Research Article

Terahertz Absorption Properties of Two Solid Amino Acids and Their Aqueous Solutions

Ping Ye [ID],[1,2,3,4] **Guoyang Wang** [ID],[1,2,3,4] **Yizhou Yang** [ID],[1,2,3,4] **Qinghao Meng** [ID],[1,2,3,4] **Jiahui Wang** [ID],[1,2,3,4] **Bo Su** [ID],[1,2,3,4] **and Cunlin Zhang** [ID] [1,2,3,4]

[1]*Key Laboratory of Terahertz Optoelectronics, Ministry of Education, Beijing 100048, China*
[2]*Beijing Key Laboratory of Terahertz Spectroscopy and Imaging, Beijing 100048, China*
[3]*Beijing Advanced Innovation Center for Imaging Theory and Technology, Beijing 100048, China*
[4]*Department of Physics, Capital Normal University, Beijing 100048, China*

Correspondence should be addressed to Bo Su; subo75@cnu.edu.cn

A new type of embedded cyclic olefin copolymer microfluidic chip was designed and combined with terahertz (THz) technology to study the effects of glycine and arginine on the THz wave absorption characteristics. This study aims to understand the interactions between solid amino acid molecules and between amino acid and water molecules and to determine the changes in their microstructure. By observing the intensity of the time domain spectra in the range of 0.2–2.6 THz, we found that, as the concentration of glycine and arginine increased, the THz transmission gradually decreased. It can be inferred that the molecular structure and quantity of different amino acids have different influence on the hydrogen bond, which affects the absorption coefficient in solution. It was also found that the terahertz technique is able to identify the solid amino acid species better, and it can also perform some species identification for liquid amino acids. These results provide a reference for future studies on the terahertz absorption properties of amino acid samples. Moreover, Gaussian16 software was used to calculate the terahertz spectra using the density functional theory, B3LYP functional, and 6-31G basis set. Additionally, Gaussian View6 video software provided the frequency values, molecular vibration modes of the theoretical absorption peaks of glycine, arginine, and its aqueous solutions in the frequency range of 0.2–2.6 THz, which offers theoretical support for future studies.

1. Introduction

Terahertz (THz) waves are a type of electromagnetic wave with frequency ranging from 0.1 to 10 THz and wavelengths ranging from 30 to 3000 μm. THz waves can show the characteristic spectrum of plasma, organisms, and biological macromolecules, such as the rotations and vibrations of macromolecules themselves or the vibration of molecular groups. Hence, the absorption characteristics in the THz region can be used to characterise the internal structure of biomolecules [1]. In addition, the energy of terahertz photons is low, which is only 4.1 meV per photon, lower than that of ordinary chemical bonds. When using THz waves for detection, it will not cause damage to biological tissues. Previous studies have confirmed that the collective vibration

modes, such as skeleton vibrations and dipole rotations, play a leading role in the structure and function of protein molecules, and apart from this, the corresponding dynamic energy level happens to fall in the THz band [2].

Because the fingerprints of many organic molecules fall in the terahertz band, terahertz technology can be used to identify them, such as proteins and amino acids. The combination of the THz time domain system, the T-SNE dimension reduction system, and the XGBoost algorithm has been confirmed to be an effective nondestructive and nonlabelled method for evaluating the conformation and activity of proteins [3]. Water is the source of life, and the formation of the protein liquid state is typically accompanied by the solvation effect of its various structures, in which the polypeptides in the chain of aqueous solution have

higher flexibility, which makes the conformational transformation of proteins more agile, and the hydrophobicity of amino acids is conducive to the formation of the stable folding structure of protein [4]. It has been confirmed that THz spectroscopy can effectively be used to analyse the relationships between amino acids, amino acid mixtures, dipeptides and identified dipeptides, and their isomers at the molecular level [5]. Kutteruf et al. [6] studied the terahertz spectra of solid-state short-chain peptide sequences and found that most of the spectral and structural information of the system was in the range of 1–15 THz. Kelley [7] studied the absorption spectra of glutamic acid, aspartic acid, glycine, alanine, and seven other amino acids in different modes by using various improved THz spectroscopy detection techniques. Yi et al. [8] established a wideband absorption spectrum database of 20 amino acids from 0.1 to 6 THz.

