Thermal and *in situ* X-ray Diffraction Analysis of a Dimorphic Co-crystal, 1:1 Caffeine-Glutaric Acid

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ABSTRACT: Spurred by the enormous interest in co-crystals from the pharmaceutical industry, many novel co-crystals of active pharmaceutical ingredients have been discovered in recent years and this has in turn led to an increasing number of reports on polymorphs of cocrystals. Hence, a thorough characterization and understanding of co-crystal polymorphs is a valuable step during drug development. The purpose of this study is to perform *in situ* structural analysis and to determine thermodynamic stability of a dimorphic co-crystal system, 1:1 caffeine-glutaric acid (CA-GA, Forms I and II). We performed thermal and structural characterizations by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), hot-stage microscopy (HSM), slurry and *in situ* variable temperature X-ray diffraction (VTXRD). For completeness, we have also re-determined crystal structures of CA-GA Forms I and II at 180 K using single crystal X-ray diffraction. Our results revealed that Form II is stable and Form I is metastable at ambient conditions. Further, the results suggest that the dimorphs are enantiotropically related and the transition temperature is estimated to be 79 °C.

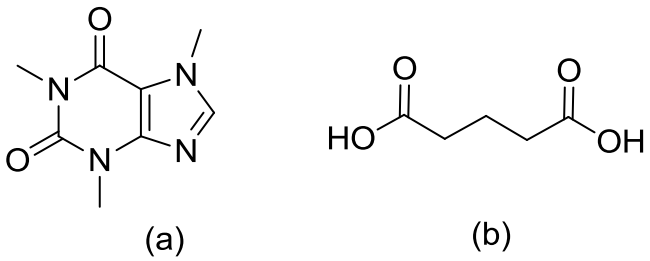
▪ INTRODUCTION

Polymorphism is defined as the ability of a solid material to exist in at least two different crystal structures.[1-4] Polymorph screening is a crucial part of the drug development process[1] because polymorphs exhibit different physicochemical properties, such as solubility, stability and bioavailability, the economic significance of novel polymorph as intellectual property is enormous.[1,2,4] The high profile cases of ranitidine hydrochloride (Zantac) and ritonoavir (Norvir) serve to illustrate the importance of polymorphs to the pharmaceutical industry.[5] It was believed

that only few multi-component crystals exist in polymorphic forms.[6] However, as per McCrone, the number of solid forms known for a given compound is proportional to the time and money spent in research on that compound.[7,8] Due to the interest of the pharmaceutical industry in co-crystals, many novel co-crystals have been discovered in recent years and this has also led to an increasing number of reported cases of polymorphism in co-crystals.[9] It was suggested that the number of co-crystal polymorphs that were reported after the year 2000 is significantly higher than the number of cocrystal polymorphs reported before. A recent database analysis in 2014[10] revealed that a total of 95 dimorphic, four trimorphic,[11-14] three tetramorphic[10] and one pentamorphic[15] co-crystals have been reported. Interestingly, the percentage of polymorphs in cocrystals is comparable to the percentage of polymorphs in single-component crystals.[9] Hence, a through characterization and understanding of polymorphs in co-crystal is as important as single-component crystals during drug development.

Caffeine (1,3,7-trimethyl-2,6-purinedione), a central nervous system stimulant, is known to exhibit instability with respect to humidity with the formation of a crystalline nonstoichiometric hydrate.[16] It is a popular model compound for pharmaceutical co-crystallization studies.[17] Jones *et al.,* prepared a series of co-crystals involving caffeine and several dicarboxylic acids viz., glutaric acid, maleic acid, malonic acid and oxalic acid and demonstrated that they could be a remedy for caffeine hydration.[18] Notably, the authors identified two polymorphic forms for the caffeine-glutaric acid (CA-GA, Forms I and II) from their liquid assisted grinding experiments and the two forms were structurally characterized. The CA-GA polymorphs varied in stability toward humidity - Form I transforms to Form II within 24 h, and Form II is stable for over three days in high humidity condition before undergoing conversion to caffeine hydrate. Yu et al. have recently used attenuated total reflectance Fourier

transform spectroscopy (ATR-FTIR) and focused beam reflectance measurement (FBRM) to monitor and control co-crystallization of CA-GA in acetonitrile.[19,20] The authors showed that by controlling the critical crystallization parameters, it is possible to eliminate the nucleation of undesirable Form I and produce considerably large particles with low proportion of fines. It has been found that in unseeded crystallization Form I nucleates first and then transforms to Form II. Seeding with Form II could avoid nucleation of Form I and produce only Form II. Recently, Thakuria *et al.,* showed that atomic force microscopy can be used to distinguish the two polymorphs of the CA-GA co-crystal on the basis of the thickness of molecular layers of the two forms and they used this difference to monitor changes at the crystal surfaces during the transformation of Form I to Form II.[21] Herein we report a thorough thermodynamic and *in situ* structural characterization study of 1:1 CA-GA co-crystal dimorphs (Scheme 1).



Scheme 1. The chemical structures of (a) caffeine and (b) glutaric acid.

▪ EXPERIMENTAL SECTION

Materials. Anhydrous caffeine (99% purity) was obtained from Fluka and glutaric acid (99%) from Alfa Aesar were used as received. The solvents were of analytical or chromatographic grade.

Liquid Assisted Grinding (LAG).[22-26] It was performed on a Retsch Mixer Mill model MM301 with 10 mL stainless steel grinding jars and one 7 mm stainless steel grinding ball at a rate of 30 Hz for 30 min. LAG screenings were carried out with stoichiometric amount of caffeine (1 mmol) and glutaric acid (1 mmol) in several solvents. 50 μL of solvent was added to 200 mg of reactants mixture prior to LAG (η = 0.25 μL mg^{-1}). The external temperature of the grinding jar at the end of grinding did not exceed 30 °C. The resulting powder samples were analyzed by PXRD.

Solution Crystallization. Caffeine (1 mmol) and glutaric acid (1 mmol) were dissolved in 10 mL of chloroform at 70 °C. The solution was allowed to evaporate slowly at ambient conditions for two days to produce the concomitant dimorphs (colorless Form I needles and Form II blocks) of CA-GA co-crystal.[27]

Powder X-ray Diffraction (PXRD). The powder diffraction data were collected in Bragg-Brentano geometry with a Bruker D8 Advance (Bruker AXS GmbH, Germany) X-ray powder diffractometer equipped with a Cu-Kα radiation (λ = 1.54056 Å) source, a Nickel-filter, 0.3° divergence slit and a linear position sensitive detector (Vantec-1). The diffractometer was operated at 35 kV and 40 mA. The sample was loaded onto a glass circular sample holder of 1

100 mm thickness and 1.5 cm diameter. The data were collected over an angle range of 5 to 50° 2θ at
101 a scanning speed of 2° 2θ per minute.

102 **Thermal Analysis.** Thermogravimetric analysis (TGA) was conducted with a SDT 2960
103 thermogravimetric analyzer (TA instruments). Approximately, 5 mg of sample was used per trial
104 in an alumina crucible. The samples were heated at a rate of 10 °C/min from 25 to 275 °C. The
105 samples were purged with a stream of flowing nitrogen throughout the experiment at 200
106 mL/min. All samples were analyzed in duplicate. Differential scanning calorimetry (DSC) of all
107 samples was performed using PYRIS Diamond DSC calorimeter (Perkin Elmer, USA). About 5
108 mg of each sample was placed in a hermetically closed aluminum pan. The sample was heated
109 from 25 to 120 °C at a rate of 10 °C/min after equilibrating the samples at 25 °C for 10 min. The
110 samples were purged with a stream of flowing nitrogen at 25 mL/min.

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112 **Hot-stage Microscopy (HSM).** Thermomicroscopic investigations were performed with an
113 optical polarizing microscope (Olympus, BX51, Olympus Optical GmbH, Vienna) equipped
114 with a Linkam hot-stage THMS 600 connected to a TMS 94 temperature controller and a LNP
115 94/2 liquid nitrogen pump (Linkam Scientific Instruments Ltd, Tadworth, Surrey, UK). The
116 microscopic images were recorded with a CCD camera attached to the microscope at 12 s time
117 interval using Soft Imaging System's Analysis image capture software. Caffeine-glutaric acid
118 Forms I and II were heated over the temperature range of 25-110 °C at a constant heating rate of
119 10 °C min^{-1} and cooled back to room temperature. The hot-stage was calibrated using USP
120 melting point standards.

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Single Crystal X-ray Diffraction. Single crystals of caffeine-glutaric acid Forms I and II were chosen under a Leica microscope and placed on a fibre needle which was then mounted on the goniometer of the X-ray diffractometer. The crystal was purged with a nitrogen gas stream at 180 K throughout the data collection. X-ray reflections were collected on a Rigaku Saturn CCD area detector with graphite monochromated Mo-*Kα* radiation (λ = 0.71073 Å). Data were collected and processed using CrystalClear (Rigaku) software. The crystal structures were solved by direct methods and SHELX-TL was used for structure solution and least-squares refinement.[28] All hydrogen atoms were fixed at idealized positions except for the acid O–H hydrogens, which were located from the difference Fourier map and allowed to ride on their parent atoms in the refinement cycles. All O–H and C−H distances are neutron normalized to 0.983 and 1.083 Å, respectively. Data collection and refinement details are given in Table 2, and the relevant hydrogen bonding interactions and their geometries are listed in Table 3.

Variable Temperature X-ray Diffraction (VTXRD). The data were collected in asymmetric reflection geometry using an Inel Equinox 3000 diffractometer (INEL, Artenary, France) equipped with a Cu-Kα source, a NiC multilayer Goebel mirror, an XRK-900 reactor chamber (Anton Paar GmbH, Graz, Austria) and a CPS-250 position sensitive detector (INEL, Artenay, France). The diffractometer was operated at 30 kV and 30 mA. All data were collected over angle range of 4 to 105° 2θ in air at an incident beam angle of 5°.

