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Marcus A. O'Mahony ǂ, Denise M. Croker ǂ, Åke C. Rasmuson ǂ, Stéphane Veesler §, Benjamin K. Hodnett ǂ.*

ǂ Solid State Pharmaceutical Cluster, Materials and Surface Science Institute, Department of Chemical and Environmental Sciences, University of Limerick, Limerick, Ireland.

§ Centre Interdisciplinaire de Nanosciences de Marseille, Aix-Marseille Université, CINAM-UMR7325, Campus de Luminy, 13288 Marseille, France.

ABSTRACT

The solubility of the stable FIII polymorph of the pharmaceutical compound carbamazepine was measured by determining its solubility gravimetrically in ethanol and methanol. Where the metastable FI polymorph was suspended in a solution of ethanol, the stable FIII polymorph nucleated immediately initiating a solution mediated transformation from FI to FIII. This meant that the FI polymorph was not in thermodynamic equilibrium with the solution as FI was continually dissolving while FIII was growing. We show that the solubility of FI can be accurately measured by *in situ* microscopy using an adaption of the bracketing method and the results show that the solubility is close to but higher than the maximum solution concentration reached during the solution mediated transformation from FI to FIII carbamazepine in both

solvents. The technique demonstrates a relatively simple and robust method for determining the solubility of a metastable crystalline phase which transforms quickly in solution.

INTRODUCTION

The solubility of a crystalline compound provides vital thermodynamic information required to design crystallization processes, engineer the crystal size distribution and understand the phases which nucleate in the case of multiple-phase systems. Polymorphs are solid phases of the same compound but with different crystal structures. These different crystal structures exist due to the different molecular arrangements of the molecules giving rise to differences in free energy and thus solubility.[1] A particular polymorph may have more favourable processing or performance characteristics over another. For example, Form II of ranitidine hydrochloride has improved filtration properties compared to Form I [2] and the orthorhombic polymorph of paracetamol is more suitable for compression and tableting than the monoclinic polymorph.[3] Whether selection of a metastable or the stable polymorph is desired for a crystallization process, knowledge of the solubility of both phases is critical.

A thermodynamically stable polymorph will not undergo a transformation and so its solubility is readily measured by allowing the crystalline phase to reach equilibrium with the solution, provided solvates are not formed in that particular solvent. However, a metastable crystalline phase in solution can often quickly transform to a more stable phase[4] via the process of solution mediated transformation, [5] a process where the metastable solid phase dissolves and the stable phase nucleates and grows independently from solution. The maximum achievable solution concentration during such a process is governed by both dissolution of the metastable phase and

growth of the stable phase and this can establish a kinetic steady-state for the solution concentration. Under such conditions, with the metastable phase dissolving the stable phase growing, the metastable phase is not strictly in equilibrium with the solution and so a strict measure of the thermodynamic equilibrium concentration for the metastable phase is not possible. This kinetic steady-state has been previously referred to as "apparent" solubility, because it represents the maximum in concentration reached by dissolution of the metastable phase.[6] The triclinic polymorph (FI) of the pharmaceutical compound carbamazepine (CBZ) is an example a metastable phase known to undergo such as solution mediated transformation. The FI polymorph quickly transforms to the stable P-monoclinic polymorph (FIII) upon addition to a solution of methanol saturated with respect to FIII.[7]

In practice, measuring the solubility of a crystalline phase (solute) and its variation with temperature is typically approached in one of two ways. The system, e.g. solute and solvent, is allowed to reach equilibrium at a specific temperature and the concentration of the solute in the system is measured via techniques such as gravimetric analysis,[8] titration,[9] UV-vis spectroscopy,[10] HPLC [11] or solution density.[12] Alternatively the system is examined for the point of equilibrium as a function of temperature, at a specific concentration of components. The latter can be achieved with techniques such as the synthetic method,[13] using differential scanning calorimetry (DSC),[14] in situ infra-red spectroscopy[15] and microscopic methods.[16]

In this paper, we present both of these approaches to measuring polymorph solubility. The gravimetric method was used to measure the solubility of the stable FIII CBZ polymorph. Due to the transformation of the FI polymorph to FIII in bulk experiments where FIII nucleated immediately with no induction time, we developed an adaption of the bracketing method [17] to measure the FI solubility using *in situ* optical microscopy.

EXPERIMENTAL SECTION

Polymorph Preparation

Pharmaceutical grade carbamazepine (FIII) having a purity >98% was obtained from POLPHARMA S.A. (Starogard Gdański, Poland) and stored in the presence of silica gel (0% humidity). ACS reagent grade ethanol (EtOH) and methanol (MeOH) were used having a purity of 99.9% and ≥99.8 %, respectively.

