"Toward Moisturizers Beyond Glycerol: Water Attraction and Additional Properties to Consider"

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1. Introduction

Water is vital for healthy skin, and the hydration of biomolecules is key to preserving their properties and enabling their full functionality [1]. Because dryness impairs skin health, maintaining optimal skin water content is a fundamental goal in skincare formulations. Humectants, ingredients that attract water, are central to achieving this goal.

Humectants have been evaluated in vitro, primarily based on their ability to attract water, typically by measuring the amount of water sorbed or lost per unit time [2]. However, considering that substances commonly used as desiccants, characterized by a large saturation absorption capacity for water [3], would likely perform well under this criterion, relying solely on this metric may result in the selection of humectants that dehydrate skin components, potentially impairing their intrinsic properties and functions. Owing to the lack of robust in vitro criteria for selecting humectants and the need to consider the retention of moisturizers on the skin in real-world applications, cosmetic researchers often rely heavily on resource-intensive in vivo testing. However, this approach has prevented a clear understanding of the most fundamental factor–the interaction between humectants and water–which would contribute to the development of useful in vitro criteria.

Among the various humectants, glycerol is widely used because of its remarkable ability to suppress water evaporation and improve skin conditions. Notably, glycerol lowers the water activity (Aw), which can be regarded as an indicator of water evaporation [4], while paradoxically enhancing the activity of skin enzymes [5, 6] that typically requires higher Aw. This paradox suggests that glycerol has ideal properties as a skin humectant, attracting water in a bioavailable state within the skin.

To clarify the manner of interaction between glycerol and water that underlies its unique properties, the present study systematically compared the effects of glycerol (GL) with those of two other polyols commonly used in cosmetics—1,3-butylene glycol (BG) and sorbitol (SB)—which have been empirically shown to exhibit lower moisturizing properties than GL, on the Aw, enzyme activity, and molecular behavior of water. By integrating cosmetological assessments and spectroscopic analyses, this study aimed to uncover previously unrecognized properties of humectants and to propose a more biologically relevant framework for future skincare formulations.

2. Materials and Methods

2.1. Test Samples

Water was deionized and distilled using an auto-stiller (WG252, Yamato Scientific Co., Ltd.) to obtain pure water. Polyol/water mixtures were prepared by mixing pure water with GL (> 99.5% purity; Fujifilm Wako Pure Chemicals Co., Ltd.), BG (> 98.0% purity; Fujifilm Wako Pure Chemicals Co., Ltd.), or SB (70% purity; Kao Corporation) to obtain a final polyol concentration of 0–80 wt% (SB up to 60 wt%).

2.2. Water Activity

The ratio of the vapor pressure of water in a sample (P) to that of pure water (P_0) is defined as Aw ($= P/P_0$) [4]. The Aw of the test samples was measured using a water activity analyzer (AW-Multi, Shibata Scientific Technology Ltd.), in which a capacitance hygrometer-equipped container with 3 mL of each sample was placed. The Aw value was recorded once the measurements stabilized.

2.3. Transglutaminase Activity

The transglutaminase (TG) activity was evaluated using a commercial kit (SensoLyte TG Activity Assay Kit, Colorimetric, AnaSpec Inc.), and the protocol was partially modified for the purpose of this study. Specifically, a portion of the reaction buffer was replaced with the test sample to achieve the desired concentration in the system, whereas the standard practice involves simply adding the sample to a fixed amount of reaction buffer. The TG activity was determined as the TG equivalent using a calibration curve that represents the relationship between the TG amount and absorbance.

