doi:10.3788/gzxb20164507.0730003

基于金属光栅的氨基酸溶液太赫兹光谱检测

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摘 要:基于太赫兹金属光栅谐振传输现象,利用金属光栅表面等离子体共振对周围介质敏感的特性,设计了一种由金属光栅、样品池和高阻硅基底组成的免标记生物传感器.利用这种传感器在太赫兹时域光谱下测量了苏氨酸和精氨酸溶液的太赫兹透射光谱.结果表明:苏氨酸和精氨酸的共振频率随着溶液浓度改变在 0.6~0.75 THz 之间出现频移,并且苏氨酸和精氨酸的混合样品的光谱并不是两者光谱的线性叠加.

关键词:生物传感;金属光栅;氨基酸溶液;太赫兹时域光谱;表面等离子增强

中图分类号:O43 **文献标识码**:A **文章编号**:1004-4213(2016)07-0730003-4

Detection of Amino Acid Solutions Using Metallic Grating for Terahertz

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Abstract: Based on the Terahertz metal grating resonant transmission phenomenon, a label free biosensor consisting of metallic grating, sample pool and high resistivity silicon substrate was designed by using the sensitive characteristic of surface plasmon resonance of metallic grating for the surrounding medium. Terahertz transmission spectrums of threonine and arginine solutions were measured by using this sensor with terahertz time-domain spectroscopy. The experimental results show that, the resonance frequencies of the threonine and arginine solutions change and shift between 0.6 THz and 0.75 THz with the increase of concentration, moreover, the spectra of the mixed samples of threonine and arginine is not linear superposition of the two spectra.

Key words: Biological sensing; Metallic array; Amino acid solutions; THz time domain spectroscopy; Surface plasmon-enhanced

OCIS Codes: 300.6495; 050.2770; 050.6624; 280.1415; 240.6680

0 Introduction

Terahertz detection technology has been developed more and more quickly in the past decades, which has been involved wide areas ranging from security detection, imaging technology to medical diagnosis, DNA(Deoxyribonucleic Acid) analysis[1-3]. At present, a biomolecular detection mainly adopts the infrared spectrum technique, which is not efficient to detect the micro molecule and side-chain of biomolecular, because the frequency range of collective vibration of the molecules is mainly in the terahertz. The refractive index and absorption coefficients of biological polymers in terahertz show a slight difference that is difficult to distinguish by normal methods, and the related reports of enzyme reaction have also been published. Owing to the biological detection demands, the applications of label-free sensing based on the terahertz waves for many biomedical molecules have become attractive and been revealed enormous potential.

Foundation item: The National Natural Science Foundation of China (No. 61575131)

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Received: Jan. 13, 2016; **Accepted**: Mar. 29, 2016

It is well known that water is very important to biomolecular, since the biological activity is only shown in the water. However, the strong terahertz absorption of water leads many biological researches to select dry sample or partially hydrated samples in terahertz field. Xu Jing researched collective vibrational dynamics of a protein in liquid water by terahertz, and they extracted absorption spectroscopy of solvated bovine serum albumin and probed differences between the solvated BSA (bovine serum albumin) and water[4]. But they only achieved amplitude information of samples spectrum and it is a challenge to apply free electron lasers and an ultrasensitive cryogenic detector they used in experiments for most researchers

Metal grating research is widely used in terahertz region in the last years. When terahertz waves transmit through a periodic subwavelength metallic hole array, Surface Plasmon Polarization (SPP) extraordinary transmission effect is observed in near interface of array[5-8]. The applications of SPP have attracted many researchers and been explored in filter, biomedical sensing, controlled modulator and other fields[9-12]. Yoshida demonstrated the high sensitivity detection of small amounts of protein horseradish peroxidase by using a thin metallic mesh, and a distinct shift of the transmission dip frequency was observed for 500 pg/mm^2 of horseradish peroxidase in their experiment[13]. This is the first to apply metallic grating to detect a protein. However the operation of printing the samples on the metallic meshes by using a commercial inkjet printer is difficult, we designed a simpler metallic grating for researchers and adopted a terahertz time domain spectroscopy not a Fourier transform infrared spectrometer.

