Infrared Physics and Technology 96 (2019) 17–21

Contents lists available at ScienceDirect

Infrared Physics & Technology

journal homepage: www.elsevier.com/locate/infrared

Regular article

# Terahertz spectral of enantiomers and racemic amino acids by time-domain-spectroscopy technology

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ARTICLE INFO

*Keywords:*
Amino acids
Isomers
Terahertz time-domain spectroscopy
Density functional theory
Collective vibration

ABSTRACT

The absorption spectra of four amino acids were studied. These spectra are obtained by terahertz time-domain spectroscopy (TDS) technique. The four amino acids are alanine, methionine, leucine, and valine, respectively. The spectra of their enantiomers (L-, D-) and racemic compounds (DL-) were investigated. Although the two isomers have very similar structures, their absorption spectra are obviously different. The absorption coefficient of each structure is calculated by the density functional theory (DFT), and the simulated spectra of each structure are obtained. It is shown that the number of the calculated peaks is in good agreement with the experimental ones. The experimental spectra were compared with the theoretical spectra. The differences between the absorption spectra of isomers are presented, and the reasons for the differences were analyzed. The vibratory spectra of biomolecules were studied, and the correlation between molecular structure and function was further understood.

## 1. Introduction

The terahertz (THz) band in the electromagnetic spectrum is between millimeter-wave and infrared, ranging from 100 GHz to 10 THz (wavelength from 3 mm to 30 μm). THz wave is a bridge between electronics and photonics. It is very sensitive to different molecular structures, even if these structures are only slightly different. Radiation in the terahertz range causes intermolecular and intramolecular vibrations, including low-frequency torsional and stretch oscillations. In recent years, there have been more and more studies on terahertz radiation mechanism [1–3], detection technique [4] and applied technology [5,6], which has led to the rapid development of terahertz technology. THz TDS technology with high signal-to-noise ratio (SNR) and high spectral resolution has been applied in agriculture [7–9], physical chemistry [10,11], material science [12], security applications [13], pharmaceutical engineering [14] and biomedical [15–19] fields. High-quality vibration spectra in low frequency and corresponding molecular mechanism information can be obtained by this technology. This technique is particularly active in biomedical applications. In recent decades, studies on amino acids such as histidine [20], alanine [21], leucine [22] and glutamic [23] have been reported. It is helpful to understand the relationship between molecular structure and function by studying the collective low-frequency vibrational spectra of biomolecules.

Different polymers have different structures, so they can be distinguished by their absorption spectra. The main factor leading to different absorption spectra of different compounds is the different molecular vibration modes. There are two modes of molecular vibration, one is the hydrogen bond vibration and the other is the intramolecular bond vibration. All the motion of hydrogen bonds serve as the bridges between molecules, while the motion of intramolecular bonds have two forms: torsional and extensional vibration. In order to better understand the internal mechanism of biological macromolecules in THz frequency band, the absorption spectra of different compounds were analyzed, and their molecular vibrational modes of each compound were simulated. In this study, we not only measured the spectral differences of each amino acid, but also carried out theoretical simulation and comprehensive comparison. The four amino acids were selected for absorption spectra study in the terahertz band 0.1–3.0 THz at room temperature. The absorption spectra of these four selected amino acids were studied in terahertz band in 0.1–3.0THz range at room temperature. At the same time, the absorption spectra of four amino acids were simulated by DFT theory encoded by DMol3 and compared with the experimental results.

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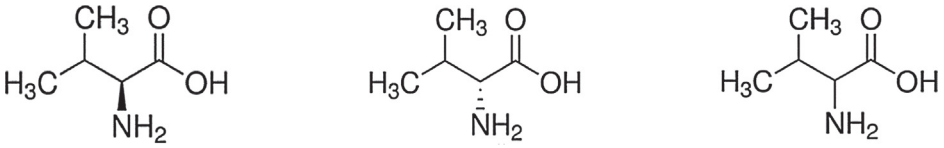
https://doi.org/10.1016/j.infrared.2018.10.026
Received 28 September 2018; Received in revised form 18 October 2018; Accepted 18 October 2018
Available online 24 October 2018
1350-4495/ 




(a) D-valine (b) L-valine (c) DL-valine

**Fig. 1.** Molecular structure of D-valine, L-valine and DL-valine. They have the same chemical formula, but their $NH_2$ position is different. The structure of D and L valine is symmetrical.

