providing flexibility and smoothness to the skin and maintaining the enzyme activity for barrier formation and desquamation.[1,2] Thus, improving and maintaining the moisture levels in the SC plays a major role in skincare formulations, and understanding the moisture level of the SC to which the formulation is applied is crucial for the development of an effective formulation. Conventionally, the moisture content of an SC is measured using electrical[3–5] and optical[6] methods. However, the electrical and optical properties of the formulation ingredients often affect the measured values. Therefore, accurate moisture measurement is challenging, especially when the formulation is applied to the SC. To solve this problem, the authors have reported the moisture measurement of SCs using terahertz attenuated total reflection spectroscopy (THz-ATR).[7,8]

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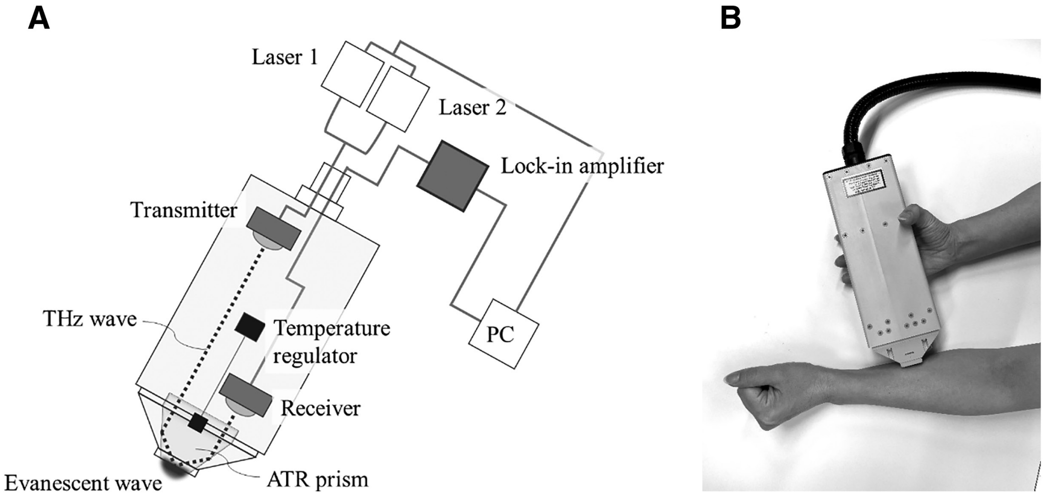

Fig. 1 Setup of the terahertz attenuated total reflection (THz-ATR) system. (A) Schematic of the THz-ATR system. (B) Photograph of the equipment used.

The THz-waves, electromagnetic waves with frequencies ranging from 0.1 to 10 THz, are strongly absorbed by water but can pass through various materials.[9] Therefore, we expected to measure the water content of the SC passing through the skincare formulations applied to the SC using the absorption intensity of the THz wave as an index. In addition, we used the ATR method, which generates an evanescent wave on the surface of a prism with a higher refractive index. This wave penetrates a sample put on an ATR prism, reducing the energy of the reflected wave based on their interaction.[10,11] The penetration depth of the evanescent wave is described as a function of an incident angle and the wavelength of the incident wave.[12] Therefore, we adjusted the penetration depth of the evanescent wave to the thickness of the SC by controlling the incident wavelength.

Previous experiments showed that the ATR signal acquired with the system correlated with the water content of the SC, and because the formulation ingredients exhibited minimal absorbance in the THz region, the ATR signal could accurately determine the water content of the SC even when a formulation was applied.[7,8] However, several tasks remain to enhance the practicality of the system. First, regardless of whether the formulation is applied, the consistency of the THz-ATR system with conventional measurements has not been elucidated, despite the fact that a number of studies on the moisture content of bare skin have provided valid results using these methods, especially when using the conductance or capacitance as an index. Second, during the practical use of the system, a thick formulation interposition between the ATR prism and the SC proved to decrease the ATR signal because it decreases the evanescent field that interacts with the SC.

In this study, we compared THz-ATR measurements with other skin moisture measurements using either the conductance or capacitance as an index to ensure consistency between these methods. In addition, we investigated strategies to mitigate the impact of thick formulations to reliably measure the moisture level of the SC, even when such formulations are applied.

