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# Single crystal growth and polarization absorption spectroscopy of theophylline anhydrous for terahertz vibrational mode assignment

Tetsuo Sasaki[a,*], Ohki Kambara[a], Tomoaki Sakamoto[b], Makoto Otsuka[c], Jun-ichi Nishizawa[d]

[a] Research Institute of Electronics, Shizuoka University, 3-5-1 Johoku, Naka-ku, Hamamatsu 432-8011, Japan
[b] Division of Drugs, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158-8501, Japan
[c] Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, Musashino University, 1-1-20 Shinmachi, Nishi-Tokyo 202-8585, Japan
[d] Jun-ichi Nishizawa Memorial Research Center, Tohoku University, 519-1176 Aoba, Aramaki, Aoba-ku, Sendai 980-0845, Japan

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ABSTRACT

In an attempt to provide a procedure for mode assignment in the terahertz (THz) frequency range, we fabricated an apparatus for single crystal growth via a temperature difference method to provide organic crystals that are sufficiently thin for wide-range THz transmission spectroscopy.

Single-crystal theophylline anhydrous (TPAH) was successfully fabricated. THz polarization spectroscopy measurements were performed on TPAH crystal samples at 70 K. Assignment of the absorption peaks was carried out by comparing measurement results with those from density functional theory (DFT) calculations under periodic boundary conditions.



## 1. Introduction

Pharmaceutical science and technology is one of the possible target fields for terahertz (THz) spectroscopy; this technique is especially sensitive to quality degradation during the manufacturing process and with respect to shelf life. For example, polymorphs and pseudo-polymorphs of organic crystals, including pharmaceuticals, can easily be distinguished using THz spectroscopy, because they have unique spectra in this frequency regime [1–4]. This suggests that THz spectroscopy can be used as a non-destructive analytical tool in pharmaceutical quality-control testing or process analysis. Defects in organic crystals could be detected as slight frequency shifts of the absorption lines or as fine structures in THz absorption spectra, depending on the defect concentration [5,6]. However, questions remain regarding the frequency shift mechanism. Assignment of each vibrational absorption peak is the first step in clarifying this effect.

Quantum chemical calculation facilitates mode assignment by comparing the vibrational frequencies and intensities of normal mode peaks with those obtained by absorption peak measurements. However, this approach is somewhat lacking, due to the inability to distinguish overlapping lines and the difficulty of the calculations. To resolve overlapping lines, spectral measurement at low temperatures is effective. From the standpoint of calculations, normal modes are analyzed at 0 K; thus, it is preferable to compare these with absorption modes at low temperature. Polarization THz absorption spectroscopy of a single crystal molecule can also increase the resolution of the spectra, as absorption peaks are observed in each direction [7,8]. In general, for transmission measurement of organic crystals in the THz frequency range, a thin plate crystal (about 10–100 μm in thickness) is necessary, while keeping mm order in the cross direction for wide-range THz measurements (~200 $cm^{-1}$). Such thin plates of biomolecular crystals are difficult to prepare by mechanical processes, such as grinding or polishing, because they are fragile.

In this study, a single crystal growth apparatus was fabricated, based on the temperature difference method in a thin cell flow path, and used to produce theophylline anhydrous (TPAH, $C_7H_8N_4O_2$, MW = 180.16) single-crystal samples. Vibrational peaks in the THz frequency range were assigned by comparing polarization-dependent THz absorption spectra measured at 70 K with results obtained from density functional theory (DFT) calculations of the crystal samples, under periodic boundary conditions.

* Corresponding author.
*E-mail address:* tsasaki@rie.shizuoka.ac.jp (T. Sasaki).

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## 2. Experiment

### 2.1. Material

Theophylline, an oral bronchodilator medicine, is known to have several types of polymorphs (Form I ~ IV) [9–13] and pseudo-polymorphs [12,14]. In general, pharmaceutical theophylline is prepared as theophylline monohydrate (TPMH), recrystallized from an aqueous solution. The crystal shape of TPMH is needle-like at first, but then converts into a TPAH micro-powder after a drying process. The TPAH used in this experiment was obtained from Wako, Japan (CAS.No. 58-55-9). There were a lot of researches applying terahertz spectroscopy on TPAH and TPMH [15–17], but they all were for powder samples.

