Invited Paper

Recent Progress of Terahertz Spectroscopy on Medicine and Biology in China

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Abstract: Terahertz (THz) spectroscopy provides a powerful tool for characterization of a great many biomolecules and tissues. Here, the fruitful work by Chinese researchers in recent years is presented, covering the THz spectroscopy on biomolecules, identification of illicit drugs and medical imaging. There are numerous challenges and problems toward the application of THz spectroscopy technique.

Keywords: Terahertz Spectroscopy, Biomolecules, Identification of illicit drugs, Medical imaging

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1. Introduction

Terahertz (THz, 1 THz=10^{12} Hz) wave, which lies in the frequency gap between the infrared and microwave, typically covers from 0.1 THz to 10 THz. However, due to the lack of effective THz wave generation and detection techniques in the past, this frequency region is largely under-utilized and referred to as the "Terahertz Gap" of the electromagnetic spectrum. Since being established in early 1990s, the THz time domain spectroscopy (THz-TDS) has been widely used in material characterization. THz-TDS uses short pulses of broadband THz radiation and the transmitted THz electric field is measured coherently. Thus, THz-TDS could provide THz broadband spectroscopic information both on amplitude and phase with high sensitivity.

THz spectroscopy has been applied to study many biological molecules and tissues, including proteins, medicines, cancer tissue, DNA [1, 2]. One important application is in the identification of biological molecules. Furthermore, THz spectroscopy could provide information about intermolecular vibrations in some chemical and organic molecules where the intermolecular vibration mode appears in the infrared region. It is expected that intermolecular vibration studies will elucidate the dynamics of large molecules. Besides that, THz imaging used for detection of some cancer is another attractive application.

Chinese scientists and researchers have done plentiful exploration about THz spectroscopy on medicine and biology. As the forerunner of China, the researchers in Capital Normal University did fruitful work on THz spectroscopy of proteins and Chinese traditional medicine. A series of work on detection of illicit drug contribute the potential application of THz spectroscopy technique in security. Research groups from other universities and the institute of Chinese Academy of Science also obtained inspiring results in THz spectroscopy and imaging. In this

paper, we review the important progress of THz spectroscopy on medicine and biology by Chinese scientists in recent years.

2. THz spectroscopy on proteins

Protein with various structures of molecular and function plays a vital role in life system. THz spectroscopy technique not only serves as a good method in identification of protein, but also is widely used in the investigation of molecular structure and dynamics [3].

