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The Impact of Crystal Structure and Molecular Conformation on the Hydration Kinetics of Fluconazole

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Supporting Information Placeholder

ABSTRACT: In this contribution the hydration kinetics of three anhydrous polymorphs (AH-A, AH-B and AH-C) of fluconazole [2-(2,4-difluorophenyl)-1,3-bis (1*H*- 1,2,4-triazol-1-yl)-propan-2-ol] were studied. The conversion kinetics from the anhydrous forms to the monohydrate (MH) were monitored at various relative humidities above the critical water activity. The studies revealed very different kinetic stabilities for the three anhydrous forms, with AH-A and AH-C converting much more easily to the MH than AH-B. Various energetic factors, which may be influencing the kinetics of hydration, were explored together with crystal structure and molecular conformation similarities between the anhydrous forms and the MH. The level of conformational and packing similarity between the anhydrous and MH structures was found to be consistent with the ease of hydration. We believe that surface similarity may be required for the nucleation of the hydrate, whilst the level of crystal packing similarity impacts the ease of growth. In terms of conformational variations, AH-B was found to require a significantly more dramatic conformational change to convert to the MH conformation than those in either AH-A or AH-C. Soft planes (low attachment energies) may allow for easier diffusion of solvent into the crystal structure to allow for solvation. The overall kinetic energy barrier of water diffusion into the lattice plus conformational change was found to correlate well with our observed hydration kinetics, indicating that both the crystal structure and the conformation play a role in the kinetic stability towards hydration of the various fluconazole polymorphs.

1. INTRODUCTION

In drug delivery, Active Pharmaceutical Ingredients (APIs) are most commonly administered as crystalline solids, formulated as a solid dosage form, such as a tablet. Crystallisation provides options to purify and control the properties of the API, such as particle size or shape.¹ A crystalline state usually provides improved physical and chemical stability over the amorphous state, which may then facilitate an acceptable shelf-life for the API and resultant solid dosage form.

A thorough understanding of the solid form landscape is a critical activity in the development of a drug product. Polymorphism is a well-known phenomenon, with the API crystallising in more than one distinguishable crystal structure, each with identical chemical composition.² The term solvate is used when a solvent molecule, in addition to the drug molecule, becomes an integral

part of the crystal lattice.³⁻⁴ When the crystal structure incorporates water the term hydrate is used, this representing by far the most common type of solvate.⁵

The physicochemical properties of a material may affect the manufacturability and stability of the API and drug product.⁶ Many physicochemical properties are related to the crystal structure and therefore a phase conversion to a hydrate has the ability to impact the properties of the drug product such as stability, dissolution, and bioavailability, potentially compromising the drug effectiveness. In general, the formation of a hydrate lowers solubility,⁷ increasing the risk that hydration will negatively impact bioavailability. As a consequence, the Food and Drug Administration recommends that hydrate formation and its effect on the drug product properties should be understood,⁸ this includes identification of any hydrated forms.⁹

The occurrence of hydrates is thought to be common, being a reported feature of approximately one in three pharmaceutical compounds.¹⁰ Since water is frequently present within the manufacturing process, e.g., as atmospheric water, via excipients or aqueous processes such as wet granulation¹¹ and tablet coating. There is a preference to avoid forms presenting a risk of hydrate formation. This is often achieved via salt or co-crystal design; however, it is not always possible to identify alternative solid forms with acceptable properties. In these cases, it is critical to understand the thermodynamic¹²⁻¹⁵ and kinetic stability¹⁶⁻¹⁸ of both the anhydrous and hydrated forms with respect to water activity and temperature.

Once the API has been formulated, hydration will likely require a solid-state conversion and the mechanism for this conversion may provide a kinetic barrier to hydration. Many studies to date have focused on channel hydrates¹⁹⁻²³ where there is little barrier to hydration, the hydrate having a similar crystal structure to the anhydrous form. Here we study a more complex system where hydration requires structural rearrangement.²⁴⁻²⁵

Fluconazole [2-(2,4-difluorophenyl)-1,3-bis (1*H*- 1,2,4-triazol-1-yl)-propan-2-ol] is a flexible drug compound used in the systemic treatment of fungal infections. This drug is on the World Health Organisation list of essential medicines. Its molecular structure (Figure 1) has been described here relative to the central, tetrahedral C12. Four distinct groups are bonded to C12: a difluorophenyl ring, a hydroxyl group and two identical –CH₂– triazole groups. Despite its relatively small molecular size (22 heavy atoms only), fluconazole is highly flexible with six rotatable bonds defining its molecular conformation.