When treating aqueous samples, this method is limited by the strong absorption of THz waves by water. Thus, the absorption of THz by liquids can be reduced by shortening the interaction distance between the THz and the liquid sample. The amount of sample required for a microfluidic chip is relatively small, and the scale can be controlled in the micron range. Fan [9] used a sandwich microfluidic chip to explore the effect of an electrolyte on the hydrogen bonding of water molecules by observing the change in the spectral intensity in a THz time domain spectroscopy system. The chip controls the thickness of the interlayer by controlling the thickness of the polydimethylsiloxane (PDMS) film, but the thickness may not be uniform, resulting in an inconsistent liquid thickness. When THz passes through a nonuniform liquid, the information about the liquid sample carried will also show some errors.

In this experiment, THz technology was combined with a new type of embedded cyclic olefin copolymer (COC) microfluidic chip to study the effects of glycine and arginine solutions on the THz wave absorption characteristics. We further revealed the interaction between solid amino acid molecules and between amino acid molecules and aqueous molecules and the changes in their molecular structures.

2. Experimental System

2.1. Experimental Apparatus and Optical System. A typical THz time domain spectral system was used in this experiment, and Table 1 shows the specific information.

Figure 1 shows the experimental optical path diagram. The femtosecond laser pulse was divided into two beams after passing through a polarisation splitter prism. One beam was used as the pump optical path, and the distance between the pump optical path and the detection optical path was made equal by extending the optical path through the reflector. The other beam, as the detection light path, passed through the delay device and then entered the optical fibre photoconductive antenna through the optical fibre coupler to detect the THz waves. The microfluidic chip containing the sample liquid was placed between the two off-axis parabolic mirrors at the focal point of the spot. The THz waves emitted by the THz antenna were focused by an off-axis parabolic mirror and then transmitted through the

microfluidic chip. After being focused by another off-axis parabolic mirror, the THz detection antenna was received and input into a phase-locked amplifier for amplification. Finally, the data were collected and processed by a computer.

2.2. Preparation of Microfluidic Chips. In this experiment, a microfluidic chip with embedded COC was used. COC has a transmittance of more than 90% for THz waves and is transparent to visible light, making it an excellent material for making microfluidic chips [9]. However, due to its high price, in order to save materials, only the terahertz transmission part uses COC materials. Figure 2 shows the manufacturing process of microfluidic chips. First, a laser-engraving machine was used for the 4 mm-thick Plexiglas to cut a 30 mm × 30 mm plexiglas block. The laser-engraving machine was then used to carve the centre of the Plexiglas to produce a 10 mm-long square groove with a depth of 1.925 mm; at the same time, a 1.925 mm deep groove is also carved at the same position on the back of the Plexiglas block. Then, a 50 μm-thick Plexiglas layer was formed between the two grooves facing each other. Finally, the 9 mm × 9 mm part in the middle of this Plexiglas layer was cut off with the laser-engraving machine to form a square border with an outer edge length of 10 mm, an inner edge length of 9 mm, and a thickness of 50 μm. The thickness of the square border was the depth of the liquid detection zone to be measured. The Plexiglas was placed vertically, and two holes at a depth of 11 mm were drilled with a drill that was 500 μm in diameter at the middle position of 11 mm from both sides of the plexiglas, which were used as the liquid inlet and liquid outlet, respectively. Two pieces of COC were cut with a thickness of 2 mm and length and width of 10 mm, and 502 glue was applied around them. Then, they are embedded into the grooves of the Plexiglas from the front and back. Finally, the gap between the Plexiglas and the COC was sealed with hot melt adhesive.

3. Simulation and Experimental Results

Because the impurities in the water will have a certain impact on the experiment, the distilled water was selected to configure the solution and perform the experiment. In this experiment, a glycine solution and arginine solution with five concentration gradients of 0.6–1.0 mol/L were configured. Then, these were injected into the microfluidic chip successively, and the THz time domain spectroscopy system was used for detection.

