

Trimorphs of a pharmaceutical cocrystal involving two active pharmaceutical ingredients: potential relevance to combination drugs

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Electronic Supplementary Information (ESI)

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1. Experimental

Materials

EA and GA were purchased from Alfa Aesar, and used as received without any further purification. Analytical grade solvents were used for the crystallization experiments.

Solution crystallizations

Crystallization experiments were carried out by dissolving a 1:1 stoichiometric ratio of EA (100 mg, 0.605 mmol) and GA (93.3 mg, 0.605 mmol) in a minimum amount of solvent (approximately 3-5 ml). A wide range of solvents, for example water, acetone, ethyl acetate, acetonitrile, ethanol, methanol, 2-propanol, diethyl ether, toluene, n-pentane, n-hexane, dichloromethane, and chloroform, were used for the experiments. If the materials were not soluble in a particular solvent (eg. water), a more polar solvent (eg. ethanol) was used to dissolve. In some instances, a mixture of two or more solvents was used. The slurry was warmed to dissolve the two materials and crystallized at RT. Single crystals suitable for X-ray diffraction were obtained within 7 days.

Grinding experiments

Grinding was performed using a Retsch Mixer Mill model MM301 with 10 ml stainless steel grinding jars with one 7 mm stainless steel grinding ball at a rate of 20 Hz for 15 min. Experiments were carried out with 100 mg of EA and an equivalent stoichiometric amount (93.3 mg) of GA. Liquid-assisted grinding or solvent-drop grinding experiments were carried out by adding two drops of a selected solvent to the reactants prior to the grinding. Water, acetone, ethyl acetate, acetonitrile, ethanol, methanol, 2-propanol, diethyl ether, 1,4-dioxane, toluene, n-pentane, n-hexane, dichloromethane, and chloroform were used for the SDG experiments. The resulting powder samples were analyzed by PXRD for the cocrystal formation. The external

temperature of the grinding jar after completion of the experiments did not exceed ca. 30 °C.

Single crystal X-ray diffraction

A good quality single crystal grown from the solution crystallization was 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 cooled nitrogen gas stream at 110 K throughout the data collection. X-ray reflections were collected on a Rigaku Saturn CCD area detector with graphite monochromated Mo-K α radiation ($\lambda = 0.71073 \text{ \AA}$). Data were collected and processed using CrystalClear (Rigaku) software. Structures were solved by direct methods and SHELX-TL was used for structure solution and least-squares refinement. The non-hydrogen atoms were refined anisotropically. All hydrogen atoms were fixed at idealized positions except for the N–H, O–H hydrogen atoms which were located from the difference Fourier map and allowed to ride on their parent atoms in the refinement cycles. The O6A–H11A bond distance which was found to be long in the normal refinement cycles was fixed using DFIX command in SHELX. Details of hydrogen bond distances in the crystal structures of cocrystal polymorphs were given in Table 1.

Despite repeated crystallization experiments, only very thin plate like crystals were obtained for Form II. This resulted in poor crystal data with higher R-factor (12%). Attempts to obtain good quality single crystals for a better crystal data were not successful. TWINOLVE was used to investigate the possible twinning; however, it was not able to find any twin relationships. Therefore the higher than usual R-factor can be attributed to poor crystal quality leading to low signal over noise ratio. The fact that the PXRD of the Form II powder matches with that of the PXRD pattern simulated from the X-ray structure further confirms the existence of Form II.

In addition to the high R-factor, the crystal structure of the Form II contains residual electron density close to O3. The fact that there was no sign of solvent inclusion from the DSC analysis rules out the possibility of included solvent in the crystals. Thus

we believe that the residual electron density could be again because of the poor X-ray data.