The terahertz spectra of amino acids are of great significance in understanding the composition and function of proteins. THz spectra can well reveal the differences of vibration modes within molecules. Different absorption peaks in the spectra come from different modes of molecular micro-vibration. The spectral characteristics of valine were focused in this paper. Valine is one of the 20 amino acids in protein. Its chemical name is 2-amino-3-methylbutyric acid. It belongs to branched chain amino acid. It works in conjunction with two other high concentrations of amino acids (leucine and isoleucine) to promote normal growth, tissue repair, blood glucose regulation, and energy requirements. At 2011, Williams et al. used TDS technology to measure the THz spectra of valine at room temperature and low temperature (78 K) respectively. In 2011, Williams et al. measured the THz spectra of valine at room temperature and low temperature (78 K) using TDS technique. The spectral characteristics of enantiomers and racemic samples were described by quantitative method, and the spectra of enantiomers and racemic samples were studied by combining theory with experiment [24]. Valine has three forms, and their structures are shown in Fig. 1. All three forms have the same chemical formula, but the different $NH_2$ positions make their structures different. L-Valine is one of the essential amino acids for proteins of human body, while D-valine is considered to be toxic to humans. DL-valine can be chemically synthesized.

The amino acids which were purchased from Sigma-Aldrich are L-alanine (≥99%), D-alanine (≥98%), DL-alanine (≥98%), L-methionine (≥98%), D-methionine (≥98%), DL-methionine (≥98%), L-valine (≥98%), D-valine (≥98%), DL-valine (≥99%), L-leucine (≥98%), D-leucine (≥97%) and DL-leucine (≥99%), respectively. The purchased amino acid crystals are large grained and need further processing. The experimental crystals were divided into two groups, one group was measured without further purification, the other group was recrystallized to obtain higher purity and more orderly samples. To minimize Mie scattering, all crystals were ground into powder with mortar and pestle of the same size as polyethylene (PE). The reliability of the experimental results is further verified at different concentrations. The mixture of amino acid crystal and PE was mixed at the ratio of 0.3: 0.7, 0.5: 0.5 and 0.8: 0.2, respectively. Then the mixture was compressed into tablets. The thickness of sample was around 1 mm with a pressure around 30 MPa and the diameter was 13 mm. The thickness of the sample is about 1 mm, the diameter is about 13 mm and the compressing pressure is 30 MPa. Each sample was tested twice in different locations. The spectra of recrystallized and higher concentration samples are superior to those of untreated samples. All the results in this paper are derived from the recrystallized samples with a concentration of 80%.

Fig. 2 shows the schematic diagram of the terahertz time-domain spectrometer. The terahertz time-domain spectrometer is based on a pulsed femtosecond laser with a spectral resolution < 1.2 GHz. It's flexible and open. It has a bandwidth of 4.5 THz, which meets the test requirements. The radiation beam of femtosecond laser is divided into two beams: one is the pump beam, which propagates to the emitter antenna, and the other is the probe beam, which propagates to the detector antenna. There is a delay in detecting beam for coherent detection with pump beam. The function of the delay line is to keep the optical path difference between the two beams unchanged. The reference signal and DL-valine signal as obtained from TDS are showed in Fig. 3. The reference signal and DL-valine signal obtained from TDS are