Approximately 100 mg of caffeine-glutaric acid Form II was filled into a sample holder by the top-fill technique and heated from 25 to 98 °C at a rate of 1 °C/min. The sample was held at 98 °C for 70 min. Subsequently, the sample was cooled to 25 °C at a rate of 1 °C/min. Quantitative analysis of solid-state transformation of Form II to Form I during heating ramp was

performed by Rietveld refinements using Topas v4.2 (Bruker-AXS GmbH, Karlsruhe, Germany). The peak shape was modeled by a convolution of a square function with a Lorentzian function, a Gaussian function and a circles function. Our re-determined single crystal structures of Forms I and II were used as structure models. The semiquantitative analysis of the melting process was also conducted by the Rietveld refinement. The structure published by Lehmann *et al.,* was used as model for caffeine.[29]

Similarly, ~100 mg of caffeine-glutaric acid Form I was heated from 25 to 95 °C and held at 95 °C for 45 min and cooled back to 25 °C. For both the heating and cooling ramps, the rates employed were 1 °C/min.

▪ RESULTS AND DISCUSSION

LAG experiments were conducted in 13 solvents and the product phases were analyzed by PXRD (Table 1). The results complemented well with the reported LAG experiments that the outcome is dependent on the polarity of solvent.[27] Herein polar solvents favored the formation of CA-GA Form II whereas in diethyl ether a mixture of CA-GA Forms I and II was obtained.

Table 1. The Outcome of Liquid Assisted Grinding of a 1:1 Molar Ratio of Caffeine and Glutaric Acid with Various Solvents.

Liquid	Cocrystal form as identified by PXRD
[a]cyclohexane, heptane, n-hexane	Form I
[a]chloroform, dichloromethane, acetonitrile, water	Form II
1,4-dioxane, toluene, mesitylene, anisole, ethylacetate, tetrahydrofuran, isopropanol, acetone, methanol, nitromethane, dimethylformamide, dimethylsulfoxide	Form II
diethyl ether	Forms I + II

[a]Presented in previous studies, see reference # 15.

Solution Crystallization

Evaporative co-crystallization of equimolar CA and GA from chloroform at ambient conditions afforded the concomitant dimorphic co-crystals (Form I and Form II) of CA-GA.[27] Due to the distinct shapes of the two forms, single crystals of each form could be easily picked out manually by visual observation.

Crystal Structure Analysis

The crystal structures of CA-GA dimorphs have been reported previously by Jones and co-workers.[18, 27] To facilitate a comparison of crystal packing and thermodynamic stabilities of CA-GA Forms I and II, single crystal X-ray diffraction at 180K were also performed for these dimorphs. Crystallographic parameters and hydrogen bonding geometries for CA-GA Forms I and II are provided in Tables 2 and 3.

Table 2. Crystallographic Data for Dimorphs of 1:1 Caffeine-Glutaric Acid.

	Form I	Form II
Empirical formula	$(C_8H_{10}N_4O_2)\cdot(C_5H_8O_4)$	
Formula weight	326.31	
Crystal system	Monoclinic	Triclinic
Space group	$P\,2_1/c$	$P\bar{1}$
T [K]	180(2)	180(2)
a [Å]	12.989(3)	8.3273(17)
b [Å]	6.5994(13)	8.6640(17)
c [Å]	17.118(3)	11.362(2)
α [°]	90.00	68.96(3)
β [°]	97.84(3)	78.54(3)
γ [°]	90.00	74.22(3)
Z	4	2
V [Å^3]	1453.6(5)	731.6(3)
D_{calc} [g cm^{-3}]	1.491	1.481
μ [mm^{-1}]	0.119	0.119
Reflections used	8749	8039
Unique reflections	2505	2546
Observed reflections	2114	2438
Parameters	212	216
R1[I > 2σ(I)]	0.1047	0.0576
wR_2 [all]	0.2921	0.1594
GOF	1.199	1.087
Crystal shape	Needle	Block

The morphologies of the CA-GA co-crystal polymorphs (Forms I and II) were distinctly different under the optical microscope i.e. needle-shaped crystals for Form I, block-shaped crystals for Form II. Form I crystallized in $P2_1/c$ while Form II in $P\bar{1}$ space group. The asymmetric units of either Form I or II consist of 1:1 stoichiometry of caffeine and glutaric acid molecules. Two dimensional crystal packing of the dimorphs shows similar hydrogen bond tapes where linear O–H···O synthons are present between the OH groups of one glutaric acid and the C=O of the adjacent glutaric acid. The other acid O-H groups of glutaric acid are connected to the imidazole ring of caffeine via O–H···N hydrogen bond to form layers (Figure 1). The alignments of molecules are slightly different in each of the polymorph meaning Form I consists of a flat layer whereas a corrugated layer can be seen in case of Form II. We plotted the overlay diagram of caffeine and glutaric acid molecules across Forms I and II (Figure 2). It depicts that

there is no appreciable change in the molecular conformations within caffeine (except for methyl C-H orientations) or glutaric acid molecules (Figure 2).

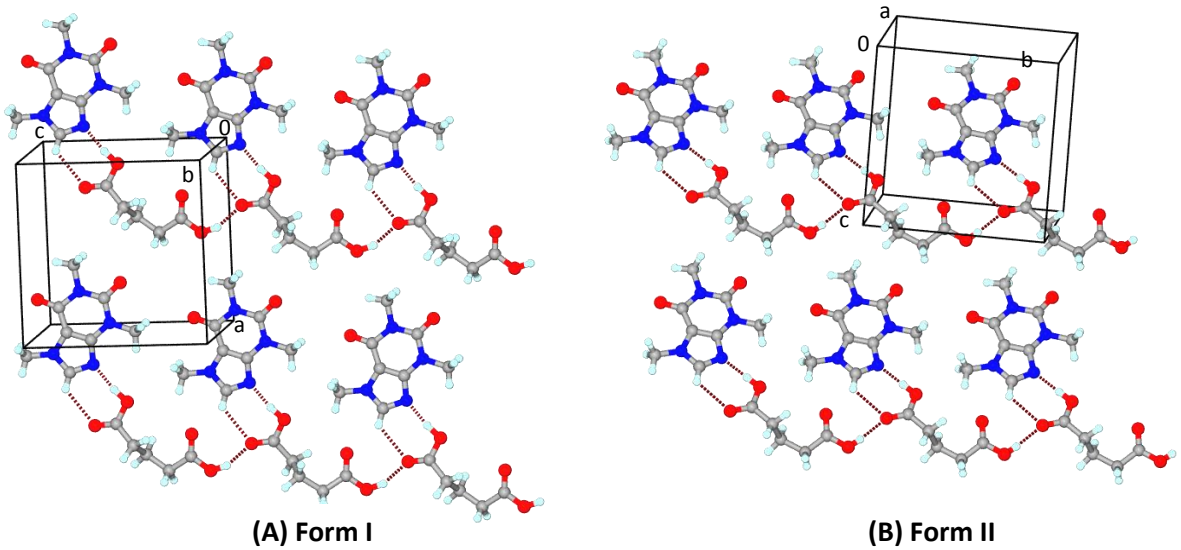


Figure 1. Crystal packing of CA-GA co-crystal dimorphs, Forms I and II. Note that these dimorphs are stabilized by similar sheet structures composed of hydrogen bonded ribbons.

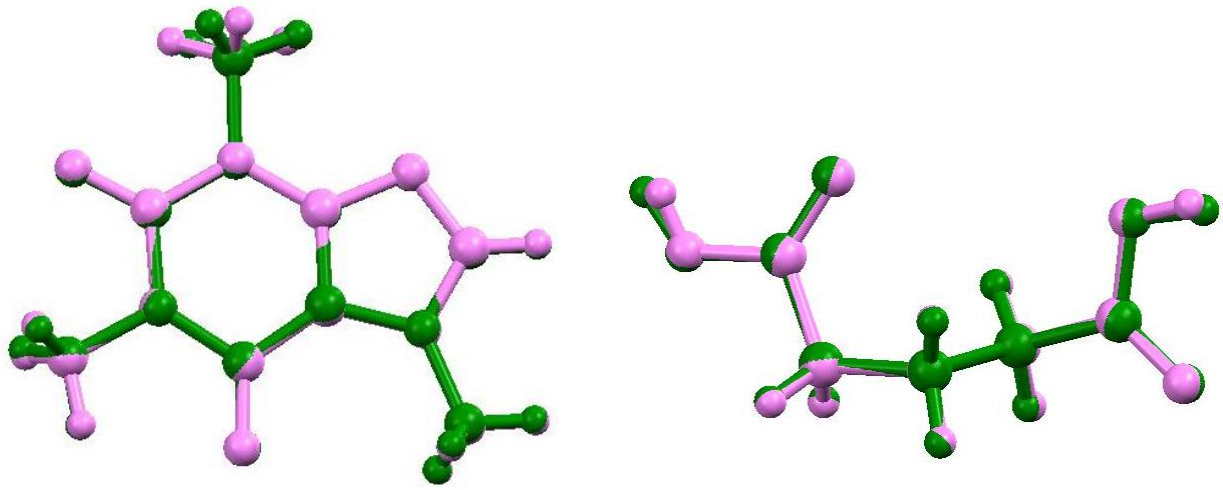


Figure 2. Overlay of conformers of CA (left) and GA (right) in Forms I and II. Color codes: Form I – green and Form II – pink.