Powder X-ray diffraction (PXRD)

The powder materials of the grinding experiments were identified by D8 Advance powder X-ray diffractometer (Bruker AXS GmbH, Germany), with Cu-K α radiation ($\lambda = 1.54056 \text{ \AA}$). The voltage and current applied were 35 kV and 40 mA, respectively. Samples were placed on the sample holder which has 1mm thickness and 1.5 cm diameter. The sample was scanned within the scan range of $2\theta = 5^\circ$ to 50° continuous scan, with a scan rate of 2 deg min^{-1} . The PXRD patterns were plotted using OriginPro 7.5 and compared with that of the standard materials to confirm the cocrystal identity.

Differential scanning calorimetry (DSC)

DSC was performed with a Perkin Elmer, Diamond DSC with Autosampler. Crystals taken from the mother liquor were blotted dry on a filter paper and ground to obtain fine powder. The powder was placed in crimped aluminium sample pans. The sample size was 2–5 mg and the temperature range was typically 30–150 °C at a heating rate of $5 \text{ }^\circ\text{C min}^{-1}$. The samples were purged with a stream of flowing nitrogen (20 ml min^{-1}). One of the DSC experiments on Form I was conducted from 30 to 220 °C.

Thermogravimetric analysis (TGA)

TGA was performed on a TA instruments, TGA Q500 thermogravimetric analyzer. Approximately 15 mg of the sample was added to an alumina crucible. The samples were heated over the temperature range of 25 to 250 °C at a constant heating rate of $5 \text{ }^\circ\text{C min}^{-1}$. The samples were purged with a stream of flowing nitrogen throughout the experiment at 40 ml min^{-1} .

Hot-stage microscopy (HSM)

Thermomicroscopic investigations were performed with an optical polarizing microscope (Olympus, BX51) equipped with a Linkam hot-stage THMS 600 connected to a TMS 94 temperature controller and a LNP 94/2 liquid nitrogen pump (Linkam Scientific Instruments Ltd., Tadworth, Surrey, UK). The microscopic images were recorded with a CCD camera attached to the Olympus BX-51 microscope (Olympus Optical GmbH, Vienna, A) at every 10 sec time intervals using Soft Imaging System's Analysis image capture software. Samples were heated over the temperature range of 25 to 150 °C at a constant heating rate of 5 °C min⁻¹.

Lattice energy calculations

The lattice energy of Forms I and II was computed by energy minimization of its crystal structure using the Forcite module in Materials Studio. COMPASS force field was used and the charges were used as assigned by the force field. The Ewald summation method was used to calculate the non-bonded interactions including the van der Waals and electrostatic contributions. Crystal lattice energies are calibrated for the number of molecules in the unit cell (per molecule).

Solubility and dissolution experiments

Aqueous solubility for **EA** and co-crystal were estimated from a solubility calibration plot of **EA**. A calibration standard graph was made by measuring the absorbance of varied concentration of **EA** in water using a Varian Cary 50 UV spectrophotometer at λ_{max} 210 nm. Saturated solutions of **EA** and co-crystal were prepared by stirring the excess powder materials of **EA** and co-crystal in water at 25 °C for 24 hrs. The solutions were filtered through a 0.2 μm syringe filter and the UV-absorbance was measured at 210 nm. Solution concentration was calculated from the **EA** calibration plot.

Samples for the dissolution experiments were prepared by compressing 500 mg of the respective powder material at 1000 psi for approximately one minute.

Dissolution experiments were performed using a Varian VK7010 dissolution apparatus equipped with a VK750D heater/circulator. Water was used as dissolution medium at 25 °C. In each experiment 900 ml of dissolution medium was used, which was

rotated at 100 rpm for uniform temperature distribution and control. The solutions were analyzed with a Varian Cary 50 UV-VIS dual-beam spectrophotometer without dilution at 25 °C in a 1.0 cm quartz cell. Absorbance readings were recorded at 10 min time interval. The solutions were analyzed at a wavelength of 210 nm. The quantity of the API and the co-crystal dissolved was then calculated using the Beer-Lambert law and the API calibration curve.