2.4. Mid-Infrared Spectrum in the HOH Bending Region

Raman and infrared absorption spectra in the HOH bending region were obtained at room temperature using a Raman spectrometer (gen2-SCA, RiverD International B.V.) and a Fourier-transform attenuated total reflection spectrometer (FT/IR-4600, Jasco Corporation) equipped with an ATR PRO ONE unit (Jasco Corporation), respectively. To investigate the peak position of the HOH bending band in the polyol/water mixtures, the position of the peak centroid ν_c was derived using the following equation:

$$\frac{\int_{\nu_1}^{\nu_c} I(\nu)\,\mathrm{d}\nu}{\int_{\nu_1}^{\nu_2} I(\nu)\,\mathrm{d}\nu} = \frac{1}{2} \quad,$$

where ν_1 and ν_2 represent the wavenumbers on the low- and high-wavenumber sides, respectively, that enclose the region where the second derivative of the HOH bending spectrum is negative, and $I(\nu)$ represents the second derivative value.

2.5. Absorbance at 1 THz

The 1 THz absorbance of the test samples was measured using a custom-built terahertz attenuated total reflection system [7]. A 2 mL sample in a 35 mm dish was brought into contact with the ATR prism maintained at 25°C and measured.

2.6. In-Vivo Test

This study was approved from an ethical perspective by the internal review board. The participants provided written consent after being informed of the study details. Five participants (20–50s) applied the samples to their inner forearms (3×3 cm area) two times per day for 5 d. Before and after application, after resting for 15 min in a room with constant temperature (22°C)

and humidity (50%), the skin water content was measured using a SKICON-200EX (Yayoi Co., Ltd.). Simultaneously, stratum corneum (SC) cells in the test areas were collected using the tape-stripping method and subjected to a cornified envelope (CE) assay.

2.7. Cornified Envelope Assay

The tape with adhered SC cells was finely cut and boiled in 1 mL of buffer (2% SDS, 20 mM DTT, 5 mM EDTA, 0.1 M Tris-HCl, pH 8.5) for 10 min. The tape substrate was removed and the residual SC cells were washed four times following the same procedure. The washed SC cells were dried on a glass slide, fixed using cold acetone, and mounted on a cover slip with Nile Red solution (3 μg/mL in 75% glycerol) to stain the CE. Fluorescence from Nile Red emitted in response to the hydrophobicity of the CE was observed.

3. Results

3.1. Aw of the Polyol/Water Mixtures

As shown in Figure 1, the Aw values of the polyol/water mixtures decrease and the differences between the polyols become more pronounced as the polyol concentration increases. Among the tested polyols, GL exhibits the strongest Aw reducing effect, followed by BG and SB.
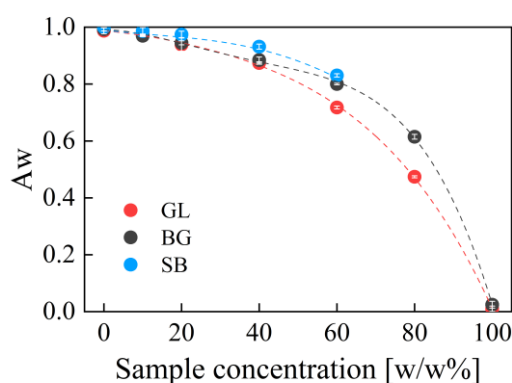
Figure 1. Aw of polyol/water mixtures. The error bars indicate the standard deviation of three measurements, and the dashed lines serve as visual guides.

### 3.2. TG Activity in the Polyol/Water Mixtures
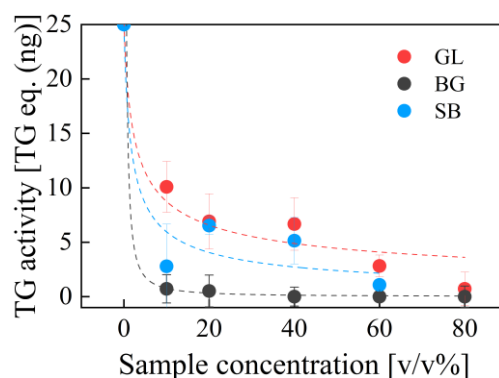
Figure 2. TG activity in polyol/water mixtures. The error bars indicate the standard deviations of three measurements, and the dashed lines serve as visual guides.

The TG activity in the polyol/water mixtures decreases as the polyol concentration increases, irrespective of the polyol species (Figure 2). Among the tested polyols, BG shows a significant inhibitory effect on the enzyme activity, even at low concentrations. In contrast, the inhibitory effects of GL and SB are moderate compared with that of BG.