Table 3. Hydrogen Bond Geometries for 1:1 Caffeine-Glutaric Acid Polymorphic Forms I and II.

CA-GA	D–H···A	*d*(H···A)/ Å	*d*(D···A)/ Å	∠(D–H···A)/ °
Form I	O–H···N	1.75	2.719(5)	169
	O–H···O	1.74	2.721(4)	177
	C–H···O	2.19	3.140(5)	145
	C–H···O	2.37	3.347(5)	150
	C–H···O	2.46	3.193(5)	124
	C–H···O	2.58	3.320(5)	125
	C–H···O	2.64	3.525(5)	139
	C–H···O	2.66	3.670(5)	155
	C–H···O	2.68	3.571(5)	139
intra	C–H···O	2.27	2.743(6)	104
intra	C–H···O	2.23	2.732(6)	106
Form II	O–H···N	1.73	2.716(2)	177
	O–H···O	1.78	2.751(2)	171
	C–H···O	2.19	3.138(3)	145
	C–H···O	2.37	3.430(3)	166
	C–H···O	2.41	3.377(3)	148
	C–H···O	2.48	3.202(3)	123
	C–H···O	2.53	3.213(3)	120
	C–H···O	2.68	3.543(3)	137
intra	C–H···O	2.19	2.698(3)	106
intra	C–H···O	2.45	2.940(3)	106

O–H and C–H geometries are neutron normalized to 0.983 and 1.083 Å. D = donor, A= acceptor.

Thermal Analysis. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were carried out for Forms I and II of CA-GA co-crystal (Figure 3 and see also Figure S1 of Supporting Information, SI). Form I showed one major endotherm with T_{onset} at 98.8 °C (heat of fusion, ΔH_f, 118 J g^{-1}) that is attributed to a melting event. In the case of Form II, two endotherms were observed. A minor endotherm (peak T_{onset} at 93.8 °C, 3.4 J/g) could be interpreted broadly as a phase transformation of Form II to Form I. But, a detailed analysis, an inset view of Form II (Figure 3), suggests that the solid-solid phase transition of Form II to Form I can be seen to occur well before a minor endotherm, which is with T_{onset} at 78.6 °C (6.7 J g^{-1}), whereas a major endotherm with T_{onset} at 98.5 °C (106 J g^{-1}) corresponding to melting of Form I.

TGA traces of CA-GA Forms I and II showed the absence of solvent molecules in these crystalline phases (Figure S1, SI). The weight loss occurred past 150 °C is attributed to degradation.

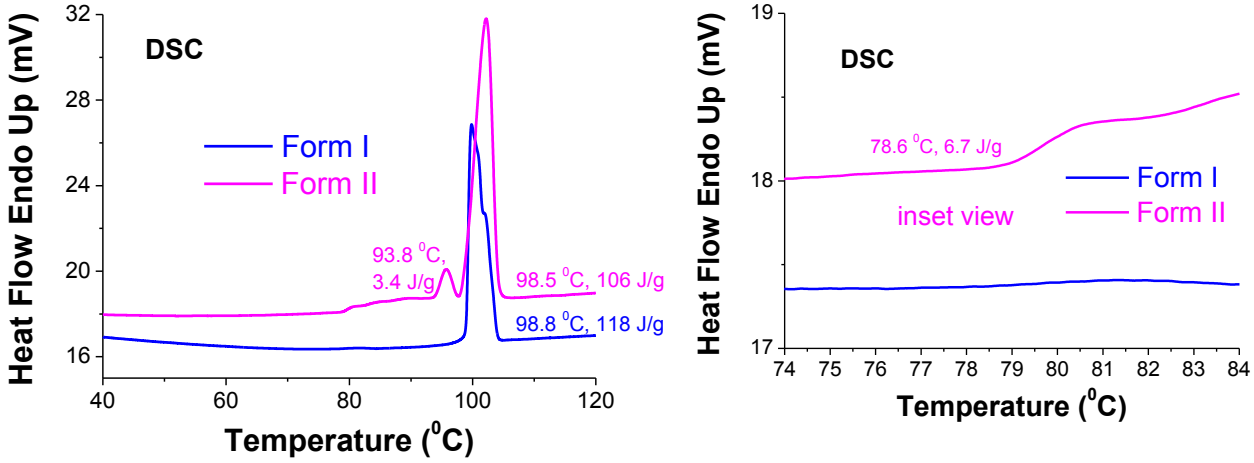
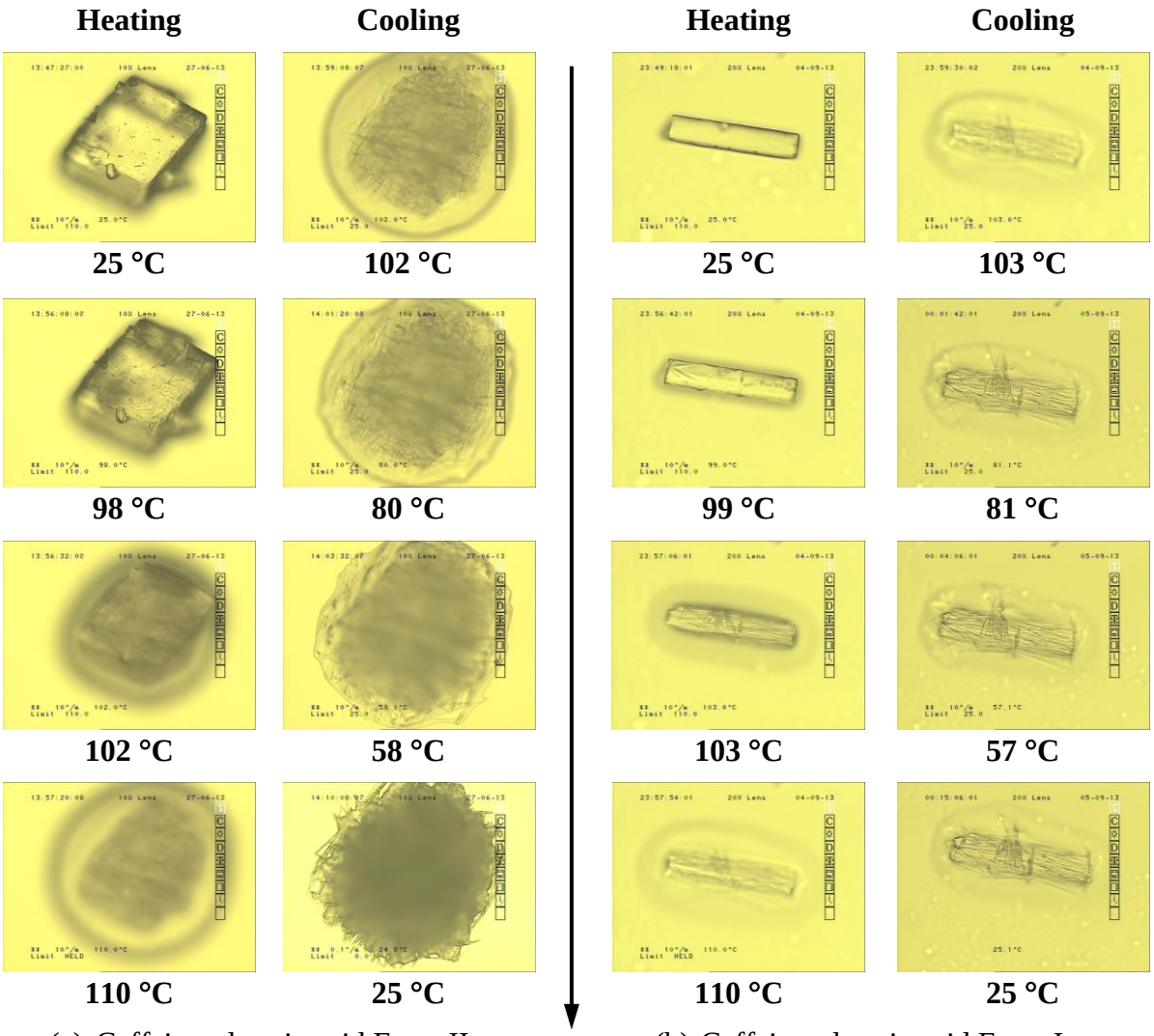


Figure 3. DSC trace for CA-GA polymorphic forms, Forms I and II. The inset view of Form II suggests that with T_{onset} at 78.6 °C (6.7 J g^{-1}) could be ascribed as solid-solid phase transformation of Form II to Form I.

Hot-Stage Microscopy (HSM)

The thermal events observed in the DSC/ TGA experiments on CA-GA Forms I and II were visualized using HSM. The photomicrographs in Figure 4 show the snapshot images of the crystals at various temperatures during the experiments. These dimorphs were heated from RT to 110 °C and cooled back to RT at a rate of 10 °C/min. The HSM results agree well with the DSC results. Melting of Form II was observed to begin from around 98 °C as evidenced from the rounding of the edge of the crystal. The transformation from Form II to Form I before melting could not be clearly observed but careful examination of the photomicrographs revealed the

appearance of small needle-shaped Form I on the surface of the Form II crystal from around 90 ^{o}C (Figure 5). The transformation of Form II to Form I then took place concurrently with melting. Upon cooling to RT, only Form I remained. For Form I, no change in morphology was observed before melting and only Form I was observed after cooling to RT.



(a) Caffeine-glutaric acid Form II. (b) Caffeine-glutaric acid Form I.

Figure 4. Photomicrographs of 1:1 caffeine-glutaric acid co-crystal polymorphic forms, (a) Form II and (b) Form I, respectively extracted from the HSM experiments.