The FIII polymorph of CBZ was prepared by crystallization from ethanol using an automatic lab reactor where 36.9 g of pharmaceutical grade CBZ was dissolved in 393 g of ethanol at 78 °C. The solution was quickly cooled to 10 °C and aged for 24hrs. The solids were harvested using vacuum filtration and dried at 25 °C in a vacuum oven for approximately 40 mins and analysed by powder X-ray diffraction (PXRD) using a Panalytical X'Pert MPD PRO X-ray diffractometer. To prepare the FI CBZ polymorph, 3 g of FIII CBZ powder was placed on a clock glass. This was then placed on aluminium foil and covered by a glass petri dish before heating the CBZ powder in the oven at 170°C for 2.5 hrs under full vacuum. Thereafter, the pressure was adjusted to ambient and the powder was held for 15-20 mins. The powder was allowed to cool slowly to 100°C before removing from the oven and placing in a dessicator to reach room temperature. The bulk powder was analysed by PXRD. Needle-like particles were grown on the underside of the pertri dish due to sublimation. Single crystal X-ray diffraction (Oxford Diffraction Xcalibur system at ambient temperature) was used to identify the needle-like particles as the triclinic FI polymorph of carbamazepine. These were used for measuring the

solubility of FI by *in situ* optical microscopy. Each polymorph was separately imaged using a Zeiss axioscope A1m imager optical microscope.

Solubility measurements

The solubility of FIII in ethanol and methanol respectively was measured by adding excess FIII in contact with approx. 30 mL of solvent in a sealed test tube. Isothermal temperature control at each temperature was achieved using a thermostatic water bath (Grant GR150, S38 stainless steel, ±0.02 °C) in which the test tube was placed. Agitation in the test tube was provided with the use of a PTFE-coated magnetic stirrer bar. After 24 hrs at each temperature the stirring was switched off to allow the suspended solid to settle for 30mins. A solution sample of ~2 mL was carefully filtered (0.22 μm filter) into a preweighed vial and then weighed to determine the solution mass. After drying in the fumehood, the vials were transferred to an air oven (Binder series VD 53) at 50°C for 24 hrs. The last hour drying was carried out under vacuum at ~300mBar. This was to assure perfect dryness and to remove any potential water of hydration, as CBZ is known to form a dihydrate structure.[18] The remaining solute was weighed and the concentration was expressed as g of solute/g of solvent. Each solution sample was performed in triplicate. Weighing was carried out using a Mettler-Toledo AX054 laboratory balance with a readability of 0.1 mg. A solid sample was also vacuum filtered at the time of solution sampling and analysed by PXRD to identify the solid phase that was present in suspension with the solution.

An attempt was made to measure the solubility of the metastable FI polymorph of CBZ in the same manner as that of FIII. To do this, an excess of FI was suspended in contact with an ethanol

solution at 10, 20 and 30 °C respectively. The ethanol was saturated with respect to FIII so as to make economic use of FI which was otherwise difficult to produce as a pure polymorph in bulk quantities. The mass of FI added in excess at each temperature was equal to the amount of CBZ already dissolved to make the solution saturated with respect FIII and as such the mass of FI added to each experiment was not the same. The excess FI was added to the clear saturated solution of ethanol and CBZ (sealed conical flask - 150mL, magnetic stirring with the same thermostatic water bath for isothermal temperature control). At specific time intervals the stirring was stopped and suspended solid was allowed to settle for 5mins and a solution sample of ~2 mL was carefully filtered and dried as described above to determine the solution concentration in g CBZ / g EtOH. Each solution sample was performed in triplicate. At the same time of solution sampling a small sample of excess solid (~50mg) was vacuum filtered and analysed by PXRD.

Microscopic Solubility Measurements

In order to measure the solubility of FI, we used an experimental set-up previously developed (ANACRISMAT) to investigate solution mediated phase transitions of an pharmaceutical compound and a protein. [6, 19] The set-up consists of a Nikon Diaphot inverted optical microscope used in conjunction with an adapted Peltier temperature control unit (±0.1 °C) designed to fit an optical holding cell. The optical holding cell was used to hold and seal a quiescent solution sample in which a single crystal of FI could be observed. An adaption of the bracketing method was used to perform the solubility measurements. [19] The bracketing method typically involves producing a solution of known concentration/composition and varying the temperature until the point of equilibrium is reached where crystals neither dissolve nor grow.[17] Our adapted bracketing method requires examining for the point of equilibrium under isothermal conditions where the solution concentration is varied for each experiment. For this method, a temperature

was chosen at which to measure the solubility of FI. A solution of known concentration was prepared in the holding cell by accurately weighing CBZ and solvent to an approximate volume of 2 mL (green point – Figure 1). The cell was heated until the CBZ was dissolved, giving a clear solution, and then cooled to the temperature of equilibrium saturation (solubility) with respect to FIII (red and blue arrow – Figure 1). The solution was then further cooled to the chosen temperature (green arrow – Figure 1), entering a region of the phase diagram supersaturated with respect to FIII, and held for ~30mins. Then a single seed crystal of FI was added and monitored over time (1 image/min using automated software) for dissolution, growth or no change in particle size before the nucleation of the more stable FIII (star point – Figure 1). This procedure was repeated for solutions of different concentrations.