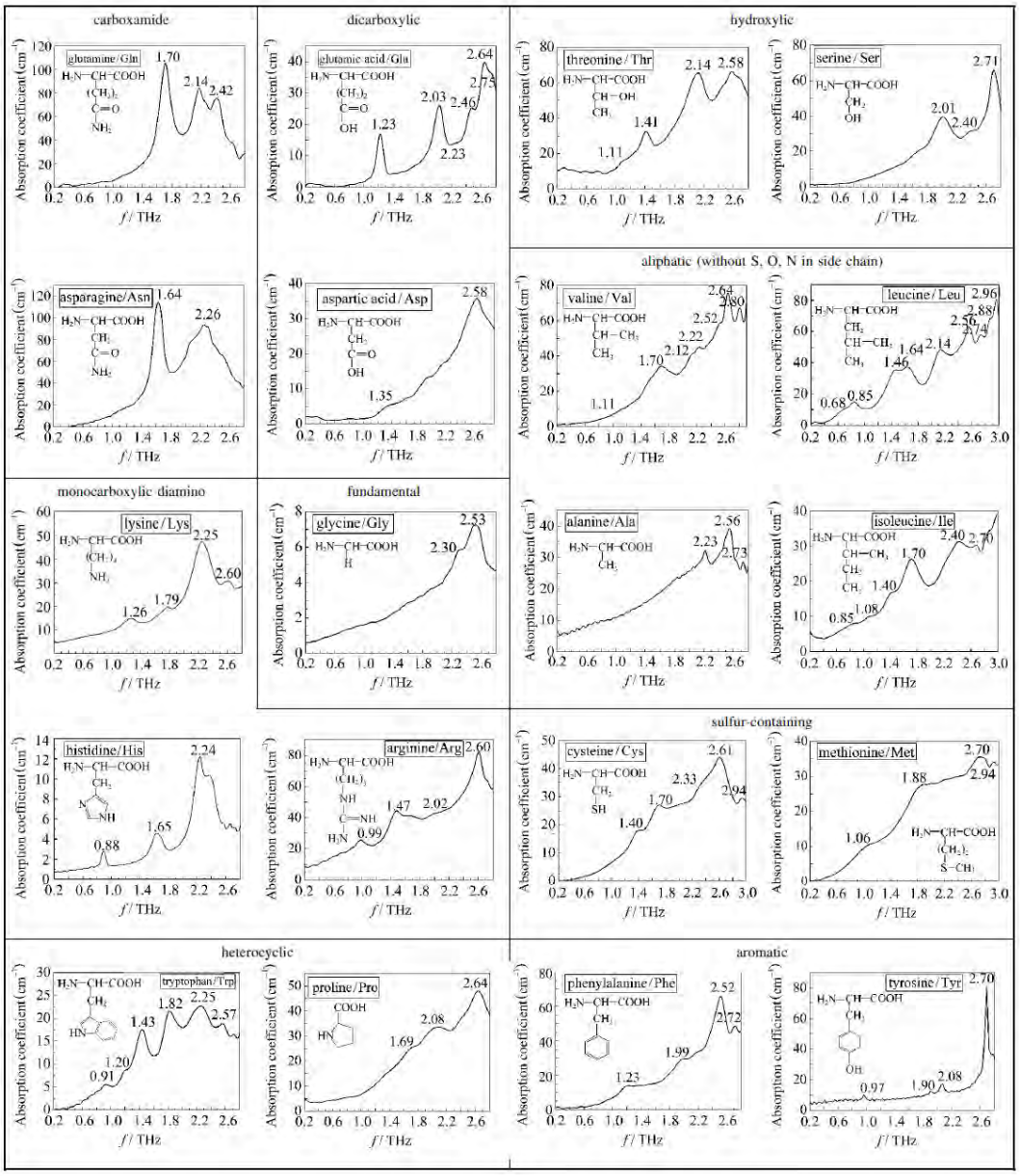

Fig. 1 THz Spectra of 20 α-amino acids.

2.1 Amino acid

The basic unit of protein is amino acid. Amino acid is strikingly sensitive to THz wave and yield a complete set of THz fingerprint spectra. As shown in Fig. 1, all 20 standard polycrystalline α-amino acids which compose human protein molecules were examined using THz-TDS at room temperature [4]. This work provided comprehensive data on characteristic absorption peaks between 0.2 and 3.0 *THz*. Through a comparative study of molecules, correlations between the THz spectral peaks and the molecular structures of amino acids are revealed, and a classification of amino acids based on both molecular structures and THz spectra was established for the first time. These correlations can be applied in identifying amino acids, and tracing some functional groups.

What is more, THz technique can be used in identification of structure isomers of amino acids. In Zheng's paper, the theoretical and experimental THz spectra of β-alanine was reported [5]. The absorption peak was found at 2.11 *THz* and the average refractive index of the pure sample was 1.96 using THz-TDS. The absorption spectrum of β-alanine was simulated by PM3 algorithm of semiempiricism. The agreement between the measured and theoretical results shows the torsion vibration modes and the structural parameters of the sample. The α-alanine and β-alanine are structure isomers, the minor difference in structure leads to distinct fingerprint spectra.

