Paper:

Hydration and dehydration processes of lactose monohydrate studied by THz time domain spectroscopy

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Terahertz spectroscopy is now extensively employed to distinguish different hydrate systems and physical characterization of pharmaceutical drug materials. In the present work, the absorption spectra of terahertz radiation is used to investigate the effect of heating process on the release of the bounding H_2O molecules present in α-Lactose monohydrate. Distinctive THz absorption spectra at various heating times were observed. The THz absorption spectra of α-lactose monohydrate and anhydrous α-lactose exhibit evident differences. The pure α-lactose monohydrate has clear absorption peaks at 0.53, 1.05, 1.11, 1.33, and 1.56 THz and a small peak at 0.8 THz. The complete dehydration of α-lactose monohydrate takes place at 145° C (418 K) in 15 minutes. Moreover, Terahertz refractive index of α-lactose monohydrate decreases during dehydration process. Application of Beer-lambert's law to dehydration process of α-Lactose monohydrate was also studied by comparing THz absorption spectra at various heating times. It was found that remaining water contents following various heating times in α-lactose monohydrate had exhibited linear relationship with absorption coefficient spectra recorded at 0.53 THz and 1.35 THz for α-Lactose monohydrate at different dehydration times.

Keywords: Terahertz Absorption, α-Lactose Monohydrate, Dehydrate.

1. Introduction

Lactose is a glucose which is linked to a galactose. It is an important disaccharide and widely used in foods and pharmaceutical applications. Lactose disaccharide powder exist as two anomers i.e. α-lactose and β-lactose. Commonly, lactose can be synthesized as crystalline α-lactose monohydrate and anhydrous β-lactose in addition to the amorphous phase of the α- and β-form mixture. Generally, Lactose is found exist in two solid forms, anhydrous form and monohydrate form which is termed as polymorphism [1-2]. Different polymorphs exhibit different product characteristics of drug such as mechanical character, biocompatibility, solubility behavior, free energy, thermodynamic activity, stability, storage and dissolution [3–8]. Addition of water of crystallization to Lactose modifies its molecular structure as well as its active pharmaceutical ingredients (API) performance behavior [9–11]. Water of crystallization molecule can act as hydrogen bond acceptor and donor and hence can affect the intermolecular interactions in Lactose crystals [12-16]. In short, the presence of the water molecules in Lactose affects the crystalline disorder. Therefore, it is essential to comprehend and analyze the hydrate and anhydrate forms of Lactose.

Various characterization techniques have been employed in the past to analyze the dehydration process of hydrates. These techniques include X-ray powder diffraction (XRPD), Raman spectroscopy, thermal analysis methods, solid-state nuclear magnetic resonance and Fourier transform infra-red spectroscopy (FTIR). However, each exhibits its own limitations [1–3]. Terahertz (THz) spectroscopy is an alternative and novel technique to study the structural as well as intermolecular interactions of solid state hydrates [12-18]. This technique is found to be highly sensitive to the lattice vibrations, hydrogen bonding, and water of crystallization [1, 12-14, 18]. THz-electromagnetic wave is easily passed through many inorganic and organic materials but easily absorbed in water compared to UV-vis-infrared light so that it's more sensitive than others. During recent years, THz spectroscopy has been employed to study the intra molecular vibrations in various compounds. Recently, Zeitler et al. [1] have used the THz pulsed spectroscopy to study different hydrate and anhydrate forms of four pharmaceutical materials including lactose, carbamazepine, piroxicam and theophylline. THz spectra of anhydrous and monohydrated glucose is studied by using THz spectroscopy and DFT theoretical calculation [19]. The characterization of L-Phe monohydrate (L-Phe·H_2O) form in THz region has been reported by Pan et al. [20]. However, the THz characterization of the cyclic processes of dehydration and rehydration has not been reported.

Table. 1 Sample specification with fraction of 7% lactose and 93% of PTFE diluent.

