Molecular networks based on noncovalent bonds have resonant frequencies in the terahertz (THz) region. THz spectroscopy is a powerful tool for identifying molecular bonds, such as intermolecular or intramolecular hydrogen bonds, in pharmaceuticals. A THz chemical imaging (TCI) system was developed by combining a THz time-domain spectrometer with a translational stage to obtain two-dimensional distributions of molecular networks in tablet samples. Since THz spectral peaks of pharmaceuticals are broad at room temperature, multicomponent chemical analysis with the TCI system has some limitations. In this paper, we describe multicomponent chemical analysis of pharmaceuticals using a sample chamber cooled by a cryostat. TCI measurement at low temperature sharpens spectral peaks and/or shifts peak frequencies, enabling us to determine the distribution of several kinds of pharmaceuticals in a tablet. The TCI system provides THz images of polymorphic form distribution of famotidine binding with D-mannitol in an over-the-counter pharmaceutical tablet. Furthermore, the molecular mechanics method was used to determine the vibrational modes of the peaks in the spectra of famotidine polymorphic forms.

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Terahertz (10^{12} \text{ Hz}) (THz) light, which occupies the region of the electromagnetic spectrum between microwaves and infrared light, has frequencies from 0.3 to 10 THz, corresponding to wavelengths from 30 to 1000 \mu m. THz light is advantageous for safe, non-destructive inspection applications because it has linear propagation characteristics and can penetrate a large variety of materials, such as plastic, paper, rubber, wood, and ceramics. THz chemical imaging (TCI) is fundamentally different from conventional X-ray imaging in that it can be used for material recognition based on spectroscopy of molecular networks.\cite{1,2} Molecular networks are created by noncovalent bonds, such as hydrogen bonds and ionic bonds, and van der Waals forces between molecules, which have a resonance frequency in the THz region. THz spectroscopy is a powerful tool for identifying intermolecular and intramolecular hydrogen bonds in biological samples, such as amino acids,\cite{3–9} polypeptides,\cite{10,11} DNA,\cite{12} protein,\cite{13,14} sugars,\cite{15,16} pharmaceuticals,\cite{17,18} and cancer cells. Molecules of amino acid and pharmaceutical crystals exhibit several peaks in THz spectra, making quantitative analyses possible. Their peak frequencies are strongly affected by the hydrogen-bond network of the molecular crystals.\cite{2,20}

Applications such as pathological examinations of tissues\cite{21,22} and identification of drugs or explosives in postal packages have received attention.\cite{24,25}

TCI has the potential to reveal not only molecular distributions but also molecular networks, which could lead to new medical diagnostic and evaluation techniques. THz light penetrates pharmaceutical tablets and enables us to inspect the homogeneity of their coating\cite{26} and identify polymorphic forms of crystals.\cite{27,28} Polymorphic forms of molecular crystals have different crystal structures comprising different types of hydrogen bonds between molecules in the crystals and thus show different chemical properties, such as solubility, hygroscopicity, and bioavailability as medicines. Bioavailability indicates the rate and extent of drug absorption, which is largely determined by the properties of the dosage form, rather than by the drug’s physicochemical properties, which determine absorption potential. Regardless of these advantages, the number of chemical species in a tablet that can be separated is limited because their spectral peaks in the THz frequency region are broad.

This paper describes multicomponent imaging of pharmaceutical crystals using a TCI combined with a vacuum chamber cooled by a cryostat. The TCI provides frequency-dependent THz images of a tablet at low temperature and enables us to determine the distribution of several kinds of pharmaceuticals within a tablet. Two-dimensional distributions of the polymorphic forms of famotidine in an over-the-counter tablet are shown by using their THz peaks which are calculated by the molecular mechanics method.

Experimental

Figure 1 is a diagram of the TCI system composed of a THz time-domain spectroscope (THz-TDS) with the vacuum chamber mounted on a three-dimensional translational stage.\cite{29} For multicomponent chemical analysis, a cryostat was added to the vacuum chamber. The sample holder inserted into the chamber has a quartz plate bottom and can be cooled down to 77 K by the cryostat using liquid nitrogen and an electric heater. The stability of the cryostat is less than 2 K. The THz—TDS consists of a 9-fs near-infrared pulse laser (Integral Pro, Femtolasers), two gallium arsenide photoconductive antennas (AISPEC), a mechanical stage delay line, and mirrors. One photoconductive antenna is an emitter and the other is a detector. A 13-fs near-infrared pulse laser (Fusion, Femtolasers) is also used for TDS measurement. The delay line is used to obtain a time-domain waveform, which is converted to a frequency-domain spectrum by Fourier transformation. The three-dimensional translational stage with 0.1-mm-step resolution and the THz-TDS are controlled by a personal computer to obtain a THz time-domain spectrum at each point in a sample tablet. The acquisition time for obtaining a 12 × 12 mm² image is about seven hours. The image consists of 60 × 60 pixels in 200-\mu m increments. The sample measurement spatial resolutions in the horizontal and vertical directions are about 0.5 and 1 mm, respectively. The number of accumulations for obtaining time-domain waveforms is 32, and no accumulations are acquired at each pixel position during THz imaging.