2. Some of the recently approved and old combination drug products and their API combinations

Brand name	APIs combination
HIV drugs	
Combivir	zidovudine and lamivudien
Truvada	emtricitabine and tenofovir
Epzicom	Abacavir and lamivudine
Atripla	emtricitabine/tenofovir and efavirenz
Trizivir	zidovudine/lamivudine and abacavir
Cardiovascular disease drugs	
BiDil	isosorbide dinitrate and hydralazine hydrochloride
Vytorin	ezetimibe and simvastatin
Caduet	amlodipine and atorvastatin
Respiratory disease drugs	
Advair	Fluticasone and salmeterol
Combivent	Ipratropium bromide and albuterol
Berodual	Ipratopium and fenoterol
Infectious disease drugs	
Kaletra	Ritonavir and lopinavir

3. ORTEP diagrams

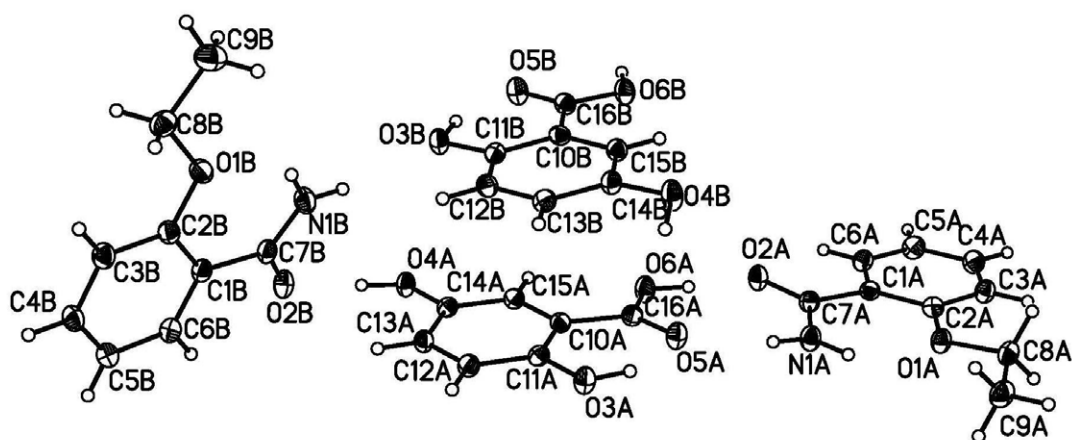


Fig. S1 ORTEP diagram for Form I showing the atom numbering. Thermal ellipsoids were drawn at 35 % probability.