Sample No.	Mass Before Heating	Duration of Heating Time	Mass After Heating	Lost Mass	Remaining Water Contents (%)
1st–S1–1m	0.4445 g	1 min	0.4097 g	0.0348 g	92.11
1st–S2–2m	0.4527 g	2 min	0.3801 g	0.0726 g	84.73
1st–S3–3m	0.4455 g	3 min	0.3411 g	0.1044 g	76.55
1st–S4–4m	0.4576 g	4 min	0.3157 g	0.1419 g	69.02

2. Experimental

2.1. Material

α-Lactose monohydrate (αL-H_2O, 4-O-β-D-Galactopyranosyl-D-glucose, D-Lactose monohydrate) ultra-pure, 99.5% (HPLC) powder was selected as an experimental material and purchased from Shanghai Macklin Biochemical Co., Ltd. China. The linear formula of α-lactose monohydrate was $C_{12}H_{22}O_{11}.H_2O$ and the molecular weight was 360.31.

2.2. Sample Preparation

In 1st set of experiments α-lactose monohydrate was mixed with the diluent and then compressed into a pellet before acquisition of the transmission spectrum. Polytetrafluoroethylene (PTFE) powder was used as diluent to mix with α-lactose monohydrate material because PTFE was transparent to terahertz radiation and it had no influence to THz radiation. The mass fraction of the α-lactose monohydrate was 7% each tablet powder was first heated at 418 K for different time durations before making a pellet. Four pellets in total were made by mixing α-lactose monohydrate and PTFE powders by using agate mortar and pestle and subsequently were heated. Details of the samples are described in **Table 1**.

In 2nd set of experiments, samples of pure (100%) α-lactose monohydrate (αL-H_2O) material were prepared without any diluent. Similarly, all the samples were heated at 418 K for a total of 15 minutes before pelletization. Two sets of samples have been used to determine the effect of diluent.

2.3. Experiment Process

The sample pellets were carefully placed into the test sample holder and then placed the sample holder into the sealed chamber. . The humidity inside the chamber was monitored by hygrometer and maintained around 3-5% by purging nitrogen gas, which is placed inside the box. To minimize the effect of humidity on a sample the experiment was started when the humidity level was around 3-5%.

One of the samples is selected to be exactly the THz radiation way by a motor-driven wheel holder. The THz spectrometer software was started to setup the spectroscopy values, the number of averages set to 3, number of points set to 2048 and step size was set to 5 (33.3 fs).To get the reference line the pulsed spectroscopy was start without sample and after getting the reference it will start on the sample pellet to get the results. In the same way all the pellets were tested in THz pulsed spectroscopy one by one. For later experiment the same process were adopted from start to until started of pulsed spectroscopy. The pellet was examined with H_2O before heating the tablet. Then the sample was heated in program control oven for 5 minutes at 145° C (418 K) temperature. After measuring the weight and the thickness of the pellet, heated it again for more 5 minutes at 418 K temperature and this process was repeated 3 times continuously. After heating the pellet for 15 minutes in total the spectroscopy was applied on the sample pellet again to analyze and determine the result.

2.4. Results and Discussion

To detect the features clearly, experiments are performed on two types of samples. One batch of samples with PTFE diluent and other type of samples with only pure α-lactose monohydrate. **Fig. 1** shows the terahertz time-domain spectroscopy waveforms of the α-lactose monohydrate for different experiments. The blue and yellow lines represented as the reference lines for both experiments. The red, purple, brown and black lines representing the 1st experiment while the green and light brown lines representing the 2nd experiment. In **Fig. 1(a)**, the reference signal and the red line sample after heating for 1 minute exhibit a higher amplitude same like as shown in **Fig. 1(b)**. The second heated sample exhibits a lower amplitude than the first one and respectively the other later heated samples have lower amplitude compared to previous ones.