In terahertz field, the spectra of amino acids powder had been researched in the related reports, but the solvated amino acids were rarely discussed. In this article, we made use of the anomalous transmission enhancement of metallic grating to detect and analyze the change of spectra of solvated amino acids with different concentration. Furthermore, the distinctions of the spectra for THz indicate the amino acids can be distinguished with metallic grating.

1 Experiment

In our experiment, the metallic array part is made by 100 μm-thick 403 stainless steel. The two dimensional array, which was placed on a high resistance silicon wafer(thickness 400μm, resistance is greater than 200Ω · m) and fabricated with subwavelength circular holes of 200μm and a period of 400μm, in both dimensions, as illustrated Fig. 1(a).

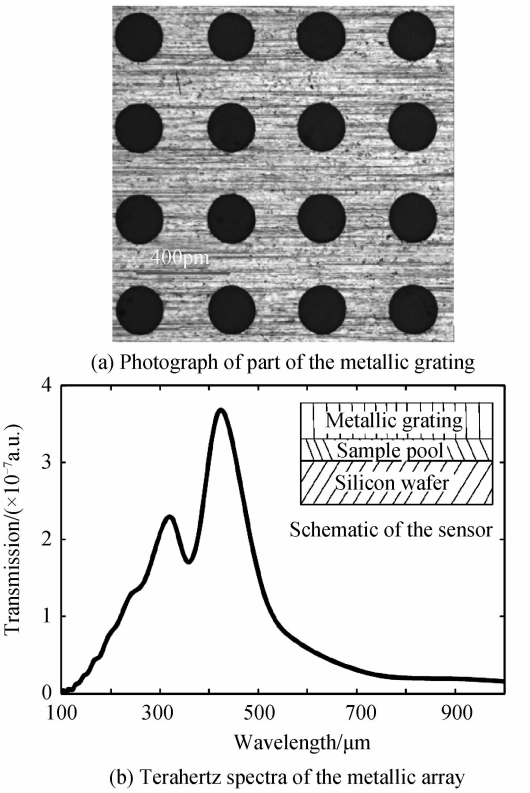


Fig. 1 Photograph and the spectra of metallic grating

L-threonine and *L*-arginine were dissolved in the distilled water to prepare different mass concentrations of 1%, 2% and 5% at a room temperature. These amino acids solutions were dripped on a high resistance silicon wafer with syringes, and then the metallic grating was placed on it. The metal grating can stick to the high resistance silicon wafer with the effect of surface tension of the solutions. The amplitude transmission is defined as $|E_s(\omega)/E_r(\omega)|$, where $E_s(\omega)$ and $E_r(\omega)$ are the Fourier transformed amplitude spectra of the terahertz waves transmitted through the sample and reference, respectively. The measurements were performed at room temperature in terahertz time-domain spectroscopy system (THz-TDS). Fig. 1(b) shows the Fourier transformed amplitude spectra of terahertz wave transmitted through the grating sensor without amino acid samples.

2 Results and discussion

When light waves are incident on the surface of a metallic array, this resonant transmission phenomenon is explained by the resonant excitation of SPPs. The resonant frequency of the SPP is expressed as[13]

$$f_{spp}^{mn}=|k_{in}+G_{mn}|\frac{c}{2\pi}\left(\frac{\varepsilon_m+\varepsilon_d}{\varepsilon_m\varepsilon_d}\right)^{1/2} \tag{1}$$

where k_{in} is the in-plane wave-vector of the incident terahertz waves, $G_{mn}=2\pi/L\cdot m+2\pi/L\cdot n$ is the reciprocal lattice vector of the periodic, L is periodic of array, m and n are integers of the SPP modes and ε_m

and ε_d are the dielectric constants of the metal and the interface medium. In the terahertz region, the dielectric constant of metals is several orders higher than the dielectric constant of dielectric media ($\varepsilon_m \gg \varepsilon_d$), then the square root of the Eq. (1) can be simplified as $\sqrt{\varepsilon_d^{-1}}$ and k_{in} is zero at normal incidence. Hence by simplified the Eq. (1), the resonance frequency of SPP at the interface can be described through the dispersion relation at normal incidence as follows