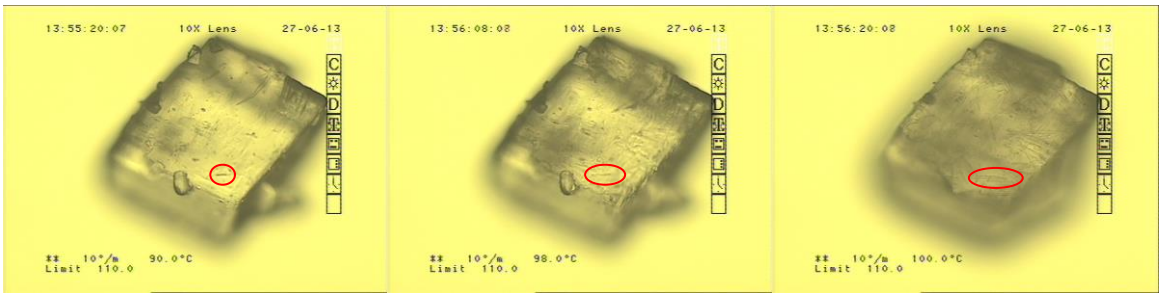


Figure 5. Nucleation and growth of needle-shaped Form I CA-GA cocrystal on the surface of Form II crystal during the HSM experiment. The red oval marks the location of one of the Form I crystals observed and its evolution in size with time as heating proceeded.

Variable Temperature X-ray Diffraction (VTXRD)

VTXRD was performed for CA-GA co-crystal dimorphs, Forms I and II, independently to understand *in situ* phase changes. Firstly, CA-GA Form II was subjected to the VTXRD from 25 °C to 98 °C at a heating rate of 1 °C/min, held at 98 °C for 60 min and cooled to 25 °C at a heating rate of 1 °C/min. The *in situ* phase changes were analyzed by means of a powder pattern taken every minute during the entire study. The heating rate employed was chosen to be much slower than that used in DSC analysis (10 ^{o}C/min) because the interval between each PXRD pattern acquisition was one minute so a slower rate would allow more detailed examination of the phase change at different temperature. Due to the slower heating rate, the phase transformation is expected to occur at a lower temperature compared to in the DSC because the system has more time to attain thermodynamic equilibrium at a slower heating rate. The VTXRD patterns were compared with that of pure CA-GA Forms I and II and also with that of caffeine itself (Figure 6 and see also Figure S2, SI). As per the PXRD patterns, Form II was intact from

25 °C to 68 °C. Notably, Form I started to appear from 69 °C alongside a major phase of Form II. The complete conversion of Form II to I was observed by 85 °C. Form I was intact from 85 to 98 °C and it melted upon being held for 2 min at 98 °C. The powder pattern of the melt phase matched to that of pure caffeine, indicating that some of the CA-GA co-crystal has dissociated into caffeine and gluartic acid. While glutaric acid phase (mp 95-98 °C) melted, the higher melting caffeine (mp 235-238 °C) remained in its solid form alongside the remaining solid Form I. During the rest of the holding period of 58 min at 98 °C, all Form I melted leaving behind solid caffeine and liquid glutaric acid. Upon cooling from 98 °C to 78 °C, Form I nucleated and further cooling led to the growth of this crystalline phase. As shown in Fig. 6, Form I phase remained even at 25 °C (See also Figure S2, SI).

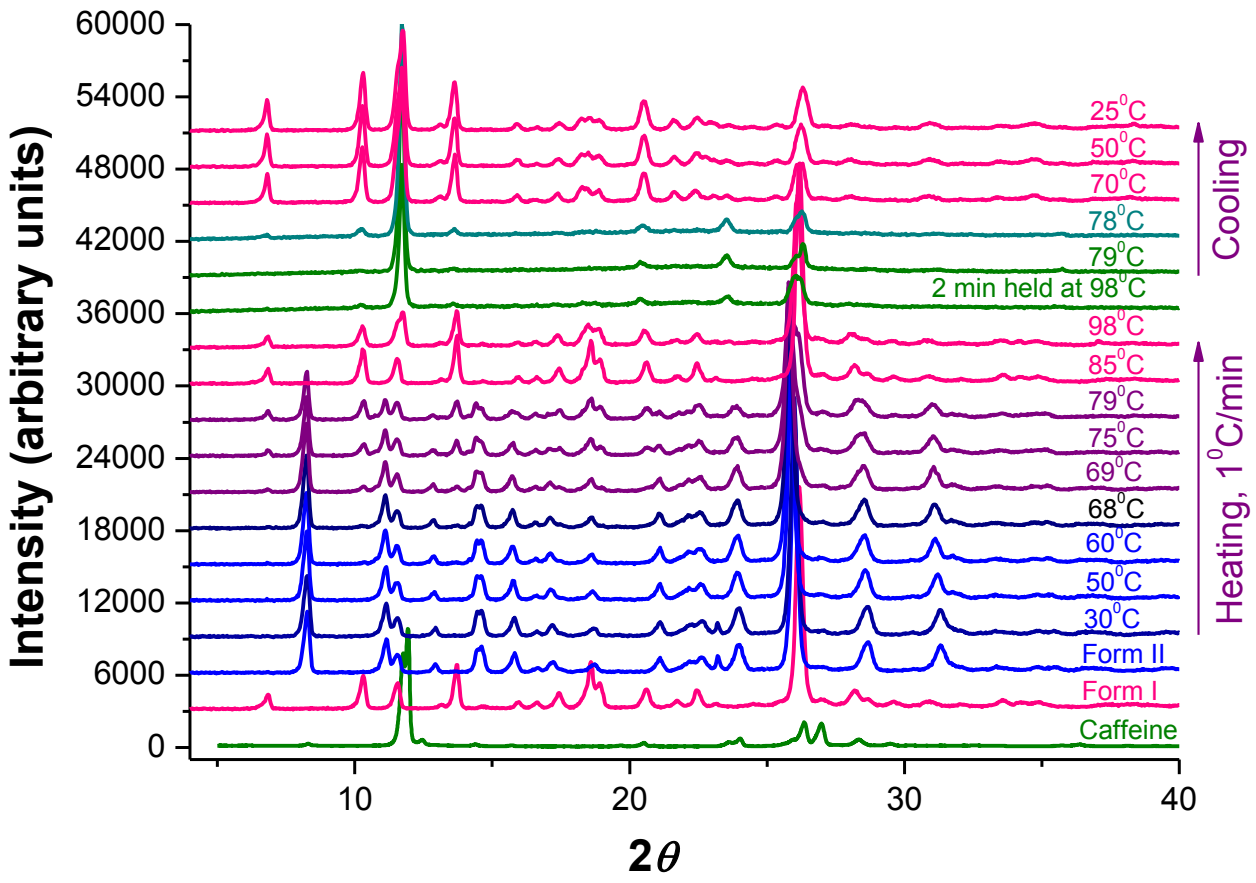


Figure 6. Variable temperature X-ray diffraction of caffeine-glutaric acid Form II from 25 °C to 98 °C at a heating rate of 1°C/min, held at 98 °C for 60 min and subsequently cooled to 25°C at a heating rate of 1°C/min. Note a start of phase change of Form II to Form I at 69 °C and exclusively Form I could be observed by 85 °C. Form I remained until 98 °C and melted upon being held for 2 min at that temperature. Upon cooling to 78 °C, Form I nucleated and was the only phase observed at the end of the cooling process at 25 °C.

The quantitative analysis of the solid-state transformation of Form II to Form I during the heating ramp was performed by Rietveld refinements using Topas v4.2 (Bruker-AXS GmbH, Karlsruhe, Germany). The peak shape was modeled by a convolution of a square function with a

Lorentzian function, a Gaussian function and a circles function. Our re-determined single crystal structures of Forms I and II were used as structure models. Rietveld refinements show that the conversion of Form II to I of CA-GA co-crystal occurred between 65 °C and 85 °C (Figure 7). Note the onset of phase transformation was observed at 66 °C, which is earlier than expected from the visual inspection of the powder patterns. At 79 °C, Form II and I exist in equal amount.