Table Legends Description:

1st–S1–1m → 1st Experiment – Sample No. 1 of 1st Experiment – 1m heating Time

1st–S2–2m → 1st Experiment – Sample No. 2 of 1st Experiment – 2m heating Time

1st–S3–3m → 1st Experiment – Sample No.3 of 1st Experiment – 3m heating Time

1st–S4–4m → 1st Experiment – Sample No. 4 of 1st Experiment – 4m heating Time

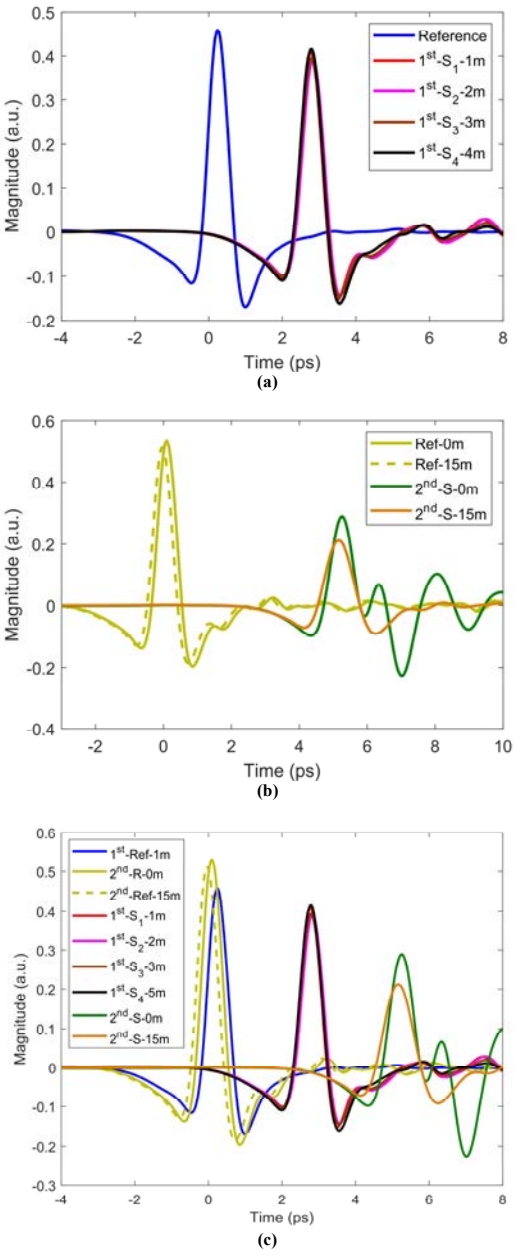

Fig. 1. Time domain spectroscopy of α-lactose monohydrate. (a) Lactose with PTFE diluent, (b) Pure (100%