Multiple polymorphic forms of fluconazole have been reported in the literature,²⁶⁻³¹ as well as various solvates³² and a monohydrate.³³ Here, we study the hydration behaviour of three fluconazole polymorphs to the monohydrate form; how particle size reduction and seed addition may influence the hydration kinetics. We put special emphasis on understanding how polymorphic changes in molecular conformations and crystal packing impact the kinetics of hydration of this important antifungal drug.

Since there is some variability in the naming convention of fluconazole polymorphs within the literature, we have summarised

the crystallographic information for the forms studied here in Table 1 together with their Cambridge Structural Database³⁴ (CSD) refcode and our adopted naming. We have used the following nomenclature throughout this contribution: MH for the monohydrate form (IVUQIZ), AH-A (Anhydrous-A) for the triclinic polymorph (IVUQOF), AH-B for the $P2_1/n$ polymorph (IVUQOF01) and AH-C for the $Z'=1$ $Pbca$ polymorph (IVUQOF02).

Table 1 Summary of the crystallographic information, naming and CSD refcode for the polymorphic and monohydrate forms of fluconazole studied.*

Form	MH	AH-A [□]	AH-B	AH-C [□]
CSD Refcode	IVUQIZ ³²	IVUQOF ³²	IVUQOF01 ³⁰	IVUQOF02 ³⁰
Crystal system	triclinic	triclinic	monoclinic	orthorhombic
Space group	$P\bar{1}$	$P\bar{1}$	$P2_1/n$	$Pbca$
a (Å)	5.626(10)	7.499(10)	6.699(4)	12.9282(9)
b (Å)	11.737(2)	7.787(10)	27.387(19)	6.0241(5)
c (Å)	12.306(3)	11.982(2)	15.290(11)	34.834(3)
α (°)	71.24(10)	84.95(10)	90	90
β (°)	79.87(10)	84.63(10)	90.32(3)	90
γ (°)	84.38(10)	75.89(10)	90	90
Volume (Å ³)	756.68	674.05	2805.09	2712.9
Packing coefficient	0.664	0.700	0.677	0.697
Density	1.423	1.509	1.45	1.5
Z	2	2	8	8
Z'	1	1	2	1
T (K)	283-303	193	100	100
R-Factor (%)	4.29	3.50	3.46	6.96

*Monohydrate (MH), Anhydrous-A (AH-A), Anhydrous-B (AH-B) and Anhydrous-C (AH-C); a form nomenclature has not been assigned to other anhydrous polymorphs found in the CSD; IVUQOF03 and IVUQOF04 as these forms were not experimentally accessible.

□For AH-A and AH-C, The CSD structure is recorded at sub-ambient conditions, in conformation of the prepared solid form an equivalent room temperature structure was used to simulate the pXRD patterns (see section 3 of the Supporting Information).

Figure 1 Molecular structure of fluconazole with atom numbering and definition of its six rotatable bonds.

2. METHODS

2.1. Materials

Fluconazole was gifted by Pfizer Global Supply, Ringaskiddy, Ireland. The various forms were first produced as pure samples in small quantities. Following this, larger batches of the various forms were prepared via seeded crystallisations.

For the synthesis of the seeds: **MH**, Fluconazole (200 mg/mL) was dissolved in ethanol: water (3:1 v/v) at 70 °C and the solution was then cooled to 10 °C at 2 °C/min. **AH-A**, Fluconazole (110 mg/mL) was dissolved in anhydrous propan-2-ol at 70 °C and the solution was then cooled to 10 °C at 2 °C/min. **AH-B**, A saturated solution containing MH and AH-A (1:1 w/w) was agitated in anhydrous butan-2-one for 7 days until pure AH-B crystals were obtained. **AH-C**, Fluconazole MH was dried under vacuum (50 mbar) at 70 °C.

