

# Use of Terahertz Time-Domain Spectroscopy for Cocrystal Characterisation and Quantitative Monitoring of Mechanochemical Cocrystal Formation

K. Lien Nguyen<sup>1</sup>, Tomislav Friščić<sup>2</sup>, Graeme M. Day<sup>2</sup>, Lynn F. Gladden<sup>1</sup>, William Jones<sup>2</sup>

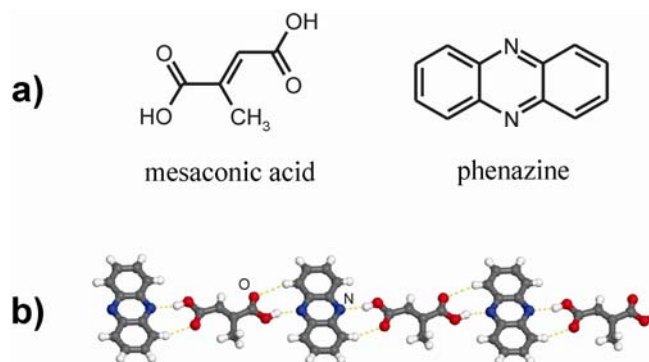
<sup>1</sup> Department of Chemical Engineering, University of Cambridge, Pembroke Street, Cambridge CB2 3RA, UK

<sup>2</sup> Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK

\* email: gladden@cheng.cam.ac.uk, wj10@cam.ac.uk

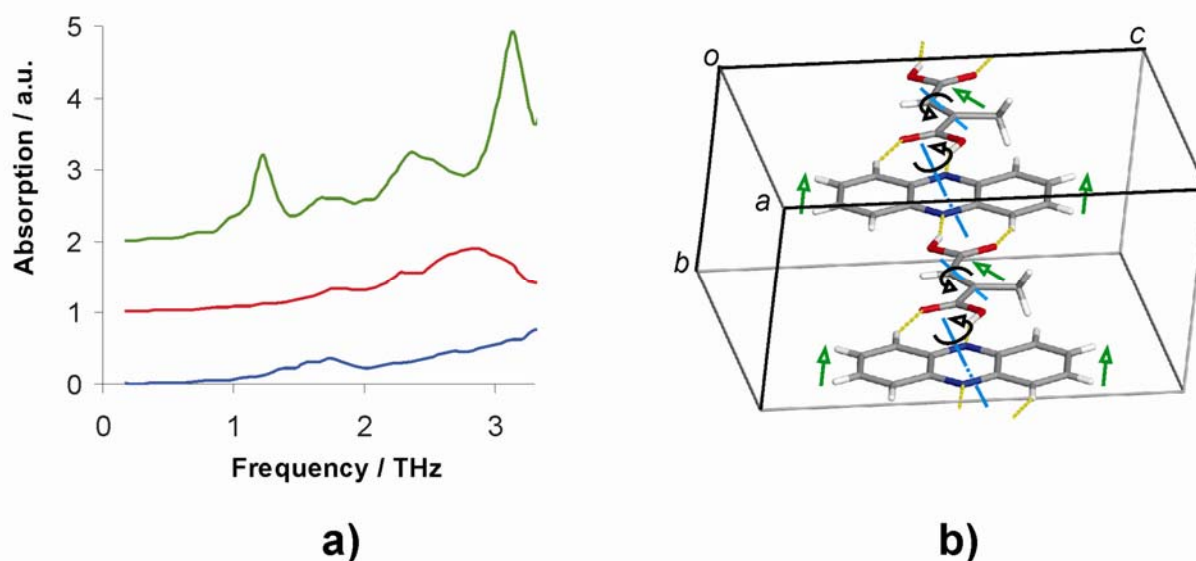
The increased availability of sources and detectors for terahertz (THz) radiation over the past decade<sup>1</sup> has brought THz spectroscopy to the attention of physicists, engineers, chemists and materials scientists. THz radiation probes intermolecular interactions through crystal lattice vibrations, allowing the characterisation of solid materials<sup>2</sup>. Thus, THz spectroscopy, employing non-ionizing radiation, is a promising alternative to current analytical methods that either use high-energy radiation (*e.g.* X-ray diffraction) or are destructive to the sample (*e.g.* thermal analysis)<sup>3</sup>. Benefits of THz spectroscopy are the feasibility of online measurement<sup>4</sup>, remote sampling<sup>5</sup> and three-dimensional imaging<sup>6</sup>, all of which are attractive for quality control or security applications. In context of pharmaceutical solids, THz spectroscopy can differentiate and quantify different forms of active pharmaceutical ingredients<sup>7,8</sup>. Inspired by growing significance of cocrystals in materials science<sup>9,10</sup>, we have used THz spectroscopy to quantitatively monitor the mechanochemical synthesis of a cocrystal, revealing the possible participation of an intermediate amorphous phase.