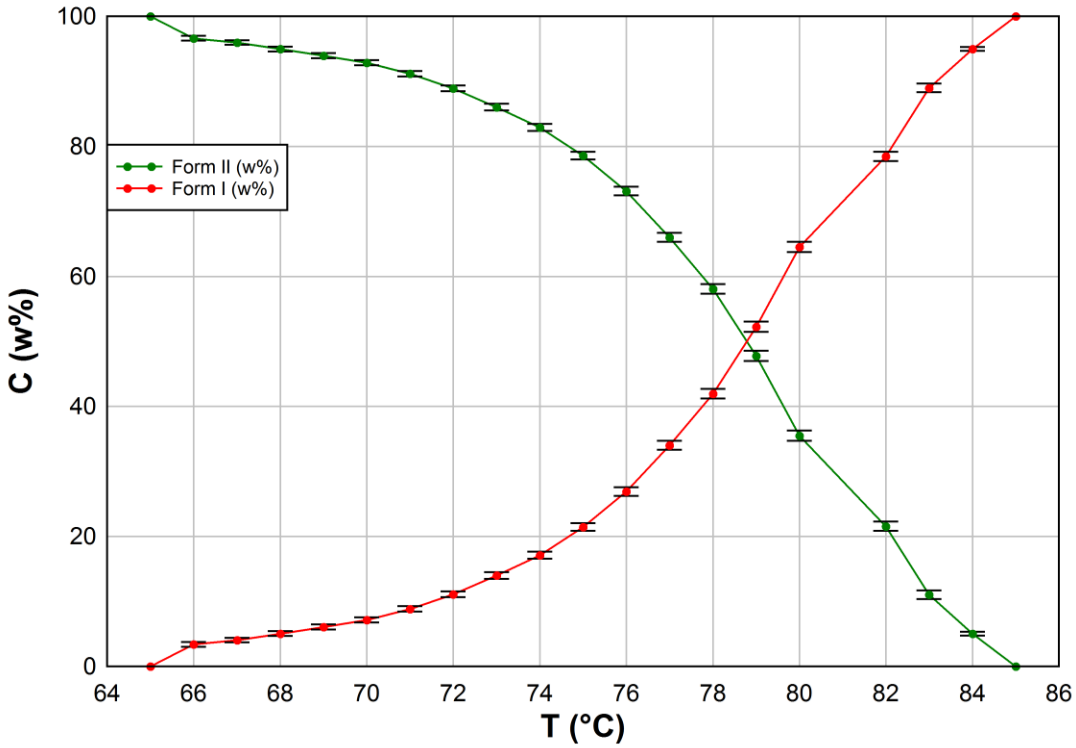
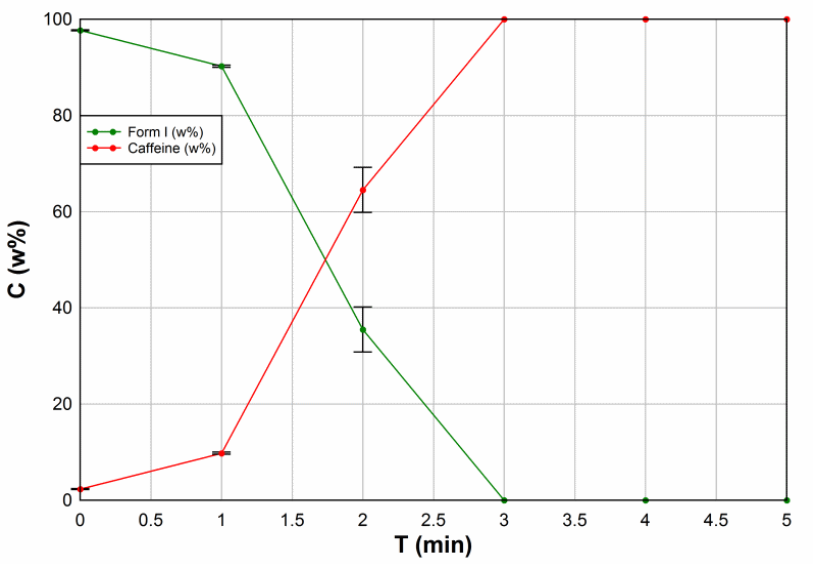


Figure 7. Reitveld refinements show that the conversion of Form II to Form I of CA-GA co-crystal occurred between 65 °C and 85 °C. Note the onset of phase transformation was observed at 66 °C, which is earlier than expected from the visual inspection of the powder patterns. At 79 °C, Form II and I exist in equal amounts.

The semi-quantitative analysis of the melting process was conducted using a combination of Rietveld refinement (to monitor the relative abundance of the crystalline phases) with the Degree of Crystallinity Method to estimate the amorphous content. The structure published by Lehmann *et al.* was used as model for caffeine.[29] The melting process can be observed between 97 and 98 °C. Most of it occurs during the first 2 minutes of the holding time at 98 °C (Figure 8). It should be noted that Figure 8 only captures the relative abundances of the two crystalline phases, co-crystal Form I and caffeine. The amorphous (melt) content is not considered in this figure. Form I was removed from Rietveld refinements when no unique peaks were observed. The strongest peak of Form I overlaps with a strong caffeine reflection and simple visual observation of the PXRD patterns could easily mistake caffeine as Form I. The evolution of amorphous content is shown in Figure 9. For this method the background was fixed to the values obtained from a refinement just before the onset of melting at 97 °C. The amorphous phase was modelled using two Split pseudo-Voigt peaks at 8.7 and 23.4° respectively while the crystalline phases were matched with a series of narrow peaks. The areas below these two peak series were integrated and the breakdown in crystalline and amorphous area gave the degree of crystallinity. The crystalline phase remaining after the first three minute corresponds to crystalline caffeine (Figure 8).



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306 **Figure 8.** Reitveld refinement shows the conversion of Form I CA-GA co-crystal to caffeine

307 occurred between 0-3 min of holding time at 98 °C.

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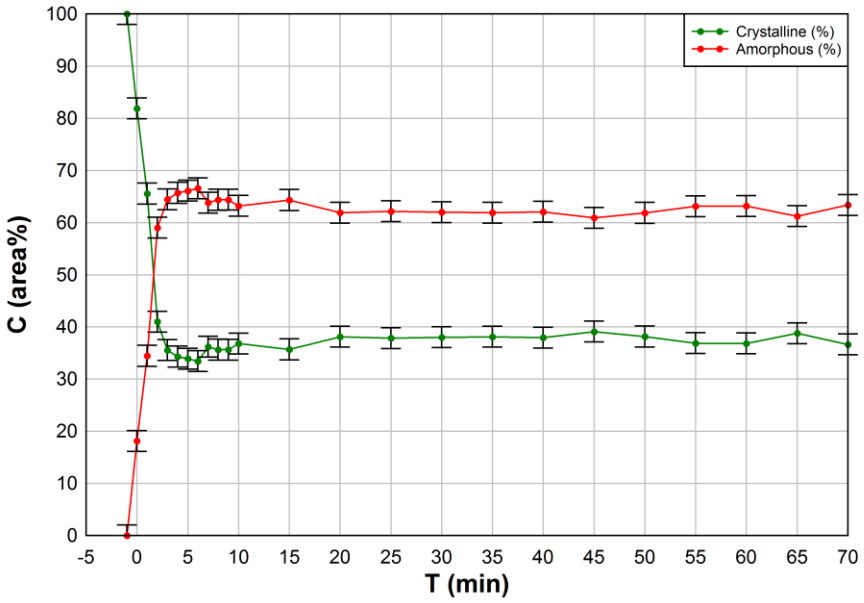

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310 **Figure 9.** Evolution of amorphous content during melting process.

Next, CA-GA Form I was subjected to VTXRD from 25 °C to 95 °C at a heating rate of 1 °C/min and held at 95 °C for 45 min and cooled to 25 °C at a cooling rate of 1 °C/min. The powder patterns were analyzed for any *in situ* phase changes (Figure 10 and see also Figure S3, SI). It reveals that Form I remained unchanged throughout the experiment. The result is in agreement with DSC and HSM results (Figures 3 and 4).

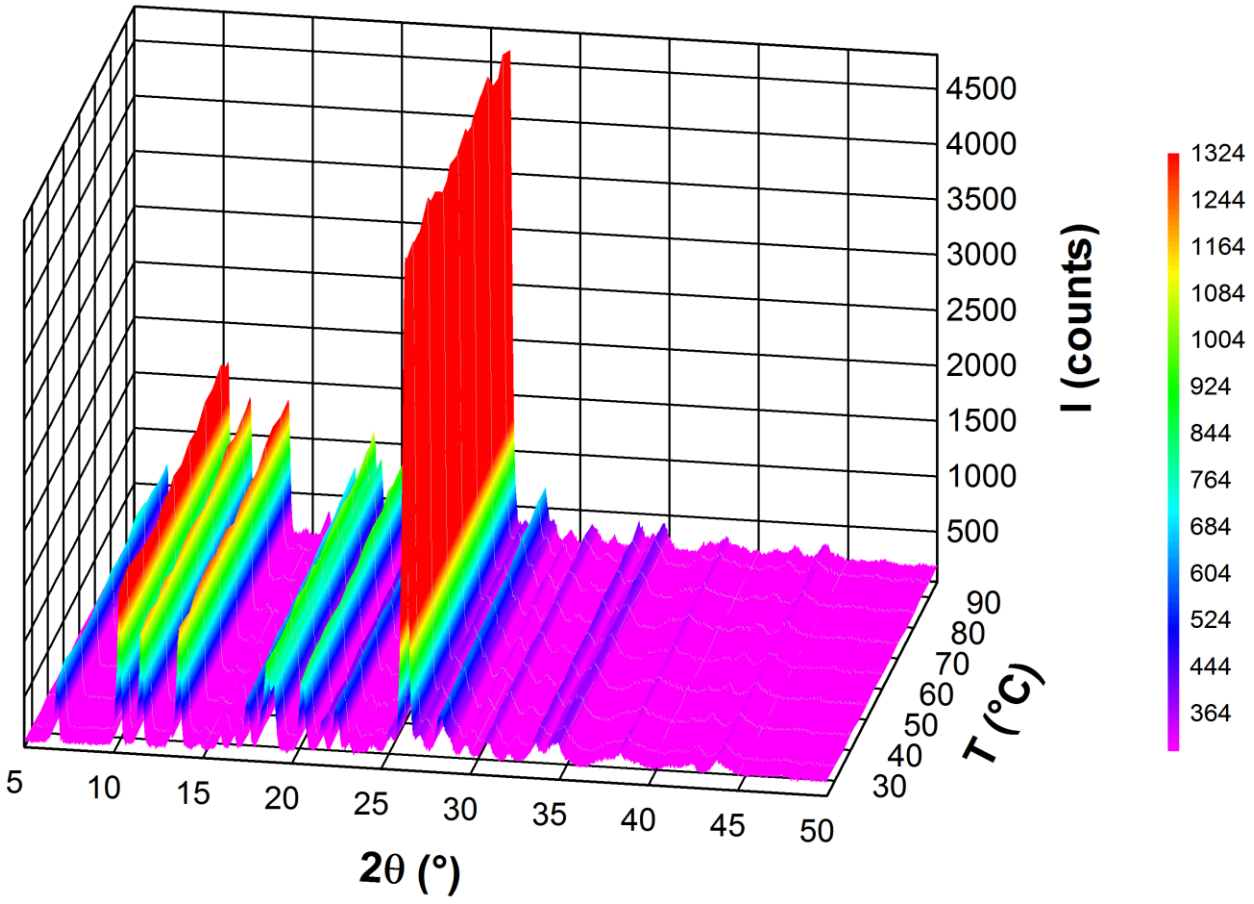

Figure 10. Variable temperature X-ray diffraction of caffeine-glutaric acid Form I. No phase change was observed during heating ramp from 25 °C to 95 °C at 1 °C/min heating rate.