3.1. Terahertz Absorption Characteristics of the Glycine and Arginine Solutions. First, glycine and arginine solutions of 0.6, 0.7, 0.8, 0.9, and 1.0 mol/L were injected into the microfluidic chips. Then, the THz time domain spectral system was used to measure the glycine and arginine solutions. Origin software was used to process the data to obtain the time domain spectrum. The THz time domain spectra of glycine and arginine solutions are shown in Figures 3 and 4 , respectively. It can be found that the THz transmission decreases with the increase of their

TABLE 1: Terahertz (THz) time domain spectral information.

| Primary unit	Brand	Performance parameters
Femtosecond laser	Self-mode-locking fibre femtosecond laser independently developed and customised by Peking University	The central wavelength was 1550 nm, the pulse width was 75 fs, the pulse power was 130 mW, and the pulse repetition frequency was 100 MHz
THz-generating device	BATOP company	bPCA-100-05-10-1550-c-f
Time delay device	Electric translation table produced by Daheng Photoelectric Co., Ltd.	This device can achieve detection with a step size of 0.02 mm, and a total of 200 data points can be recorded
THz radiation detection device	BATOP company	bPCA-180-05-10-1550-c-f
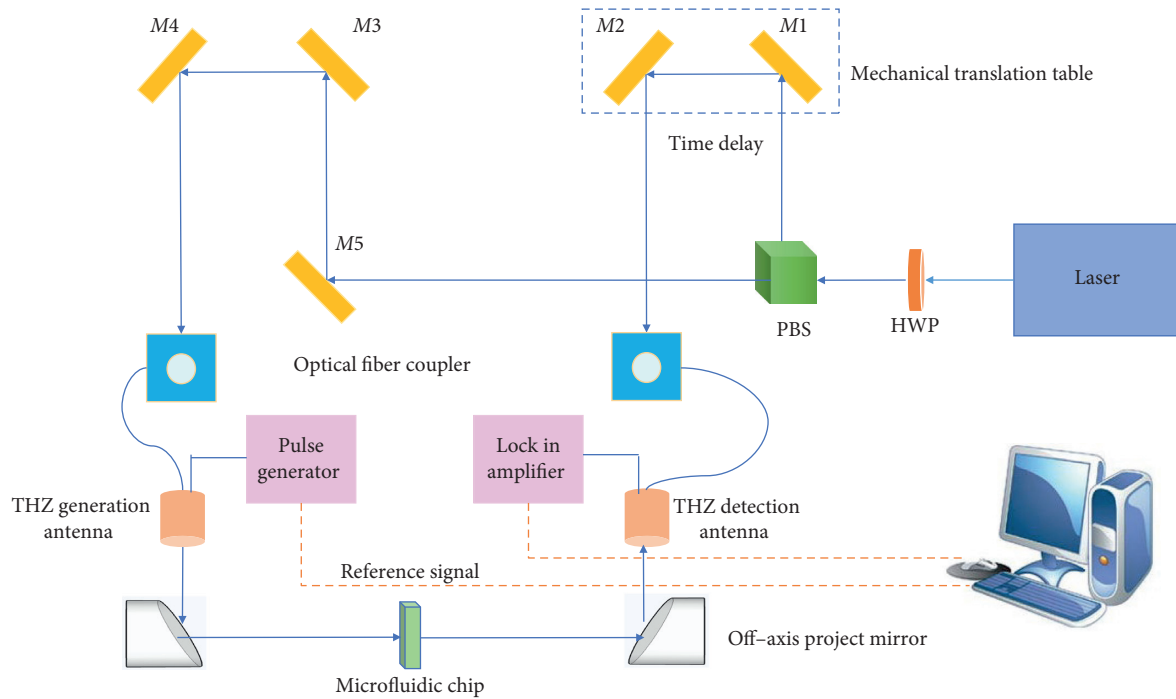
FIGURE 1: Experimental light path. (M1–5: reflector, PBS: polarisation beam splitter, HWP: 1/2 wave plate).
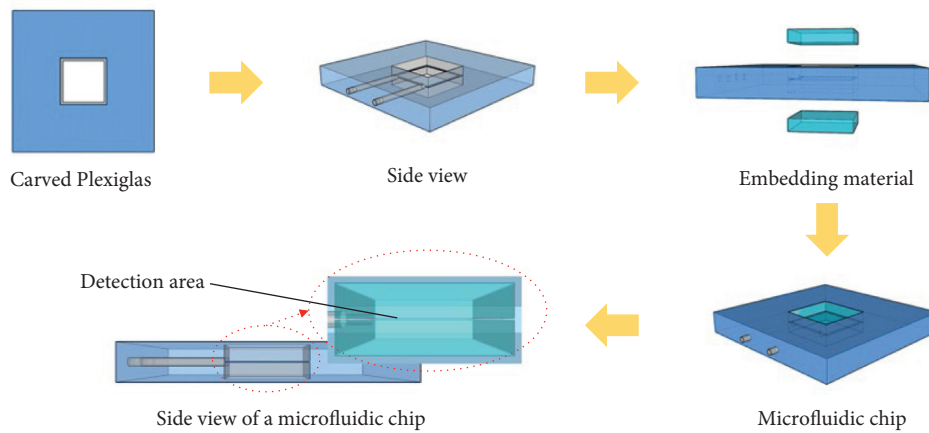