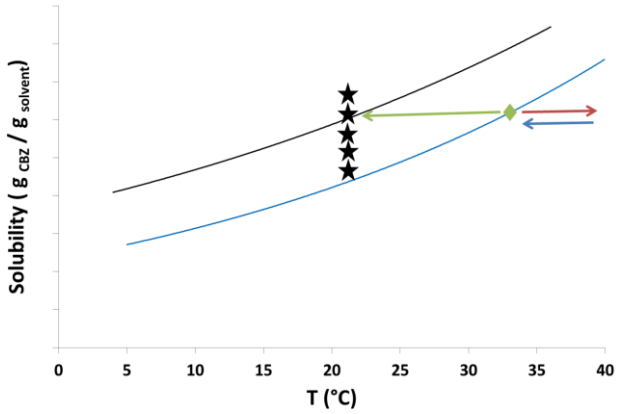
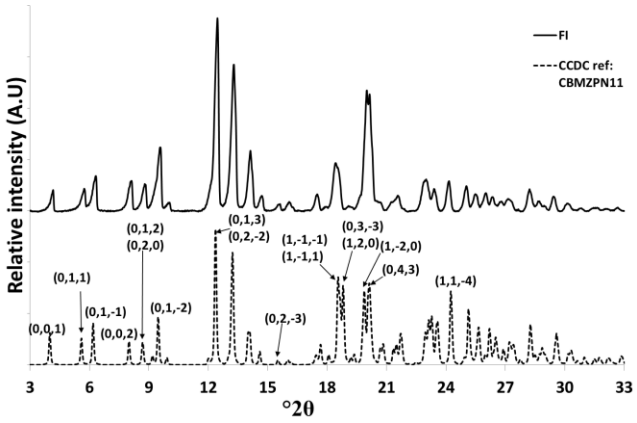


Figure 1. Schematic illustration of the adapted bracketing method used to measure the solubility of the FI polymorph of CBZ with *in situ* microscopy. — Stable FIII solubility, — putative metastable FI solubility →: heat to complete dissolution, ←: cool to saturation and ← cool further to the chosen seeding temperature. Stars illustrate different solution concentrations tested at the chosen seeding temperature.

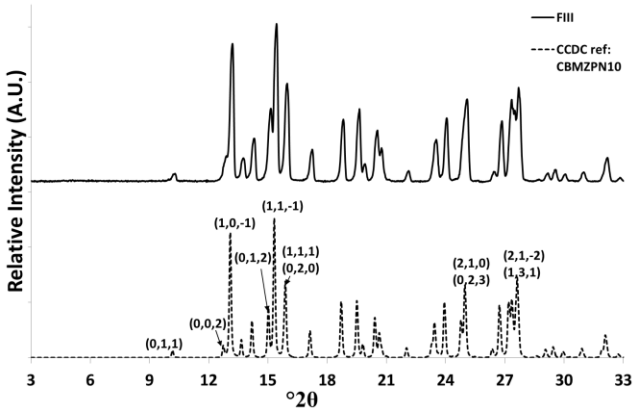
The solubility was the average of the concentrations where no change in the particle size of FI was observed, before the nucleation of FIII. This method was then repeated to measure the solubility of FI at other temperatures. The mass of the FI crystal particle (<10 μg) was negligible by comparison to the solution concentration and so it was safe to assume that the solution concentration remained constant during growth or dissolution of FI (observable to a resolution of 10 μm) before the nucleation of FIII. The nucleation and growth of FIII was easily detected in the cell by checking the cell for the distinct granular habit of FIII.

RESULTS AND DISCUSSION

The bulk polymorph preparation of FI and FIII was analysed by PXRD and compared to the theoretical PXRD patterns generated from the .cif files from the Cambridge Crystallographic Data Cente (CCDC) – Figure 2. CCDC Database codes: FI – CBMZPN11 and FIII - CBMZPN10. The PXRD patterns for each polymorph agree with those reported experimentally in the literature [20] and with their respective theoretical PXRD patterns. FIII peaks at 15.1 and 15.4 °2θ were used to check for the presence of FIII in samples taken from bulk experiments where FI was suspended in excess with a solution of ethanol and CBZ. The crystal habit of each polymorph, identified by optical microscopy, is shown in Figure 3.



(A)



(B)

Figure 2. PXRD of the pure polymorphs FI (A) and FIII (B) of CBZ compared to theoretical PXRD pattern generated from the .cif file from the CCDC database.