Thermodynamic Stability

In this part, we aim to understand the stability relationships of Forms I and II. Thermal analysis and *in situ* X-ray diffraction formed an excellent basis for the construction of a qualitative energy vs temperature diagram (Figure 11). The physical properties of the CA-GA polymorphs are given in Table 4. The heat of fusion value for Form II was estimated using Hess's law of heat summation[30] ($\Delta H_f + \Delta H_t$) as no direct melting was observed for Form II. From our DSC analysis, the phase transition from Form II to Form I is endothermic. According to heat of transition rule,[31,32] the dimorphs of CA-GA co-crystal are enantiotropically related. VTXRD heat-cool studies suggested that these enantiotropic dimorphs are irreversible in the employed conditions that Form I was not observed to transform to Form II upon cooling. However, given time, CA-GA Form I would transform to Form II in solid state but the solid-state transformation rate depends on the environment that the crystals are in, which ranges from less than a day at high humidity to over weeks at 0% RH.[18] In any case their free energies become equal at the transition temperature $T_p^{II–I}$. From *in situ* VTXRD information we estimate that the transition temperature could be at 79 °C. Enantiotropic transitions are by definition thermodynamically reversible with temperature and pressure.[33,34] To gain further understanding of the CA-GA dimorphs, DSC heat-cool-heat studies were performed for Form II where Form II was heated from room temperature to melting and the melt was cooled to below room temperature i.e. -5 °C and then the sample was heated until its melting point at 10 °C/min heating rate (Figure S4, SI). As expected, heating cycle 1 results were similar to DSC events shown in Figure 3 whereas heating cycle 2 profile resembled by and large heating cycle 1 results meaning there was a solid-state conversion of Form II to Form I phase that was followed by melting event. The results indicate that upon

heating cycle 1, i.e. during cooling of the melt, Form I was formed (see also VTXRD results) and the phase was retained until room temperature, however, further cooling to -5 °C led to the solid-state phase transformation of Form I to II. Hence, DSC heat-cool-heat results suggest that CA-GA dimorphs are enantiotropically related. Herein the enantiotropic reversible conversion has been noted with hysteresis, meaning cooling beyond room temperature was required in accordance with findings elsewhere.[33-34]

Slurry transformation studies[35] are a practical way to determine the thermodynamic stabilities of different solid forms. A 50%+50% (w/w) mixture of Forms I and II were added to acetonitrile and stirred at ambient conditions for 24 h. The analysis of PXRD and DSC of the product phase indicated that Form I had transformed to Form II. This result confirmed that the Form II is thermodynamically stable form and Form I is metastable at the ambient conditions.

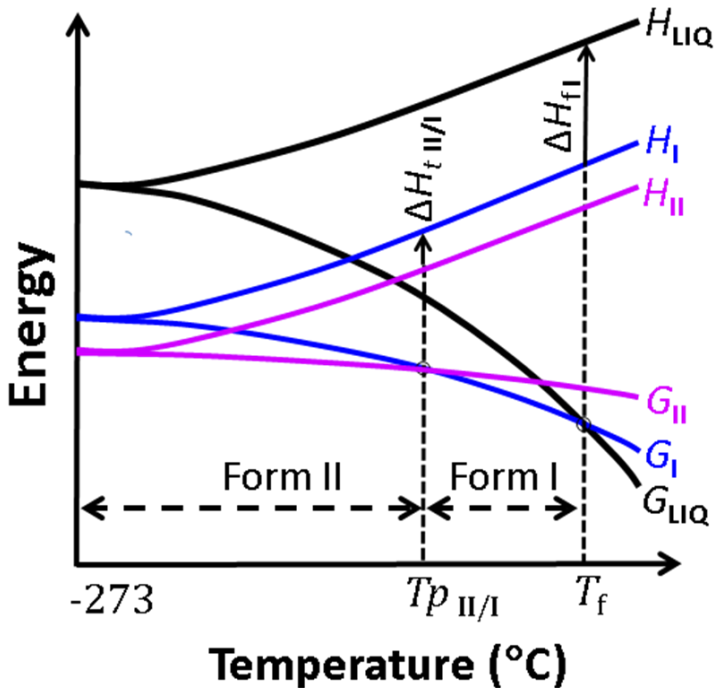

Figure 11. Energy versus temperature (E–T) diagram of an enantiotropic dimorphic cocrystal of caffeine-glutaric acid: G = Gibbs free energy, H = enthalpy, liq = liquid phase, T_p= transition point and T_f = melting temperature.

The calculated densities and packing fractions were obtained from the single crystal structures determined at 180 K (Table 4). The density and packing fraction of Form I are higher than those of Form II which is in contrary to the density rule that a polymorph with a lower density is metastable and a polymorph with a higher density is stable at room temperature.[32] But, exceptions to the density rule are reported in literature.[36-38] Density rule could be more applicable to the molecular crystals where van der Waals interactions are predominantly involved in the crystalline lattice.[1] In cases where hydrogen bonding interactions play a significant role, such as with CA-GA Forms I and II, density rule may not be strictly followed.

Table 4. Physical Properties of Caffeine-Glutaric Acid Polymorphs, Forms I and II.

Modification	Form I	Form II
M.p. (°C) (DSC onset temperature)	98.8	-
T_p (^{o}C) (DSC onset temperature)	-	78.6
Heat of fusion, ΔH_f/ J g^{-1}	118	124.7[a]
Heat of transition, ΔH_t/ J g^{-1}		6.7
Density (g cm^{-3})	1.491	1.481
Packing coefficient (C_k*)	74.0	73.5
Stability at 25 °C	Metastable	Stable

[a] Calculated by Hess's law of heat summation

▪ CONCLUSIONS

In conclusion, the dimorphs, Forms I and II, of 1:1 caffeine-glutaric acid cocrystal were unequivocally characterized by LAG, thermal, single crystal and *in situ* X-ray diffraction and slurry transformation studies. LAG in polar solvents favored the formation of Form II. Thermal (DSC TGA and HSM) and slurry studies suggest that Form II is thermodynamically stable and Form I is metastable at ambient conditions and they are enantiotropically related. VTXRD results reveal interesting insights about the thermal behavior of these dimorphs. Form II converted to Form I at an onset temperature of 66 °C and pure Form I was observed by 85 °C which then melted at ~98°C. Upon cooling, Form I nucleated at 78 °C and was the only form observed at the end of the cooling process at 25^{o}C. Rietveld analysis showed that the transition temperature for this dimorphic pair could be at 79 °C where both forms were found to be present in equal proportion. On the other hand, VTXRD of Form I showed no phase changes throughout the experiment. It should be noted that Form I transformed to the thermodynamically stable Form II with hysteresis at temperature below the transition point during cooling suggesting that the CA-GA dimorphs Forms I and II are enantiotropically related. A schematic energy-temperature diagram was constructed for the two polymorphs based on the results obtained.

▪ ASSOCIATED CONTENT

Supporting Information. It contains crystallographic information files (CIFs) and additional figures of variable temperature X-ray diffraction studies. CCDC reference numbers 1402960 and

1402961 contain the supplementary crystallographic data for this paper. This information is available free of charge via the Internet at http://pubs.acs.org/ .

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▪ REFERENCES

1. Bernstein, J., *Polymorphism in Molecular Crystals*. Oxford University Press: New York, 2002.
2. Brittain, H. G., *Polymorphism in Pharmaceutical Solids*. 2nd ed.; Informa Healthcare: New York, 2009; Vol. 192.
3. Byrn, S. R.; Pfeiffer, R. R.; Stowell, J. G., *Solid State Chemistry of Drugs*. 2nd ed.; SSCI, Inc.: West Lafayette, Indiana, 1999.
4. Hilfiker, R., *Polymorphism in the Pharmaceutical Industry*. Wiley-VCH Verlag: Weinheim, 2006.
5. Peterson, M. L.; Hickey, M. B.; Zaworotko, M. J.; Almarsson, Ö., Expanding the scope of crystal form evaluation in pharmaceutical science. *J. Pharm. Pharm. Sci.* **2006,** *9*, 317-326.
6. Vishweshwar, P.; McMahon, J. A.; Peterson, M. L.; Hickey, M. B.; Shattock, T. R.; Zaworotko, M. J., Crystal engineering of pharmaceutical co-crystals from polymorphic active pharmaceutical ingredients. *Chem. Commun.* **2005,** 4601-4603.