FIGURE 2: Schematic of microfluidic chip bonding.

concentration. The absorption of THz wave by water mainly comes from hydrogen bond in water. Therefore, it can be preliminarily considered that different concentrations of amino acid solution have different effects on hydrogen bond in water, which can be indirectly reflected in the intensity of THz spectrum.
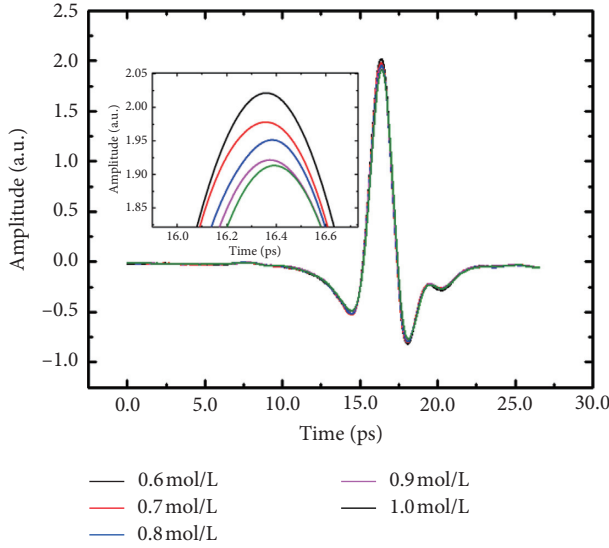
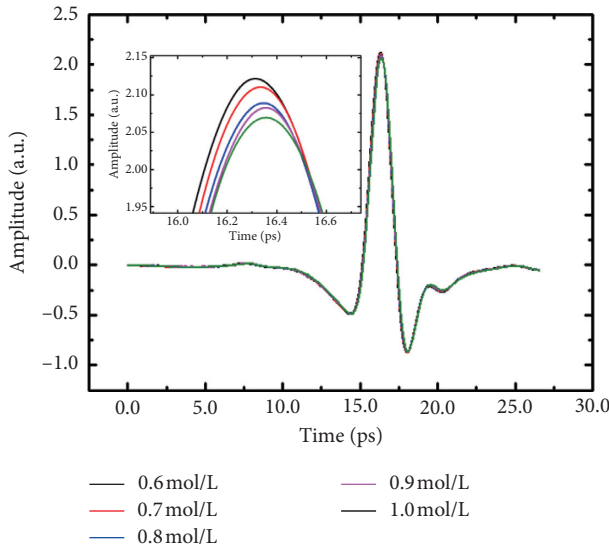Figure 3: Time domain spectra of glycine solutions of different concentrations.

Figure 4: Time domain spectra of arginine solutions of different concentrations.