3.3. HOH Bending Band of the Polyol/Water Mixtures

In the HOH bending band observed in the Raman spectrum, only the BG/water mixtures exhibit a distinct peak shift with increasing concentration (Figure 3). In the infrared absorption spectra, the peak shift observed in the BG/water mixtures remains the largest, and the GL/water mixtures also show a moderate shift (Figure 4).

(a)   (b)
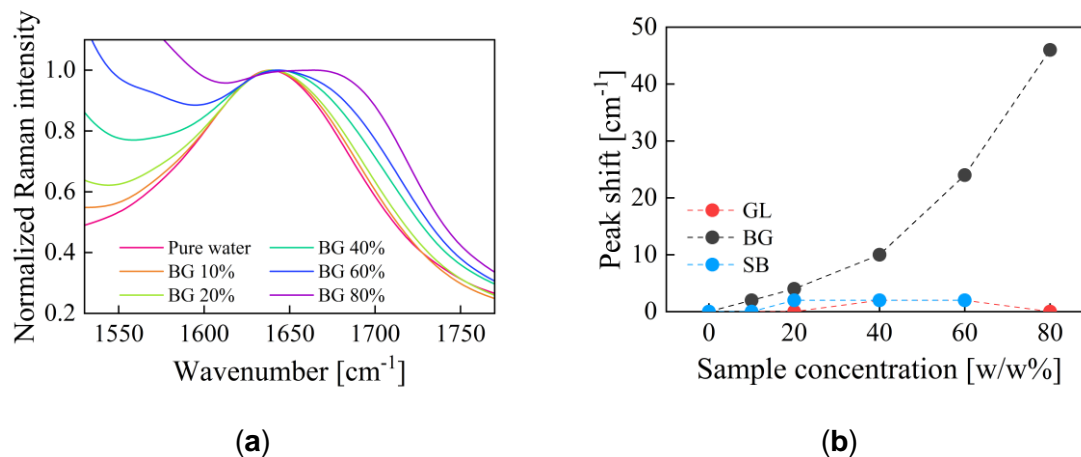
Figure 3. (a) Raman spectra of the HOH bending of the BG/water mixtures; (b) Peak shift of the HOH bending of the polyol/water mixtures. The dashed lines serve as visual guides.

(a)   (b)
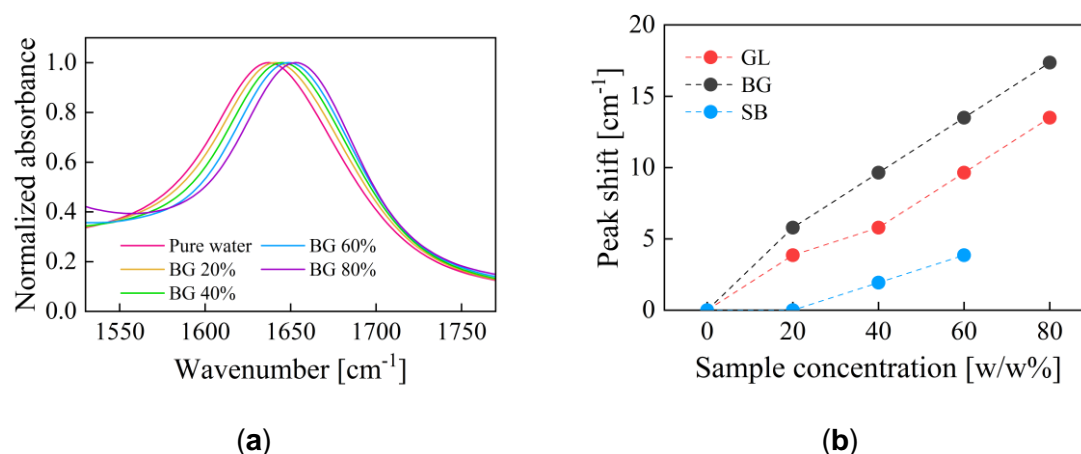
Figure 4. (a) Infrared absorbance spectra of the HOH bending of the BG/water mixtures; (b) Peak shift of the HOH bending of the polyol/water mixtures. The dashed lines serve as visual guides.