The single crystal particles grown by sublimation were analysed by single crystal XRD at room temperature and identified the crystals as having a triclinic unit cell with lattice parameters: a = 5.2728(6), b = 20.6510(3), c = 22.3094(1) Å, α = 84.22(6) °, β = 87.78(1) ° and γ = 84.93(6) ° confirming the particles as single crystals of FI CBZ.[20]

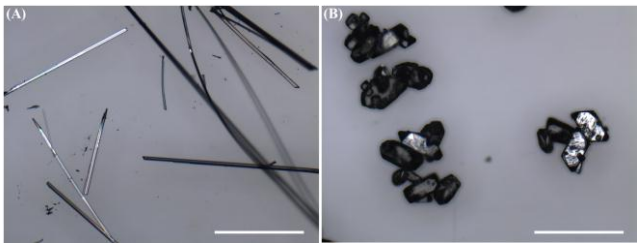


Figure 3. Optical microscopy used to identify the acicular habit of FI (A) and and granular habit of FIII (B). Inset: scale bar 500 μm

The solubility of FIII in ethanol and methanol are presented in Table 1. For each solubility measurement, PXRD identified FIII as the only phase present in solution at the time of sampling. The temperature was also observed to be accurate to within ±0.05 °C during solution sampling. The solubility of FIII increases with temperature in both solvents and agrees well with the solubility data of Liu et al. measured for FIII in these solvents using the synthetic method.[13]

Table 1. FIII polymorph solubility data as measured gravimetrically with standard deviations (n=3) in the temperature range from 5 – 35 °C.

T (°C)	SOLVENT	Solubility (g CBZ / g EtOH)	SOLVENT	Solubility (g CBZ / g MeOH)
5.0	Ethanol	0.0155 (±0.0001)	Methanol	0.0549 (±0.0004)
10.0		0.0184 (±0.0003)		0.0630 (±0.0001)
15.0		0.0217 (±0.0003)		0.0721 (±0.0001)
20.0		0.0259 (±0.0002)		0.0834 (±0.0001)
25.0		0.0307 (±0.0004)		0.0970 (±0.0004)
30.0		0.0363 (±0.0003)		0.1126 (±0.0004)
35.0		0.0433 (±0.0003)		0.1334 (±0.0004)

The results of the bulk experiments that attempted to measure the solubility of FI in ethanol gravimetrically at 10, 20 and 30 °C are presented in Figure 4, where excess FI was added at t = 0 mins and the solution and solid phase were sampled over time.

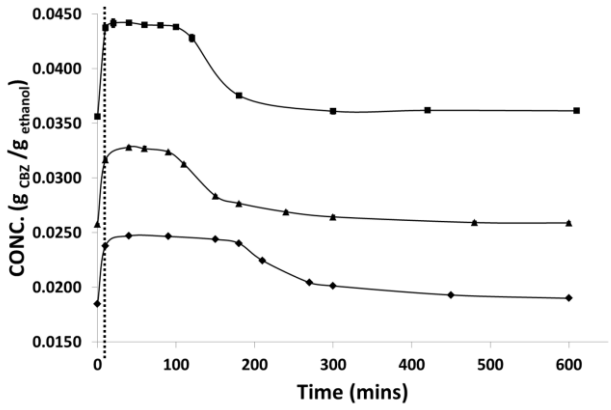


Figure 4. Solution concentration profiles for the attempted measurement of FI solubility in ethanol at ♦10 °C, ▲20 °C and ■ 30 °C. The profiles indicate the transformation of FI to FIII and the vertical dashed black line indicates where FIII is first detected by PXRD. Lines are added as a guide for the eye.

The solution concentration profiles show the solution mediated transformation of FI to FIII in ethanol at the different temperatures. As the mass of FI added in excess to each experiment varied with temperature the profiles are not directly comparable. The solution concentration at the end of the each experiment corresponded to the solubility of FIII in ethanol at each temperature previously measured, confirming the transformation to FIII. The transformation was also confirmed by the solid samples taken over time which were analysed by PXRD. An example of this is shown in Figure 5 for the experiment at 20 °C in ethanol where the transformation of the excess suspended solid sampled over time is presented. The presence of FIII in the excess solid samples is detected by the peaks at 15.1 and 15.4 °2θ corresponding to the (012) and (11-1) peaks of FIII, respectively. These peaks are distinct from a very minor (02-3) FI peak at 15.6

°2θ. FIII was detected in the excess solid after 10 mins (Figure 5B). The presence of FIII was also detectable after 10 mins at 10 and 30 °C when FI was suspended in excess in ethanol.

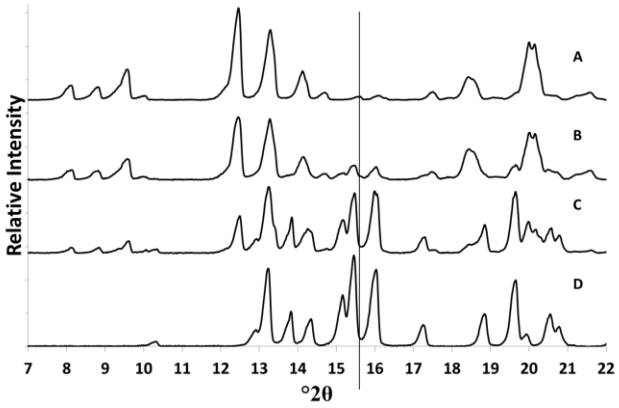


Figure 5. The transformation from FI to FIII in the excess suspended solid in ethanol at 20 °C as indicated by PXRD. (A) 0 mins – FI, (B) 10mins, (C) 90mins, (D) 150mins – FIII. The vertical line indicates the position of the (02-3) peak of FI at 15.6 °2θ and is distinct from the (012) and (11-1) peaks of FIII at 15.1 and 15.4 °2θ, respectively. The FIII peaks at are detectable in (B) after 10mins.