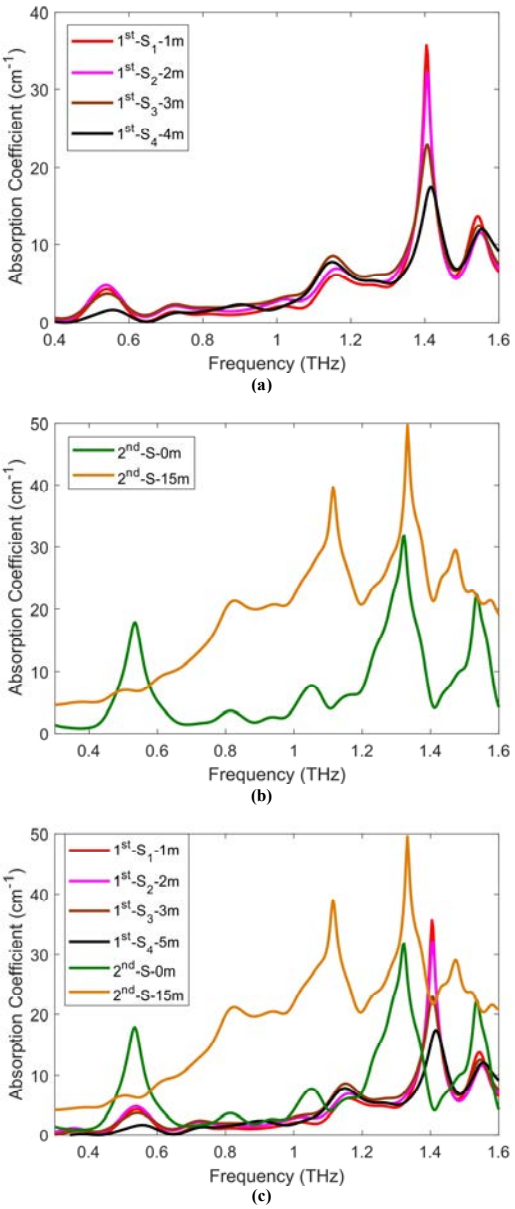

Fig. 2. Terahertz absorption spectra of α-lactose monohydrate. (a) 1st experiment samples with different concentrations of H_2O (b) 2nd experiment sample with different concentrations of H_2O (c) Comparison of Figure 2(a) and 2(b)

The experiment result features in the frequency domain are shown in **Fig. 2**, in which their baselines have been deleted by an exponential function [21]. **Fig. 2(a)** reveals that for samples with PTFE diluent, same absorption peaks are obtained for different sample thicknesses, indicating that these absorption characteristics are not from the etalon effect of samples. The mixture α-lactose monohydrate with PTFE diluent, heated from 1-4 minutes, clearly reveals the strong absorption peaks at 0.53, 1.14, 1.41, and 1.56 THz and a weak peak at 0.7 THz. **Fig. 2(b)** shows the THz absorption spectra of α-lactose monohydrate and anhydrous α-lactose (following heating of α-lactose monohydrate for 15 minutes) with the positions of the distinguishable absorption peaks marked. The two absorption spectra have evident differences, which are supposed to result from the different phonon modes of the anhydrous and hydrated α-lactose [12-16]. The pure α-lactose monohydrate has clear absorption peaks at 0.53, 1.05, 1.11, 1.33, and 1.56 THz and a weak peak at 0.8 THz. In the case of pure α-lactose monohydrate, the spectrum partially agrees with the observation by Jin et al. [12] who measured the spectra of α-lactose monohydrate with PTFE diluent. However, the peak at 1.23 THz in Jin et al. work is not observed in our case.