2. Materials and Methods

This study was approved by the Naris Cosmetics Co. Ltd research committee. Informed consent was obtained from all volunteers before participation.

2.1. Instrumentation

The THz-ATR measurements were performed using the system shown in Fig. 1. A THz continuous wave was generated by photomixing two distributed feedback lasers (a wavelength-changeable light source mainly for communication use) with different oscillating wavelengths on a uni-traveling-carrier photodiode.[13,14] The THz wave was incident on an ATR prism made of high-resistance silicon at an incident angle of 50°. The reflected wave was received by a Fermi-level-managed barrier diode, which enabled wave detection with high sensitivity and low noise,[15,16] and was amplified with a lock-in amplifier. The ATR prism was kept at a similar temperature with the SC surface, 32°C,[17] using a temperature controller. As shown in Fig. 1B, the system from the transmitter to receiver was enclosed in a housing connected to lasers through flexible optical fibers, which enabled the ATR prism to adhere to subjects in variable postures.

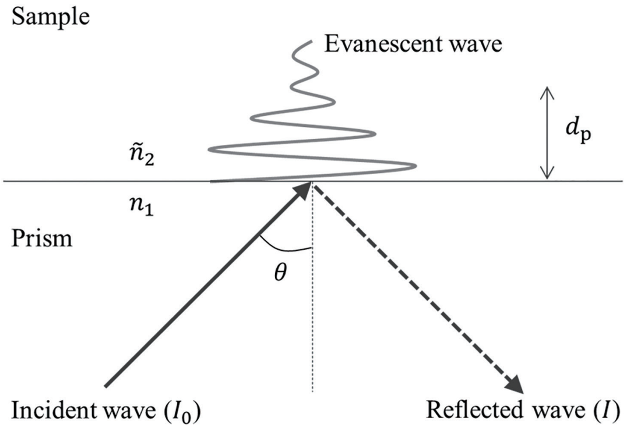

Fig. 2 Conceptual diagram of the THz-ATR system, where I_0 and I indicate the intensities of the incident and reflected waves, respectively; n_1 and $\tilde{n}_2$ refer to the refractive indices of the prism and sample, respectively; θ corresponds to the incident angle; and d_p corresponds to the penetration depth of the evanescent wave.

2.2. Concept of THz-ATR spectroscopy

As shown in Fig. 2, THz-ATR spectroscopy was used to observe the attenuation of the incident wave owing to the interaction between the evanescent wave and the sample attached to the ATR prism. When the sample is the skin which is topically applied cosmetic formulation, the degree of attenuation of the incident wave can serve as an indicator of the water content of the SC, provided that water, rather than the components of the formulation or SC components, is the primary absorber. The principle of this spectroscopy, along with the introduction of the parameters to be considered, is explained as follows.

When the electromagnetic wave enters a sample from a prism with a relatively higher refractive index at above an angle called the critical angle, the wave experiences total reflection with generating an evanescent wave which penetrates to the sample with a lower refractive index.[18] The amplitude of the evanescent wave decays exponentially with increasing distance from the surface of the prism, and the distance from the surface at which the intensity of the evanescent wave decays to $1/e$ of its initial intensity is defined as the d_p:

$$\begin{aligned} d_p &= \frac{\lambda}{\sqrt{2}\pi n_1 \sqrt{\sqrt{\xi^2+\mu^2}+\xi}} \\ \xi &= \sin^2\theta - \left(n_2/n_1\right)^2 + \left(\kappa_2/n_2\right)^2 \\ \mu &= 2n_2\kappa_2/n_1^{\,2} \end{aligned} \tag{1}$$

where θ is an incident angle, λ is the wavelength of the incident wave, and n_1 is the refractive index of the ATR prism. Considering the absorbance of the sample, its refractive index of the sample is expressed as a complex refractive index, $\tilde{n}_2 = n_2 + ik_2$, where n_2 is the refractive index and k_2 is the extinction coefficient, measured using THz time-domain spectroscopy. Equation (1) shows that the d_p can be modulated using θ and λ. Because the amplitude of the evanescent wave decays in the depth direction, the d_p slightly exceeding the thickness of the SC[19,20] interacts with the SC in areas with a strong electric field.