### 3.4. Absorbance of the Polyol/Water Mixtures at 1 THz

Although the 1 THz absorbance of the polyol/water mixtures decreases as the polyol concentration increases (Figure 5a), that of the BG/water mixtures decreases more steeply than those of the GL/water and SB/water mixtures. When the 1 THz absorbance is shown as a function of the molar concentration of water (Figure 5b), the absorbance of the BG/water mixtures is less likely to increase with increasing concentration.
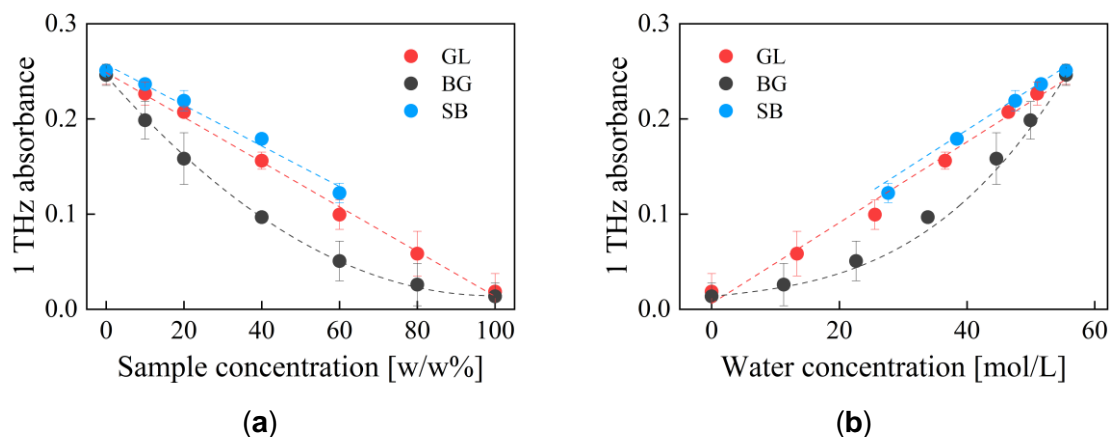
Figure 5. Absorbance of polyol/water mixtures at 1 THz: (a) as a function of polyol concentration, and (b) as a function of water molar concentration. The error bars indicate the standard deviations of three measurements, and the dashed lines serve as visual guides.