Sample tablets studied were made of famotidine, which is a histamine H2-receptor antagonist for the prevention and treatment of stomach and intestinal ulcers. Two polymorphic crystalline forms of famotidine form A and form B were obtained by recrystallization in hot water and hot methanol aqueous solution, respectively.\cite{29} Original famotidine reagent was purchased commercially (ICN Pharmaceuticals). The polymorphic form was determined by differential scanning calorimetry (SSIC-5200, Seiko Instruments), which measured heat capacity at the melting point each form. D-mannitol of analytical grade (Sigma-Aldrich) was used without further purification. D-mannitol

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is sometimes used in famotidine tablets to control solubility in the body. The crystals were crushed into fine powder and then diluted with polyethylene powder (Sigma-Aldrich). Tablets were then formed with a mechanical compress machine. The diameter and thickness of each 100-mg tablet were about 10 and 1.5 mm, respectively. For the test tablets used in imaging measurements, a piece was cut from each tablet and multiple tablet pieces were compressed within additional polyethylene powder to form a tablet 10 mm in diameter and about 2-mm thick. The amount of famotidine in an over-the-counter tablet purchased from a drug store was 10 mg.

For the molecular mechanics method, calculation for famotidine form A and form B, we used CONFLEX ver. 6 and BARISTA ver. 1 (Conflex Corp), with the crystal structure data measured by X-ray diffraction.

Results and Discussion

Figure 2 shows the concentration-dependent spectra of (A) famotidine form A, (B) famotidine form B, and (C) D-mannitol. Each spectrum measured at room temperature in a vacuum chamber was accumulated 32 times to obtain a better signal-to-noise ratio. The form-A spectrum shows peaks at 0.90 and 1.63 THz, and the form-B has peaks at 1.07, 1.20, and 1.32 THz in the range of 0.3 to 3.3 THz as shown in Figs. 2A and 2B. D-mannitol, one of the pharmaceutical excipients generally used in over-the-counter pharmaceutical tablets, shows peaks at 1.11 and 1.48 THz at room temperature. The peaks at less than 2 THz are indicated by asterisks. The number of famotidine peaks above 2 THz is greater than those at less than 2 THz, and broad spectral peaks above 2 THz overlap. Therefore, for the use of the TCI technique, peaks at less than 2 THz are very useful for determining various kinds of pharmaceuticals. Both form-A and form-B crystals are composed of the same molecule; however, their crystal forms differ depending on the synthesis method used. Since THz spectroscopy detects vibrational modes of molecules in crystals, the two forms of famotidine show different spectral peak positions. A set of plots showing the average optical density at the peaks at 0.90 and 1.63 THz in the famotidine form-A spectrum against its concentration is shown in Fig. 3A. Linear fits for all plots are shown as solid lines. Similarly, two sets of plots showing the average optical density at the famotidine form-B and D-mannitol spectral peaks are shown in Figs. 3B and 3C, respectively. The optical density, or absorbance, is proportional to the concentration of famotidine form A and B at the peaks in the 0.3- to 2-THz region; however, it is not proportional to the concentration of D-mannitol because of the D-mannitol spectrum exhibits a baseline, which increases with increasing THz frequency. The origin of the baseline is probably related to scattering from the powder and is still under investigation. Furthermore, the spectral peaks of the three pharmaceuticals are too close together to determine their distribution in an image at 1.07 THz for famotidine form B or 1.11 THz for D-mannitol. In order to solve this problem, a cryostat was added to the vacuum chamber in the TCI system.

Figure 4 shows a set of temperature-dependent THz spectra of famotidine forms A and B and D-mannitol, which were measured at (A) 298, (B) 220, (C) 120, and (D) 77 K. The concentrations of all pharmaceutical tablets are 10 wt%. Peaks in the spectra of both famotidine forms are sharpened at low temperature and their frequencies are shifted very little. However, peaks in the spectrum of D-mannitol are greatly shifted, indicating that the neighboring peaks of famotidine form B and D-mannitol can be separated at 220 K.

Figure 5 shows a set of temperature-dependent THz spectroscopic images of a compressed tablet containing pieces of famotidine polymorphic forms A and B and D-mannitol measured at 220 and 298 K after calibration with an image at 0.5 THz. The peak frequencies of the famotidine-B and D-mannitol spectral peaks are close to each other at 298 K, or room temperature. Therefore, the 298-K-image at 1.07 THz, which is one of the peak positions of famotidine form B, shows both famotidine form-B and D-mannitol pieces. However, only the famotidine form-B distribution is observed at 1.08 THz in the image measured at 220 K. As a result, the two-dimensional distribution of the three pharmaceuticals is almost completely determined at 220 K. This result indicates multicomponent chemical analysis of pharmaceuticals is possible when a cryostat is added to the vacuum chamber in a TCI system.