Calculating the d_p in the SC based on a preceding study[7] by substituting our system parameters ($\theta = 50°$, $n_1 = 3.42$) into Eq. (1), a frequency range of 850–1000 GHz was determined to be suitable for the incident wave, as it generates an evanescent wave with a d_p of 20–25 μm. Therefore, the measurements were performed with an incident wave in this frequency range.

ATR spectroscopy determines the absorbance of an electromagnetic wave at the sample surface by measuring the attenuation of the reflected wave relative to the incident wave caused by the interaction of the evanescent wave with the sample. The ATR signal, representing the degree of attenuation, is defined as follows:

$$\text{ATR signal} = -\log_{10}(I/I_0) \tag{2}$$

where I_0 and I represent the intensities of the incident and reflected waves, respectively.

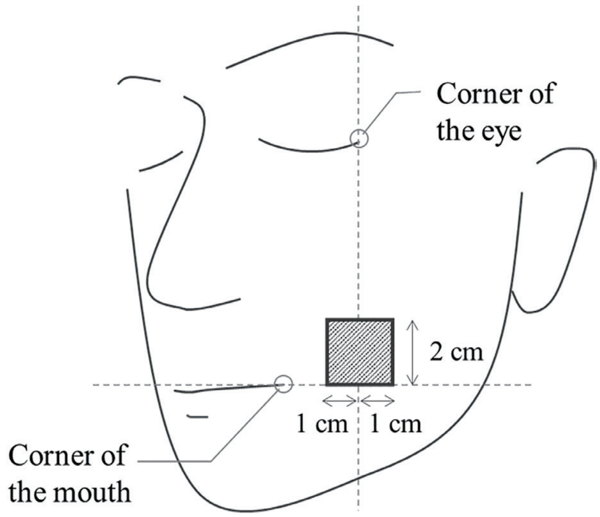

Fig. 3 Measured area used to evaluate the hydration state of the stratum corneum (SC).

Table 1 Thin films used as obstacles.

Thickness (μm)	Material	Manufacturer
5	Polyethylene terephthalate	Nitto Denko Corporation
10	Polyethylene	Ube Film, Ltd.
15	Polyethylene	Takeda Corporation
20	Polyethylene	Tsukasa Chemical Industry Co. Ltd.

2.3. Relationship between the ATR signal and the water content of the SC samples

Human SCs were collected from the heels of 28 healthy volunteers using a heel shaver (KAI CORPORATION) and mixed to prepare SC samples. SC samples with variable water contents were prepared by adding water in a stepwise manner. The ATR signals of the samples were obtained using a THz-ATR system with the sample attached to the ATR prism. The water content of the samples was determined using a Karl Fischer moisture meter MKV-710 (Kyoto Electronics Manufacturing Co. Ltd.).

2.4. Evaluation of the moisture levels in the SC

The moisture levels of the SCs of 53 healthy women (22–64 years old) were measured using the THz-ATR system, SKICON 200-EX (YAYOI), and Corneometer CM-825 (Courage + Khazaka) after the participants washed their faces and rested in a test room at 22°C with 50% humidity for 15 min. The measurement was conducted in a 2 cm × 2 cm-area above the intersection of a line drawn down from the corner of the eye and across the corner of the mouth on the left cheek of each participant (Fig. 3).

2.5. Measurement of the ATR signal through obstacles between the ATR prism and the sample

The ATR signal was measured when a butylene glycol–water mixture (25–100 wt% of water with an interval of 25 wt%) was placed over the 5–20 μm-thick obstacle shown in Table 1 on the ATR prism. The frequency of the incident wave was set to 850 GHz as the frequency at which the estimated penetration depth of the evanescent wave is above the thickness of the obstacle in this experiment. The 50 wt% butylene glycol–water mixture was used when the change in ATR signal as a function of the obstacle thickness was measured.

2.6. Measurement of the moisture level of the SC passing through a coating formulation

The moisture level of the SC of a healthy woman (46 years old) was measured using the THz-ATR system after applying 12 μL of a 10 wt% glycerin–water mixture and petrolatum to a 3 cm × 4 cm area on her inner forearm in a room at 25°C and 70% humidity. When the applied volume is simply divided by the area, the thickness is 10 μm.