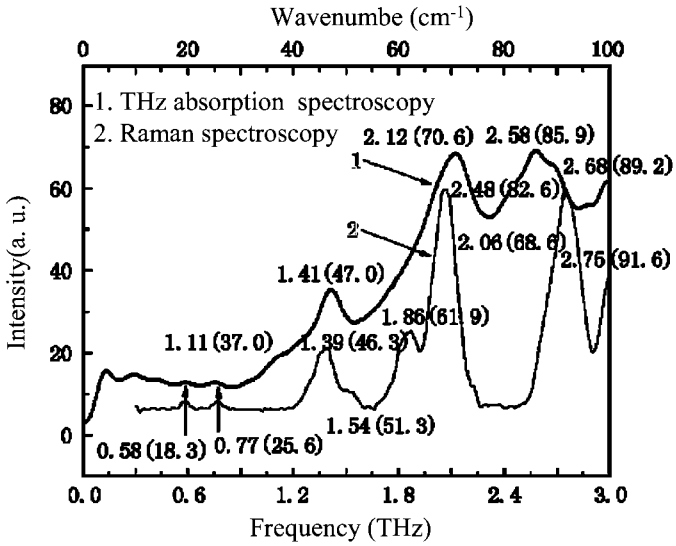

Fig. 2 THz absorption spectroscopy and Raman spectroscopy of polycrystalline L-threonine.

Besides THz absorption spectroscopy, Raman spectroscopy is another effective technique that provides the information of vibration mode in molecular. The THz absorption spectroscopy and Raman spectroscopy are different in selection rule, so their roles in studying the structure of molecular are quite complementary. In Wang's paper, both THz-TDS and Raman spectroscopy are used to study polycrystalline L-threonine [6]. Two kinds of spectra are shown in Fig. 2. Based on the theory of space group and the comparison of two kinds of spectra, the polar and the nonpolar vibrational modes of the polycrystalline molecule are discriminated. With further calculation based on DFT theory and Gaussian08 Software, all the experimental THz absorption peaks and low frequency Raman scattering peaks were assigned. This comprehensive and

creditable method serves as good reference to study THz spectra of other biological molecules.

2.2 Short-chain peptides

Peptides are polymer of amino acids. The THz spectroscopy technique also could be used to identify peptides. The THz-TDS was applied to study the absorption peaks and optical characteristics of reduced glutathione and Carnosine [7, 8]. The density functional theory (DFT) was used to predict the cause of absorption peak, different absorption peaks correspond with different vibration modes of the molecule. The theoretic predictions show satisfactory agreement with the experimental results except somewhat blue shift.

2.3 Conformational dynamics of proteins

THz-TDS technique can offer direct insight into the configurational and conformational dynamics of proteins. Chen and co-workers adopted THz-TDS technique to study the denaturation process of chlorophyll protein 43 (CP43) and CP47 treated by guanidine (Gu) HCl [9]. The distinct changes in absorption spectra not only proved the feasibility of THz technology in sensing protein denaturation, but also provided evidence for studying conformation changes. It was found that the conformation change was induced by the C=O group of chlorophyll*a*, interacting with the N–H group of GuHCl to form hydrogen bonds.

3. THz spectroscopy on detection and identification of illicit drugs

Illicit drugs seriously impair both human body and mind, so the inspection and detection of illicit drugs are very demanding for public security. Many limitation lies in conventional inspection technique, including x-ray scanning, trace detector and infrared imaging. THz spectroscopy is proposed to be a significant technique in detection and identification of illicit drugs.

The researchers at Capital Normal University and Ministry of Public Security of China have done a series of fruitful work in this developing field. The characteristic absorption peaks in THz band of ketamine hydrochloride and 3, 4-methylenedioxymethamphetamine (MDMA) [10], ketamine [11], methyleriedioxyamphetarnine (MDA), methamphetamine (MA) [12] and, methylenedioxy amphetamine [13] were measured by THz-TDS. The theoretical calculation based on DFT showed qualitative agreement with the experiment data. As shown in Fig. 3, three obvious absorption peaks were observed in THz spectra for MDMA and calculated results are in good accordance [10].