The seeds were confirmed to be phase pure by comparison of experimental and simulated powder X-ray diffraction patterns (see section 3 of the Supporting Information) and were then used in the preparation of larger scale batches of the various forms (8 – 10 g). For the **MH**, Fluconazole (200 mg/mL) was dissolved in ethanol: water (1:1 v/v) at 70 °C, the solution was cooled to 50 °C at 1 °C/min and seeded with MH (1 % w/w); cooling continued at 5 °C/min to 20 °C. For **AH-A**, Fluconazole (160 mg/mL) was dissolved in anhydrous propan-2-ol at 70 °C, the solution was cooled to 60 °C at 1 °C/min and seeded with AH-A (1 % w/w), cooling continued at 5 °C/min to 20 °C. For **AH-B**, Fluconazole (150 mg/mL) was dissolved in anhydrous grade butan-2-one at 70 °C. The solution was cooled to 60 °C at 2 °C/min and seeded with AH-B (1 % w/w), cooling continued at 0.2 °C/min to 20 °C, the product then held at 20 °C for 3 days. For **AH-C**, A hot (filtered)

solution of fluconazole (anhydrous propan-2-ol, 60 °C, 85 mg/mL) was crash cooled into a flask (on ice) containing AH-C seed (5 % w/w). All anhydrous forms were isolated within a glove box held at ≤ 5 %RH.

2.2. Determination of Critical Water Activity

The critical water activity for an anhydrate and hydrate system is the water activity value, at a defined temperature and pressure, at which the anhydrous and hydrate forms are in equilibrium. Conducting competitive studies, at defined water activities (A_w) allows conversion to the more stable form via a solution mediated conversion pathway and therefore minimises the kinetic barriers associated with solid state conversion processes.¹²⁻¹³ ASPEN³⁵ (a process simulation software package) was used to calculate the water activity of water solvent mixtures through application of the non-random two-liquid model³⁶ (NRTL). This model correlates the activity coefficients of a solvent with its mole fractions in the liquid phase. Solvent mixtures were prepared using propan-2-ol (anhydrous grade) and water. The water activity was confirmed using an Aqualab TDL (at 25 °C) which measures the relative humidity of a samples head space using a tuneable diode laser, calibrated against water activity standards. Solvent systems were initially saturated with the anhydrous form of interest, at or above the analysis temperature and excess anhydrous and MH forms were added to the resultant solution (1 mL) to achieve an approximate 1:1 w/w mixture of anhydrous and MH forms. These slurries were held at 25 °C and the form present monitored using powder X-ray diffraction. The sample (slurry) was held on a low-background silicon wafer mount and covered with an X-ray transparent (polyimide) film; this added to prevent a change in the solvent composition and therefore form during the analysis. AH-A slurries were tested within 2 hours of mixture preparation with AH-B slurries being left for 1 week prior to analysis.

2.3. Sample Preparation and Hydration

Hydration was monitored using a gravimetric approach, involving manually weighing samples stored in a controlled humidity atmosphere. Samples were weighed over a period of six months, initially weekly decreasing in periodicity commensurate with the rate of hydration (see section 6.1 of the Supporting Information). This approach was used firstly because given the duration of the study it was impractical to use automated equipment and secondly because each anhydrous form was known to be non-hygroscopic (AH-A and AH-B adsorb < 0.005 % w/w and AH-C = 0.06% w/w water at 80 %RH, see section 5.5 of the Supporting Information) therefore any weight gain could be directly attributed to hydration. Full hydration requires the absorption of one mole of water, equating to 5.88% w/w relative to the anhydrous molecular weight. The conversion fraction (α) was calculated relative to this expected weight gain, therefore $\alpha = \text{\%weight gain}/5.88$. No secondary quantification methodology was used during the sample weight monitoring period, as this could detrimentally affect the sample weight; however, the final form composition was confirmed on conclusion of the study, using powder X-ray diffraction.

Samples (35 mg each) were held at ambient temperature (19 – 23 °C) and controlled humidity (in a sealed vessel). The humidity was maintained using saturated salt solutions of either $\text{Mg}(\text{NO}_3)_2$ (54.5 %RH) or NaCl (75.3 %RH).³⁷ The sample weight was monitored using a Sartorius MC5 microbalance. The impact of particle size reduction and addition of MH seeds on the hydration process were also assessed. Particle size reduction was achieved using a pestle and mortar. To minimise the impact of grinding on the crystallinity, the grinding time and applied pressure were kept to a minimum. Multiple 10-second grinding periods were applied.