3. Results

3.1. Relationship between the ATR signal and the water content of the SC samples

As shown in Fig. 4, the ATR signal and water content of the SCs exhibited a sigmoidal correlation, where the ATR signal monotonically increased along with the water content.

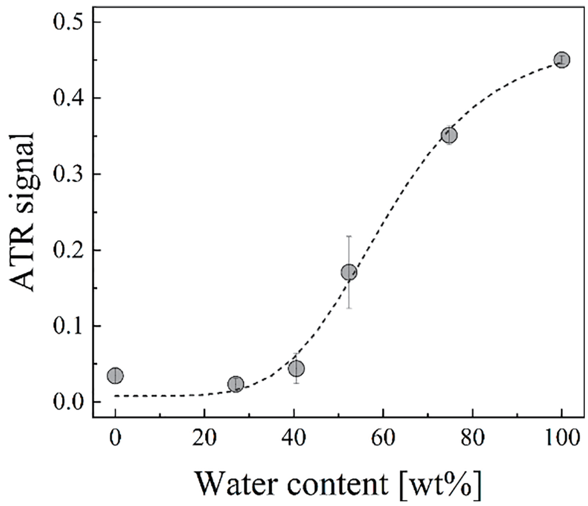

Fig. 4 ATR signal as a function of the water content in the SC samples. The error bars indicate the standard deviation of three measurements, and the dashed line is shown as a guide to the eye.

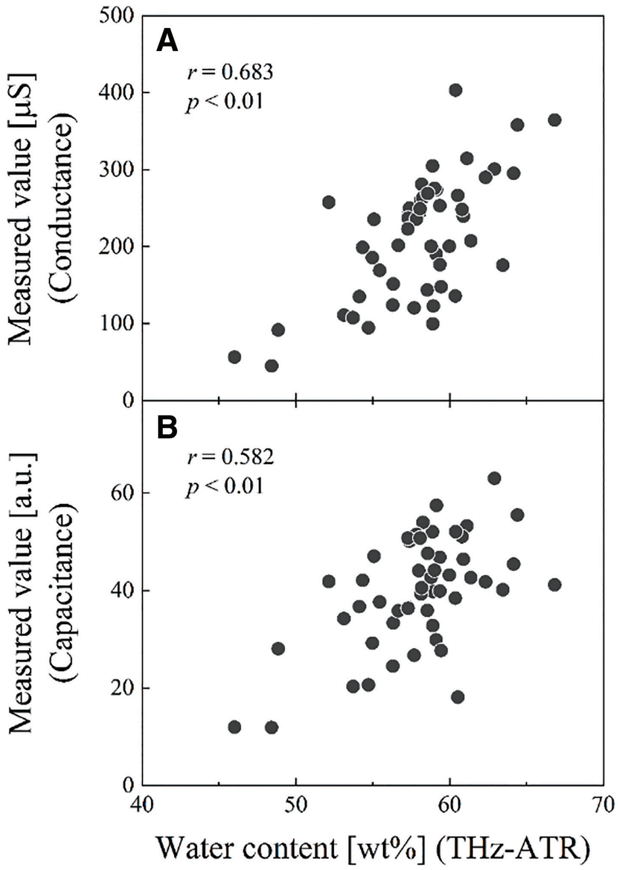

Fig. 5 Correlation between the moisture levels measured using conventional methods and the THz-ATR system. Pearson's correlation coefficient, r, and the significance probability, p, are shown in each figure.

3.2. Comparison of the THz-ATR and two conventional methods for measuring the moisture levels of SC

The ATR signal measured using the THz-ATR system showed a statistically significant correlation with the conductance and capacitance measured using a SKICON 200-EX and Corneometer CM-825, respectively (Fig. 5). The correlation strength was moderate for both based on a general understanding of the correlation coefficient.