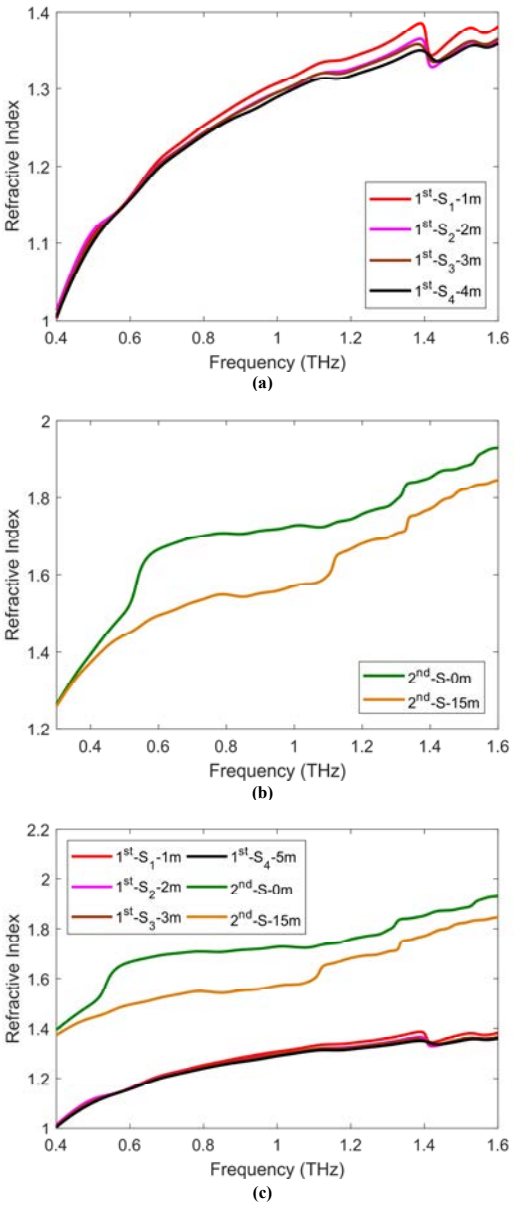

Fig. 3. Terahertz refractive index of α-lactose monohydrate. (a) 1st experiment samples with different concentrations of H_2O (b) 2nd experiment sample with different concentrations of H_2O (c) Comparison of Figure 3(a) and 3(b)

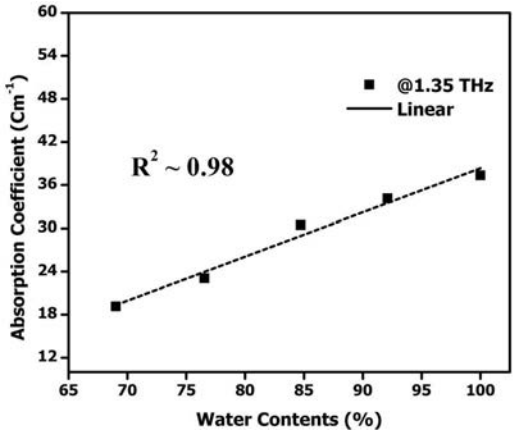

Fig. 4. The absorption coefficient of α-lactose monohydrate with PTFE diluent at different dehydration times at 1.35 THz

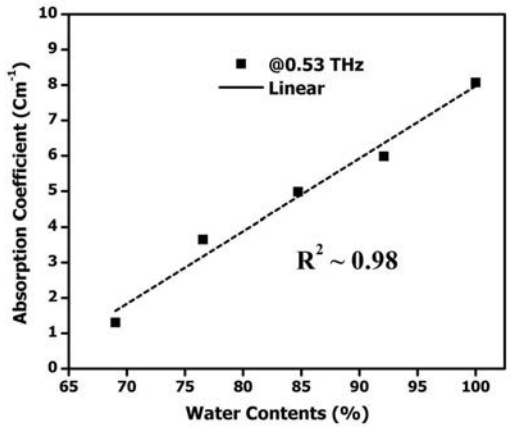

Fig. 5. The absorption coefficients of α-lactose monohydrate with PTFE diluent at different dehydration times at 0.53 THz

Figure 2(b) shows that upon heating, the α-lactose monohydrate starts to lose its water and the anhydrous α-lactose forms. Therefore, the absorption peaks of α-lactose monohydrate in the range of 0.53-0.55, 1.11-1.14 and 1.33-1.40 THz decrease and the absorption peaks of anhydrous α-lactose at slightly higher frequencies appear due to the reduction of α-lactose monohydrate and the increase of anhydrous α-lactose, respectively. It is obvious that the increased absorption peaks reach to the maximum after a certain time, indicating the completion of dehydration after around 15 minutes. **Fig. 2(c)** indicates that THz spectral differences between anhydrous and monohydrated α-lactose are obvious and the reason may be ascribed to their different crystalline structures due to the existence of water molecule. That's free space other than crystal lattice. Danylov et al. [18] have concluded that peaks in the range of 0.53-0.55 THz are due to atmospheric humidity and all others peaks at higher frequencies are due to binding water contents. Binding water contents are same for all the frequency range in our work but higher absorption at 1.3 and 1.4 THz compared to peaks at lower frequencies may be ascribed to the high sensitivity to binding water contents in our samples. It is concluded that THz