$$f_{spp}^{mn} = cL^{-1}(m^2+n^2)^{1/2}(\varepsilon_d)^{-1/2} \quad (2)$$

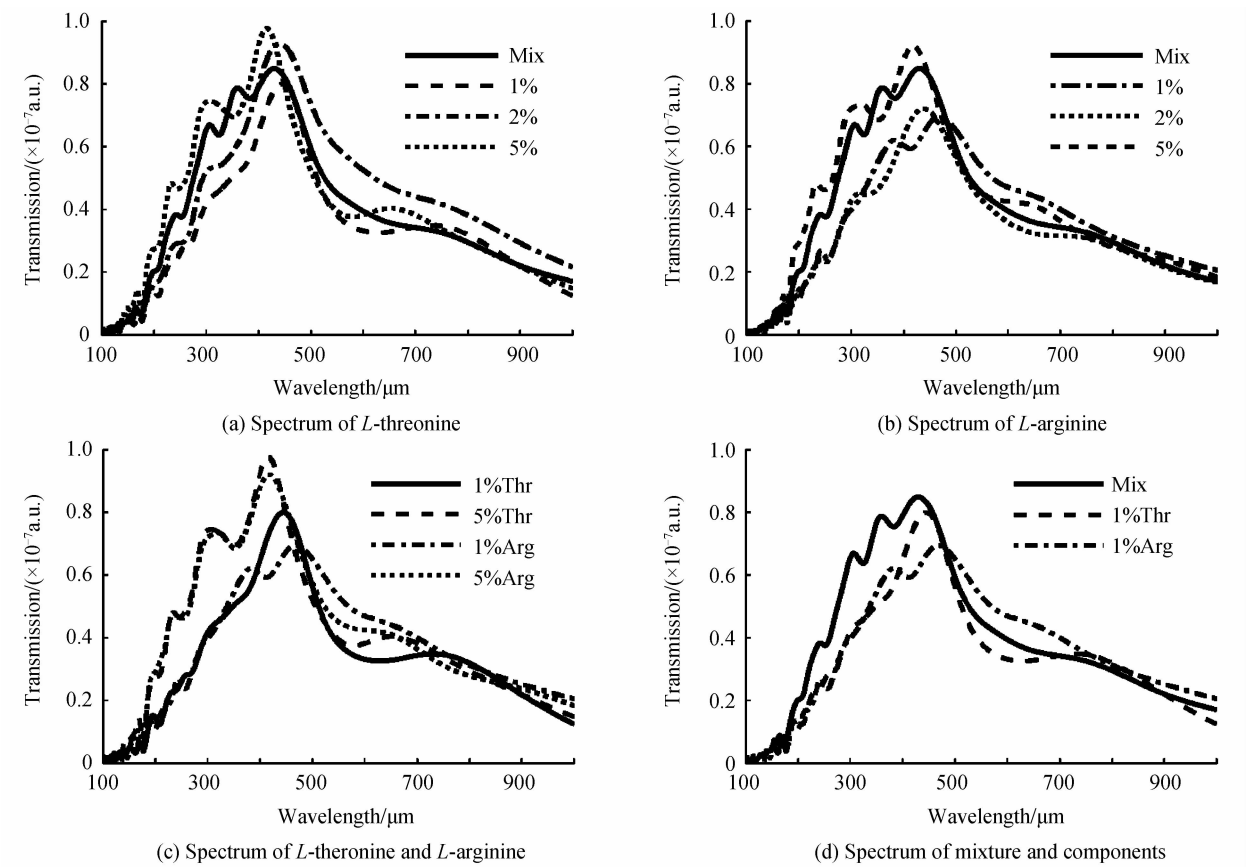
According to Eq. (2), the refractive index of dielectric media n_d ($n=\sqrt{\varepsilon}$) is inversely proportional to the resonance frequency f_{spp}^{mn}, which decreases with the increase of n_d.

