

Terahertz spectra of vitamins studied by terahertz spectroscopy and density functional theory

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Abstract—We have investigated the terahertz spectra of biological molecules of vitamins such as L-ascorbic acid, thiamine hydrochloride, biotin, pyridoxine, riboflavin, and vitamin K2, measured by terahertz time-domain spectroscopy (THz-TDS) and Fourier transform infrared spectroscopy (FTIR). The measured absorption spectra were demonstrated to be in good agreement with the results simulated by Density Functional Theory (DFT) using hybrid functional B3LYP with basis set of 6-31G (d). The study suggests that the effect of intermolecular and intramolecular interaction on the absorption bands in THz region.

I. INTRODUCTION

To date, the terahertz (THz) spectroscopy has been widely applied in various fields of research to understand the THz frequency dynamics of biological molecular systems related to intermolecular vibrations and large-amplitude intramolecular modes [1]. Extensive spectroscopic studies in this region have been carried out, and THz spectroscopy has demonstrated that this method is complementary to other spectroscopic techniques.

Fourier transform infrared spectroscopy (FTIR) is a useful method for evaluating the optical properties of materials and biological molecules in infrared range, which has been demonstrated to have similar sensitivity as THz time domain spectroscopy (TDS) in the low frequency THz region [2]. Beside low-frequency THz range, the far-infrared at higher frequencies, middle-infrared and near-infrared region are very important to study the molecular vibrations and determine the presence of certain characteristic groups in biological molecules [3,4], which can be measured by FTIR spectroscopy.

L-ascorbic acid (Vitamin C), thiamine hydrochloride (Vitamin B1), biotin, pyridoxine, riboflavin, and vitamin K2 are important vitamins for human health. Using THz-TDS spectroscopy, the absorption spectra of the vitamins were studied. Yu Bin *et al.* studied the THz spectra of the L-ascorbic acid and the thiamine hydrochloride [5]. However, the theoretical calculation results were not in agreement with the measurement results. The same study was done in the Vitamin C by using THz-TDS, but showing different absorption peaks [6, 7]. D. G. Allis *et al.* investigated the assignment of the low-frequency THz absorption signatures in the biotin [8]. T. M. Korter and David F. Plusquellic *et al.*

employed continuous-wave THz spectroscopy to study the impact of mechanical anharmonicity and temperature dependence on the absorption spectra of the biotin [9, 10]. Above research mainly focuses on the investigation of low-frequency characteristic of the biotin below 3 THz, and made few study on the mechanism of intramolecular and intermolecular modes. It is necessary to investigate the identity of signatures and analyze the spectra at higher THz frequencies using THz spectroscopy in combination with density functional theory (DFT). Mayuri Srivastava *et al.* compared the experimental and theoretical results of vibrational spectrum and molecular structure of the pyridoxine in IR and Raman bands [11, 12]. Yu Bin *et al.* studied the THz spectra of the pyridoxine at 0-2.2 THz, only one absorption peak appeared at 1.71 THz. The theoretical simulation was based on single molecule structure [5]. The detailed investigation of the crystalline pyridoxine throughout THz region is not done until now. Therefore, it is necessary to demonstrate the experimental and theoretical results for these vitamins, and build the vitamin fingerprint database in terahertz region.

In our work, we employed FTIR spectroscopy to investigate the THz spectra of the vitamins experimentally and theoretically. We study the effect of mixing proportion of the biotin and pyridoxine samples with high density polyethylene powder on the absorption spectra. The spectrum difference indicate that the absorbance of samples increase with the frequency. The experimental results are in good agreement with the theoretical calculations based on density functional theory (DFT) embedded in Gaussian software (G09W Rev D. 01) [13].

II. SAMPLE PREPARATION AND MEASUREMENT METHOD

The vitamin samples measured in our experiment were purchased from Sigma Aldrich Co. and used without further purification. Samples were prepared by weighing 50-100 mg of each solid and homogenizing the material in a mortar and pestle. This procedure ensured particle sizes sufficiently smaller than THz wavelengths to reduce baseline offsets at higher frequencies arising from non-resonant light scattering. The samples were pressed as a pellet in a 13 mm diameter vacuum die at the lowest possible pressures to minimize decomposition from transient heating. The pellets have

thickness between 0.5 and 1 mm which is suitable for the measurement of the THz-TDS and FTIR spectroscopy. The samples were diluted with polyethylene powder for different ratios to observe the vibration of absorption peaks.