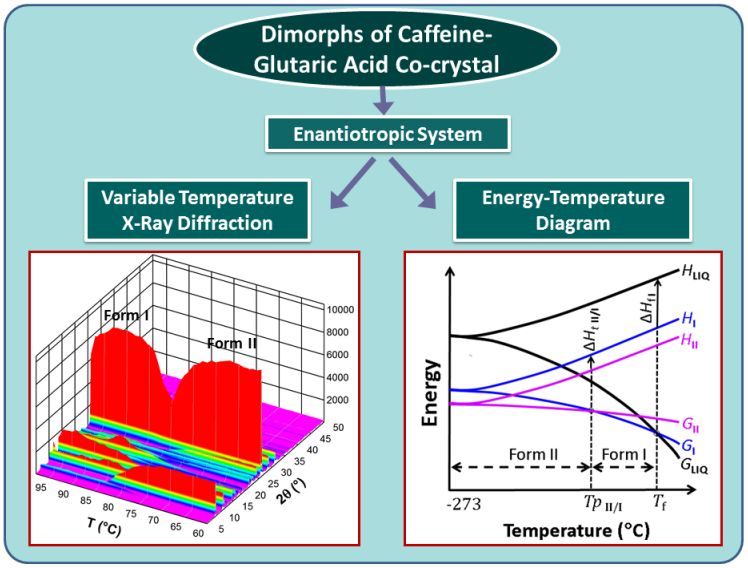
7. Haleblian, J.; McCrone, W., Pharmaceutical applications of polymorphism. *J. Pharm. Sci.* **1969,** *58* (8), 911-929.
8. McCrone, W. C., *Polymorphism in Physics and Chemistry of the Solid State*. Interscience: New York, 1965; Vol. 2, pp 725-767.
9. Aitipamula, S.; Chow, P. S.; Tan, R. B. H., Polymorphism in cocrystals: a review and assessment of its significance. *CrystEngComm* **2014,** *16*, 3451-3465.
10. Bolla, G.; Mittapalli, S.; Nangia, A., Celecoxib cocrystal polymorphs with cyclic amides: synthons of a sulfonamide drug with carboxamide coformers. *CrystEngComm* **2014,** *16*, 24-27.
11. Aitipamula, S.; Chow, P. S.; Tan, R. B. H., Trimorphs of a pharmaceutical cocrystal involving two active pharmaceutical ingredients: potential relevance to combination drugs. *CrystEngComm* **2009,** *11*, 1823-1827.
12. Braga, D.; Palladino, G.; Polito, M.; Rubini, K.; Grepioni, F.; Chierotti, M. R.; Gobetto, R., Three polymorphic forms of the co-crystal 4,4′-bipyridine/pimelic acid and their structural, thermal, and spectroscopic characterization. *Chem. Eur. J.* **2008,** *14*, 10149-10159.
13. Clarke, H. D.; Arora, K. K.; Bass, H.; Kavuru, P.; Ong, T. T.; Pujari, T.; Wojtas, L.; Zaworotko, M. J., Structure−stability relationships in cocrystal hydrates: Does the promiscuity of water make crystalline hydrates the nemesis of crystal engineering? *Cryst. Growth Des.* **2010,** *10*, 2152-2167.
14. Gryl, M.; Krawczuk, A.; Stadnicka, K., Polymorphism of urea-barbituric acid co-crystals. *Acta Cryst.* **2008,** *B64*, 623-632.
15. Ueto, T.; Takata, N.; Muroyama, N.; Nedu, A.; Sasaki, A.; Tanida, S.; Terada, K., Polymorphs and a hydrate of furosemide–nicotinamide 1:1 Cocrystal. *Cryst. Growth Des.* **2012,** *12*, 485-494.
16. Griesser, U. J.; Burger, A., The effect of water vapor pressure on desolvation kinetics of caffeine 4/5-hydrate. *Int. J. Pharm.* **1995,** *120*, 83-93.
17. Cambridge Structural Database (CSD) survey conducted using ConQuest 1.16 (November 2013, Build RC5) on cocrystals involving caffeine: 87 neutral pharmaceutical/organic multi-component crystals were found, of which 54 hits constitute cocrystals and 33 hits belong to co-crystal hydrates/solvates. Among the 54 hits for cocrystals, four pairs of dimorphic co-crystals were identified - caffeine-glutaric acid, caffeine-maleic acid, caffeine-4-hydroxybenzoic acid and caffeine-pterostilbene.
18. Trask, A. V.; Motherwell, W. D. S.; Jones, W., Pharmaceutical cocrystallization: Engineering a remedy for caffeine hydration. *Cryst. Growth Des.* **2005,** *5*, 1013-1021.
19. Yu, Z. Q.; Chow, P. S.; Tan, R. B. H., Operating regions in cooling cocrystallisation of caffeine and glutaric acid in acetonitrile. *Cryst. Growth Des.* **2010,** *10*, 2382-2387.
20. Yu, Z. Q.; Chow, P. S.; Tan, R. B. H.; Ang, W. H., Supersaturation control in cooling polymorphic co-crystallization of caffeine and glutaric Acid. *Cryst. Growth Des.* **2011,** *11*, 4525–4532.
21. Thakuria, R.; Eddleston, M. D.; Chow, E. H. H.; Lloyd, G. O.; Aldous, B. J.; Krzyzaniak, J. F.; Bond, A. D.; Jones, W., Use of in situ atomic force microscopy to follow phase changes at crystal surfaces in real time. *Angew. Chem. Int. Ed.* **2013,** *52*, 10541-10544.
22. Delori, A.; Friscic, T.; Jones, W., The role of mechanochemistry and supramolecular design in the development of pharmaceutical materials. *CrystEngComm* **2012,** *14*, 2350-2362.

23. Friščić, T.; Childs, S. L.; Rizvi, S. A. A.; Jones, W., The role of solvent in mechanochemical and sonochemical cocrystal formation: a solubility-based approach for predicting cocrystallisation outcome. *CrystEngComm* **2009,** *11*, 418-426.
24. Shan, N.; Toda, F.; Jones, W., Mechanochemistry and co-crystal formation: effect of solvent on reaction kinetics. *Chem. Commun.* **2002,** 2372-2373.
25. Friščić, T.; Jones, W. Recent advances in understanding the mechanism of cocrystal formation via grinding. *Cryst. Growth Des.* **2009**, *9*, 1621-1637.
26. Losev, E. A.; Boldyreva, E. V. The role of a liquid in "dry" co-grinding: a case study of the effect of water on mechanochemical synthesis in a "L-serine-oxalic acid" system. *CrystEngComm* **2014**, *16*, 3857-3866.
27. Trask, A. V.; Motherwell, W. D. S.; Jones, W., Solvent-drop grinding: green polymorph control of cocrystallisation. *Chem. Commun.* **2004,** 890-891.
28. Sheldrick, G. M. *SHELXS-97 and SHELXL-97*, University of Göttingen: Germany, 1997.
29. Lehmann, C. W.; Stowasser, F., The crystal structure of anhydrous β-caffeine as determined from X-ray powder-diffraction data. *Chem. Eur. J.* **2007,** *13*, 2908-2911.
30. Hess's law of heat summation: The enthalpy change accompanying a transformaion is the same whether the process occurs in one or many steps and total enthalpy change is the summation of all steps.
31. Burger, A.; Ramberger, R., On the polymorphism of pharmaceuticals and other molecular crystals. II. *Mikrochim. Acta* **1979,** *72*, 273-316.
32. Burger, A.; Ramberger, R., On the polymorphism of pharmaceuticals and other molecular crystals. I. *Mikrochim. Acta* **1979,** *72*, 259-271.
33. Herbstein, F. H. On the mechanism of some first-order enantiotropic solid-state phase transitions: from Simon through Ubbelohde to Mnyukh. *Act Cryst.* **2006**, *B62*, 341-383.
34. Kawakami, K., Reversibility of enantiotropically related polymorphic transformations from a practical viewpoint: Thermal analysis of kinetically reversible/irreversible polymorphic transformations. *J. Pharm. Sci.* **2007,** *96*, 982-989.
35. Hu, Y.; Wikström, H.; Byrn, S. R.; Taylor, L. S., Estimation of the transition temperature for an enantiotropic polymorphic system from the transformation kinetics monitored using Raman spectroscopy. *J. Pharm. Biomed. Anal.* **2007,** *45*, 546-551.
36. Thirunahari, S.; Aitipamula, S.; Chow, P. S.; Tan, R. B. H. Conformational polymorphism of tolbutamide: A structural, spectroscopic, and thermodynamic characterization of Burger's forms I–IV, *J. Pharm. Sci.*, **2010**, *99*, 2975-2990.
37. Sun, C. C. Thermal expansion of organic crystals and precision of calculated crystal density: A survey of Cambridge crystal database. *J. Pharm. Sci.*, **2007**, *96*, 1043–1052.
38. Nelyubina, Y. V.; Glukhov, I. V.; Antipin, M. Y.; Lyssenko, K. A., "Higher density does not mean higher stability" mystery of paracetamol finally unraveled. *Chem. Commun*., **2010**, *46*, 3469-3471.

For Table of Contents Use Only

Thermal and *in situ* X-ray Diffraction Analysis of a Dimorphic Co-crystal, 1:1 Caffeine-Glutaric Acid

*Venu R. Vangala,**[†,‡] *Pui Shan Chow,* *[†] *Martin Schreyer,*[†] *Grace Lau,*[†] *and Reginald B. H. Tan*[†,¶]



A thorough characterization and understanding of co-crystal polymorphs is a valuable step during drug development. Thermal and *in situ* structural analyses of caffeine-glutaric acid co-crystal dimorphs revealed that Form II is stable while Form I is metastable at ambient condition. Dimorphs are enantiotropically related and the transition temperature is estimated to be 79 °C.

Supporting Information (SI)

Thermal and *in situ* X-ray Diffraction Analysis of a Dimorphic Co-crystal, 1:1 Caffeine-Glutaric Acid

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Figure S1. TGA trace of for CA-GA polymorphic forms, Forms I and II. Note the absence of solvent molecules in these crystalline phases.