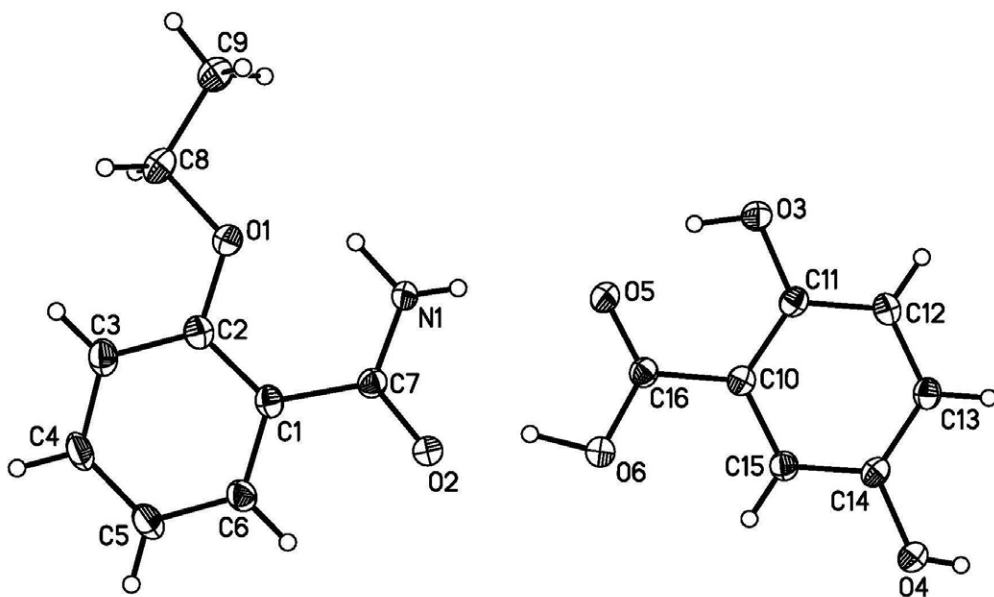


Fig. S2 ORTEP diagram for Form II showing the atom numbering. Thermal ellipsoids were drawn at 35 % probability.

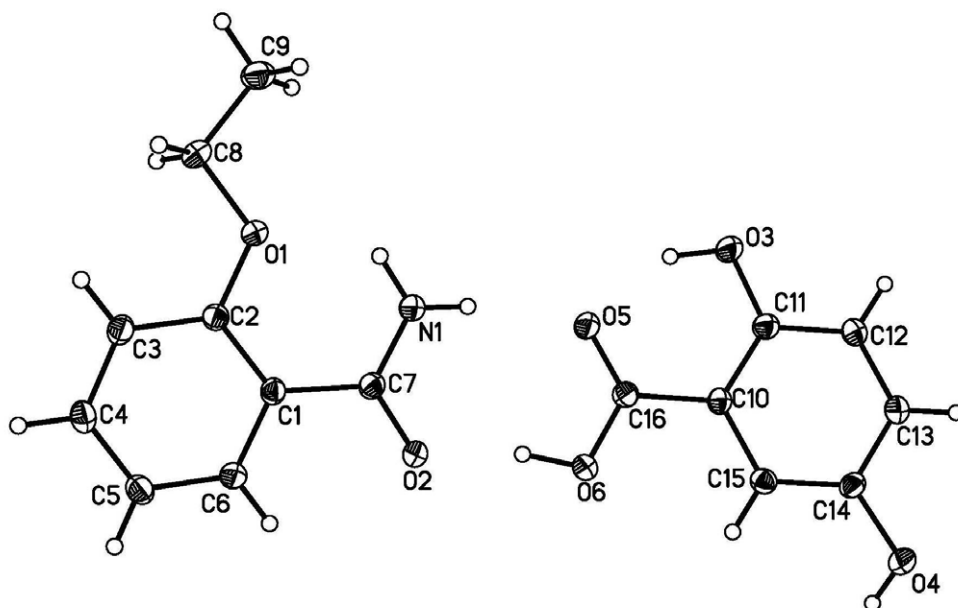


Fig. S3 ORTEP diagram for Form III showing the atom numbering. Thermal ellipsoids were drawn at 35 % probability.

4. Packing diagram for Form III

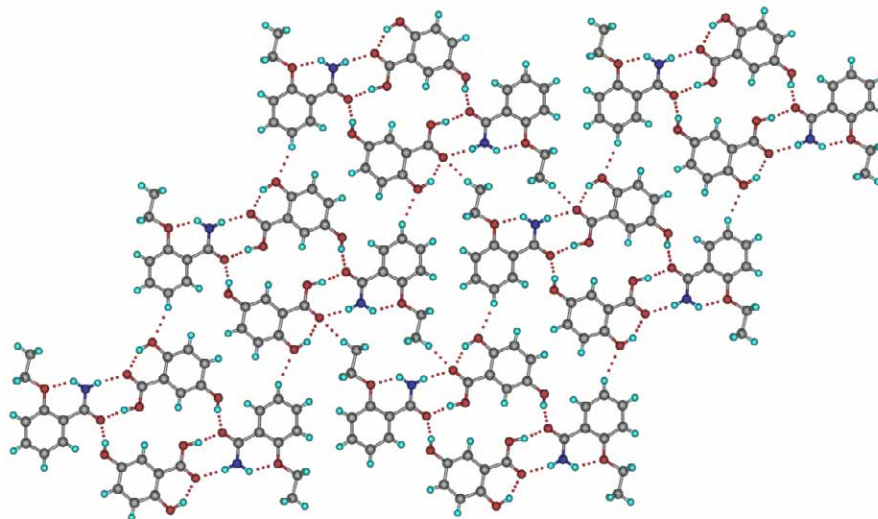


Fig. S4 Packing diagram of Form III showing the auxiliary C-H...O interactions between the hydrogen bonded tetrameric motifs.

