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Terahertz Time-Domain Spectroscopy to Identify and Evaluate Anomer in Lactose

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ABSTRACT

Lactose powder consisting of α-D-lactose monohydrate and anhydrous β-D-lactose was nondestructively and quantitatively evaluated by transmission-type Terahertz time-domain spectroscopy (THz-TDS). An absorption with peak at 39.7 cm^{-1} (1.19 THz) was assigned to be derived from anhydrous β-D-lactose, in addition to the absorptions due to α-D-lactose monohydrate with peak at 17.1 cm^{-1} (0.53 THz) and 45.6 cm^{-1} (1.37 THz). After deconvolution of the spectra using Lorentzian, integrated intensities of the absorptions with peak at 39.7 cm^{-1} and 45.6 cm^{-1} were uniquely dependent on the weight composition ratio of the α- and β-lactose powder. As a result, the net molar-ratio of the α- and β-lactose in lactose powder could be precisely evaluated by the integrated intensity ratio. Further, anomer content in lactose powder extracted from lactose solution was evaluated and the refined and unrefined features were shown by the evaluation method.

Keywords: THz-TDS; Lactose-Powder; Anomer; α-Lactose; β-Lactose

1. Introduction

Lactose comprising a glucose linked to a galactose abundantly present in milk of most mammals is an important disaccharide used in foods and pharmaceutical applications. Two anomers (α-lactose and β-lactose) commonly exist in the disaccharide powder. Evaluation and control of the ratio are important to use lactose in foods and drugs because the α- and β-form show significantly different physicochemical properties, *i.e.* solubility (β-form is more soluble than α-form) and hardness (α-form is harder than β-form), and crystallized shape [1]. Commonly, lactose can be prepared as α-lactose monohydrate and anhydrous β-lactose crystalline in addition to the amorphous phase of the α- and β-form mixture. The α- and β-crystals are usually formed in supersaturated lactose solution, where α-lactose can be crystallized with preventing the β-crystallization at room-temperature but the β-lactose is condensed above 93.5°C with extracting of the α-crystal [2]. Amorphous lactose fabricated by freeze-drying or spray-drying is crystallized into several crystal forms such as α-lactose monohydrate [3], anhy-

drous β-lactose [4] and anhydrous crystal with α- and β-lactose in molar ratios of 5:3 and 4:1 [5]. The crystallization which may enhance both physical and chemical deterioration [6] is dependent on the composition ratio, drying process, storage temperature, period and humidity [7], where the precise evaluation of crystallized α/β-form ratio is so important to study the crystallization feature.

Commonly, the α-lactose/β-lactose ratio and the crystallization behavior are analyzed by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) [8,9], Raman spectroscopy [10], Fourier transform infrared spectroscopy (FTIR) [11], X-ray diffraction (XRD) [7,12] etc. However, the TGA and DSC bring to destruction of sample, and the Raman spectroscopy, FTIR and XRD can evaluate in significantly thin region limited within dozens of micrometers. In contrast, THz-spectroscopy is expected to be useful for nondestructive characterization of materials with the thickness in millimeter-order because THz-electromagnetic wave in very-far-infrared region is notably absorbed in water but easily passed through most inorganic and organic materials comparing to UV-vis-infrared light. Further, it has been recognized that THz-absorptions based on the intra-mo-

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lecular vibrations in organics and/or the inter-molecular dynamics incorporated hydrogen-bonding are significantly dependent on molecular- and crystal-structure as demonstrated for DL-alanine racemic compound comparing to D- and L-alanine [13], L-phenylalanine comparing to L-tyrosine [14], and three different retinal isomers with polyene chain [15]. Many monosaccharides and disaccharides are also interesting biomolecules for the THz-spectroscopy since they show the spectral fingerprints with relatively narrow-bands in THz-region [16,17]. α-D-lactose also shows the typical absorptions with narrowbands in THz-region below 3 THz [18] as well as the other disaccharides. However, THz-spectroscopy has not been used to determine the anomer content because the absorption spectra derived from β-D-lactose has not been identified.

In this study, transmission-type THz time-domain spectroscopy (THz-TDS) is applied to characterize lactose. Firstly, we focus to identify THz-absorption due to β-D-lactose, then achieve quantitative evaluation of net molar ratio of α- and β-lactose in the mixtures using integrated intensity ratio of the absorptions due to α-D-lactose and β-D-lactose.