As our first entry into the investigating cocrystals and cocrystal formation through THz spectroscopy, we have selected the previously studied system of phenazine (**phen**) and mesaconic acid (**mes**)<sup>11</sup>. Grinding of an equimolar mixture of the components yields a two-component cocrystal with the composition (**phen**)(**mes**). The cocrystal is made up of infinite chains held together by pairs of O-H $\cdots$ N and C-H $\cdots$ O hydrogen bonds (Figure 1)<sup>12</sup>. A system of fairly rigid molecules was chosen with the intention of using rigid body lattice dynamics calculations to assign the observed spectra<sup>13</sup>. The particular system was selected so that the reactant crystals would produce relatively featureless THz spectra, while the product cocrystal should show more distinct features. The symmetry of the **phen** crystal structure limits the number of THz active rigid body lattice modes to three and, due to the zero net dipole moment of the **phen** molecule, the observed intensities of these modes are expected to be low<sup>14</sup>. Our calculations suggested only three THz active lattice modes for the **mes** crystal in the frequency range of interest (0-3 THz) and twelve THz active lattice modes for (**phen**)(**mes**) in this spectral range. Therefore, we felt that there was a good chance that we would observe a characteristic (**phen**)(**mes**) peak in the THz spectrum that could be used for quantification of cocrystal formation.



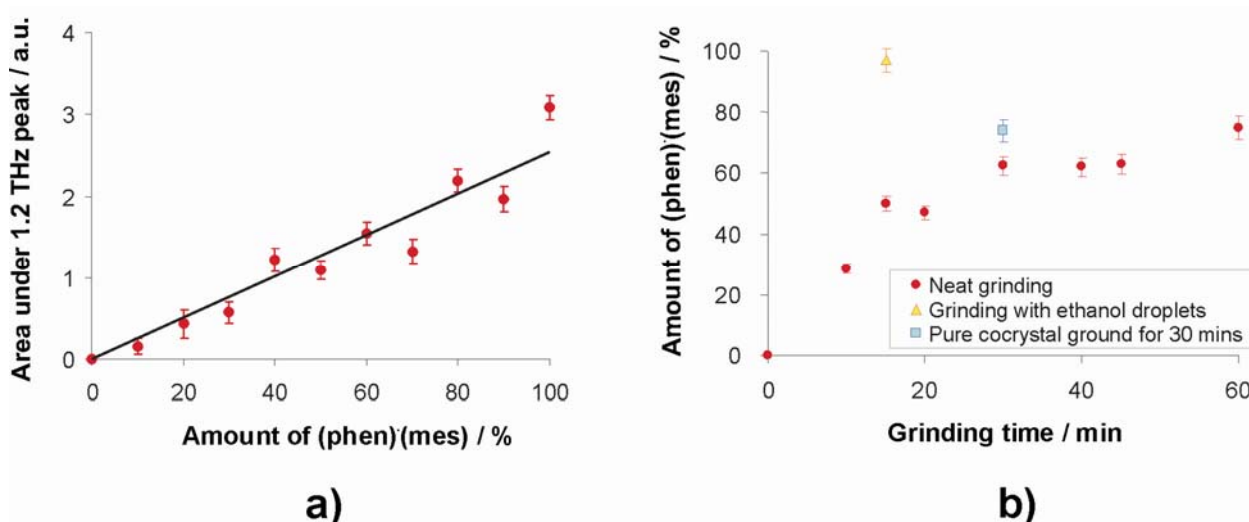
**Figure 1. Representations of involved molecules.** **a**, schematic representations of **mes** and **phen**; **b**, a ball-and-stick representation of a fragment of **(phen)(mes)** cocrystal, demonstrating the presence of O-H $\cdots$ N and C-H $\cdots$ O bonds.