The particle size was monitored (microscopy) during grinding and once no further size change was observed (~ 3 repetitions) grinding was stopped. Approximately 0.5 g of each anhydrous polymorph, seeded with 5 % w/w of ground MH was prepared. Monohydrate seeds were added to each anhydrous form in the following manner. The monohydrate was ground, as described above and ~ 25 mg accurately weighed into a 1 cm diameter, pressed aluminium foil weighing boat. (Grinding did not impact the MH hydration state, see section 5.3 of the Supporting Information.) The quantity of anhydrous polymorph required to deliver a MH seed level of 5 % w/w, relative to the pre-weighed MH seed, was calculated and then accurately weighed into a 15 mL glass vial. The ground MH was added to the surface of the anhydrous powder and the vial gently rolled to mix the contents. This was followed by multiple, short (5 x 3 second) periods of vortex mixing, tapping and rolling the vial between each. The MH seed weight was accounted for in the conversion fraction calculation. Replicates (5) of each sample type were weighed, this allowing for sample variability to be assessed.

2.4. Microscopy

The appearance of the MH crystals formed on hydration of AH-A, AH-B and AH-C were compared to those of the anhydrous forms. Powder was dispersed in a drop of silicone oil and viewed using a Nikon LV100 polarising light microscope, fitted with a range of objective lenses and a camera. Images were captured using Linkam Scientific LINK software³⁸ v1.05.

2.5. Calculation of Conformational Energies

Adjustment and conformer energies³⁹ for the various conformations of fluconazole, as found in the crystal structures of the MH, AH-A, AH-B and AH-C, were calculated. Density functional theory (DFT) calculations were carried out using the standalone functional B97-D (which includes Grimme's dispersion corrections⁴⁰) together with the 6-31G+d(d,p) basis set using the quantum mechanics package Gaussian 09. This choice of functional was made on the basis that the model would accurately account for the intramolecular dispersion.

For the molecular energy calculations, initial molecular geometries were retrieved from the known crystal structures, as available in the CSD. Molecules were placed in isolation in the gas-phase and the geometries were relaxed, firstly keeping the torsion angles fixed (E_{crys}) and secondly without any torsional constraints (E_{gas}). All geometry optimisations were carried out using tight convergence criteria. Each conformation was optimised in an independent simulation. These relaxations take the crystal conformation to the nearest equilibrium conformer (local or global). The adjustment energy (E_{adj}) was calculated for each system by subtracting the electronic energy of the relaxed conformation in the gas-phase from the electronic energy of the constrained molecule in the gas-phase. Thus, the adjustment energy for the molecule of fluconazole in the AH-A form would be calculated as $E_{\text{adj}}[\text{AH-A}] = E_{\text{crys}}[\text{AH-A}] - E_{\text{gas}}[\text{AH-A}]$. The adjustment energy represents the amount of conformational stability lost upon placing a particular fluconazole conformer from the gas-phase into the crystal structure. The relative conformer energy (ΔE_{gas}) was calculated as the difference between a given gas conformer and the most stable gas conformer of those calculated. Three different conformers were identified in the fluconazole form, (C1, C2 and C3) C1 being the most stable. Thus, for AH-A, ΔE_{gas} is calculated as the difference between $E_{\text{gas}}[\text{AH-A}]$ and $E_{\text{gas}}[\text{C1}]$. The total conformational energy penalty of the molecules in the different crystal structures is then calculated as the sum of the adjustment and the conformer energies, thus $E_{\text{conf}} = E_{\text{adj}} + \Delta E_{\text{gas}}$. All energy values are reported in kJ/mol.

After all geometry optimisations were completed the potential energy surface (PES) of fluconazole in the gas phase and water (using a continuum solvation model based on density, SMD, as implemented in Gaussian09) were calculated as a function of rotation of τ_{2-A} , τ_{2-B} and τ_{OH} from 0° to 360° in steps of 10°. The pertinent areas of those PESs were then put together and the conformational energy barriers, to convert from the conformers found in the different crystal structures (AH-A, AH-B, AH-C and MH), were calculated.