2. Experimental

2.1. THz-TDS System

Transmission-type THz-TDS system as shown in **Figure 1** was used to characterize lactose-powers. After a femto-second laser-light (peak wavelength at 782 nm, half-width of 87 fs, repetition rate of 48 MHz) was split into a pump light and a probe light, the pump light chopped at 1 kHz was focused and irradiated on a THz emitter consisting of a dipole antenna with 10 μm-gap space fabricated on LT-GaAs layer and attached on a hemispherical Si-lens, in which the antenna was biased at 10 V to generate transient current in pico-second order. The radiated THz-pulse through the Si-lens was focused
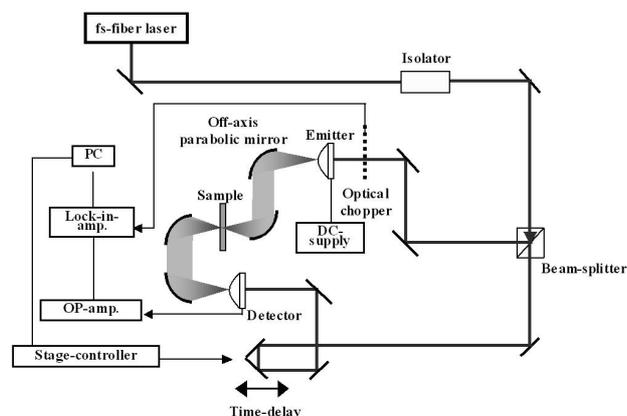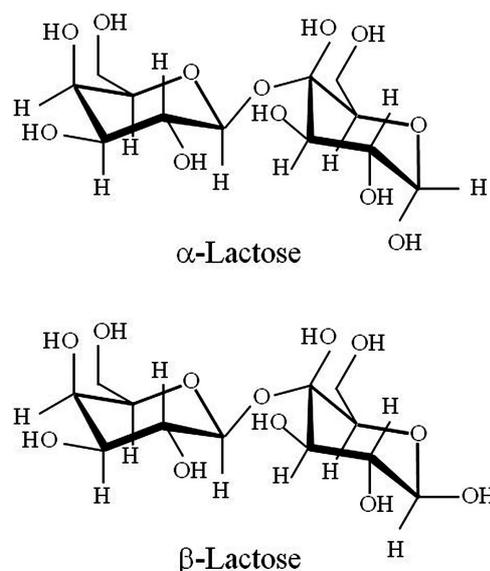
and normally incident to the sample using two off-axis parabolic metal mirrors. Then the THz-pulse passed through the sample was introduced to the detector with the same antenna configuration of the emitter by two off-axis parabolic mirrors and a hemispherical Si-lens. When the THz-pulse was introduced to the antenna on the detector, the probe laser light was simultaneously irradiated to detect the pulse by sampling technique, where time-delay of the probe light was controlled by a retro-reflector and a micro-step stage controller with the step length of 1 μm. The sampling data were recorded in a PC after signal-amplification, lock-in noise reduction and A/D conversion. The recorded pulse data was processed by a DFT after a Gaussian-window was superposed on the pulse data to remove aliasing. Humidity and temperature in the THz-TDS system was carefully controlled below 5% and at 20°C, respectively, by a dehumidifier and a heater system to prevent THz-absorptions of water vapor [19] and thermal fluctuation of samples.

2.2. Preparation of Lactose Sample

Two types of lactose powders purchased from Sigma-Aldrich Co., Ltd. were used for source materials. The one was α-D-lactose monohydrate ($L_\alpha \cdot H_2O$: O-β-galactopyranosyl-(1→4)-α-D-glucopyranose monohydrate ($C_{12}H_{22}O_{11} \cdot H_2O$)) and the other was anhydrous β-D-lactose (L_β: O-β-galactopyranosyl-(1→4)-β-D-glucopyranose ($C_{12}H_{22}O_{11}$)), in which a hydrogen- and a hydroxyl-coordination in the glucose-unit are spatially different in each other as shown in **Figure 2**. It should be noted here that the lactose powders were including the anomer. The anomer content in the $L_\alpha \cdot H_2O$ powder (content of L_β) and the L_β powder (content of $L_\alpha \cdot H_2O$) were about 4% and

Figure 1. Schematic configuration of transmission-type THz time-domain spectroscopy (THz-TDS) system.