On the field of detection and identification of illicit drugs by the THz-TDS, the detection must be based on the identification from the THz spectra of samples. The THz imaging technique [11] and artificial neutral network (ANN) technique [14, 15] were suggested to identify illicit drugs. For terahertz imaging technique, image and absorption spectra of sample were obtained from point-by-point scanning with THz-TDS in ambient air. Then a component spatial pattern analysis method was adopted to calculate the spatial distribution of samples and identify samples.

Compared with conventional technique, this method no longer depends on characteristic absorption peak and dry surrounding environment. Besides that, spatial pattern of each chemical could be extract from the THz image as shown in Fig. 4.

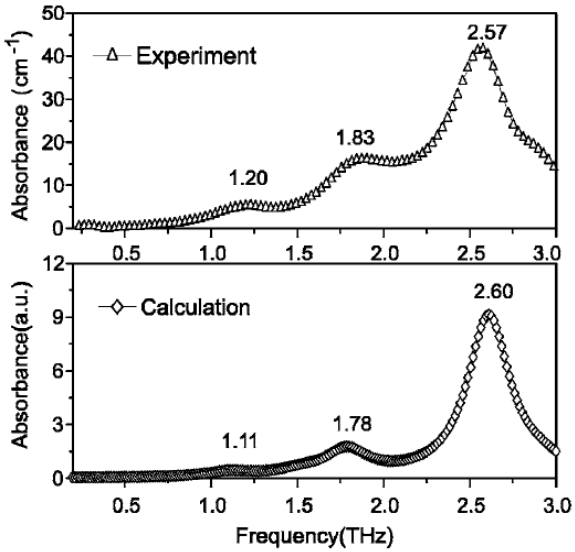

Fig. 3 Comparison of experimental and calculated characteristic absorption peak in THz band for MDMA.

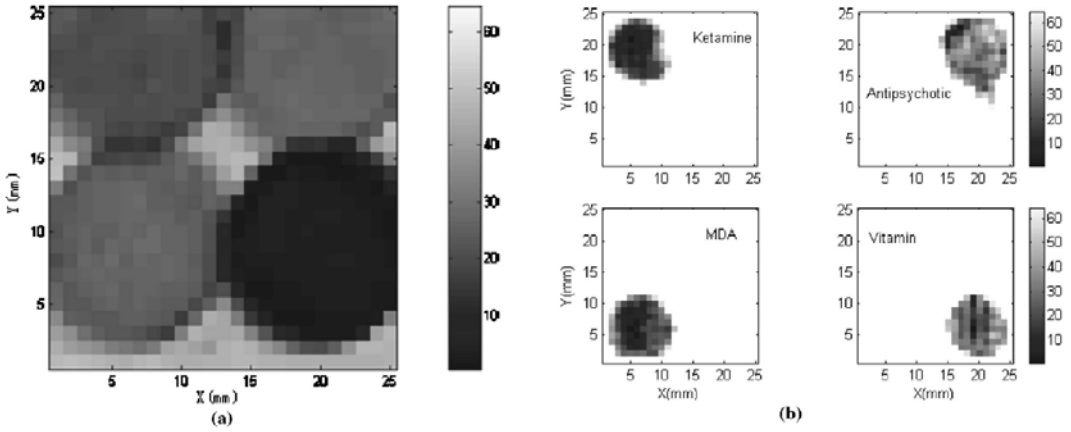

Fig. 4 (a) A scanning transmitted terahertz image (b) The extracted spatial patterns of ketamine, MDA, vitamin, and antipsychotic. Two different illicit drugs and other chemicals contained in an envelope are clearly separated and corresponding spatial patterns obtained.