### 2.2. Single crystal growth

Fig. 1 shows the custom-designed single crystal growth apparatus used to fabricate TPAH single crystal directly. All of the components of the growth apparatus were housed inside of a thermally insulated box; the temperature was controlled by a heater and a fan and monitored with a chromel-alumel thermocouple (TC). A saturated aqueous solution of theophylline was obtained by dissolving TPAH in distilled water in a bottle set on a magnetic stirrer. The solution was fed through a silicone tube by a pump into a cell constructed from two cyclo olefin copolymer (COC) plates; these plates exhibited high transparency with respect to THz frequencies, especially at higher frequencies. The thickness of the flow cell was adjusted to 0.03 mm by a spacer. The midpoint of the flow path was cooled by a Peltier cooler, under TC monitoring control. Because the saturated solubility of TPMH is lower than that of TPAH in the temperature range below 345 K, the TPMH crystal was grown at lower temperatures approaching normal room temperature [18]. Although TPMH crystal is known to transform TPAH by stored in low humidity condition long time at room temperature [19], large size TPAH single crystal could not be obtained because they turned into small flaky pieces only by storing in dry air. Thus, the temperature conditions were maintained at 355 K, but adjusted to 351 K for TPAH single-crystal growth. Although the shape of the TPAH single crystal becomes needle-like in free space, a plate-like shape was obtained in the restricted cell. Single crystals at 0.03, 0.05 and 0.1 mm thickness were grown with different spacer thickness and we found 0.03 mm was suitable to obtain wide range spectra at last. The size of the grown crystal was 1.0 × 5.0 × 0.03 mm.

### 2.3. X-ray diffraction (XRD) analysis

Crystal structures were confirmed by powder XRD measurements for a crushed sample of grown single crystals. Crystal orientation was confirmed with XRD pole figure measurements of each crystal using a PANalytical Empyrean System (Spectris Co. Ltd., Tokyo, Japan).

### 2.4. THz measurement

THz transmission spectroscopy was performed using a gallium phosphide (GaP) continuous wave (CW) THz-absorption spectral measurement system [20]. The THz light source for the system is based on difference frequency generation of two infrared (IR) laser beams in the GaP crystal via excitation of phonon–polariton modes under small-angle non-collinear phase-matching conditions. One of the pump beams was delivered from a distributed feedback laser, and the other was delivered from an external-cavity laser diode; each beam was amplified by optical fiber amplifiers. By tuning and locking the wavelengths of both lasers to the interferometric wavelength meter precisely, THz measurement in the range of 16.7–183 $cm^{-1}$ (0.5–5.5 THz) in 0.03 $cm^{-1}$ (1 GHz) step increments was performed, with accuracy well below 0.03 $cm^{-1}$ (100 MHz) for each measurement point. The temperature of the crystal on the cryostat stage, which varied from 70 to 300 K, was controlled by a heater and nitrogen gas intake (as a spray). The THz waves had high polarization purity; thus, polarization-dependent spectra were obtained by rotating the sample.

### 2.5. DFT calculation

DFT calculations were performed using the CRYSTAL09 software package [21]; periodic boundary conditions were assumed to optimize the crystal structure and to obtain harmonic frequencies and IR absorption intensities. Calculations were performed using

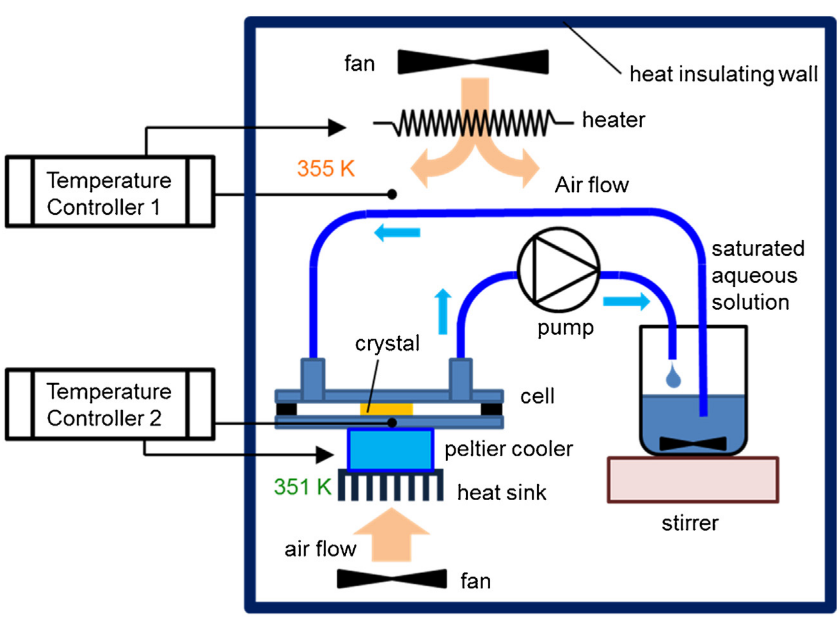


**Fig. 1.** Schematic diagram of the custom-designed single crystal growth apparatus by temperature difference method.