The results of Figures 4 and 5 show that the stable FIII polymorph nucleates immediately in solution with no detectable induction time in these bulk experiments as FIII was detectable by PXRD after only 10 mins. Subsequent solid samples for the solution mediated transformation from FI to FIII identified the PXRD peaks associated with FIII to be increasing and those of FI to be decreasing until only FIII remains (Figure 5). This reflected a situation whereby the solution concentration remained relatively unchanged with the stable FIII growing and the metastable FI dissolving, establishing as a kinetic steady-state. Under these conditions, the maximum

concentration achievable is dependent on a balance between the overall dissolution and growth rates of FI and FIII, respectively. FI is constantly dissolving as FIII grows and FI is not in thermodynamic equilibrium with the solution. This is likely to obstruct the strict measurement of the thermodynamic equilibrium concentration for the metastable FI polymorph. Recently, the solubility of FI in methanol at 10 °C was reported as 0.0874 g (±0.0001) g CBZ / g MeOH in an experiment performed by O'Mahony *et al.*[7] This value was the maximum solution concentration measured gravimetrically where excess FI was added to a solution of methanol and where FI transformed quickly to FIII with no detectable induction time for the nucleation of FIII (similar to the case in ethanol described in Figures 4 and 5). This reported solubility of FI again represents a balance between dissolution of FI and growth of FIII and is not a strict measure of the thermodynamic equilibrium concentration of the FI polymorph.

The solvent methanol was chosen for the in situ microscopy experiments to enable the solubility of FI to be determined for a number of temperatures in a relatively efficient timeframe. CBZ has a higher solubility in methanol relative to ethanol and the processes of growth and dissolution (necessary to observe for concentrations outside of the FI equilibrium concentration) are generally faster in the solvent with higher solubility.[21] Using *in situ* optical microscopy, the solubility of FI in methanol was measured by continuously observing a single crystal of FI in a solution of known concentration and at a constant temperature, where the variation in temperature was observed to be within ±0.1 °C. In conditions where the solution concentration was undersaturated with respect to FI, dissolution was evident and when supersaturated with respect to FI, growth was evident, before the nucleation of FIII. Some *in situ* microscopy images illustrating these phenomena are presented in Figure 6.

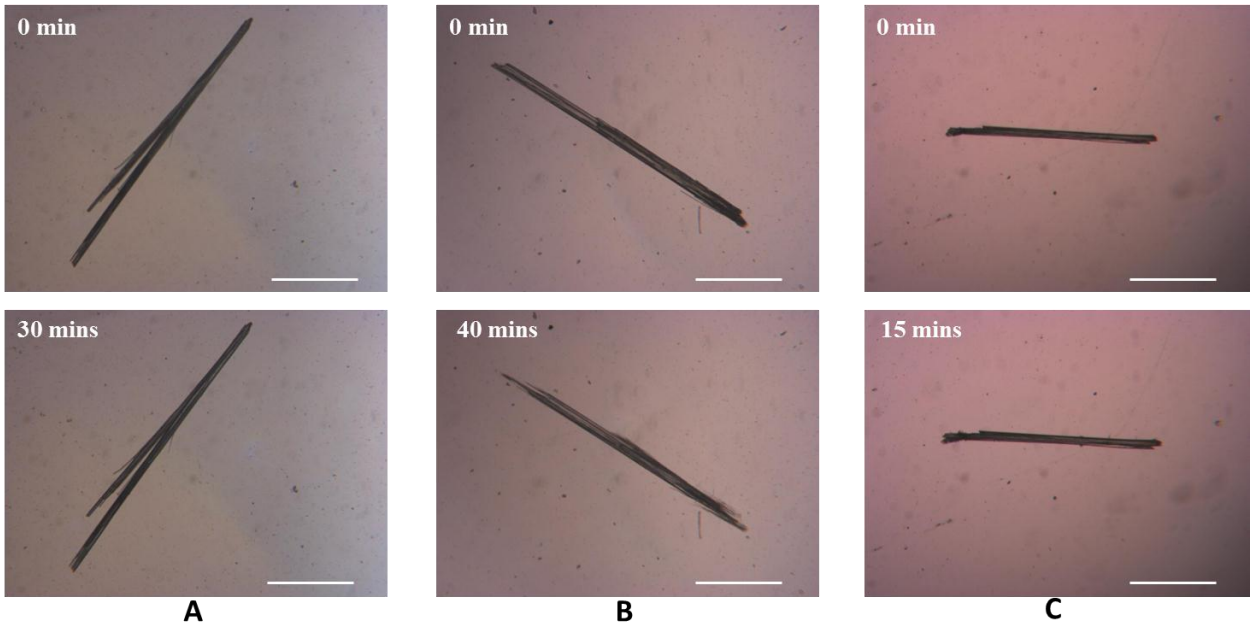


Figure 6. In situ optical microscopy images of FI crystal particles before the nucleation of FIII. A: no change in particle size (0.0883 g CBZ / g MeOH at 10°C), B: dissolution (0.1352 g CBZ / g MeOH at 30°C) and C: growth (0.1568 g CBZ / g MeOH at 30°C). Inset: scale bar 500 μm.