The great ability of self-leaning and self-adaption makes ANN a suitable method in identifying THz absorption spectra. Two types of ANN, Back Propagation (BP) and Self-Organizing Feature Map (SOM) neural networks were employed to do the identification of absorption spectra of illicit drugs [14, 15]. The spectra of various drugs and antibiotics were used to train a BP neural network and then the absorption spectra measured in different times were identified by the trained BP neural network with an average identification rate of 76%. Different from that, the SOM neural network is an unsupervised neural network which clusters the identical patterns

according to the similarity of the patterns. In Liang's work, SOM neural network was used for clustering the spectra of six illicit drugs measured at different times [14]. After the well-clustered network training process, the tested spectra were entirely discriminated according to effective distance.

In order to put the THz technique into application, many conditions should be considered, i. e. the drugs may be delivered by post, and the drug is mixed with other chemicals. The feasibility of THz technique in detection of illicit drugs was explored further. Li and co-workers measured THz spectra of MA and MDA using THz-TDS in two conditions, within and without conventional envelopes [12]. The results show that the characteristic absorption peak was almost the same in these two conditions. This work validates the reliability of THz-TDS spectroscopy in inspection the illicit drug in post. Jiang and co-workers proposed a method to quantitatively inspect the mixtures of illicit drugs with THz-TDS technique [16]. The mixture of MA and a normal chemical, flour, was selected for experiment. The mass percentages of all components in a mixture can be obtained by linear regression analysis from THz absorption spectra. Experimental results were in significant agreement with actual content, which suggested that it could be an effective method for quantitative identification of illicit drugs. Despite of that, this method depends on that all components in the mixture and their absorption features are known, thus the range of its applicability remains to be improved in the future work.

4. THz spectroscopy on Chinese traditional medicine

The effective components of Chinese traditional medicine are complex, so the identification is very important for its development. In recent years, the THz spectroscopy provides an effective method of identification. However, characteristic fingerprint spectra technique is not available for a lot of Chinese traditional medicine. In Chen's paper, Support Vector Machine (SVM) method is introduced for identification of two kinds of similar medicine [17]. In this work, the THz absorption spectra of three groups of Chinese traditional medicines were measured and used to construct the SVM. And a good identification rate was obtained through SVM method. These results indicate that SVM method is a efficacious way for classification of Chinese traditional medicines.

Besides that, THz spectroscopy technique can provide conformational properties of Chinese traditional medicine that can be used to investigate its interaction with bimolecular and pharmaceutical mechanisms. In Wang's work，THz spectra of 1,4-naphthoquinone and its four derivatives were studied with THz-TDS [18]. These almost identical molecules could be identified much more easily using their distinctive THz spectra than conventional spectroscopy. Furthermore, through comparative analysis, some possible correlations between THz spectra and molecular structures were revealed, which will be of great help in studying the therapeutic mechanisms of naphtoquinones.

5. THz spectroscopy on sugar

THz-TDS is sensitive to sugar, and THz spectroscopy technique can be used to study the molecular structure and vibration mode of sugar. In Jin's paper, THz-TDS and ab-initio

simulation based on DFT were used to study THz vibration spectrum of molecules in the crystalline phase [19]. Fig. 5 shows THz absorption spectra of α-lactose monohydrate predicted by the precise ab-initio calculations. The results demonstrate two kinds of vibration modes. One is a pure inter-molecular hydrogen bonding vibration mode, and the other is dominated by inter-molecular and intramolecular hydrogen bonds. These results are very useful to understand the physical and chemical properties of the molecular crystals.

6. THz medical imaging

THz radiation is non-ionizing, and thus is not expected to damage tissues, unlike X-rays. Some frequencies of THz radiation can penetrate several millimeters of tissue with low water content and detect differences in water content and density of a tissue. THz imaging is regarded as a safer and less painful method for effective detection of cancer.