3.5. Relationship Between Skin Condition and the Effects of the Test Samples on the Water Molecular Behavior

The Aw values and 1 THz absorbance of various samples were measured to identify a test sample that could reduce Aw more effectively than glycerol, without hindering the water molecular behavior (Figure 6a). The identified sample is indicated by the green dot in Figure 6a. As expected, the effect of the sample on the TG activity is equivalent to that of GL (Figure 6b). The in-vivo comparison of GL, the sample, and BG in Figure 6b indicates that the skin water content in the area where the BG/water mixture is applied is comparable to that of water. In contrast, the selected sample results in a skin water content higher than those resulting from the GL and BG/water mixtures, although the difference from GL is not significant. In the CE assay results presented in Figure 7, the CE formation in the area where the sample/water mixture is applied is enhanced compared to that of the GL/water mixture, whereas CE formation is enhanced in the area where the BG/water mixture shows minimal CE formation.
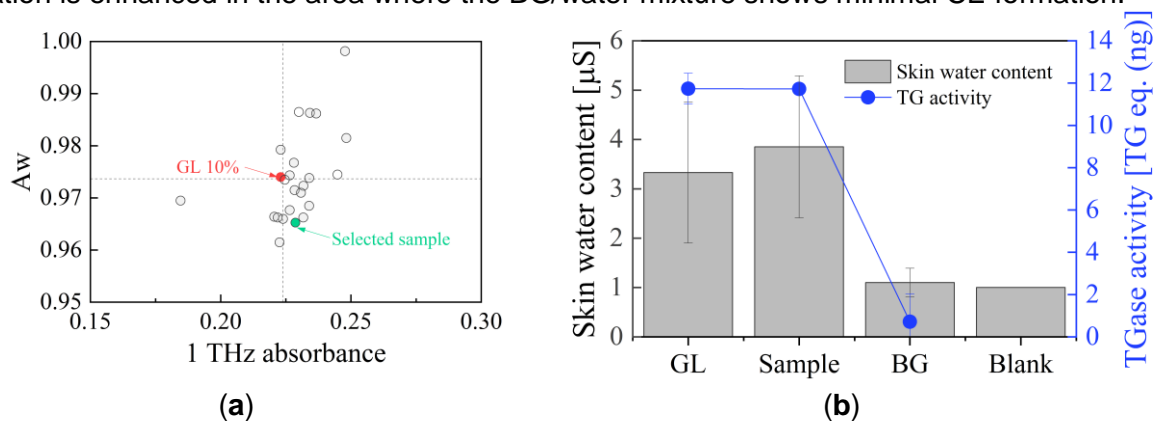
Figure 6. (a) Scatter plots of Aw and 1 THz absorbance; (b) Skin water content 8 h after applying each sample and TG activity in the mixture. The error bars indicate the standard deviations of five participants and three measurements, respectively.
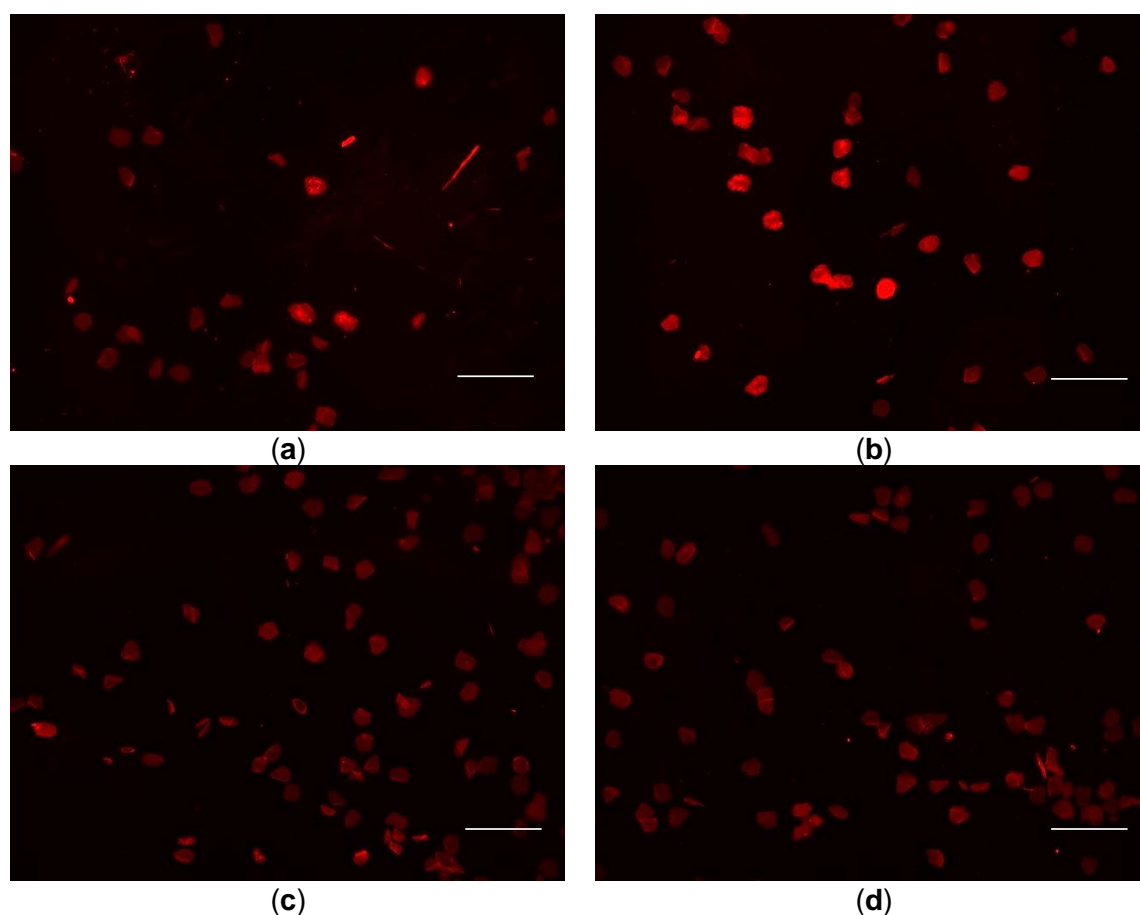
Figure 7. Representative observations of the CE stained with Nile red (red). SC cells from (a) GL-, (b) sample-, (c) BG-, and (d) pure water-treated areas. Scale bar = 200 μm.