Figure 2. Molecular structures of α-D-lactose (α-lactose) and β-D-lactose (β-lactose).

below 30% in the commercially specifications, respectively. The lactose powers were mixed with various weight ratios and milled, then filled in a metal aperture with a hole and compressed to form pellet of 6 mm-diameter and 0.7 mm-thickness with parallel surfaces. The pellet was placed with the aperture in the THz-TDS system and THz-wave was normally incident to the pellet surface. After the THz-TDS measurement, weight of the lactose-pellet was measured by an electronic weight-scale.

2.3. Extraction from Lactose Solutions

Two types of extract processes (refine and unrefined process) were used to examine the extracting behavior of $L_\alpha \cdot H_2O$ from lactose solution, where the $L_\alpha \cdot H_2O$ powder was dissolved in ultra-pure water with the resistivity above 18.2 MΩcm. As the refine process, lactose was crystallized by seeding of the $L_\alpha \cdot H_2O$ powder (10 mg) into a supersaturated solution at 20°C (1 g-$L_\alpha \cdot H_2O$/3 cc-water) for 24 hrs and dried at 60°C for 72 hrs in an incubator after removal the residual solution, then milled and pressed in the aperture. On the other, lactose was crystallized from an unsaturated solution (0.3 g-$L_\alpha \cdot H_2O$/3 cc-water) without the seeding, where water in the solution was gradually vaporized in a dehumidifier for 24 hrs to form wet powder then milled and pressed in the aperture after dry at 60°C for 72 hrs in an incubator.

3. Results and Discussions

3.1. Absorption Property of Lactose Powders

Figures 3 show **(a)** transmission and **(b)** absorption spectra of $L_\alpha \cdot H_2O$ powder (denoted as α_P) and L_β powder (β_P), where the incident THz-wave (Blank) without the lactose sample is also shown in **(a)**. In these spectra, any absorption due to water vapor could not be observed in sufficiently low humidity (<5%) and spectrum fluctuation due to aliasing was successfully removed by Gaussian window superposed on the THz-pulse. Since THz-wave from 0.2 to 3 THz could be observed in this system but the intensity was exponentially decreased with the frequency, the frequency useful to analyze the lactose was limited below 1.7 THz because of absorption due to lactose-pellet. Typical narrow-bands and broad-band increasing with frequency were observed in the absorption spectra as shown **Figure 3(b)**. Intense absorptions with peak at 0.53 and 1.37 THz were observed in the $L_\alpha \cdot H_2O$ powder (α_P), which were in good agreement to other reports by THz-TDS [18] and assigned to lactose-active modes originated from L_α-molecular rotations in $L_\alpha \cdot H_2O$ crystal as shown by first-principles calculations [20]. The absorptions were significantly decreased in the L_β powder (β_P) but the other absorption with peak at peak 1.19 THz was clearly observed. **Figure 4** shows the absorption spectra