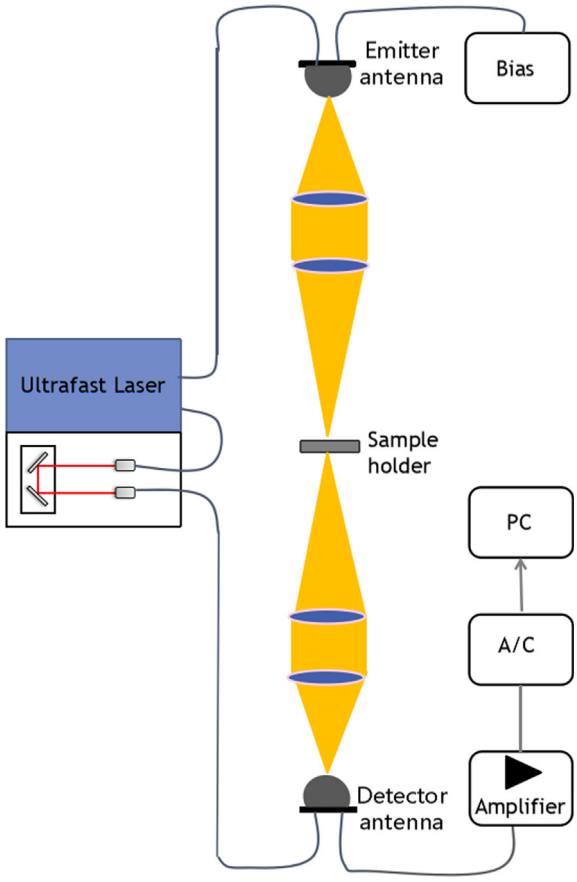



**Fig. 2.** Schematic of the Terahertz Time domain Spectrometer. The emitter antenna is based on a bias semiconductor structure with a low carrier lifetime. The detector antenna works as a dipole. The input terahertz pulse generates a small current when the femtosecond pulse is excited.

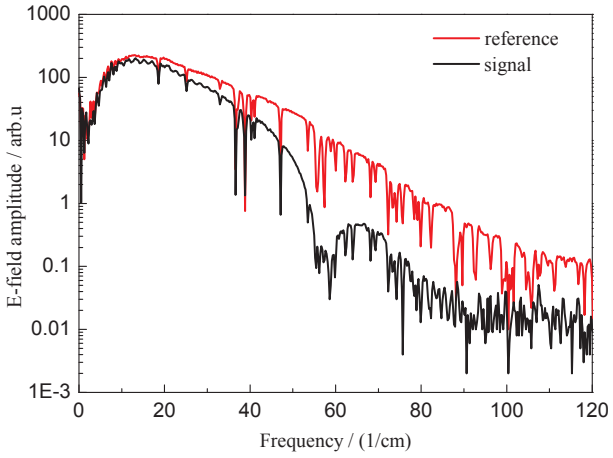



**Fig. 3.** The representative frequency domain map is obtained by using the system. E-field amplitude converted with Fast Fourier-transform (FFT) as a frequency function.

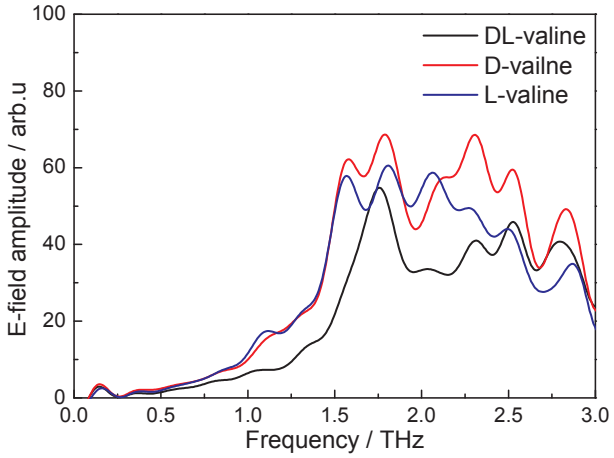



**Fig. 4.** The absorption spectra of L-valine, D-valine, DL-valine.

shown in Fig. 3. The spectral baseline increases with the increase of frequency due to the scattering effect of two-phase medium [26].

The refractive index $n$ and absorption coefficient $\alpha$ are derived from the following formulas:

$$n(\nu) = 1 + \frac{c}{2\pi\nu d}(\Phi_{sam}(\nu) - \Phi_{ref}(\nu))$$

$$\alpha(\nu) = -\frac{d}{2}\ln\left[\frac{E_{sam(\nu)}}{E_{ref(\nu)}}\frac{(n(\nu)+1)^2}{4n(\nu)}\right]$$

where $E_{sam}$ and $E_{ref}$ are the electric field of sample and reference (air only) respectively, $\nu$ is the frequency, $d$ is the thickness of the sample, and $\Phi$ is the phase.