The absorption spectra in the 0.1-4 THz range were obtained with the THz-TDS system working in transmission mode, provided by Advantest Co. [14]. Figure 1 shows the schematic of THz-TDS measurement system (type: TAS7500SP). The THz-TDS spectroscopy employs two ultra short pulse fiber lasers self developed by Advantest, which are ensured to synchronized control. These pulses are centered at 1550 nm with maximum output power of 50 mW, and provides extremely short pulse width less than 50 fs and low jitter below 50 fs. The THz-TDS system achieves the sampling rate with 8 ms per scan and ultra-wide frequency band extending to 4 THz. The whole system purged by dry-air has sufficient sensitivity to generate high quality spectra in the frequency range of 0.1-4 THz. The spectral resolution of 0.25 cm^{-1} were obtained and averaged 2048 times for one point.

The infrared absorption spectra covering far-infrared to near-infrared were measured by Bruker v80 Fourier transform infrared spectrometer (FTIR) [15]. In the far-infrared band, we employed low temperature silicon bolometer as detector working at 4-kelvin instead of DTGS room temperature

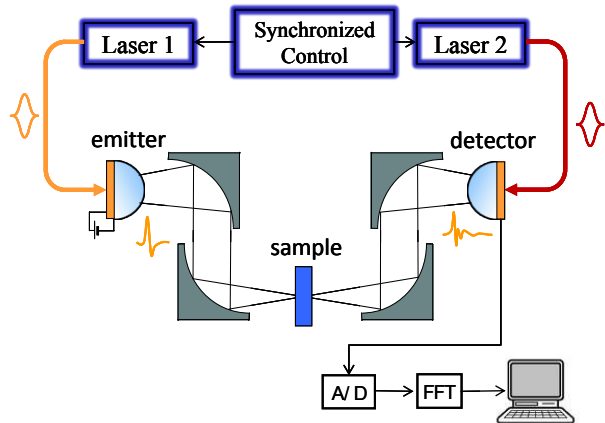


Fig. 1 Schematic of THz-TDS measurement system.

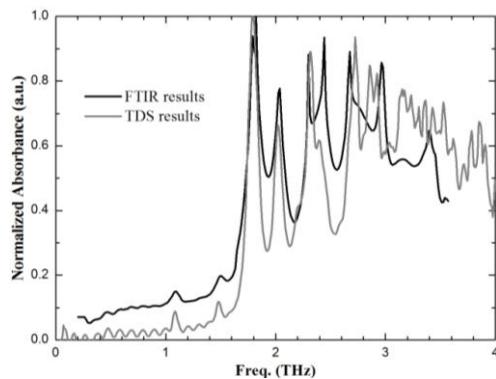


Fig. 2 THz spectra of L-ascorbic acid measured by the FTIR and THz-TDS.

detector in order to enhance the SNR of system. The vacuumized FTIR spectroscopy provides 2 cm^{-1} spectral resolution for the far-infrared band and 4 cm^{-1} for the middle-

infrared band. Reproducibility of spectral features was demonstrated well by measuring several pellets with various