The solubility of FI measured by in situ microscopy using the adapted bracketing method is summarised in Table 2.

Table 2. FI polymorph solubility data as measured in methanol by in situ microscopy using the adapted bracketing method. Standard deviations are included, 10 °C n = 6, 22 °C n = 4 and 30 °C n= 3. Also included is the FIII polymorph solubility and chemical potential between FI and FIII calculated as RTlnS - product of the gas constant and temperature (K) and the log of the solubility ratio (mole fraction) between both polymorphs.

SOLVENT	T (°C)	Solubility FI (g CBZ / g MeOH)	Solubility FIII (g CBZ / g MeOH)	RT lnS (J/mol)
Methanol	10.0	0.0940 (±0.0048)	0.0630 (±0.0001)	933.0
	22.0	0.1221 (±0.0048)	0.0896 (±0.0000)[a]	749.5
	30.0	0.1480 (±0.0043)	0.1126 (±0.0004)	678.2

a - the solubility data for FIII at 22 °C in methanol was calculated by interpolation of the data in Table 1

The data in Table 2 show that the FI solubility increases with temperature and is greater than that of FIII. The chemical potential difference between each polymorph, approximated by calculating RTlnS (shown in Table 2), decreases with increasing temperature. This indicates the existence of a temperature at which the chemical potential of FI will equal that of FIII, known as the thermodynamic transition temperature (T_t) and above this temperature the thermodynamic stability order of the polymorphs changes, where FI will be the more stable polymorph. Using a straight line approximation, a van't Hoff plot of the solubility data of FI and FIII in methanol was extrapolated to determine this temperature. A transition temperature of 77 °C was calculated from the van't Hoff plot in Figure 7. This temperature lies below the melt temperature of all polymorphs of CBZ (189-191 °C) [1] indicating an enantiotropic relationship between the FI and FIII polymorph of CBZ.

Numerous other studies have attempted to estimate T_t for the FI and FIII polymorphs of CBZ based on thermodynamic calculations and/or solubility data in 2-propanol with T_t estimates ranging from 71 °C to 96 °C.[14, 22-25] However, extensive slurry experiments performed by Getsoian *et al.* in cumene for mixtures of FI and FIII CBZ over the range of temperatures spanning the estimated T_t has experimentally verified the T_t for FI and FIII polymorphs of CBZ to exist between 79-82 °C.[26] This value agrees well with the T_t of 77 °C calculated for this work.

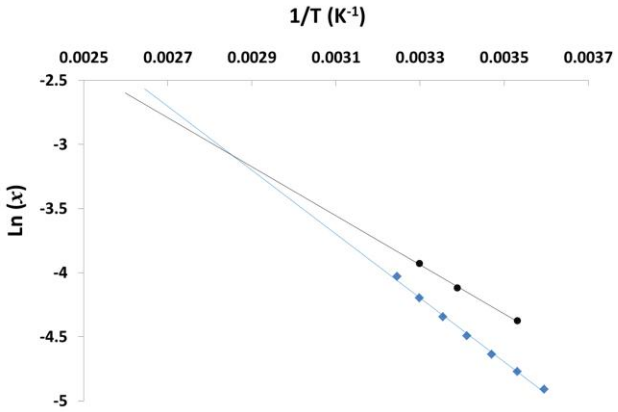


Figure 7. An extrapolated van't Hoff plot of the solubility (in mole fraction) of FI ● and FIII ♦ in methanol used to estimate the thermodynamic transition temperature of 77 °C for the polymorphs. Data were fitted with linear functions.

The solubility of FI at 10 °C is 0.0940 (±0.0048) g CBZ / g MeOH as measured in Table 2. This value is close to but significantly higher than the previously reported solubility value of 0.0874 (±0.0001) g CBZ / g EtOH measured gravimetrically where a kinetic steady-state was established as FIII nucleated with no detectable induction time and FI dissolved while FIII grew.[7] To investigate if this previously reported solubility value, a solution concentration of 0.0874 g CBZ / g MeOH was prepared and the method with *in situ* microscopy, previously described, was used to examine for growth, dissolution or no change in particle size of FI at 10 °C in the prepared solution. In Figure 8 time-lapsed *in situ* microscopy images show the dissolution of FI prior to the nucleation and growth of FIII at the surface of FI. The dissolution of FI prior to the nucleation of FIII confirmed that the maximum solution concentration achieved during the transformation from FI to FIII in methanol at 10 °C [7] was below the solubility of FI. The nucleation of the stable FIII polymorph at the surface of FI was a common feature observed in all microscopy experiments where FIII had nucleated.