Figures Legends Description:
Ref–0m → Reference Signal of without heating sample
Ref–15m → Reference Signal of heating after 15 minutes
1st–S1–1m → 1st Experiment–Sample No.1–1m Heating Time
1st–S2–2m → 1st Experiment–Sample No.2–2m Heating Time
1st–S3–3m → 1st Experiment–Sample No.3–3m Heating Time
1st–S4–4m → 1st Experiment–Sample No.4–4m Heating Time
2nd–S–0m → 2nd Experiment–Sample of 2nd Experiment–Before Heating
2nd–S–15m → 2nd Experiment–Sample of 2nd Experiment–After heating 15 minutes

Table. 2 Absorption coefficients (cm-1) of α-lactose monohydrate with PTFE diluent at different dehydration times at eight frequencies (THz) around 1.35 THz.

Absorption Coefficients (Cm^{-1})	Frequency (THz)								
	1.3447	**1.3462**	**1.3477**	**1.3500**	**1.3515**	**1.3535**	**1.3547**	**1.3563**	**1.3505**
1st–S0–0m	38.9620	38.1561	37.7578	37.3566	37.0492	36.7409	36.4588	36.1679	37.3346
1st–S1–1m	35.6421	34.7422	34.4236	34.1014	33.8263	33.6462	33.3098	33.1473	34.1402
1st–S2–2m	31.9521	31.4612	31.2977	31.087	30.7069	29.5581	28.9071	28.4865	30.4320
1st–S3–3m	23.9332	23.7881	23.5594	23.2283	22.9007	22.6341	22.3929	22.0234	23.0575
1st–S4–4m	18.6930	18.9109	19.0743	19.1739	19.2934	19.1836	19.4863	19.3452	19.1450

Table. 3 Absorption coefficients (cm-1) of α-lactose monohydrate with PTFE diluent at different dehydration times at eight frequencies (THz) around 0.53 THz.

Absorption Coefficients (Cm^{-1})	Frequency (THz)								
	0.5192	**0.5247**	**0.5280**	**0.5308**	**0.5336**	**0.5364**	**0.5392**	**0.5409**	**0.5319**
1st–S0–0m	7.0132	8.2363	8.3513	7.9052	8.4621	8.5009	8.5321	8.5503	8.0695
1st–S1–1m	4.9986	5.8895	5.9550	5.7886	5.9238	5.8519	5.9312	5.9820	5.7775
1st–S2–2m	4.7177	4.9980	5.8341	31.087	5.0504	4.6880	4.9612	4.9719	5.0906
1st–S3–3m	3.5119	3.5116	3.6058	5.5237	3.7058	3.6456	3.7508	3.7805	3.6447
1st–S4–4m	1.2068	1.3418	1.6126	1.4767	1.6120	1.6101	1.6063	1.5814	1.3044

spectroscopy is highly sensitive to different specimens. Anhydrous α-lactose and its monohydrate can be distinguished and identified readily according to their unique fingerprints.

Figure 3 indicates the refractive indexes of α-lactose monohydrate with and without PTFE diluents. The refractive index decreases gradually in as shown in **Fig. 3(a)**, and **(b)**. Upon heating for long duration the samples exhibit significant differences. It shows that the speed of light increases and pass through the samples, when the sample is heated as the H_2O in the α-lactose monohydrate vaporize after heating. Beer–Lambert law provides the basis for quantitative analysis of various spectrophotometric methods. The derivation of the expression of Beer–Lambert law in the terahertz band can be seen in previous work [22]. According to this law, for a given material sample, path length and concentration of the sample are directly proportional to the absorbance of the light.