3.2. Terahertz Absorption Coefficients of Glycine and Arginine Solutions.

We calculated the refractive indices and absorption coefficients of amino acid samples with the aid of THz-TDS system and using the following equations [10, 11]:

$$n(\omega) = \frac{c\varphi(\omega)}{d\omega} + 1, \tag{1}$$

$$n(\omega) = 1 - \frac{c\varphi(\omega)}{d\omega}, \tag{2}$$

$$\alpha_s = \frac{2}{d} \ln \frac{n_s (n_1 + 1)^2}{\rho(\omega) \cdot (n_1 + n_s)^2}, \tag{3}$$

where $\varphi(\omega)$ is the phase difference between the sample signal and the reference signal, $\rho(\omega)$ is the amplitude ratio between

the sample signal and the reference signal, ω is the angular frequency of THz, c is the speed of light, n_1 is the COC refractive index, n_s is the sample refractive index, d is the thickness of the sample, and α_s is the absorption coefficient of the sample. Equation (1) is used to calculate the refractive index of COC. Equation (2) is used to calculate the sample refractive index. Equation (3) is used to calculate the sample absorption coefficient.

Figures 5 and 6 show the absorption spectra of glycine and arginine solutions at 0.6 mol/L, respectively. Since water has a strong absorption for terahertz, the absorption coefficients of glycine and arginine solutions were compared with those of water. By observing the 0.2–2.6 THz band, it was found that the absorption peak of the glycine solution was mainly reflected at 1.79 THz, and the rest of the absorption peaks were from water. However, the absorption peak of the arginine solution was all from water.

3.3. Terahertz Absorption Coefficients of Solid Glycine and Arginine.

In order to better reflect the absorption peaks of the glycine and arginine molecules themselves, their solid samples were also subjected to absorption spectroscopy in this experiment. In the experiment, the samples were homogeneously mixed with polyethylene in the ratio of 1 : 5, pressed into tablets using a force of 6 tons, and finally placed in a vacuum system for measurement. The experimental results are shown in Figures 7 and 8 . In the range of 0.2–2.6 THz, the absorption peaks of solid glycine and solid arginine were at 2.38 THz and 0.97, 1.46, 1.68, and 2.07 THz, respectively.

3.4. Theoretical Calculation of the Absorption Peaks of Solid Glycine and Arginine and Their Aqueous Solutions.

To provide a deeper understanding of the absorption characteristics of the two amino acids in the THz band, Gaussian16 software can be used to conduct a theoretical simulation of the two amino acids using density functional theory, the B3LYP functional, and the 6-31G basis set for the terahertz spectral calculations. First, the structure was optimised to obtain the configuration of the minimum energy. Then, a frequency calculation was performed to obtain the theoretical spectrum. The different vibration modes of the absorption peaks of the two amino acids were assigned.

We used Gaussian View6 video software for solid glycine and arginine and aqueous solutions in the frequency range of 0.2–2.6 THz for evaluating the modes of vibration for identification of the absorption peaks. Figures 9(a) and 9(b) indicate the vibration model for solid glycine and its aqueous solution. The solid state of glycine at 2.32 THz absorption peak corresponds to collective molecular vibration caused by oscillating amino and methylene groups. The absorption peak of the aqueous glycine solution at 1.72 THz also has a contribution from the vibration of the hydrogen atom in the carboxyl group.

Since the absorption peaks of the substance itself are not clearly reflected in the arginine solution, the theoretical vibrational modes of the absorption peaks are attributed only for solid arginine. Figure 10 shows the vibration modes
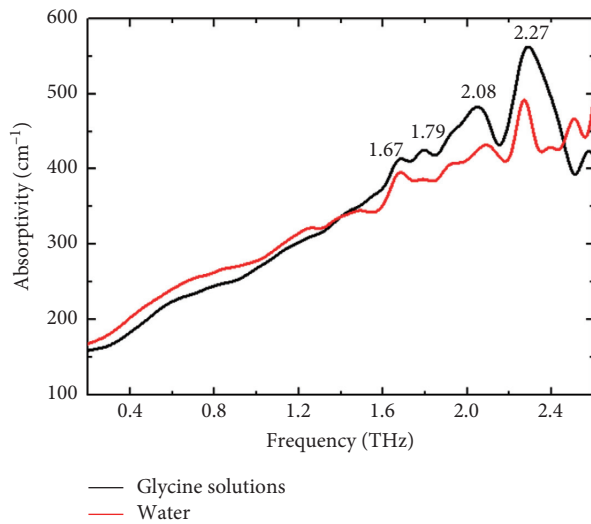
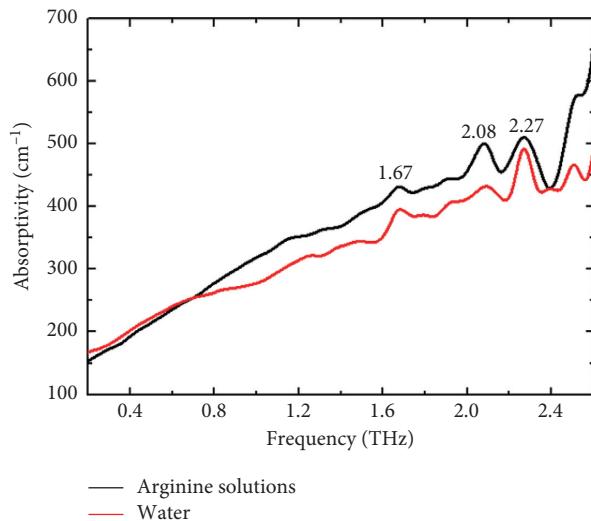FIGURE 5: Absorption spectra of glycine solutions.