## 2. Results and discussion

The THz absorption spectra of amino acid crystals were measured at room temperature (temperature 298 K, humidity 36.7%), and the pulse signals in air were used as reference signals. The absorption spectra of D-valine, L-valine and DL-valine are shown in Fig. 4. There is a certain frequency spectrum vibration in the range of 0.1–1.5 THz, and the baseline increases with the increase of frequency. The causes of this phenomena are the internal reflection [25] and scattering effects of the two-phase medium [26]. The effective spectral width is 0.1–3.0 THz. Although the structures of the three valine are similar, their absorption spectra are obviously different. As can be seen from the figure, their amplitudes are obviously different. Because of the symmetry of the enantiomeric structure, the number of peaks is basically the same. However, the difference between enantiomers and racemic compounds lies not only in the number and position of the peaks, but also in FWHM [27] and amplitude of the peaks. D-valine has six absorption peaks at 1.65 THz, 1.80 THz, 2.18 THz, 2.35 THz, 2.50 THz and 2.77 THz, while L-valine has six absorption peaks at 1.60 THz, 1.85 THz, 2.23 THz, 2.30 THz, 2.50 THz and 2.80 THz. The positions of peaks of DL-valine are at 1.75 THz, 2.35 THz, 2.25 THz and 2.75 THz, but there is no absorption peak at 1.60 THz and 2.20 THz. The characteristic absorption peaks of valine in the range within 2.50 THz are in good agreement with the experimental results published by Williams [24]. But he didn't study the range beyond the 2.5 THz. There are also absorption peaks at 2.50 THz and 2.80 THz, but the absorption peaks at 2.55 THz and 2.85 THz have no effect on the identification of valine. Since all the measured spectral images are similar on the absorption peaks at 2.55 THz and 2.85 THz, it is probably caused by other factors. Specific factors need to be further studied. The number and amplitude of absorption peaks may be the main factors of valine type identification.

As shown in Fig. 5, the absorption spectra of L-valine, D-valine and DL-valine are calculated at BLYP-D2 level. Before calculation, the molecular structure is optimized in geometry to minimize the energy and relax the lattice parameters and atomic coordination. The energy convergence tolerance is set to 2.0e − 5 eV/atom. Compared with the calculated results, the number of absorption peaks is consistent, which verifies the feasibility of the experiment. However, compared with the experimental results, the overall position has occurred a blue shift. Through investigation and analysis, it is found that there are three main reasons for this phenomenon. First, the DFT computing system overestimates the frequency of the normal mode [28]. Second, the simulation method is not yet mature. The main reason is the lack of experimental data. Third, the experimental conditions are different with calculation. The calculation is carried out in vacuum condition, while the experiment is carried out in air. As shown in Fig. 6, the absorption spectra are the result of the intermolecular and intramolecular vibration modes. The energy required for the vibrations between the points is less than that for the molecules [23,29].

There are two main vibratory modes of the molecule that make the spectra different. They are the external deformation of the intramolecular functional groups and the collective vibration mode of polymer structure. The molecules in the polymer are arranged periodically, and the movement direction of individual molecules is consistent. Therefore, the collective vibration mode means that the whole motion direction of a large number of molecules is the consistent. Different polymers have different molecular structures, so they have different vibration modes. The vibratory modes of valine molecules are derived from carboxyl (−COOH), amino (−NH2), hydroxyl (−OH), methyl (−CH3) and methylene (−CH2−). The telescopic vibration modes of C−C, C−O and C−H bonds are also the main mode of vibration. The reverse oscillation at both ends of the molecule causes the torsion of the branched backbone. As shown in Fig. 6, there is a reverse movement trend between L-valine and D-valine, and DL-valine is different from any of them. The intermolecular interaction has great influence on the shape and position of absorption peaks.