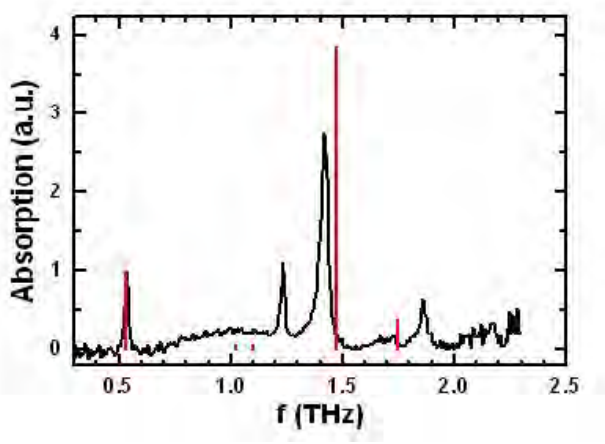

Fig. 5 THz absorption spectra of α-lactose monohydrate predicted by the precise ab-initio calculations.

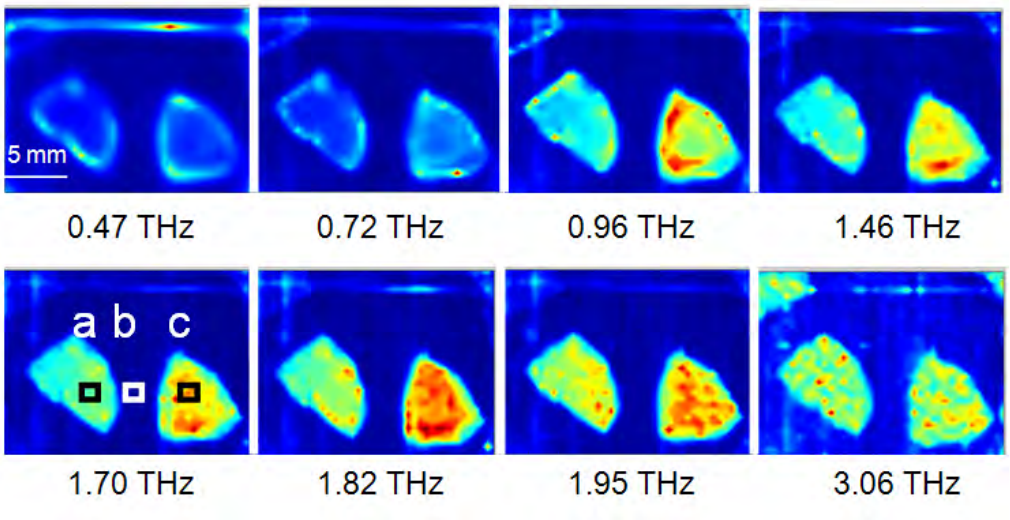

Fig. 6 THz absorption imaging of diseased (left) and normal tissue (right) at several frequencies.

Zhang and co-workers used THz-TDS imaging technique to study the subcutaneous tumors, inflamed liver and their corresponding normal tissues. [20] Fig. 6 shows the THz absorption imaging of diseased and normal tissues at several frequencies. It was found that at certain frequency range, most tumors and serious inflamed livers have lower absorption than normal tissues, which means THz-TDS imaging could obviously distinguish the tumors and normal tissues, serious inflamed and normal livers, but could not obviously distinguish the grade of tumors and inflammation.

7. Conclusions

The study on THz spectroscopy on biology and medicine has undergone dramatic developments over the recent years in China. THz spectroscopy serves as useful technique in identifying biological molecules and medicine, studying molecular structure and discerning some diseased tissues. Despite of that, a great many problems remain to be challenging. Though THz spectroscopy and DFT could reveal the correlation of absorption spectra and molecular structure, the challenge present in predicting material's physical and chemical properties based on structure. In addition, there is a large gap toward the application of THz spectroscopy in biology, security and medical imaging. Besides that, the research work about Chinese tradition medicine should gain more support and encouragement in order to preserve and develop our culture.