FIGURE 6: Absorption spectra of arginine solutions.
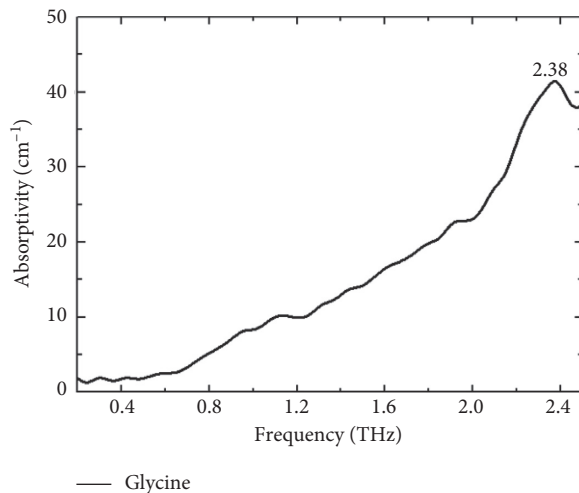

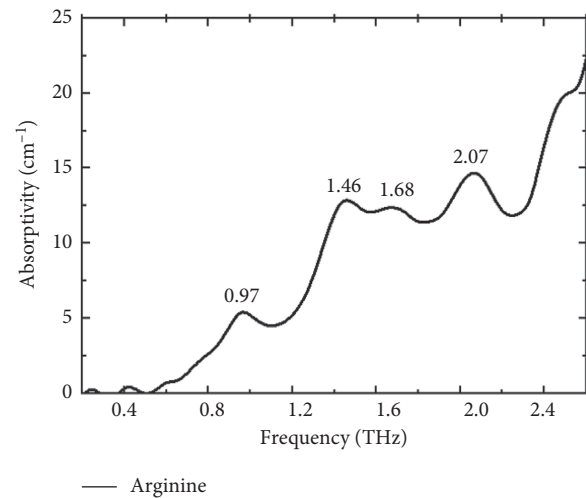FIGURE 7: Absorption spectra of solid glycine.

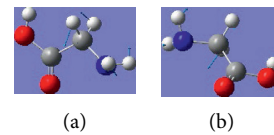FIGURE 8: Absorption spectra of solid arginine.

FIGURE 9: Vibration simulation diagram of the theoretical absorption peaks of solid glycine and glycine solution. (a) Solid glycine vibrational mode at 2.32 THz. (b) Glycine solution with the vibration mode at 1.72 THz.
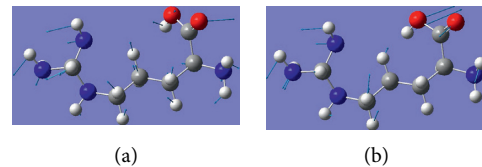

FIGURE 10: Vibration simulation diagram of the theoretical absorption peaks of solid arginine. Vibration modes at (a) 1.07 THz and (b) 1.71 THz.

of solid arginine. The absorption peaks of solid arginine correspond to the collective vibration of the molecule caused by the vibration or twisting of the amino and imino groups, carboxyl groups, and the hydrogen atoms on the carbon skeleton. Because the atomic vibration orientation changes at different frequencies and the number of atomic vibrations is different, the theoretical absorption peaks in the frequency range of 0.2–2.6 THz appear at multiple values.