4. Discussion

This study focused on the unique water-attracting mechanism of GL, which lowers Aw while enhancing the enzyme activity in the skin, which typically requires high Aw. To improve understanding of the water-attracting mechanism of GL that underlies this paradox, this study compared three polyols (GL, BG, and SB) based on their water-attracting capacity, their effects on skin enzyme activity, and the molecular behavior of water observed by spectroscopy.

The Aw of the polyol/water mixtures was measured as an index of the water-attracting capacity (Figure 1). All samples showed lower Aw at higher concentrations, and the extent of the reduction followed the order of GL, BG, and SB. Because a lower Aw indicates a higher water-attracting capacity, the water-attracting capacity follows the same order. A study directly comparing these three polyols simultaneously could not be found; however, the results presented in Figure 1 agree well with previously reported Aw values for individual polyols [8, 9].

The activity of TG was used as an indicator of the skin enzyme functionality. TG is an enzyme critical for CE formation in the SC, and its enzymatic activity has been reported to correlate with relative humidity [10]. Thus, the activity of this enzyme is considered to indicate the water availability in the surrounding environment. As shown in Figure 2, the TG activity of the polyol/water mixtures decreased with increasing polyol concentration. Given that the water

content in the mixture decreased as the polyol concentration increased, the corresponding reduction in the enzyme activity, which requires water, is reasonable. However, the TG activity-lowering effect of BG was significant, and the effect appeared at such a low concentration that it could not be accounted for by water loss alone. Previous articles have reported that a greater amount of hydration water surrounds a BG molecule than a GL molecule [11]. Therefore, the present results suggest that the molecular state of water may serve as a useful indicator of water availability to biomolecules.

The hydration of a solute is induced by factors such as hydrogen bonding between the solute and water molecules, as well as the hydrophobicity of the solute [1], which affects the vibrational and rotational behaviors of the water molecules. To investigate the impact of each polyol on the water molecules, the differences in the molecular behavior of water among the polyol/water mixtures were analyzed. Specifically, this study aimed to understand the extent to which each polyol influenced the water molecules. Raman spectra were analyzed to elucidate the changes in water molecular vibrations with respect to the polyol species and concentration of the polyol/water mixtures (Figure 3). In mid-infrared Raman spectra, signals from OH stretching and HOH bending vibrations are observed [12]. However, the OH groups of each polyol overlap with the OH stretching region of water molecules, making it difficult to isolate the changes specific to the water molecules experimentally. Therefore, this study focused on the HOH bending region. In the Raman spectra, only the BG/water mixtures exhibited a distinct peak shift from the peak position of water. Because a peak shift of the HOH bending band to a higher wavenumber has been reported to be induced by strong hydrogen bonding and the isolation of water molecules in the mixture [13, 14], the peak shift observed in the BG/water mixtures suggests that BG affects the environment surrounding water molecules more strongly than GL and SB. Considering that the Raman spectra show relatively weak HOH bending signals, the infrared absorption spectra of the polyol/water mixtures were also examined, and these signals were more clearly observed (Figure 4). The significant changes caused by BG were the most pronounced with this method as well. Using this approach, a peak shift was also observed in the GL/water mixtures; thus, these results suggest that GL may not be completely absent from interactions with water, including hydrogen bond formation.