References

[1] B. Ferguson and X. C. Zhang, "Materials for terahertz science and technology", *Nature Materials* 1, 26-33, (2002).

[2] M. Tonouchi, "Cutting-edge terahertz technology", *Nature Phontonics* 1, 97-105, (2007).

[3] Y. Wang, W. Yu, F. Zhou, et al. "Application of Terahertz (THz) Spectroscopy in Biological Macromelecules", *Progress in Biochemistry and Biophysics* 37 (5), 484-489, (2010).

[4] W. Wang , H. Li, Y. Zhang, et al., "Correlations between Terahertz Spectra and Molecular Structures of 20 Standard α-Amino Acids", *Acta Physico-chimica Sinica* 25 (10), 2074-2079, (2009).

[5] W. Wang, "Terahertz and Raman spectra of L-threonine", *Acta Physica Sinica* 58 (11):7640-7645, (2009).

[6] Y. Zheng, Y. Li and W. Wang, "THz Time Domain Spectrum of β-alanine", *Acta Chimica Sinica*, 65(1), 72-76, (2007).

[7] H. Yan, W. Wang, W. Yue, et al., "THz spectrum of reduced glutathione", *Science in China Ser. G Physics, Mechanics & Astronomy* 48 (5), 585-592, (2005).

[8] H. Yan and W. Wang, "Experimental and Theoretical Study of Carnosine in THz Range", *Chinese Physics Letters*, 22, 3179-3181, (2005).

[9] H. Chen, L. Wang, Y. Qu, et al., "Investigation of guanidine hydrochloride induced chlorophyll protein 43 and 47 denaturation in the terahertz frequency range", *Journal of Applied Physics* 102, 074701, (2007).

[10] G. Wang, J. Shen, and Y. Jia, "Vibrational spectra of ketamine hydrochloride and 3, 4-methylenedioxymethamphetamine in terahertz range", *Journal of Applied Physics* 102, 013106, (2007).

[11] M. Lu, J. Shen, N. Li, et al., "Detection and Identification of Illicit Drugs Using Terahertz Imaging", *Journal of Applied Physics* 100, 103104, (2006).

[12] N. Li, J. Shen, M. Lu et al., "Non-destructive inspections of illicit drugs in envelope using terahertz time-domain spectroscopy", *Proceedings of SPIE* 6047, 60472Y, (2006).

[13] G. Wang and J. Shen, "Terahertz spectroscopic investigation of methylenedioxy amphetamine", *Proceedings of SPIE* 6840, 68400W, (2007).

[14] M. Liang, J. Shen and G. Wang, "Identification of illicit drugs by using SOM neural networks", *Journal of Physics D: Applied Physics* 41, 135306, (2008).

[15] J. Shen, J. Yan, M. Liang, et al., "Identification of THz absorption spectra of chemicals using neural networks", *Proceedings of SPIE* 6695, 66951F-1, (2007).

[16] D. Jiang, S. Zhao and J. Shen "Quantitative analysis of the mixtures of illicit drugs using terahertz time-domain spectroscopy", *Proceedings of SPIE* 6840, 68400U, (2007).

[17] Y. Chen, Y. Liu, G. Zhao, et al., "Chinese Traditional Medicine Recognition by Support Vector Machine (SVM) Terahertz Spectrum", *Spectroscopy and Spectral Analysis,* 29(9), 2346, (2009) .

[18] W. Wang, H. Li, X. Luo, et al., "THz Spectra of 1,4-Naphthoquinones and its Four Derivatives", *Proceedings of SPIE* 6840, 68400O, (2007).

[19] B. B. Jin, Z. X. Chen, Z. Li, et al., "Mode assignment of terahertz spectrum of α-lactose monohydrate", The 34th International Conference on Infrared, Millimeter, and Terahertz Wave, September, (2009).

[20] C. Zhang, "Research about The Application of THz Spectroscopy on Various Materials", PhD. thesis, Nanjing University, (2010).