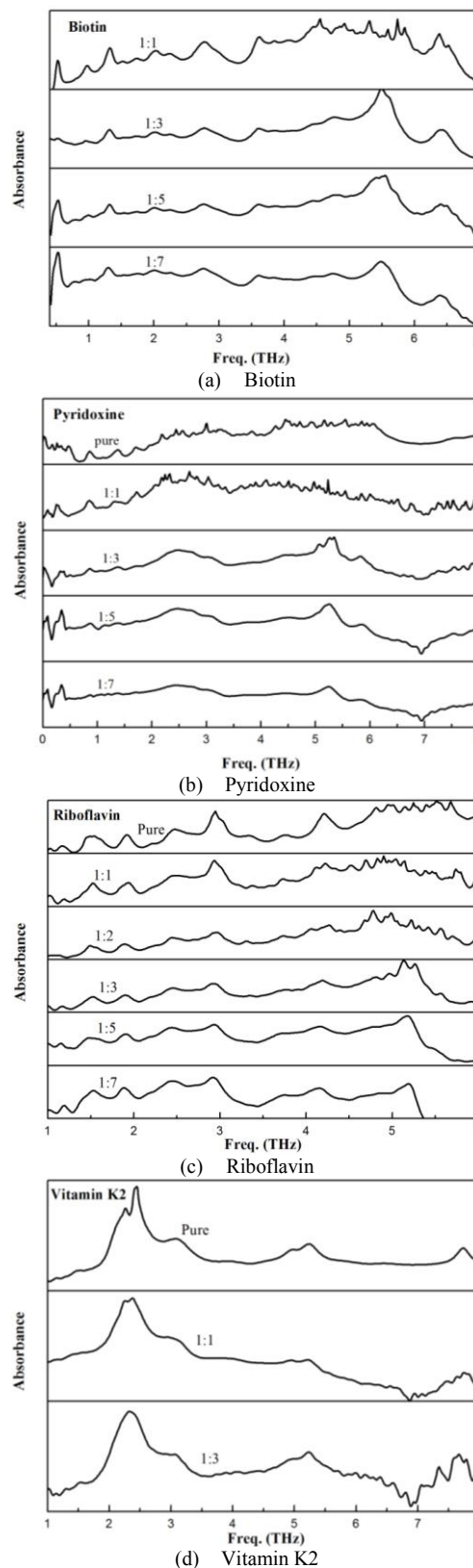


Fig. 3 Experimental THz absorption spectra (FTIR) for biotin (a), pyridoxine (b), riboflavin (c), and vitamin K2 (d) with various proportional mixing with polyethylene powder. Here "pure" means pure sample without mixing the polyethylene.

amounts of the samples for both of the THz-TDS and FTIR.

TABLE I
Measured THz absorption frequencies of vitamins

Measured absorption frequencies by FTIR (THz)					
L-ascorbic acid	thiamine hydrochloride	biotin	pyridoxine	Riboflavin	Vitamin K2
0.25	0.48	0.56	0.37	1.00	1.04
1.10	1.11	1.00	0.85	1.17	1.29
1.50	1.57	1.34	1.39	1.25	1.52
1.82	1.75	1.79	2.43	1.47	2.32
2.03	1.92	2.05	2.83	1.91	3.09
2.30	2.08	2.29	3.03	2.19	3.73
2.44	2.31	2.80	3.78	2.45	3.87
2.67	2.53	3.65	4.48	2.94	4.10
2.97	2.69	3.86	5.24	3.35	4.44
3.12	2.85	4.09		3.73	4.95
3.40	3.12	4.45		4.17	5.25
	3.22	4.78		4.76	
	3.31			4.97	
				5.18	

TABLE II
CALCULATED THz ABSORPTION FREQUENCIES OF VITAMINS

Measured absorption frequencies by FTIR (THz)					
L-ascorbic acid	thiamine hydrochloride	biotin	pyridoxine	Riboflavin	Vitamin K2
0.23	0.37	0.53	0.29	0.63	0.64
0.66	0.77	1.25	0.39	0.90	1.07
1.11	1.23	1.46	0.98	1.30	1.41
1.41	1.41	2.10	1.43	1.55	1.73
1.83	1.68	2.37	1.94	1.92	2.33
1.98	1.89	2.94	2.11	2.25	2.77
2.25	2.10	3.29	2.28	2.72	3.26
2.61	2.25	3.61	2.53	3.78	3.66
2.99	2.61	3.85	2.96	4.45	4.04
3.22	2.80	4.24	3.55	4.95	
3.64	3.56	4.68	3.81		
4.46	4.43	4.82	4.56		

III. MEASUREMENT RESULTS

Absorption measurement using the THz-TDS was conducted under a continuous flow of dry air, and the FTIR measurement was done in vacuum condition. The effect of water absorption was eliminated for both of measurement. The measured THz spectra of the L-ascorbic acid by THz-TDS and FTIR systems are shown in Fig. 2. The absorption frequencies measured by the FTIR are almost consistent with these measured by the TDS in the low-frequency range.

To study the effect of polyethylene powder on the absorption frequency band, we mixed the vitamin samples without and with the polyethylene for various proportions between 1:1 and 1:7, and homogenized the mixture in a mortar and pestle. The absorption spectra of the vitamin samples were obtained by using the polyethylene powder as reference background, as shown in Fig. 3.

The measured terahertz absorption frequencies are listed in Table I for the vitamins.