$$A = \log_{10} Io/I = \in lC$$

I_0 and I are the intensity of the incident light and the transmitted light, respectively. The constant ε is called molar absorptivity or molar extinction coefficient. l is optical path length (thickness) and C is molar concentration. Water of crystallization is very sensitive to terahertz waves. In the present work we also find a relationship between remaining water contents in lactose monohydrate following heating for different times and absorption coefficient by comparing the terahertz absorption spectra of samples heated for different times [**Fig. 4** and **5**]. As the heating time increases the absorbance coefficient values at 0.53 THz and 1.35 THz tend to decrease. The shapes of the curves are basically the same for all heating times and consequently remaining water contents. This means qualitative analysis of loss of water contents in lactose monohydrate can be performed with terahertz spectrum. Changes in intensities of absorption coefficient give the quantitative analysis for loss of water contents. Here we take all data points near 0.53 THz and 1.35 THz. **Tables 2** and **3** show four groups of different dehydration times recorded nearby 0.53 THz and 1.35 THz. The last column in both tables is the calculated average of these eight frequency points and the corresponding absorbance coefficient mean. **Fig. 4** and **5** clearly indicate that there is linear relationship between remaining water contents (**Table 1**) and absorption coefficients. It implies that loss of water contents is directly proportional to absorbance justifying the applicability of Beer–Lambert law.

3. Conclusions

THz absorption spectra of α-lactose monohydrate as fingerprints were used to identify the dehydration and hydration process in α-lactose monohydrate based on Beer–Lambert law. A linear relationship between remaining water contents following heating for different times and absorption coefficients were identified by THz imaging. The peaks at 0.53, 0.54 and 0.55 THz due to atmospheric humidity and all other peaks are due to binding water contents in the samples. The dehydration process or evaporation of H_2O contents from lactose through heating the sample can significantly change the absorption spectra for α-lactose monohydrate. In the present work, the terahertz pulse spectroscopy work has made good use of the sensitivity of THz spectroscopy and THz imaging to quantify the water contents in α-lactose monohydrate showing that THz technology is indeed a helpful and alternative tool for chemical, biological, and pharmaceutical applications.

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References:

[1] J. A. Zeitler, K.Kogermann, J.Rantanen, T. Rades, P.F. Taday, M. Pepper, J. Aaltonen and C.J. Strachan, "Drug hydrate systems and dehydration processes studied by terahertz pulsed spectroscopy," Int. J. Pharm., vol. 334, no. 1–2, pp. 78–84, 2007.

[2] T. Sasaki, T. Sakamoto, and M. Otsuka, "Detection of Impurities in Organic Crystals by High-Accuracy Terahertz Absorption Spectroscopy," Anal. Chem., vol. 90, no. 3, pp. 1677–1682, 2018.

[3] E. P. J. Parrott and J. A. Zeitler, "Terahertz Time-Domain and Low-Frequency Raman Spectroscopy of Organic Materials," Appl. Spectrosc., vol. 69, no. 1, pp. 1–25, 2015.

[4] K. J. Tielrooij, R. L. A. Timmer, H. J. Bakker, and M. Bonn, "Structure Dynamics of the Proton in Liquid Water Probed with Terahertz Time-Domain Spectroscopy," Phys. Rev. Lett., vol. 102, no. 19, p. 198303, 2009.

[5] D. S. Venables and C. A. Schmuttenmaer, "Spectroscopy and dynamics of mixtures of water with acetone, acetonitrile, and methanol," J. Chem. Phys., vol. 113, no. 24, pp. 11222–11236, 2000.

[6] D. S. Venables and C. A. Schmuttenmaer, "Far-infrared spectra and associated dynamics in acetonitrile–water mixtures measured with femtosecond THz pulse spectroscopy," J. Chem. Phys., vol. 108, no. 12, pp. 4935–4944, 1998.

[7] P. Dutta and K. Tominaga, "Obtaining Low-Frequency Spectra of Acetone Dissolved in Cyclohexane by Terahertz Time-Domain Spectroscopy," J. Phys. Chem. A, vol. 113, no. 29, pp. 8235–8242, 2009.

[8] P. Dutta and K. Tominaga, "Terahertz time-domain spectroscopic study of the low-frequency spectra of nitrobenzene in alkanes," J. Mol. Liq., vol. 147, no. 1–2, pp. 45–51, 2009.

[9] P. Dutta and K. Tominaga, "Dependence of low frequency spectra on solute and solvent in solutions studied by terahertz time-domain spectroscopy," Mol. Phys., vol. 107, no. 18, pp. 1845–1854, 2009.