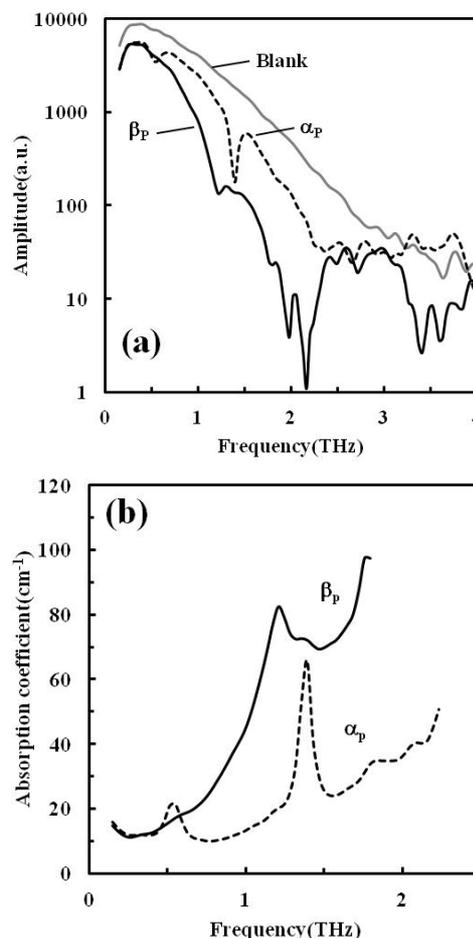**Figure 3.** (a) Transmission and (b) absorption spectra of commercially available α-D-powder (denoted as "α_P") and β-D-lactose powder (denoted as "β_P"). The spectrum denoted as "Blank" in (a) was obtained without lactose sample.
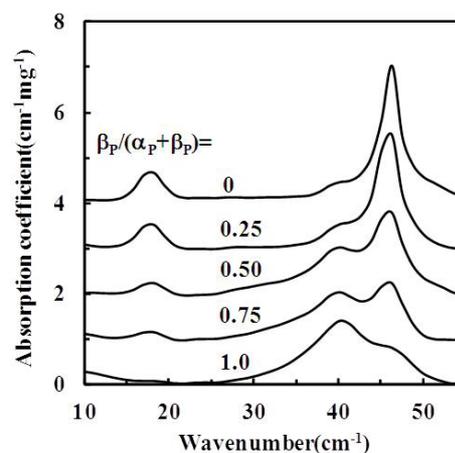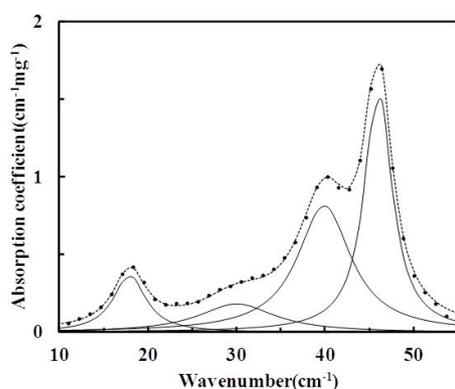
Figure 4. Absorption spectra of the α-D-lactose powder and the β-D-lactose powder mixtures with various β-D-lactose powder mixed weight-ratio, where the absorption coefficient was normalized by the sample weight in mg and the background broad-band was removed by polynomial function.

of lactose-pellets consisting of the $L_\alpha \cdot H_2O$ powder and L_β powder with various weight-content of the L_β powder ($\beta_P/(\alpha_P + \beta_P)$) in the pellets, where the broad-band was removed by polynomial cubic function. It is noted that the coefficient should be used in the molar coefficient but was normalized by the weight of pellet because of the uncertain anomer ratio in the $L_\alpha \cdot H_2O$ powder and the L_β powder. The intense absorption with peaks at 17.5 cm^{-1} (0.53 THz) and 45.6 cm^{-1} (1.37 THz) observed in the $L_\alpha \cdot H_2O$ powder were gradually decreased with the L_β-content whereas the absorption with peak at 39.7 cm^{-1} (1.19 THz) was gradually increased with the rate. It is not difficult to recognize from the results that the absorption dominated in L_β powder is derived from L_β. Further, it is expected that the net anomer content in the powders can be evaluated from the intensity ratio of the absorptions derived from $L_\alpha \cdot H_2O$ and L_β. For the purpose, the absorption spectra as shown in **Figure 4** have to be deconvoluted to each spectrum. Previously, it was reported that the lowest-lying absorption with peak at 17.5 cm^{-1} observed in $L_\alpha \cdot H_2O$ powder can be successfully fitted by Lorentzian [21]. In this work, not only the lowest-lying absorption but also the other absorptions below 55 cm^{-1} were successfully fitted by Lorentzian as shown in **Figure 5**, where the dot-line depicts a fit by four spectra with peak at 17.5, 30.0, 39.7 and 45.6 cm^{-1} (solid-lines) with the experimentally obtained absorption for a lactose pellet of 50 wt% L_β powder (solid-circles). In the spectral feature, the absorption with peak at 39.7 cm^{-1} due to L_β (FWHM around 7.5 cm^{-1}) was significantly broader than the two spectra due to $L_\alpha \cdot H_2O$ (FWHM below 4 cm^{-1}). The absorption around 30 cm^{-1} was also broad with the FWHM of 7.8 and slightly observed in the pellet with the L_β powder weight-ratio above 25%, but the absorption was not originated from anhydrous L_β as discussed in Section 3.3.