The inspection of THz spectra of **phen**, **mes** and the cocrystal **(phen)(mes)** revealed that the cocrystal exhibits a distinctive absorption peak at 1.2 THz (Figure 2a). Therefore, the peak was selected as a reference to monitor the formation of **(phen)(mes)**. To achieve a full understanding of the origin of the characteristic peak, lattice dynamics calculations were performed on the **(phen)(mes)** cocrystal, providing tentative assignments of observed THz features. The agreement between the calculated and observed THz spectra was not sufficient to characterise the entire **(phen)(mes)** spectrum. However, only one THz active lattice mode was calculated in the 1-1.5 THz range, at 1.31 THz, allowing reasonable confidence in assigning the calculated mode to the 1.2 THz absorption peak. The calculated mode corresponds to a combination of molecular translations and librations (Figure 2b), which give rise to an asymmetric stretch, with some out-of-plane twist, of the O-H $\cdots$ N and C-H $\cdots$ O hydrogen bond pairs within each **(phen)(mes)** hydrogen-bonded chain.



**Figure 2. THz spectra and representation of the chain distortion leading to a THz absorption peak.** **a**, spectra of **phen** (blue), **mes** (red) and **(phen)(mes)** (green) are shown, vertically offset for clarity; **b**, the distortion of the hydrogen-bonded chain in **(phen)(mes)** that results in the absorption peak at 1.2 THz is a combination of wagging motions of mes and phen components in each chain. The chains propagate parallel to the crystallographic {110} direction.

In order to obtain quantitative information on the formation of **(phen)(mes)**, we first constructed a calibration curve by recording THz spectra of physical mixtures of **phen**, **mes** and **(phen)(mes)** at known compositions, corresponding to different levels of conversion to the cocrystal. The integration of the 1.2 THz peak correlates to the amount of the cocrystal in a linear fashion (Figure 3a). The progress of the solid-state reaction of **phen** and **mes** was then followed by measuring THz spectra of mixtures of **phen** and **mes** ground at increasing time periods up to the total grinding time of 90 min. Assignment of the integrations of the peak at 1.2 THz to the calibration curve revealed that the reaction rate follows a first-order reaction law and the reaction yield leveled off at 70 % after approximately 60 min of grinding (Figure 3b). Further grinding beyond 60 min reduced the amount of **(phen)(mes)** detected by THz spectroscopy. Powder X-ray diffraction pattern of the sample after 60 min of grinding provides no signal that could be attributed to either **phen** or **mes**, suggesting a complete conversion to **(phen)(mes)**. In a related experiment involving 30 min grinding of a sample of pure **(phen)(mes)** prepared from solution, THz spectroscopy revealed an apparent reduction in **(phen)(mes)** content from 100 % to 70 % (Figure 3b), whilst powder X-ray diffraction pattern remained unchanged.



**Figure 3. Results of quantification experiments.** **a**, the calibration curve, based on a best-fit line through all experimental data points ( $R^2 = 0.915$ ), demonstrating a linear correlation between the concentration of **(phen)(mes)** and the area under the peak at 1.2 THz; **b**, dependence of the yield of **(phen)(mes)** on different grinding conditions: using neat grinding or liquid-assisted grinding.

We also observed the increase in the yield of **(phen)(mes)**, calculated from the THz data, upon standing of the samples at room temperature. For example, room-temperature ageing of the sample ground for 20 min over a period of 120 h led to an increase in the yield from 50 % to 80 %, close to the yield observed immediately after 60 min of grinding. We extended the study further and used THz spectroscopy to observe the cocrystallisation in the presence of a drop of liquid. Liquid-assisted grinding was previously demonstrated to provide the crystalline product at a much faster pace than neat grinding<sup>15</sup>. As shown in Figure 3b, the THz spectrum of a sample of **phen** and **mes**, ground for 15 min with a single drop of ethanol achieved complete conversion to the cocrystal. The result was qualitatively supported by X-ray powder diffraction pattern which exhibited only sharp signals of **(phen)(mes)**, corresponding to high level of crystallinity.