3.3. Measuring the ATR signal of samples through obstacles between the ATR prism and the sample

The ATR signal measured using the THz-ATR system increased with the water content of the sample, and the trend was the same with and without the obstacle (Fig. 6A). However, when the moisture content was varied while keeping the

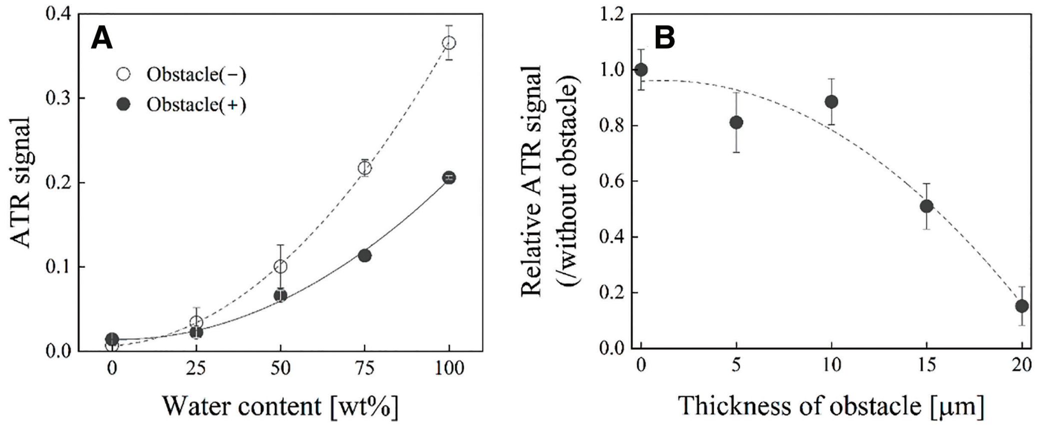

Fig. 6 ATR signals of the samples measured through obstacles between the ATR prism and sample. (A) ATR signal as a function of the water content. The error bars indicate the standard deviation of three measurements. The dashed and solid lines are shown as guides to the eye for the ATR signals in the absence (obstacle (−)) and presence (obstacle (+)) of an obstacle, respectively. (B) ATR signal of 50 wt%-butylene glycol–water mixture as a function of the obstacle thickness. The error bars indicate the standard deviation of three measurements, and the dashed line is shown as a guide to the eye.

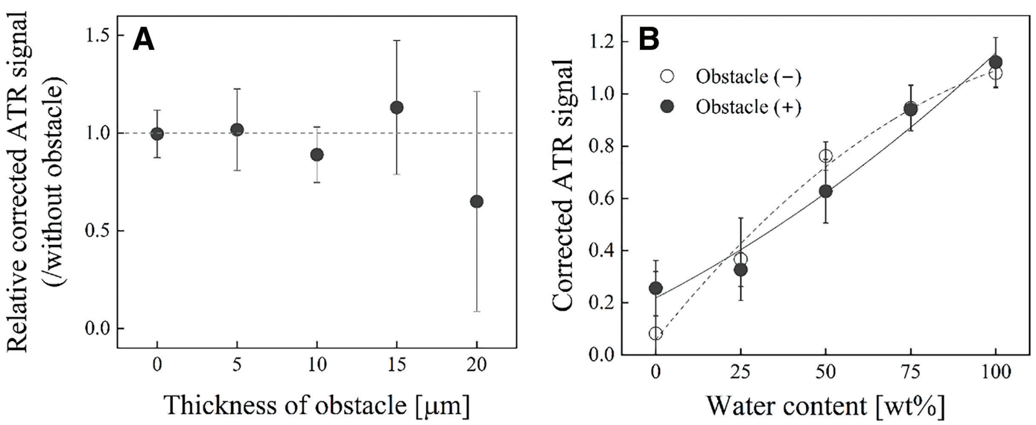

Fig. 7 Corrected ATR signals of the samples measured through obstacles between the ATR prism and sample. (A) Relative corrected ATR signal as a function of the obstacle thickness. The error bars indicate the standard deviation of three measurements. The dashed line represents the ideal correction value. (B) Corrected ATR signal as a function of the water concentration. The error bars indicate the standard deviation of three measurements. The dashed and solid lines are shown as guides to the eye for the ATR signals in the absence (obstacle (−)) and presence (obstacle (+)) of an obstacle, respectively.

obstacle thickness constant at 10 μm, the measurement through an obstacle yielded a lower ATR signal than that without an obstacle, and the difference increased with the moisture content of the sample. When the water content of the sample was constant, the decrease in the ATR signal depended on the thickness of the obstacle (Fig. 6B).