Figs. 7–9 show the absorption spectra of alanine, leucine and methionine. The spectra of alanine enantiomers and their racemic compounds vary greatly in the position and amplitude of the peaks. L- and D-alanine have two absorption peaks at 2.25 THz and 2.58 THz. DL-alanine has one absorption peak at 1.28 THz. The characteristic absorption peaks of alanine are in keeping with the experimental results reported by Yamaguchi [21]. For leucine, its enantiomers have different THz absorption spectra. Among them, the peak position, peak number, peak amplitude and the absorption peak of racemic compounds are obviously different. The absorption spectra of leucine is in keeping with the experimental results reported by Williams and Wang [22,30]. Similarly, the absorption spectra of methionine have the same features.

## 3. Conclusion

The molecular vibration characteristics of amino acid crystals valine, alanine, leucine and methionine were studied by THz TDS. The absorption coefficient was measured in the range of 0–3.0 THz. Compared with the theoretical simulation, although the position of the peaks is slightly different due to the different circumstances, the number of peaks is the same. It is indicated that THz TDS is a powerful technique to distinguish molecule structure of materials. Therefore, terahertz spectroscopy is of great significance in the study of structural characteristics and dynamics of biomolecules. The main purpose of spectral research is to obtain the characteristic spectrum of the object of study. There are relatively few studies on the four amino acids mentioned in this paper at home and abroad. These four amino acids are of great significance to the study of the structure and function of biological molecules in the life sciences. The study of vibration spectrum of amino acids helps to understand the correlation between molecular structure and function. The main difficulty in interpreting the terahertz spectrum lies in the lack of the corresponding molecular vibration model in the terahertz band.

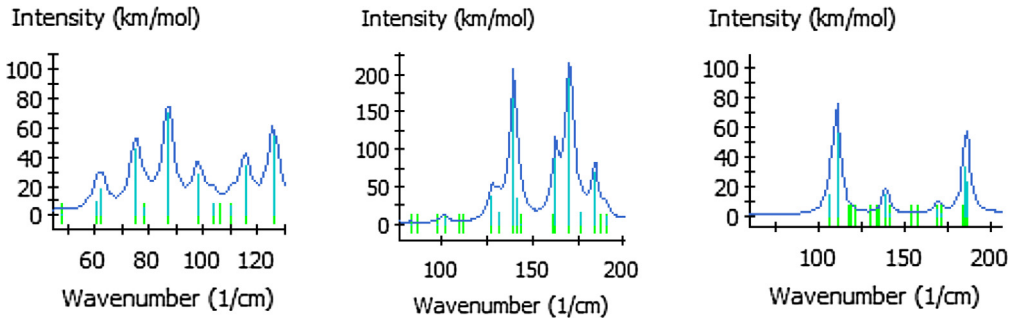



**Fig. 5.** The spectra obtained by simulation. From left to right, L-valine, D-valine and DL-valine. The calculation process adopts DFT at BLYP-D2 level.

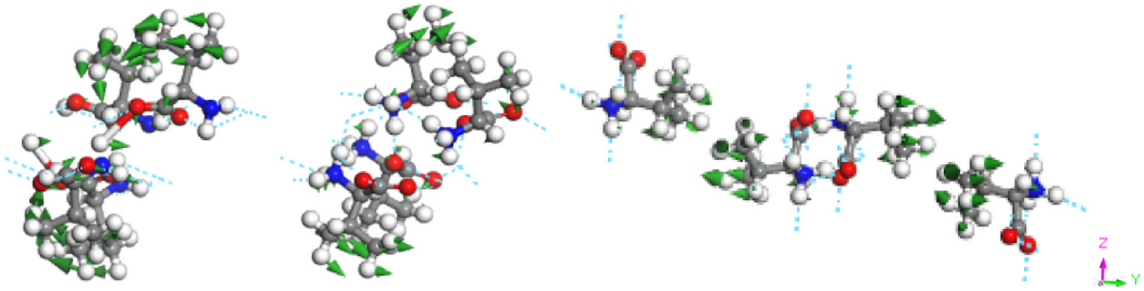


**Fig. 6.** The molecular vibration model. These three pictures represent L-valine, D-valine and DL-valine, respectively. Among them, the white ball represents the hydrogen atom, red ball represents oxygen atom, gray ball represents carbon atom and blue ball represents nitrogen atom.