5. DSC analysis and TGA analysis of Form I and II

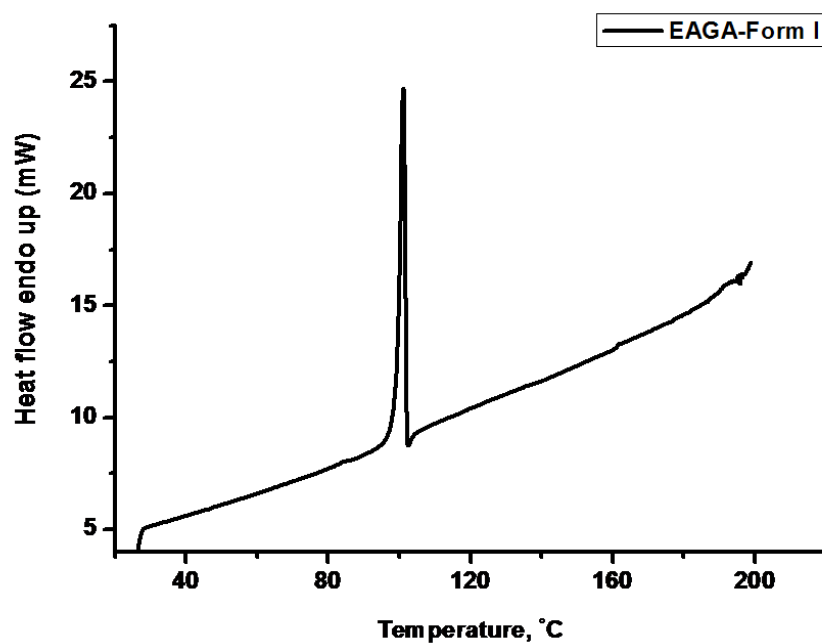


Fig. S5 DSC curve of Form I from 25-200 °C. Heating rate was 5 °C min⁻¹.

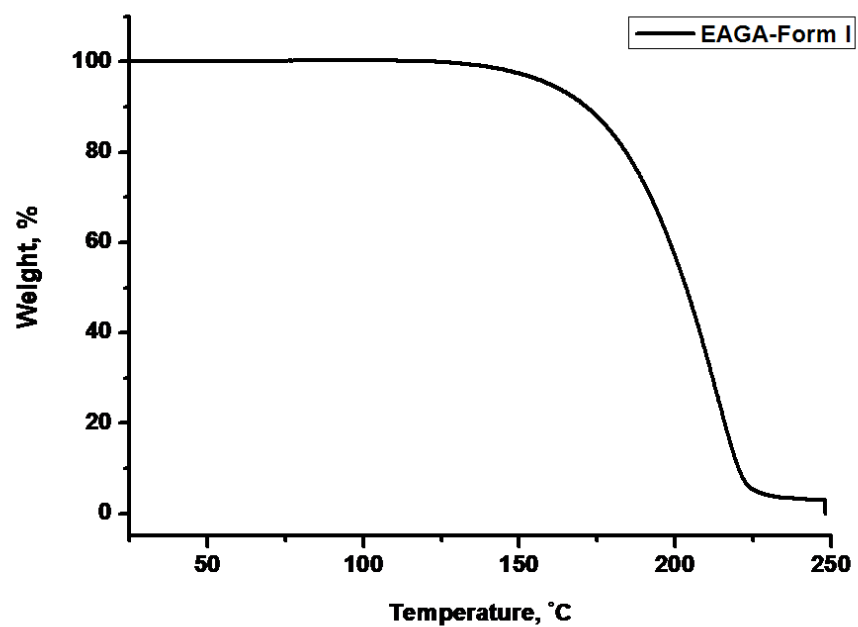


Fig. S6 TGA analysis of Form I. The cocrystal decomposes completely between 135 and 225 °C. Heating rate was 5 °C min⁻¹.