The ATR signal of the sample with a constant water content of 50 wt% was measured at two different frequencies, 850 and 1000 GHz, and the ratio (A_{850}/A_{1000}) was calculated as a corrected ATR signal. The relative ratio of the corrected ATR signal measured through an obstacle to that without an obstacle was approximately 1 in the obstacle thickness range of 0–15 μm, whereas the corrected ATR signal was conspicuously small when the obstacle thickness was 20 μm (Fig. 7A). When the moisture content was varied while keeping the obstacle thickness constant at 10 μm, the difference between the cases with and without an obstacle was smaller in the corrected ATR signal (Fig. 7B) than that in the ATR signal (Fig. 6A).

3.4. Measuring the moisture level of the SC passing through a coating formulation

As shown in Fig. 8, the ATR signal of the glycerol solution-treated SC was greater than that before application. However, the ATR signal decreased after the application of petrolatum. By contrast, the corrected ATR signal increased with glycerol solution application but did not show a definite change with petrolatum application.

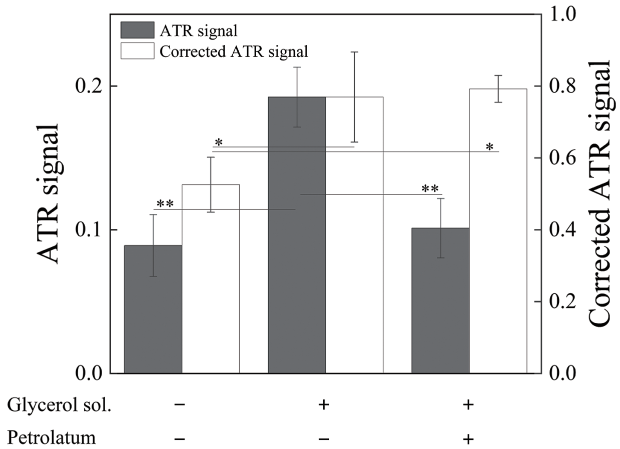

Fig. 8 Normal and corrected ATR signals of the SC passing through the coating formulation. The error bars indicate the standard deviation of three measurements. * $p < 0.05$, ** $p < 0.01$ (Tukey–Kramer's test).

4. Discussion

The evaluation of the moisture levels of SCs is important for assessing the effectiveness of cosmetic formulations. Conventionally, cosmetic chemists must wash off the formulation from the SC surface before measuring the moisture level to avoid interference from the physical properties of the formulation. However, this practice makes it challenging to accurately evaluate the effects of the formulation on the SC when applied. To solve this problem, we previously reported the usefulness of a THz-ATR system for measuring the water content of the SC.[7,8] Nevertheless, two issues emerged that required resolution: first, the consistency of the THz-ATR system with conventional skin hydration measurements has not been clarified; second, the impact of a thick formulation interposition could not be ignored because it decreased the evanescent field interacting with the SC. Therefore, we compared THz-ATR measurements with a skin moisture meter based on conductance or capacitance as an index using a newly built THz-ATR system and investigated a method to mitigate the influence of a thick formulation.

To compare measurements using different apparatuses, the measurement conditions must be uniform. In particular, the posture of the participants affects their sweating.[21] However, because the fixed ATR prism of our desktop THz-ATR system[7,8] compels the participants to tilt their heads to the side, asking them to maintain the same posture during some types of measurements is not practical. Thus, we built the THz-ATR system shown in Fig. 1, whose ATR prism was held in a flexible housing so that the participants only needed to sit comfortably during the measurement. This new instrumental design allowed us to compare measurements using the THz-ATR system with those using conventional systems in a sitting position. Before comparing the different apparatuses, the relationship between the ATR signal provided by the THz-ATR system and water content of the SC sample was investigated to determine whether the system could evaluate the moisture level. The ATR signal increased with increasing water content of the SC sample (Fig. 4), indicating that we could estimate the water content of the SC sample based on the relationship between the two.