3.2. Determination of Anomer Content

The integrated intensities of absorptions with peak at 45.6 cm^{-1} (open-circles and denoted as "I_α") and 39.7 cm^{-1} (closed-circles and denoted as "I_β") were linearly dependent on the L_β powder weight-content as shown in **Figure 6**, in which the correlative square-factors on the least squares method were 99.8% and 99.6% for I_α and I_β respectively. The results indicate the intensities of I_α and I_β for the L_β powder weight-ratio can be shown by the below relationships.
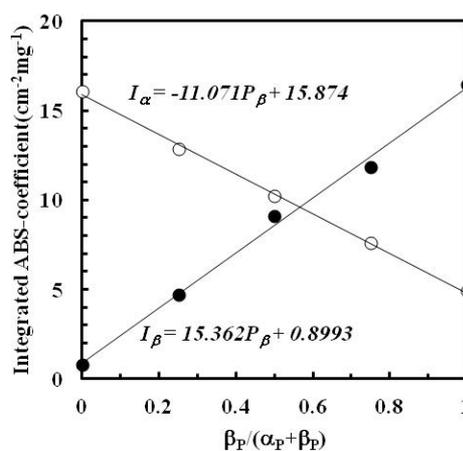
$$
\begin{aligned}
I_\alpha &= P_\alpha x_\alpha \alpha_w + P_\beta (1 - x_\beta) \alpha_w \\
&= -\alpha_w (x_\alpha + x_\beta - 1) P_\beta + \alpha_w x_\alpha \quad (1) \\
&= -r_\alpha P_\beta + \alpha_w x_\alpha
\end{aligned}
$$

$$
\begin{aligned}
I_\beta &= P_\alpha (1 - x_\alpha) \alpha_w + P_\beta x_\beta \beta_w \\
&= \beta_w (x_\alpha + x_\beta - 1) P_\beta + \beta_w (1 - x_\alpha) \quad (2) \\
&= r_\alpha P_\beta + \beta_w (1 - x_\alpha)
\end{aligned}
$$

where P_α and P_β are mixed weight-ratio of the $L_\alpha \cdot H_2O$ powder and the L_β powder ($P_\alpha + P_\beta = 1$), x_α and x_β are net anomer weight-content in the $L_\alpha \cdot H_2O$ powder and the L_β powder, α_w and β_w are absorption coefficient per weight of $L_\alpha \cdot H_2O$ and L_β, r_α and r_β are rate constant of I_α and I_β for L_β weight-ratio, respectively. Then, the rate constant ratio (r) of r_α/r_β for the L_β-powder weight-content can be reduced from the Equation (1) and (2) to the follow,

$$
r = \frac{r_\alpha}{r_\beta} = \frac{\alpha_w}{\beta_w} = \frac{360 \alpha_M}{342 \beta_M} = 1.05 \frac{\alpha_M}{\beta_M} \quad (3)
$$

where α_M and β_M are molar-absorption coefficient of $L_\alpha \cdot H_2O$ and L_β, and 360 and 342 are molecular weight of


Figure 5. Absorption spectrum of the *α*-D-lactose powder and the *β*-D-lactose powder mixture with the *β*-D-lactose powder mixed weight-ratio of 50%, where the experimentally obtained data, the deconvoluted spectra and a fit by the spectra are shown by solid-circles, solid-lines and a dotted-line, respectively.

Figure 6. Integrated intensity of absorption derived from *α*-D-lactose monohydrate (peak at 45.6 cm^{-1}; open circles) and anhydrous *β*-D-lactose (peak at 39.7 cm^{-1}; solid-circles) for the *β*-D-lactose powder mixed weight-ratio in the *α*- and *β*-lactose powders mixture, where the relationships for I_α and I_β were obtained by the least-squares method.

L$\alpha\cdot$H$_2$O and L$_\beta$. The rate constant ratio of r could be determined as 0.721 since the r$_\alpha$ and r$_\beta$ were experimentally obtained by the results in **Figure 6** as 11.07 and 13.36 respectively. On the other, the integrated absorption ratio of I$_\alpha$(z$_\alpha$) and I$_\beta$(z$_\beta$) in lactose-powder including z$_\alpha$-mol L$\alpha\cdot$H$_2$O and z$_\beta$-mole L$_\beta$ in the THz-wave pathway should be described as below,