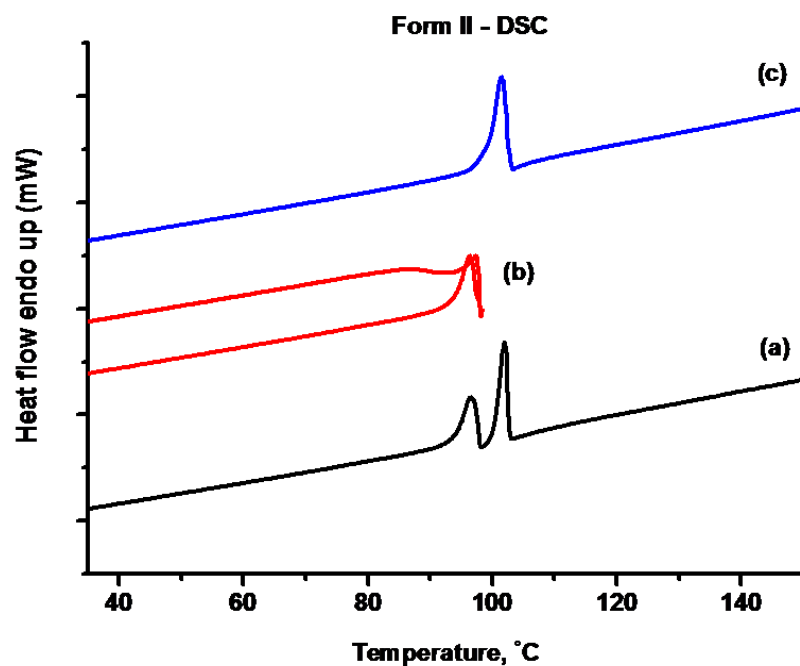


Fig. S7 DSC curves of Form II, (a) heating from RT to 150 °C, (b) heating to 98.5 °C and cooling to RT, (c) reheating to 150 °C. Note that reheating the sample results only one endotherm corresponding to the melting point of Form I (100.49 °C). This observation further suggests that Form II converts in to Form I at 98.5 °C. Heating rate was 5 °C min⁻¹.