Table 2 shows the theoretical and experimental values of the partial absorption peaks of glycine and arginine. As shown in the table, the difference between the theoretical and experimental values is small. There was a difference in the absorption peaks between the solid sample and the aqueous solution. This was because the addition of amino acids alters the acid base environment of the pure aqueous solution, thus changing the interaction of amino acids with bound water [12] and possibly affecting the number of hydrogen bonds formed between amino acid molecules and water molecules. Moreover, solid arginine has a more

TABLE 2: Theoretical and experimental values of the partial absorption peaks of glycine and arginine.

Sample	Theoretical absorption peak (THz)	Experimental absorption peak (THz)	Error (THz)
Solid glycine	2.32	2.38	0.06
Glycine solution	1.72	1.79	0.07
	1.07	0.97	0.10
	—	1.46	—
Solid arginine	1.71	1.67	0.04
	—	2.07	—

complex molecular structure, which may result in more absorption peaks.

4. Theoretical Analyses and Discussion

Whether the THz absorption spectrum is dominated by intermolecular or intramolecular vibrations is determined by factors such as temperature, acidity, and molecular size [13]. The mechanism of action is more complex. When glycine and arginine molecules are incorporated into water, they cause collective molecular rotation and weak intermolecular interactions, including local molecular translation, oscillation, twisting, and intramolecular and intermolecular hydrogen bonding [14]. Xu et al. [15] found that when amino acids are added to a solution of water molecules, the water molecules can easily form another hydrogen bond with the amino and carboxyl atoms. It is also possible to form a double hydrogen structure when the water molecule is in a position where it interacts strongly with the amino hydrogen atom. Thus, the higher the concentration of an amino acid solution, the more amino acid molecules, the stronger the interaction effect, and the easier it is to form hydrogen bonds with water molecules. Macroscopically, for the same type of amino acids, the higher the concentration, the smaller the THz transmission and the lower the spectral peak in the time domain.

The properties of solid substances are relatively stable and can better reflect the inherent properties of molecules. However, in real life, most substances exist in liquid form. Therefore, the detection of liquid samples is crucial. When a substance is dissolved in water, water has a strong absorption effect for terahertz. Therefore, the absorption peaks in liquid samples often reflect the absorption peaks of water, thus covering the absorption peaks of the solid substance itself. We found that the absorption peak of glycine solution was 1.79 THz and that of solid glycine was 2.38 THz. The absorption peak was significantly shifted due to the addition of water, which caused changes in the acidity and alkalinity of the liquid sample, the temperature, and the intra- and intermolecular forces. However, for the arginine solution, all the absorption peaks of the substance itself were absorbed by water.

To further understand the absorption characteristics of these two amino acids in the THz band, Gaussian16 software was used to calculate the terahertz spectra of theoretical amino acid molecules. The results showed that the absorption peaks in the THz band are mainly in the collective vibrational mode of the molecules caused by the wobble or twist vibrations of the radicals. The THz absorption spectrum is a concentrated representation of intramolecular

atomic and atomic group interactions, intermolecular hydrogen bonds, and van der Waals forces [16]. Different absorption peaks have different atomic vibration patterns, but most of the atoms participate in the vibration.

Theoretical spectrum is a theoretical simulation of a single amino acid molecule, and it can only reflect the vibration caused by the intramolecular interaction. However, the measured spectrum also contains the vibration caused by intermolecular interactions and lattice vibrations. Moreover, the ambient temperature in the experiment and the acidity and alkalinity of the solution may be different from those in the theoretical simulation. The above factors are the main reasons for the difference between the theoretical spectrum and the actual spectrum.