$$\frac{I_\beta\left(z_\beta\right)}{I_\alpha\left(z_\alpha\right)} = \frac{\beta_w z_\beta}{\alpha_w z_\alpha} = \frac{1.05}{r} \cdot \frac{z_\beta}{z_\alpha}. \quad (4)$$
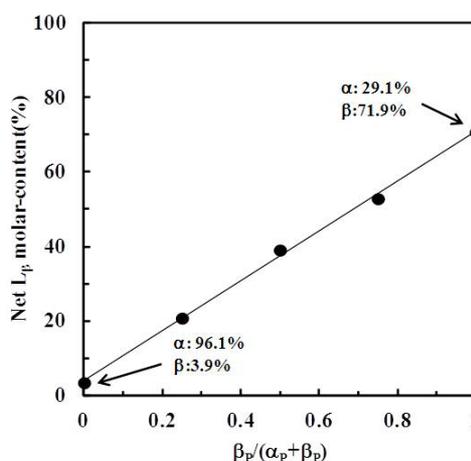
As a result, the net mole-ratio (z$_\beta$/z$_\alpha$) in the sample can be determined by the integrated absorption ratio of I$_\beta$(z$_\beta$)/I$_\alpha$(z$_\alpha$) and the r-value (0.721). **Figure 7** shows net L$_\beta$ molar-content in the pellets with various L$_\beta$ weight-content evaluated by the Equation (4) using the integrated absorption ratio (I$_\beta$/I$_\alpha$) and the r-value. In the result, correlative square-factors by the least squares method for the dependence on the weight-ratio showed an excellent value as high as 99.8%. The net L$_\beta$ molar-content uniquely increased from 3.9% to 70.9% with the L$_\beta$-powder weight-ratio indicated anomer ratio was 3.9% in the L$\alpha\cdot$H$_2$O powder and 29.1% in the L$_\beta$ powder. The anomer ratios in the preliminary used powders were in good agreement to the commercial specifications (L$_\beta$ about 4% in the L$\alpha\cdot$H$_2$O powder, L$\alpha\cdot$H$_2$O below 30% in the L$\alpha\cdot$H$_2$O powder). The quantitative coincident can be concluded the analysis using the absorptions with peak at 45.6 cm^{-1} and 39.7 cm^{-1} were suitable to determine the anomer content in lactose powders. It should be mentioned that the evaluation can be applied to lactose-including samples with uncertain thickness and density because the ratio can be determined by the integrated intensity ratio in the THz-absorption spectrum and the r-value.
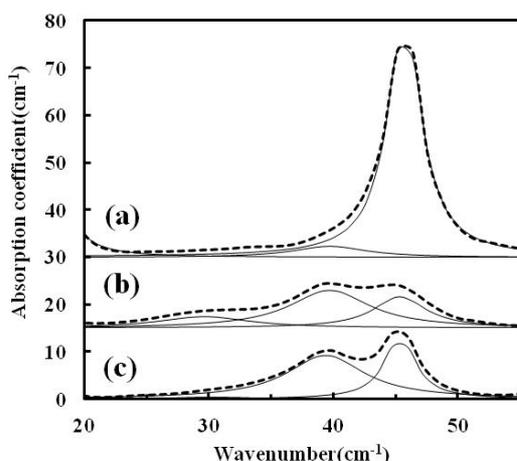
3.3. Anomer Content in Extracted Lactose Powders

As a demonstration to determine the α-lactose/β-lactose molar-content ratio, lactose powders extracted from lactose solution were examined by THz-TDS as shown in **Figure 8**, in which the absorption spectra after removal background broad-band and deconvoluted spectra are shown by dot-lines and solid-lines respectively. It is noted that the absorption coefficient is shown in cm^{-1} without normalization by the lactose-weight. Commonly, it has been recognized that L$\alpha\cdot$H$_2$O can be crystallized in supersaturated lactose solution [22]. **Figure 8(a)** shows THz-absorption spectrum of lactose extracted from a supersaturated solution (1.0 g-L$\alpha\cdot$H$_2$O powder/3 cc-water) by seeing of 10 mg-L$\alpha\cdot$H$_2$O powder including L$_\beta$ with 3.9 %. The lactose was extracted at 20˚C for 24 hrs and dried at 60˚C for 72 hrs after removal the solution and then milled and pressed in the aperture. Absorption spectrum consisting of intense L$\alpha\cdot$H$_2$O-absorption and weak L$_\beta$-absorption showed a net L$_\beta$-lactose molar-content as low as 2.8%, which showed the L$\alpha\cdot$H$_2$O was refined. In contrast, the absorption by lactose powder gradually extracted from lactose solution without the seeding showed quite different behavior as shown in **Figures 8(b)** and **(c)**, where **(a)** the water of 3 cc in unsaturated lactose solution (0.3 g-L$\alpha\cdot$H$_2$O powder/3 cc-water) was gradually vaporized at 25˚C for 24 hrs in a dehumidifier and then **(b)** dried at 60˚C for 72 hrs. For the spectrum of L$\alpha\cdot$H$_2$O-absorption with peak at 45.6 cm^{-1}, the FWHM was decreased from 5.1 cm^{-1} to 3.1 cm^{-1} by the post-anneal. In contrast, any decrease of the FWHM of L$_\beta$-absorption with peak at 39.7 cm^{-1} was not substantially observed after the post-annealing. The significant difference of the