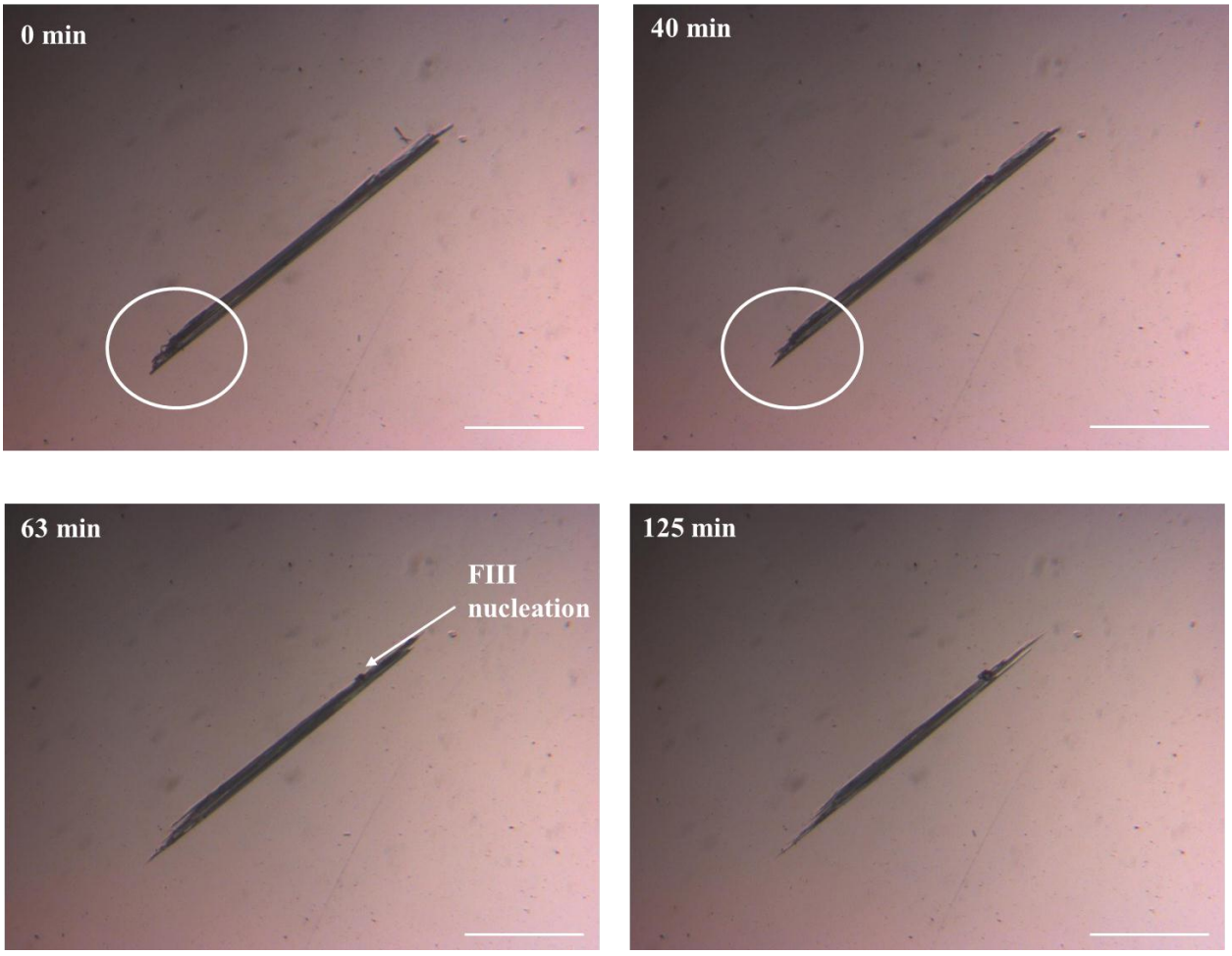


Figure 8. Time-lapsed in situ microscopy images of an FI crystal particle added to a solution of methanol (0.0874 g CBZ / g MeOH) at 10 °C. The dissolution evident at the end of the FI needle-like particle from 0-40 min is circled, after 63 min FIII nucleated on the surface of FI and this is also highlighted. Inset: scale bar 500 μm.

The FI and FIII polymorph solubility ratio, measured in methanol (Table 2), were used to calculate the solubility of FI in ethanol. Assuming the activity coefficient, γ, is independent of small changes in concentration, particularly for a compound with relatively low solubility, the approximation was made that the polymorph solubility ratio was independent of the solvent. [27] γ

was considered constant for the case of a relatively small change in solubility in going from methanol to ethanol (approximately 3 times for FIII solubility data – Table 1). The solubility of FI in ethanol was calculated using the measured solubility of FIII in ethanol, from Table 1, and the solubility ratio in methanol (in mole fraction). The calculation was made by converting the g CBZ / g MeOH data for FI and FIII solubility to mole fraction of CBZ and using the solubility ratio to calculate the solubility of FI in ethanol, first in mole fraction then in g CBZ / g EtOH. The calculated solubility of FI and the measured solubility of FIII in ethanol is presented in Figure 9. The maximum solution concentration reached for the attempted gravimetric measurement of FI solubility in ethanol (Figure 4) is also indicated in Figure 9. The data indicate that the early solution concentrations established during the attempted gravimetric measurement of FI solubility (Figure 4) resulted from a kinetic steady-state being established and lies below the solubility of FI.