Figure 6 shows a set of temperature-dependent THz spectroscopic images of an over-the-counter tablet containing 10 mg of famotidine. A set of THz absorbance images from 1.07 to 1.65 THz measured at 220 and 298 K are shown after calibration with an image at 0.5 THz. The peaks at 1.65, 1.08, and 1.14 THz, measured at 220 K correspond to famotidine polymorphic forms A and B and D-mannitol, respectively. The image acquisition time for the tablet was about three
Figure 3. Plots of average optical density of famotidine (A) form A, (B) form B and (C) D-mannitol against their concentration. A linear fit for each plot is shown as a solid line.

Figure 4. Temperature-dependent THz spectra of famotidine form A (blue line), form B (red line) and D-mannitol (green line). The measurement temperatures were (A) 298, (B) 220, (C) 120, and (D) 77 K. The concentrations of all pharmaceutical tablets were 10 wt%.

Figure 5. Temperature-dependent THz spectroscopical images of a compressed tablet containing pieces of famotidine polymorphic forms A and B and D-mannitol, measured at 220 K (upper) and 298 K (lower). The tablet is 10 mm in diameter and 2-mm thick.
Figure 6. Temperature-dependent THz spectroscopical images of an over-the-counter tablet containing 10 mg of famotidine, measured at 220 K (upper) and 298 K (lower). The peaks at 1.65, 1.08, and 1.14 THz, measured at 220 K correspond to famotidine polymorphic form A, form B, and D-mannitol, respectively.

good agreement with the factors in the mid-infrared region, which are also listed at the bottom of Table I. The typical vibrational modes in Table I that were used for imaging are shown in Fig. 7. Figs. 7A and 7B show the vibrational modes of the peaks of famotidine form A at 1.65 THz and famotidine form B at 1.08 THz measured at 220 K. The intermolecular hydrogen bonds with oxygen atoms in the sulfamoyl and the ones with nitrogen atoms in the amidino group are in the stretching mode.

Conclusions

A terahertz spectroscopic imaging (TCI) system, consisting of a THz time-domain spectrometer and a translational stage, was constructed for the detection of two-dimensional molecular networks in pharmaceutical tablets. For multicomponent chemical analysis, a cryostat was added to the vacuum chamber in the TCI system. At low temperature enables us to identify three pharmaceutical chemicals — famotidine polymorphic forms A and B and D-mannitol crystals — in a compressed tablet. Even though the frequencies of the THz peaks of famotidine B and D-mannitol are close to each other at room temperature, their two-dimensional distribution was obtained at a lower temperature, where the peaks are sharpened and/or shifted in frequency. This result indicates that multicomponent chemical analyzes using a TCI system has the potential to open new avenues in nondestructive pharmaceutical evaluation techniques.

Table I. Vibrational modes calculated by the molecular mechanics method in the frequency range from 0.3 to 2.7 THz. The factor is defined as observed frequency divided by the calculation frequency. Some frequencies in the mid-infrared region of famotidine are also included.

| Observed (ν_{obs} THz) | Observed (ν_{obs} cm\(^{-1}\)) | Calculated (ν_{calc} cm\(^{-1}\)) | Factor (ν_{obs}/ν_{calc}) |
|-------------------------|-------------------------------|---------------------------------|--------------------------|
| 0.90 [1,*1,*3]          | 30.0                          | 37.89                           | 0.79                     |
| 0.97 [1,*4]             | 32.3                          | 37.89                           | 0.85                     |
| 1.07 [2,*3]             | 35.6                          | 32.21                           | 1.11                     |
| 1.08 [2,*4]             | 36.0                          | 32.21                           | 1.12                     |
| 1.20 [2,*3]             | 40.0                          | 48.85                           | 0.82                     |
| 1.20 [2,*4]             | 40.0                          | 48.85                           | 0.82                     |
| 1.32 [2,*3]             | 44.0                          | 55.33                           | 0.79                     |
| 1.43 [2,*4]             | 47.6                          | 55.33                           | 0.86                     |
| 1.53 [2,*4]             | 50.9                          | 62.80                           | 0.81                     |
| 1.63 [1,*3]             | 54.3                          | 57.20                           | 0.95                     |
| 1.65 [1,*4]             | 54.9                          | 59.83                           | 0.92                     |
| 1.87 [1,*4]             | 62.3                          | 72.74                           | 0.86                     |
| 2.11 [1,*4]             | 70.3                          | 83.43                           | 0.84                     |
| 2.42 [1,*4]             | 80.6                          | 92.10                           | 0.87                     |
| 2.55 [1,*4]             | 84.9                          | 97.17                           | 0.87                     |
| 1147 [2,*3,*5]          | 1416                          |                                  | 0.81                     |
| 1533 [2,*3,*5]          | 1689                          |                                  | 0.91                     |
| 1601 [2,*3,*5]          | 1770                          |                                  | 0.90                     |
| 1639 [2,*3,*5]          | 1794                          |                                  | 0.91                     |

*1: Famotidine form A  *2: Famotidine form B  *3: Measured at room temperature  *4: Measured at 77 K  *5: Mid-infrared measurements

Figure 7. Typical vibrational modes of the peaks of (A) famotidine form A at 1.65 THz and (B) famotidine form B at 1.08 THz, measured at 220 K.
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