IV. THEORETICAL CALCULATION

In order to better understand the origin of the absorption features observed in our experimental spectra, a complementary series of calculations were performed to extract a picture of the intramolecular and intermolecular modes of vibration and rotation for the vitamin samples. The unit cell geometries for the L-ascorbic acid, thiamine hydrochloride, biotin, and pyridoxine were obtained from CSD databases [16]. Crystal cell parameters of the room temperature L-ascorbic acid were taken from Ref. [17]: Space group $P2_12_12_1$ ($Z=4$), $a=17.299$ (8) Å, $b=6.353$ (3) Å, $c=6.411$ (3) Å, $\alpha=90.00$, $\beta=102.18$ (13), $\gamma=90.00$. Room temperature thiamine hydrochloride were taken from Ref. [18]: Space group $P21/n$ ($Z=14$), $a=7.099$ (<1) Å, $b=19.808$ (2) Å, $c=11.638$ (1) Å, $\alpha=90.00$, $\beta=101.53$ (<1), $\gamma=90.00$. Room temperature biotin were taken from Ref. [19]: Space group $P2_12_12_1$ ($Z=4$), $a=5.196$ (<1) Å, $b=10.302$ (1) Å, $c=20.943$ (2) Å, $\alpha, \beta, \gamma=90.00$. Room temperature pyridoxine crystal cell parameters were taken from Ref. [20]: Space group $Pn21a$ ($Z=4$), $a=12.377$ (2) Å, $b=4.652$ (1) Å, $c=14.112$ (2) Å, $\alpha, \beta, \gamma=90.00$. The unit cell geometries of crystalline sample were optimized by DFT method using the hybrid functional B3LYP and a standard double-zeta basis set augmented with one set of polarization functions 6-31G (d). This combination produces reliable force fields not only for covalent bonds but also for weaker interactions like hydrogen bonds, which has been often used in the THz spectroscopic calculation of biological molecules [21]. All starting geometries were taken from solid-state X-ray measurements and were subject to structural optimizations. The ground state of these structures has been confirmed by using conformational analysis. We calculated the THz absorption spectra based on the optimized structures. Note that there is no imaginary frequency appears for all calculations. The structure parameters were included as Gaussian calculation setup in the Gaussian 09 software. The calculated absorption spectra were shown in Fig. 4. We employ unit cell geometry for the L-ascorbic acid, thiamine hydrochloride, biotin, and pyridoxine samples, and single

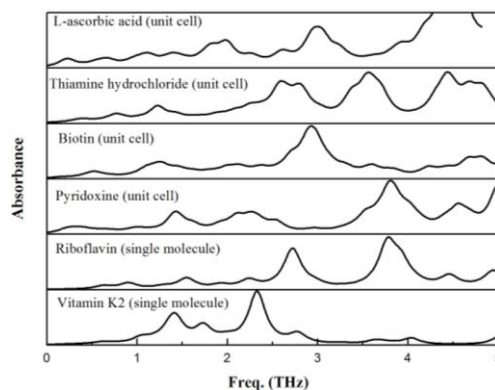


Fig. 4 THz spectra of vitamins calculated by Gaussian 09.

molecule geometry for the riboflavin and vitamin K2. The calculated absorption frequencies are shown in Table II.

The solid FTIR spectra of the vitamin samples are consistent with the calculations in the frequency region of 0-5THz except with slight frequency shift and few frequencies missing. The discrepancy between calculation and experiment results originates from that the molecular structure used in the calculation is gas phase molecular model, however the measured samples is solid state.

V. CONCLUSIONS

The THz spectra of the L-ascorbic acid, thiamine hydrochloride, biotin, pyridoxine, riboflavin, and vitamin K2 were studied using THz spectroscopy and DFT theory. The measurement results were demonstrated to be in good agreement with theoretical simulation by Density Functional Theory (DFT). We investigate the proportional effect of the samples mixing with polyethylene powder on the absorption spectra. Experiment demonstrates that the low ratio of 1 to 7 provide better reproducibility because of higher sensitivity in upper frequencies. The solid FTIR spectra of the vitamin samples are consistent with the calculations in the frequency region of 0-5THz except with slight frequency shift and few frequencies missing. The discrepancy between calculation and experiment results originates from that the molecular structure used in the calculation is gas phase molecular model, however the measured samples is solid state.

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