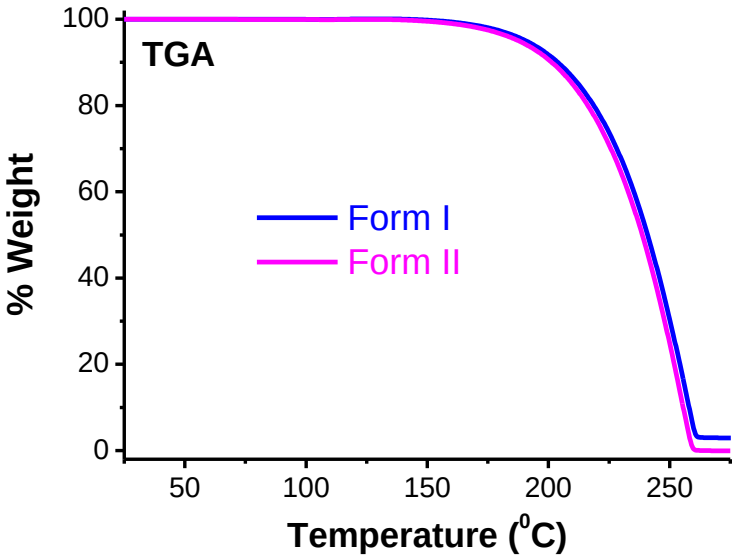
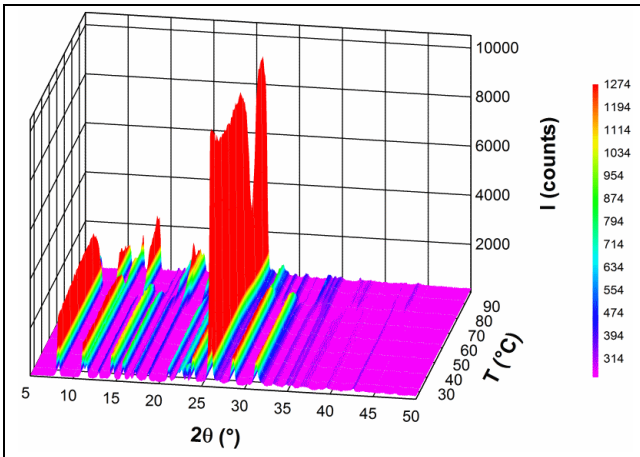
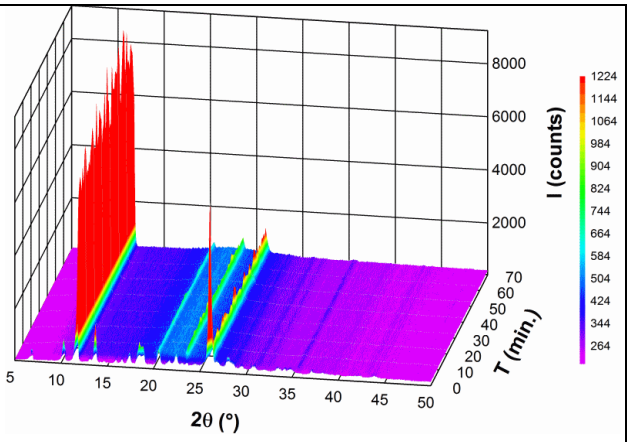


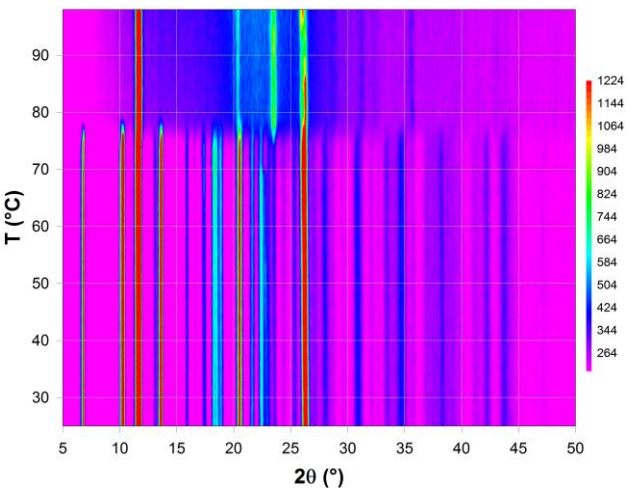
Figure S2. Variable temperature X-ray diffraction patterns of caffeine-glutaric acid Form II are shown in details.



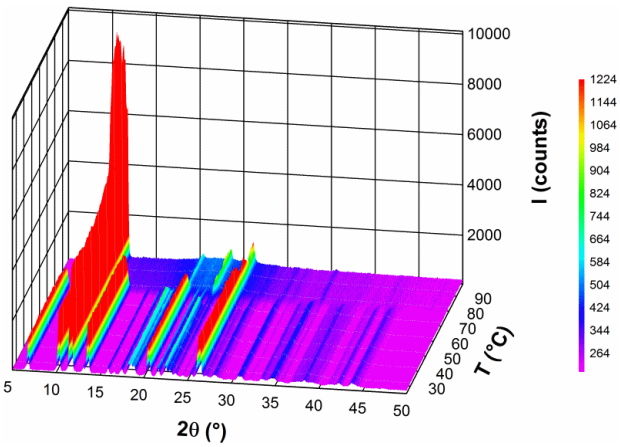
(a) 3D plot showing the events occurred during heating from 25 °C to 98 °C at the heating rate of 1 °C/min. Note the phase change of Form II to Form I.



(b) Holding ramp at 98 °C for 60 min. Note that Form I melts within the first 2 minutes. The observed crystalline phase after the melting matches to that of pure caffeine.

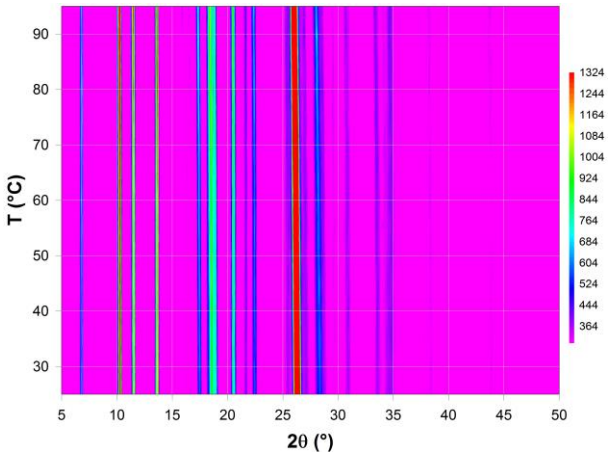


(c) 2D plot of cooling ramp from 98°C to 25 °C at the heating rate of 1 °C/min. Notice the phase change of caffeine to Form I, caffeine-glutaric acid co-crystal, at 78 °C.

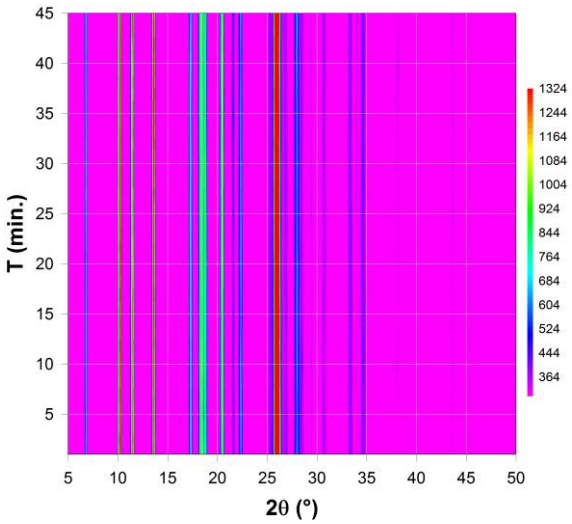


(d) 3D plot of cooling ramp from 98°C to 25 °C at the heating rate of 1 °C/min. Notice the phase change of caffeine to Form I, caffeine-glutaric acid co-crystal, at 78 °C.

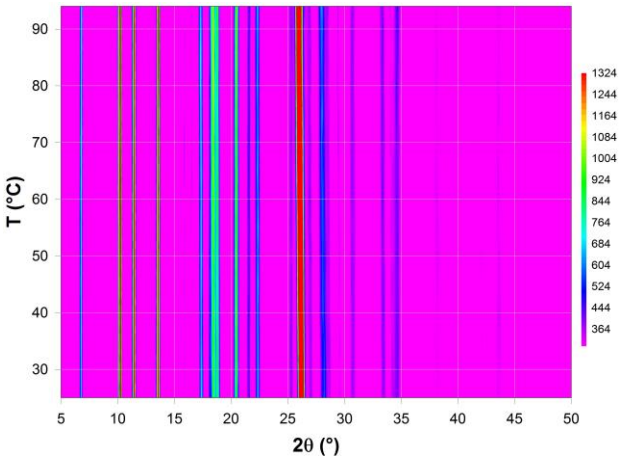
Figure S3. Variable temperature X-ray diffraction of caffeine-glutaric acid Form I from 25 °C to 95 °C at 1 °C/min. Next, it was held at 95 °C for 45 min. Subsequently, the sample was cooled to 25 °C at 1 °C/min.



(a) 2D plot shows that there were no phase changes occurred during heating ramp.



(b) 2D plot shows that there were no phase changes during holding ramp.



(c) 2D plot depicts that there were no phase changes occurred during cooling ramp.

Figure S4. DSC heat-cool-heat profiles of CA-GA Form II at 10 °C/min heating rate. Heating cycle 1 involved heating of sample from room temperature to 170 °C and the melt was subjected to cooling cycle 1 (170 °C to -5 °C) , which was followed by heating cycle 2 (-5°C to 170 °C). The heating cycle 2 results suggest that Form II transformed to Form I during heating followed by melting of Form I at 98.2 °C which indicates that during cooling cycle 1, initially Form I was formed (VTXRD results support this) and upon further cooling to -5 °C, Form I could have converted to Form II. Hence, CA-GA dimorphs, Forms I and II, are enantiotropically related.