5. Conclusion

In this study, by observing the intensity of the time domain spectra in the range of 0.2–2.6 THz, we found that the THz transmission decreased with increase in the concentrations of glycine and arginine. For solid amino acids, different types of amino acids are well identified using the terahertz technique. For liquid amino acid solutions, the microfluidics-based terahertz technique is able to identify glycine better, but the absorption peak of arginine is completely covered by water. Additionally, at the end of this study, Gaussian16 software was used to calculate the theoretical terahertz spectra of the amino acid molecules in the frequency range of 0.2–2.6 THz, and Gaussian View6 video software was used to assign the vibration modes. In the future, we will probe deeper into the liquid amino acid solution absorption peaks and compare them with solid amino acids. Therefore, THz techniques are used to identify liquid amino acids in solution whenever possible.

Data Availability

The authors declare that they have no conflicts of interest.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

References

[1] M. Tang, M. Zhang, S. Yan et al., "Detection of DNA oligonucleotides with base mutations by terahertz spectroscopy and microstructures," *PLoS One*, vol. 13, no. 1, p. e0191515, 2018.

[2] C. Cao, *Identification of Protein Conformation and Characterization of Intermolecular Interactions Based on Terahertz Spectroscopy*, University of Science and Technology Beijing, Beijing, China, 2021.

[3] C. Cao, Z. H. Zhang, X. Y. Zhao et al., "Terahertz spectroscopy and machine learning algorithm for non-destructive evaluation of protein conformation," *Optical and Quantum Electronics*, vol. 52, no. 39, 2020.

[4] B. Ferguson and X.-C. Zhang, "Materials for terahertz science and technology," *Nature Materials*, vol. 1, no. 1, pp. 26–33, 2002.

[5] Y. J. Bian, X. Zhang, Z. Q. Zhu et al., "Investigation of the correlations between amino acids, amino acid mixtures and dipeptides by terahertz spectroscopy," *Journal of Infrared, Millimeter, and Terahertz Waves*, vol. 42, no. 1, 2020.

[6] M. R. Kutteruf, C. M. Brown, L. K. Iwaki et al., "Terahertz spectroscopy of short-chain polypeptides," *Chemical Physics Letters*, vol. 375, no. 3, pp. 337–343, 2003.

[7] M. Kelley, *Terahertz Time Domain Spectroscopy of Amino Acids and Sugars*, California Institute of Technology, Pasadena, CA, USA, 2013.

[8] W. Yi, J. Yu, Y. Xu et al., "Broadband terahertz spectroscopy of amino acids," *Instrumentation Science & Technology*, vol. 45, no. 4, pp. 423–439, 2017.

[9] N. Fan, B. Su, Y. X. Wu et al., "Sandwich terahertz microfluidic chip," *Spectroscopy and Spectral Analysis*, vol. 38, no. 5, pp. 1362–1367, 2018.

[10] F. M. Al-Douseri, Y. Q. Chen, and X.-C. Zhang, "THz wave sensing for petroleum industrial applications," *International Journal of Infrared and Millimeter Waves*, vol. 27, no. 4, pp. 481–503, 2006.

[11] T. D. Dorney, R. G. Baraniuk, and D. M. Mittleman, "Material parameter estimation with terahertz time-domain spectroscopy," *Journal of the Optical Society of America A*, vol. 18, no. 7, pp. 1562–1571, 2001.

[12] Y. Y. Fu, *Study on Terahertz Spectroscopy of Interaction Between Amino Acids and Water*, Zhejiang Sci-Tech University, Hangzhou, China, 2019.

[13] W. N. Wang, H. Q. Li, Y. Zhang, and C. L. Zhang, "Terahertz spectra of 20 α-amino acids and their correlation with their molecular structures," *Journal of Physical Chemistry*, vol. 25, no. 10, pp. 2074–2079, 2009.

[14] S. Q. Zong, *Study on the Application of Terahertz Spectroscopy to Alkali Metal Inorganic Salt and Amino Acid Salt*, East China University of Science and Technology, Shanghai, China, 2018.

[15] P. C. Xu, *Amino Acid Hydration and H_2O, H_2 Study on Hydrogen Bond of S, HF Dimer*, University of Jinan, Jinan, China, 2017.

[16] F. Yan, W. Li, and Z. C. Wang, "Terahertz vibration modes of amino acid functional groups," *Spectroscopy and Spectral Analysis*, vol. 40, no. 2, pp. 397–402, 2020.