Figure 7. Net anhydrous β-D-lactose molar-content for the β-D-lactose powder weight-ratio in the α- and β-lactose powders mixtures, where the net molar-content in the β-D-lactose powder (β_P/($\alpha_P + \beta_P$) = 1) and the α-D-powder (β_P/($\alpha_P + \beta_P$) = 0) were evaluated as 71.9% and 3.9% respectively.

Figure 8. Absorption spectra of (a) refined lactose in a supersaturated lactose-solution, (b) as-extracted lactose by gradual evaporation of water from unsaturated lactose solution and (c) post-annealed lactose of the sample for (b), where the dot-lines and the plain-lines show experimentally obtained spectra and the deconvoluted spectra.

two absorption behaviors by the post-annealing suggested that α-lactose can be crystallized into the hydrates with excess water although anhydrous crystal is formed by β-lactose. The peak intensity ratio of $L_\beta/L_\alpha \cdot H_2O$ was decreased but the integrated intensity ratio was increased from 46.2% to 62.7% by the post-annealing because the absorption of $L_\alpha \cdot H_2O$ was narrowed by the annealing. As a result, the net L_β-molar ratio was increased from 46.2% to 62.7% after the annealing.

The evaluated net L_β-molar ratio of 46.2% in the as-extracted powder was higher than that in the refined powder but significantly low comparing to the equilibrium ratio of 62.7% in solution [23], which could be recognized by the solubility of L_α lower than of L_β [24]. The increased of net L_β-molar ratio after the post-annealing indicated non-crystallized lactose was crystallized in mostly L_β during the annealing. It was reported that collapsed lactose formed from non-collapsed spray-dried amorphous lactose by exposure in 50% RH for long time is crystallized in mostly L_β with some presence of $L_\alpha \cdot H_2O$ at relatively low temperature of 70°C [8]. Crystallization of the extracted powder in this work seemed to be similar to the crystallization of collapsed lactose, that is, collapsed lactose was included in the as-extracted lactose powder. It should be mentioned that the broad-band around 30 cm^{-1} observed in the as-dried lactose was almost disappeared after the annealing. The decrease of the absorption and the preference crystallization into L_β during the annealing speculates the broad-band is derived from collapsed lactose.

4. Conclusion

Transmission-type THz-TDS was applied to non-destructive evaluation of anomer content in lactose powder. Absorption spectra of lactose-pellets consisting of $L_\alpha \cdot H_2O$ and L_β powders were significantly dependent on the mixed rate. The absorption with a peak at 39.7 cm^{-1} (1.19 THz) was assigned to be originated from L_β in addition to the fingerprint of $L_\alpha \cdot H_2O$ at 17.5 cm^{-1} (0.53 THz) and 45.6 cm^{-1} (1.37 THz). Since the integrated values of the absorption coefficient were uniquely dependent on the powder mixed rate of $L_\alpha \cdot H_2O/L_\beta$, the net anomer content in lactose powder could be precisely determined such as 3.9% and 70.9% for $L_\alpha \cdot H_2O$ and L_β powders, respectively. Low L_β-content of 2.8 mol% in lactose powders evaluated by the THz-TDS analysis showed the refined feature of $L_\alpha \cdot H_2O$ in supersaturated lactose solution using $L_\alpha \cdot H_2O$-seeds. In contrast, L_β-content in lactose powder extracted from unsaturated lactose solution by gradual water evaporation was increased from 34.4% to 45.3% by post-annealing at 60°C. A broad-band with a peak at 30.5 cm^{-1} was speculated to collapsed-lactose absorption by the behavior crystallized in mostly L_β after the annealing.

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