When we compared the moisture measurement results from the THz-ATR system in this study with those from two conventional skin moisture meters based on conductance or capacitance, the results from the THz-ATR system correlated with those from both meters. Several studies using Raman spectroscopy have reported that the water content in the SC has a slope of 30–65 wt% in the depth direction.[6,20] Our results that the water content measured by THz-ATR system scattered in the range of 40–70 wt% is consistent, considering that it measured collectively without resolution in the depth direction. Skin moisture meters based on conductance or capacitance have been widely used and shown to detect differences in water content in several studies, such as comparisons between measurements of SCs artificially moistened with water and those without water,[3] and investigations of the relationship between water content and measured values on cellulose filters with varying levels of moisture.[4] Thus, the correlation shown in Fig. 5 presents the reliability of the measurement results of the THz-ATR system. The moderate degree of correlation was attributed to differences in measurement indices and depths between the devices. Unlike the THz-ATR system,[22] which is unaffected by electrolytes or lipids,[23] conductance and capacitance measurements are affected by them. In addition, the measuring depths of conductance and capacitance devices were reported to be 15–40 and 15–20 µm,[4,5] respectively, compared with an estimated depth of approximately 20–25 µm in our THz-ATR system. Cosmetic chemists should select the appropriate one depending on the purpose of the experiment.

As the consistency of our system with conventional skin hydration measurements was confirmed, we attempted to solve the problem derived from the interposition of a thick formulation between the ATR prism and SC. As shown in Fig. 6 using obstacles instead of formulations, the obstacles between the ATR prism and SC decreased the ATR signal as the obstacle thickness increased. Given that THz waves exhibit little absorption in substances other than water,[9] the results in Fig. 6 were caused by obstacles that reduced the interaction volume between the evanescent field and the sample. The weak signal at a 20-μm thick obstacle and no signal at 30 μm (data not shown) support this theory because the d_p of the evanescent wave in this experiment was set at approximately 20–25 μm. For practical use of the system in SC moisture evaluation, methods to reduce the impact of thick obstacles must be applicable regardless of the presence or thickness of these obstacles, as measuring their exact thickness for every measurement is not feasible. Referring to the spectrum of absorption coefficient, the value that is proportional to the ATR signal, of pure water in the frequency range of around 1 THz,[24,25] a frequency-dependent increase in the slope of the absorption coefficient is observed. Thus, if an obstacle is responsible for the attenuation of the ATR signal at a similar rate around the frequency, the ratio of the ATR signals at the two frequencies would be constant with or without an obstacle. However, this ratio would still be variable owing to the composition of the sample. In addition, substances other than water do not exhibit any apparent absorption in this range.[8] Hence, the slope observed in the ATR spectrum presumably correlates with the water content of the sample. The calculation of the ratio at two frequencies, namely, 850 and 1000 GHz (A_{850}/A_{1000}), as a corrected ATR signal, reduced the difference with and without an obstacle (Fig. 7A) and mitigated the thickness-dependent decrease in the signal (Fig. 7B). Considering the ratio was clearly smaller than 1 at a thickness of 20 μm, the corrected ATR signal could be used to evaluate hydration up to a thickness of 15 μm. To verify the applicability of the corrected ATR signal for SC moisture evaluation, measurements were performed on skin coated with model cosmetic samples. Consequently, both the normal and corrected ATR signals were higher on skin coated with a glycerol solution than those of uncoated skin. However, when the petrolatum was overlaid, the ATR signal decreased, whereas the corrected ATR signal was maintained (Fig. 8). There should be little or no change in the moisture content of the SC immediately after application of a sample that does not contain water. As calculated in section 2.6, the thickness of the petrolatum, which would remain rather longer on the skin, is roughly estimated to be 10 μm, thinner than the maximum thickness of the corrected ATR signal that could be applied. Thus, these results are considered to have resulted from successful correction, and of the two indices, the corrected ATR signal is more useful for evaluating the moisture content of SCs coated with cosmetics.

5. Conclusion

To achieve an accurate moisture evaluation of SCs with various coatings, we introduced a THz-ATR system to measure the water contents of the SC samples. Our study demonstrated that this system was consistent with conventional skin moisture meters and introduced a method to mitigate the influence of formulation thickness, although there was a limit to how much the thickness could be corrected. In future, to make moisture measurements using the THz-ATR system more practical, we intend to develop a system that can easily acquire multiple ATR signals according to the object being measured.

Abbreviations: SC, stratum corneum; THz-ATR, terahertz attenuated total reflection spectroscopy

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