To investigate the change in water molecular rotation, the 1 THz absorbance of the polyol/water mixtures was measured (Figure 5). Referring to the spectrum of the dielectric loss, which is related to the absorption coefficient, of the GL/water mixture [14, 15], the signal dispersion of pure water is centered at approximately 20 GHz, corresponding to its rotational motion, whereas the center exhibits a low-frequency shift with increasing GL concentration. The resulting spectrum is considered to be a combination of the signal dispersion of GL and hydration water, both centered at frequencies lower than that of pure water, and that of residual bulk water, whose signal center remains unchanged from that of pure water. Because the tail of the signal dispersion of bulk water extends to around 10 THz [16], the 1 THz absorbance of the polyol/water mixtures is considered to approximately reflect the presence of bulk water, although contributions from other relatively minor dielectric relaxation processes near 1 THz may also be present. As shown in Figure 5b, the relationship between the molar concentration of water and the 1 THz absorbance exhibited a concave curve in the BG/water mixtures, whereas

in the GL/water and SB/water mixtures, the relationship was nearly linear, connecting the points at 0 mol/L (pure polyol) and 55.5 mol/L (pure water). These results indicate that, in the BG/water mixtures, bulk water decreases more than the change in the number of water molecules in the mixture. Considering that hydration water exhibits a peak at a frequency lower than that of pure water, the low 1 THz absorbance in the BG/water mixture is attributed to the generation of hydration water in the mixture. Conversely, the almost linear increase in 1 THz absorbance observed in the GL/water and SB/water mixtures is likely indicative of a smaller amount of hydration water in the mixtures. Taken together, these results suggest that, among the three polyols, BG has the strongest impact on the water molecular behavior.

Hydrogen bonding interactions with water molecules are often associated with the water-attracting properties of humectants [2, 17]. Indeed, there are cases—such as SB—where both the ability to attract water and the interaction with water molecules are minimal. Nevertheless, the present results revealed that the interaction between GL and water molecules is not particularly pronounced, contrary to expectations based on its high water retention capacity. Instead, BG, which is less effective than GL in suppressing water evaporation, induces more significant changes in the state of water molecules. Considering the strong inhibition of TG activity observed in BG/water mixtures, the ability of GL to attract water with fewer interfering water molecules could be an important factor in achieving the fundamental goal of skin moisturization: maintaining the functionality of molecules, such as proteins, including TG, within the skin. These findings suggest that combining the Aw and water molecular behavior offers a new criterion for selecting effective humectants.

To evaluate the validity of this criterion, a sample that lowers Aw more effectively than GL without reducing the 1 THz absorbance was selected (Figure 6a), and its effects on the skin were examined (Figure 6b). In this screening, observation of the HOH bending band was not suitable for application to all samples because the characteristic signals of the candidate samples sometimes overlapped on the band. As expected, the selected sample demonstrated a superior moisturizing effect, with a low inhibitory effect on TG, ultimately promoting CE maturation. These results indicate that the aforementioned criteria are valid and useful for introducing a robust framework for evaluating humectants and advancing skincare formulations.

5. Conclusion

This article highlights the paradoxical properties of GL, which attracts water without impairing the function of skin components, suggesting its unique interactions with water. Compared to other polyols such as BG and SB, although GL inhibits water evaporation, its impact on the molecular behavior of water is notably small. These results suggest the importance of considering the molecular behavior of water in addition to water attraction.

This study proposes a new framework for evaluating humectants by integrating the Aw and water molecular behavior, which offers a more comprehensive criterion for selecting ingredients that maintain skin water content and support biological functions. Furthermore, because the Aw and indicators of the molecular behavior of water, unless the peaks overlap, can be used to assess the effects of mixtures and chemicals with undefined structures, such as plant

extracts, this approach can improve skincare formulations by enabling the selection of humectants that preserve skin functionality.

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