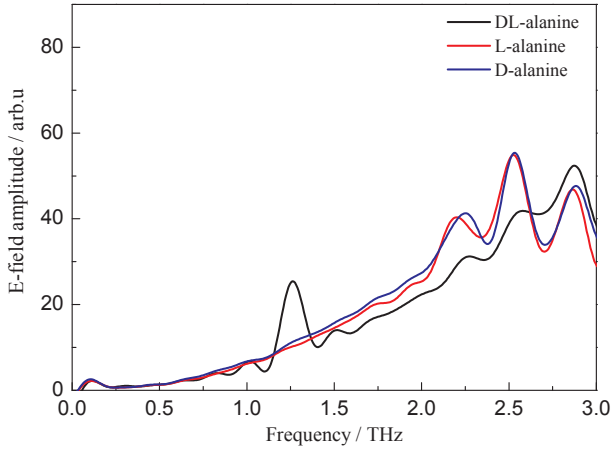



**Fig. 7.** The absorption spectra of L-alanine, D-alanine, DL-alanine.

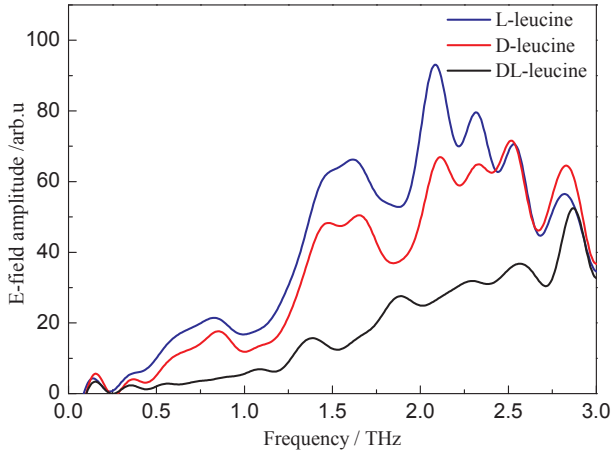



**Fig. 8.** The absorption spectra of L-leucine, D-leucine, DL-leucine.

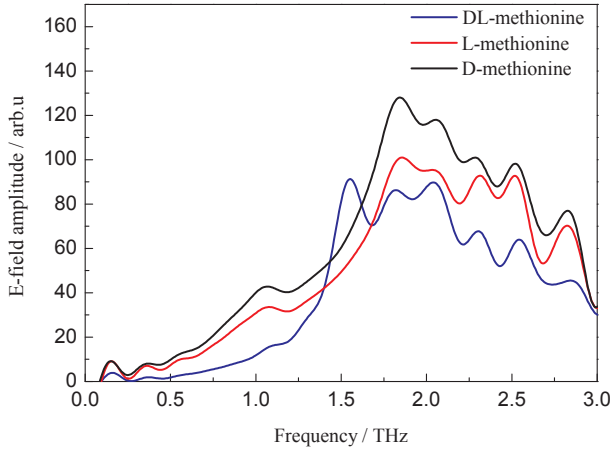



**Fig. 9.** The absorption spectra of L-methionine, D-methionine, DL-methionine.

## Conflict of interest

The authors declare that they do not have any conflict of interest of this manuscript entitled "Terahertz Spectral of Enantiomers and Racemic Amino Acids by Time-Domain-Spectroscopy Technology".

## Acknowledgment

This work was supported by National Key R&D Program of China (2017YFF0106302), the 973 Program of China (Grant No. 2014CB339803), the National Natural Science Foundation of China (Grant Nos. 61775229, 61704181, 61575214, 61574155, and 61604161), and the Shanghai Municipal Commission of Science and Technology (Project Nos. 15JC1403800, 15DZ0500103, 17YF1429900, 17YF1430000).

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