6. Photomicrographs from HSM experiments

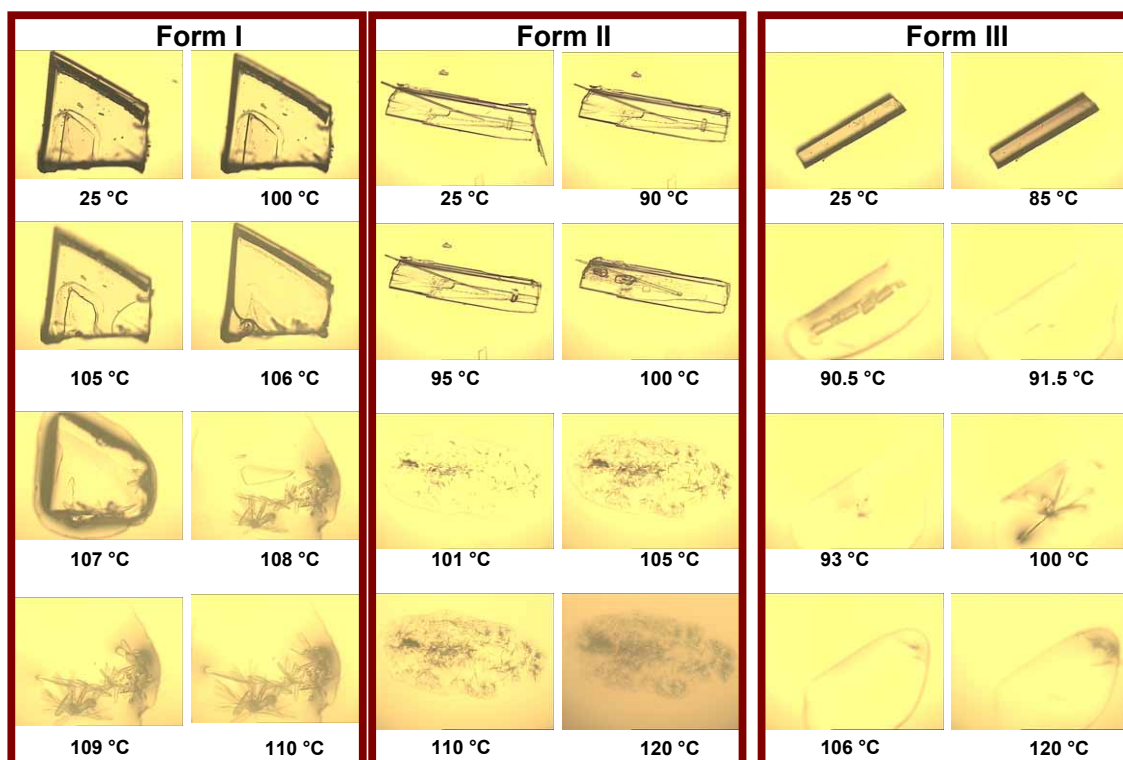


Fig. S8 Photomicrographs of Forms I-III at various temperatures in the HSM experiment, showing the series of events upon heating. The difference in the observed melting point in DSC and HSM could be because the DSC was recorded on powder samples but the HSM experiments were done on a single crystal. All three polymorphs show nucleation of needle shaped crystals from the melt, however, attempts to obtain a suitable single crystal for structural analysis were not successful.

7. PXRD patterns from slurry, SDG, and melting experiments

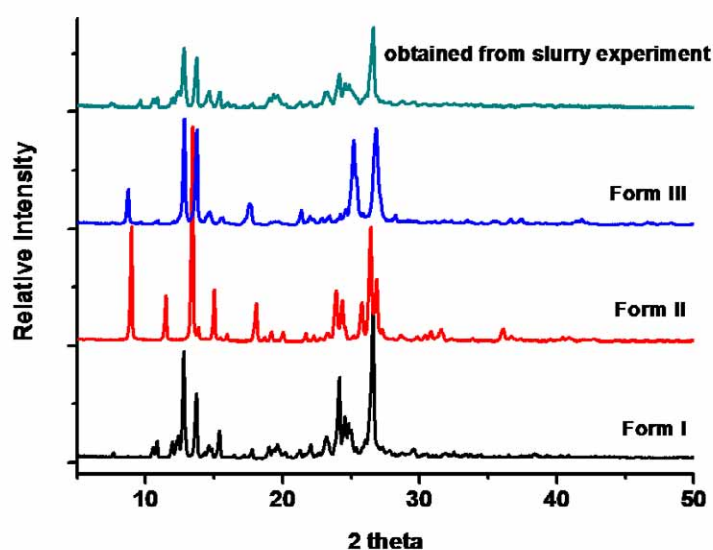


Fig. S9 Comparison of the PXRD patterns of the powder material obtained from slurry experiment in *n*-hexane. Notice that the pattern matches perfectly with Form I.