[10] K.-J. Tielrooij, J. Hunger, R. Buchner, M. Bonn, and H. J. Bakker, "Influence of Concentration and Temperature on the Dynamics of Water in the Hydrophobic Hydration Shell of Tetramethylurea," J. Am. Chem. Soc., vol. 132, no. 44, pp. 15671–15678, 2010.

[11] J. H. Smith, S. E. Dann, M. R. J. Elsegood, S. H. Dale, and C. G. Blatchford, "Aα-Lactose monohydrate: A redetermination at 150 K," Acta Crystallogr. Sect. E Struct. Reports Online, vol. 61, no. 8, pp. 1–18, 2005.

[12] B. B. Jin, Z. X. Chen , Z. Li, J. L. Ma, R. Fu, C. H. Zhang, J. Chen and P. H. Wu, "Mode assignment of terahertz spectrum of α-lactose monohydrate," 34th International Conference on Infrared, Millimeter, and Terahertz Waves, 2009.

[13] S.Warnecke, J.X. Wu, Å. Rinnan, M. Allesø, F. V. D. Berg,P. U. Jepsen, S. B. Engelsen, "Quantifying crystalline α-lactose monohydrate in amorphous lactose using terahertz time domain spectroscopy and near infrared spectroscopy," Vibrational Spectroscopy, vol. 102, pp. 39–46, 2019.

[14] S.Yamauchi, S. Hatakeyam, Y. Imai, M. Tonouchi, "Terahertz Time-Domain Spectroscopy to Identify and Evaluate Anomer in Lactose," American Journal of Analytical Chemistry, vol. 4, pp. 756–762, 2013.

[15] J.H. Kirk, S.E. Dann, C.G. Blatchford, "Lactose: A definitive guide to polymorph determinationInternational Journal of Pharmaceutics," vol. 334, pp. 103–114, 2007.

[16] S. Garnier, S. Petit and G. Coquerel, "Dehydration mechanism and crystalline behaviour of Lactose," Journal of Thermal Analysis and Calorimetry, vol. 68, pp. 489–7502, 2002.

[17] S. Saito, T. M. Inerbaev, H. Mizuseki, N. Igarshi, R. Note, Y. Kawazoe, "First principles calculation of terahertz vibrational modes of a disaccharide monohydrate crystal of Lactose," Japanese Journal of Applied Physics, Vol. 45, No. 43, pp. L1156–L1158, 2006.

[18] A. Danylov, "THz Laboratory Measurement of Atmospheric Absorption Between 6% and 52% Relative Humidity," Submillimeter-Wave Technology Laboratory University of Massachusetts, Https://www.uml.edu/docs/UML_STL_WaterVaporSept2006_tcm19-42128. vol. 175, no. 44, pp. 1–7, 2006.

[19] Z. Zheng, W. Fan, H. Li, J. Tang, "Terahertz spectral investigation of anhydrous and monohydrated glucose using terahertz spectroscopy and solid-state theory," Journal of Molecular Spectroscopy, 296 (2014) 9–13 vol. 296, pp. 9-13, 2014.

[20] T. Pan, S. Li, T. Zou , Z. Yu, B. Zhang, C. Wang, J. Zhang, M. He, H. Zhao, "Terahertz spectra of L-phenylalanine and its monohydrate," Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, vol. 178, pp.19–23, 2014.

[21] X. Jiang, Z. Zhang, X. Zhao, Y. Yin, K. Ajito and H. Song, Journal of Physics(C), 276(1), 1 (2011).

[22] S.Hai-Xia, Z Zhao-Hui, Z. Xiao-Yan, L. Zhi, Y. Fang, Z. Han, " The Lambert-Beer's law characterization of formal analysis in tetrahertz spectrum quantitative testing," spectroscopy and spectral analysis, vol. 33, pp.3180–3186, 2013.