We recognize that the observed differences between the content of **(phen)(mes)** in neat ground samples suggested by X-ray powder diffraction and THz spectroscopy can be accounted for by either a particle size effect or partial amorphisation of the sample upon grinding. A reduction in particle size induced by grinding would be expected to decrease the intensity of the THz signal, due to scattering effects. A similar outcome would be expected by the formation of an amorphous phase from crystalline **(phen)(mes)**. The relative importance of the effects of particle size and amorphous content on THz spectra is not clear at this moment. Preliminary scanning electron microscopy (SEM) imaging of samples obtained by neat and liquid-assisted grinding revealed that liquid-assisted grinding provides significantly larger particles. The finding suggests that the difference in yields of samples obtained by neat and liquid-assisted grinding could be attributed to a particle size effect. However, SEM images did not reveal any significant change in particle size upon standing of the samples over a period of 4 days, suggesting that the increase in reaction yield upon ageing may be attributed to the crystallization of an amorphous phase.



**Figure 4. SEM images of ground samples of (phen)(mes).** a, a typical sample obtained via neat grinding for 30 min; b, a typical sample obtained via liquid-assisted grinding over a period of 30 min using ethanol as a liquid phase.

We have for the first time demonstrated the use of THz spectroscopy to characterise a two-component cocrystal and monitor its solid-state formation. The ability to assign a lattice vibration mode to a feature in the cocrystal THz spectrum encourages us to further develop lattice dynamics calculations in order to achieve a molecular-level understanding of THz spectra<sup>16,17</sup>. Particularly, we regard developments in computational methods will provide a means to design molecular solids that can readily be monitored by THz spectroscopy. In that context, we have made preliminary investigations of a set of related cocrystals: **phen** with each of fumaric, maleic and citraconic acids. Despite chemically similar constituents, each cocrystal has a characteristic THz spectrum, reflecting the differences in molecular packing and exhibiting distinctive features that could be used for quantification. We anticipate that further use of THz spectroscopy to probe solid-state materials and processes, aided by computational efforts, will establish THz spectroscopy as a viable alternative to X-ray diffraction in characterising organic solids. Clearly,

the effects of particle size and amorphous content on THz spectra need further investigation. Full understanding of such effects on THz spectra could result in applications related to monitoring particle size and amorphous content in solids.

## METHODS

All materials and reagents were purchased from Sigma-Aldrich, UK and were used without further purification. Cocrystal of pure **(phen)**(**mes**) were obtained by cocrystallisation from ethyl acetate as described previously<sup>11</sup>. Scanning electron microscopy images have been recorded on a JEOL JSM-5510 LV scanning electron microscope, using the accelerating voltage of 20 kV.

## MECHANOCHEMICAL EXPERIMENTS

Grinding experiments were performed on 100 mg samples of a solid equimolar mixture of **phen** and **mes**. Each sample was ground in a 10 mL stainless steel container using two 7 mm stainless steel balls. The grinding was carried out in a Retsch MM200 mixer mill, operating at a frequency of 30 Hz.

## TERAHERTZ TIME-DOMAIN SPECTROSCOPY (THz-TDS)

Spectra were taken on pellets prepared by embedding the powder solid sample within a polyethylene matrix transparent to THz radiation. A typical sample was made using 31.0 mg of the ground sample and 35 mg of polyethylene. The sample and polyethylene were thoroughly mixed using a Reax-top vortex mixer (Heidolph Instruments). The obtained mixture was manually pressed in a mould to form a pellet of 8 mm in diameter and approximate thickness of 0.8 mm.

THz radiation was obtained using a Ti:sapphire laser (Femtolasers) pumped by a Millennia Xs CW laser (Spectra Physics), producing 12 fs laser pulses centred at approximately 800 nm (bandwidth > 100 nm). The laser beam was split into a pump beam and a probe beam used to generate and detect THz radiation, respectively. The probe beam was guided to an electro-optic ZnTe crystal detector. The pump beam was guided to a biased semi-insulating GaAs emitter, generating THz pulses of 0.1-3.0 THz bandwidth. The THz beam reflected from the emitter surface was collimated and focused onto the sample pellet using a pair of parabolic mirrors. THz radiation transmitted through the sample pellet was subsequently collected and focused onto the ZnTe crystal detector by a different set of parabolic mirrors. The THz pulses and the laser probe beam arriving at the ZnTe detector allowed the detection of THz radiation *via* an electro-optic scheme. The entire path of the THz beam was contained in a chamber continuously purged with dry nitrogen gas. The acquisition time was approximately 30 s. Each spectrum was based on an average of 30 acquisitions. The spectral resolution was 75 GHz ( $2.5 \text{ cm}^{-1}$ ).

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## COMPETING FINANCIAL INTERESTS

The authors declare that they have no competing financial interests.