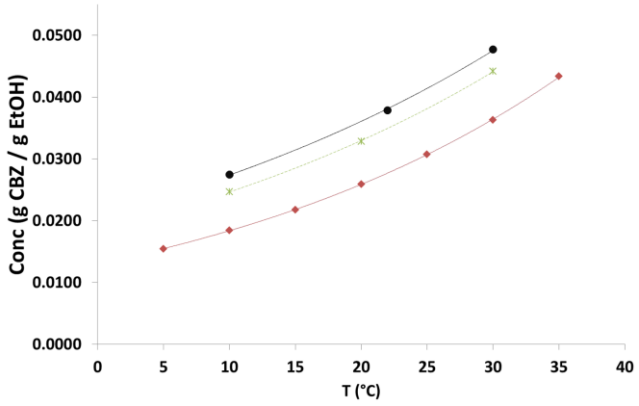


Figure 9. Solubility of FIII CBZ ♦ in ethanol as measured gravimetrically and calculated solubility of FI CBZ ● in ethanol. ж marks the maximum solution concentration reached where FI was suspended in excess with a solution of ethanol and CBZ – see Figure 4. Data were fitted with an exponential function.

To examine the validity of the calculated solubility of FI in ethanol, a solution of the same concentration as the calculated solubility of FI in ethanol at 30 °C (0.0476 g CBZ / g EtOH) was prepared and seeded with a single crystal particle of FI as before. The adapted bracketing technique with in situ microscopy previously described was used to examine for growth, dissolution or no change in particle size of FI. This solution showed no growth or dissolution under the microscope before the nucleation of FIII, confirming that the solution concentration was the solubility of FI in ethanol. A solution was prepared having the same concentration as the maximum concentration reached in the attempted gravimetric measurement of FI solubility (0.0442 g CBZ / g EtOH – see Figure 4) at 30 °C. A single crystal of FI was added to this solution and examined for growth, dissolution or no change in particle size of FI. This solution showed dissolution of FI before the nucleation of FIII. This result demonstrates that the maximum concentration reached for the 30 °C concentration time-profile in Figure 4 where FIII nucleated immediately with no induction time is lower than the solubility of FI. As expected, a solution prepared with a higher concentration than the calculated solubility of FI (0.0519 g CBZ / g EtOH) showed growth of FI under the microscope.

It is well understood that the presence of a plateau in concentration for the transformation profiles in Figure 4 show that the dissolution of FI is faster than the growth of FIII.[5] This would explain why the maximum solution concentration reached during the transformation is close to the solubility of FI. However, for a strict measure of the FI solubility, FI must be in equilibrium with the solution and this requires balance between dissolution and growth rates occurring at the surfaces of the FI crystal. The nucleation and growth of FIII on the surfaces of FI may potentially disrupt this balance which may obstruct the solution from reaching thermodynamic equilibrium with FI.

The absence of the immediate nucleation of FIII for the *in situ* microscopy measurements is a distinct difference compared to the bulk experiments which attempted to measure the FI solubility gravimetrically. It is this difference between the methods that facilitates the accurate measurement of FI solubility. A more precise measurements of FI solubility may be possible by this method if a larger magnification was used to measure the growth or dissolution of a specific crystal face.[28] However, due to the crystallization of FIII at concentrations close to the solubility of FI, this approach may prevent the ability to observe FIII nucleation and growth if it does not occur on the specific face being observed during the experiments and thus such an approach could potentially introduce errors into the FI solubility measurements. Our approach, using lower magnifications, presents a robust method for observing FI dissolution, growth or no change in crystal particle size while simultaneously checking for the crystallization of the more stable FIII polymorph. This has enabled the measurement of the solubility of the FI polymorph of CBZ which otherwise quickly transforms under bulk experimental conditions.

CONCLUSION

The solubility of FIII CBZ was measured gravimetrically in ethanol and methanol. The attempted gravimetric measurement of the solubility of FI CBZ in ethanol resulted in the immediate nucleation of FIII with no detectable induction time and subsequent transformation to FIII - similar to a previous experiment in methanol. An adaption of the bracketing method was used with *in situ* microscopy to measure the solubility of FI CBZ in methanol, before the nucleation of FIII. Further investigations using the in situ microscopy method showed that where the maximum concentration was reached as FI transforms to FIII in solution and where FIII had nucleated with no detectable induction time, this concentration lies close to but below the

solubility of FI. By avoiding the immediate nucleation of the stable phase, the use of *in situ* microscopy with the adapted bracketing method described here, demonstrates a relatively simple and robust method for determining the solubility of a metastable crystalline phase which readily transforms in solution.

AUTHOR INFORMATION

Corresponding Author

* marcus.omahony@ul.ie

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