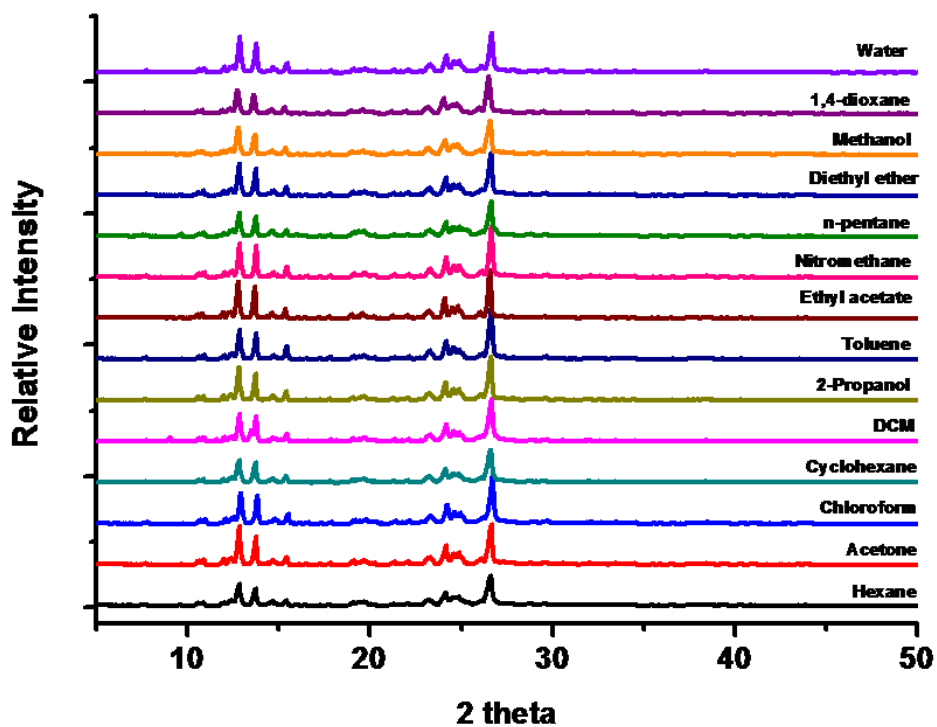


Fig. S10 Comparison of the PXRD patterns of the powder materials obtained from the SDG experiments. Notice that all the patterns match perfectly with Form I (refer Fig. S7 for Form I PXRD pattern).

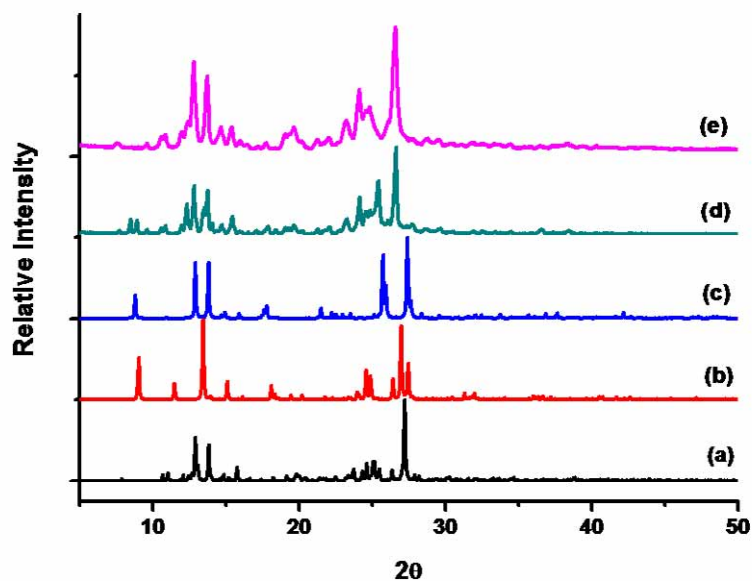


Fig. S11 Comparison of the PXRD patterns of (a) Form I, simulated from X-ray structure, (b) Form II, simulated from X-ray structure, (c) Form III, simulated from X-ray structure, (d) the powder obtained from the melting experiment, and (e) the powder obtained from stirring Form I and II in *n*-hexane for 1 day at RT. Notice that the PXRD patterns of (d) and (e) match with Form I.