The broad transmission peak is observed at 0.7 THz and the transmission dip around peak frequency is observed clearly from Fig. 1(b). The discrepancy between the observed peak frequency 0.7 THz and the resonant frequency of SPP 0.75 THz expected from the Eq. (2) appears in our experiment. This is attributed to the coupling effect between the terahertz waves transmitted directly through metal openings and the SPP excited on the metal surface[13-14]. This effect leads to the asymmetric spectral shape and the transmission dip around the resonant frequency, which is a reason why two peaks are observed from Fig. 1(b), and the frequency shift of the transmission peak with respect to the SPP resonant frequency[13].

An evanescent wave excited by SPP exists inside the metal and the dielectric in the vertical direction of the interface[15]. When the media around the interface change, the propagation of evanescent will affect the resonance frequency. Due to the limitation of the propagation depth, the evanescent wave can penetrate through the thin dielectric (sample) near the array and reach the silicon wafer, and the resonance frequency is partly influenced by dielectric at this moment. With the increase of the thickness of the dielectric, one part of the evanescent wave existing in the dielectric gradually will rise, other part of evanescent wave penetrating through dielectric will decrease, and so the effective refractive index, which affects the change of the resonance frequency, will change gradually. At last, the effective refractive index exactly is the refractive index of sample when the evanescent wave completely exists inside the dielectric.

In addition, different kinds of dielectrics have different refractive indices, which are correspond to the different resonance frequency. So the resonance frequency can be considered as the characteristic index to distinguish dielectric substances.

Fig. 2(a), (b) shows the transmission spectrum



(a) Spectrum of *L*-threonine (b) Spectrum of *L*-arginine

(c) Spectrum of *L*-theronine and *L*-arginine (d) Spectrum of mixture and components

Fig. 2 The measured amplitude transmission spectrums of metallic array with *L*-threonine and *L*-arginine of different concentrations 1%, 2%, 5% and mixture

through the sensor with samples of *L*-threonine and *L*-arginine with the concentration of 1%, 2% and 5%. The resonance frequencies of *L*-threonine are 0.664 THz, 0.691 THz, 0.723 THz and *L*-arginine are 0.638 THz, 0.684 THz, and 0.716 THz with the increase of concentration. The frequency shifts of the transmission peak of two kinds of amino acids reach 59 GHz and 78 GHz, respectively, in concentration ranging from 1% to 5%. This remarkable distinction in the terahertz spectra demonstrates that dozens of nmol concentration measurement used here is feasible. Under the same concentration, the resonance frequencies shift between *L*-threonine and *L*-arginine also can reach 26 GHz. It indicates two kinds of amino acids are clearly distinguished in our experiment. Fig. 2 (c) shows the spectrums of amino acids are separated.

The resonance frequency of mixture sample with 1% *L*-arginine and 1% *L*-threonine in concentration is 0.697 THz. Compared with *L*-arginine spectrum and *L*-threonine spectrum, the mixture sample spectrum is not a superposition of their single spectrum, so we can infer that the components of mixture not only interact with water, but also interact with each other. Fig. 2 (d) shows the measured amplitude transmission spectra of metallic array with mixture, 1% *L*-threonine and 1% *L*-arginine.

From Fig. 2, we can find the transmission peak frequency shows a tendency to increase with the increase of sample concentration. However, it is not consistent with the result reported in Ref. [13]. It is quite likely that the evanescent reach silicon wafer through the sample and then the effective refractive index that affect the resonance frequency is modulated by the sample and the silicon wafer. Because the refractive index of Si is greater than the samples, when the refractive index of samples increase with the concentration, the effective index will slightly decrease.

3 Conclusions

In summary, we probed the terahertz spectroscopy of *L*-threonine and *L*-arginine, and demonstrated the sensitivity in detecting solvated amino acids by using a metallic array. A characteristic index that is the resonance frequency of SPP can be used to identify the spectrum. The transmission peak frequency increased with the concentration of samples and the transmission dip frequency is observed clearly. Mixture appeared different resonance expression caught our attention, and we infer *L*-threonine and *L*-arginine interact with each other in water. Therefore, we not only use metallic to make qualitative analysis, but use it to research interaction between amino acid and water. The identification approach has a promising potential in accurately identifying biomedical substances. As a next challenge, we aim to make quantitative analysis by accurately controlling the thickness of sample.

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