2.6. Calculation of Hydrogen Bond Interactions

Crystal Explorer version 17.5⁴¹ was used for computing the hydrogen bond energies of fluconazole dimers and chains (as found in the IVUQOF and IVUQOF02 crystal structures respectively). The CE-B3LYP model for intermolecular energies was used as derived from B3LYP/6-31G(d,p) electron densities.⁴²⁻⁴³

2.7. Growth Morphology

Crystal structures of the different forms were retrieved from the CSD and were geometry optimised within the Forcite tool available within BIOVIA Materials Studio.⁴⁴ Calculations were carried out using the COMPASSII (v1.2) forcefield with forcefield assigned partial atomic charges. All atom-atom dispersion-exchange-repulsion calculations were computed to a maximum interatomic distance cut-off of 15.5 Å. Electrostatic interactions were calculated using the Ewald summation method to an accuracy of 0.0001 kcal/mol. Lattice geometry optimizations were carried out using the 'Smart' algorithm with convergence limits of 0.0001 kcal/mol for energy and 0.005 kcal/mol/Å for force. Unit cell and atomic coordinates were all allowed to optimise freely. The optimised crystal structures were then used as input files for the calculation of crystal morphologies using the attachment energy method as implemented within the Growth Morphology tool, applying the same forcefield parameters used in the geometry optimisation.

2.8. Scanning Electron Microscopy (SEM)

A Carl Zeiss Supra 40VP field emission scanning electron microscope (FE-SEM) operated at high vacuum was used. Secondary electron images were recorded for each sample at an accelerating voltage of 3 kV and a working distance of 12 mm. Powder samples were adhered to an individual sticky-tab, mounted on a 12.5 mm diameter aluminium pin stub. Excess powder was removed by tapping and then dusting with particle free compressed air. Prior to analysis the prepared samples were sputter coated with approximately 10 nm of platinum using a Quorum Technologies Ltd., Q150TS coater operated at 20 mA for 1 minute. Images were collected using Zeiss Smart SEM software v5.06.⁴⁵

2.9. Differential Scanning Calorimetry (DSC)

A TA Instruments Discovery DSC (heat-flux system), with an auto-sampler, was used. Samples of 1 to 3 mg were heated in an aluminium T0 sample pan with a crimped lid. Samples were heated from 20 to 200 °C at a rate of 10 °C/min. Data was collected and analysis performed using TA Instruments TRIOS software.⁴⁶

2.10. Powder X-ray Diffraction (pXRD)

The solid form of the monohydrate and each anhydrous polymorph used in the hydration studies was confirmed using a Bruker-AXS Ltd., D8 Advance, powder X-ray diffractometer, fitted with a theta-theta goniometer, automatic beam divergence slit and a LynxEye detector. Samples were prepared for analysis by filling a 0.7 mm diameter borosilicate capillary. The specimen was rotated whilst being irradiated with copper K-alpha₁ X-rays (wavelength = 1.5406 Å) with the X-ray tube operated at 40kV/40mA. The analyses were performed with the goniometer running in continuous mode from 2 – 55° 2 θ , step size 0.011° 2 θ ,

step time 12 seconds. Data was collected using DIFRAC plus XRD Commander v2.6.1.

At the end of the hydration study the resultant solid form was confirmed using a Bruker AXS Ltd., D8 Endeavor, powder X-ray diffractometer, fitted with a theta-theta goniometer and a LynxEye XE-T detector. The primary motorized slits were set at a constant illumination of 11 mm and anti-scattering slit set at a constant distance of 2.2 mm. Samples were prepared for analysis on a low background cavity, silicon wafer mount. The wafer mount was rotated at 15 rpm, whilst being irradiated with copper K-alpha₁ X-rays with the X-ray tube operated at 40kV/40mA. The analyses were performed with the goniometer running in continuous mode from 2 – 55° 2 θ , step size 0.019° 2 θ , step time 0.10 seconds. Data was collected using DIFRAC Measurement Centre v7.3.1. Analysis was performed using Bruker AXS DIFRAC.EVA v4.2.1.10.⁴⁷

3. RESULTS

3.1. Physicochemical Characterisation of Anhydrous Polymorphs

Characterisation was carried out using DSC, pXRD and SEM on both initial and ground samples to establish the impact of particle size reduction on the particle properties. From a comparison of the measured and simulated (from the crystal structure) pXRD patterns, each solid form was confirmed to be a single phase; however, AH-C was found to contain just detectable levels (typically detection limit 1%) of AH-A (see section 5 of the Supporting Information).

It is commonly accepted that milling crystalline materials can induce surface disorder, this in turn affecting the physicochemical properties exhibited.⁴⁸⁻⁴⁹ Surface disorder can act as nucleation points for form conversion. When comparing data for initial and ground samples there was no detectable loss of crystallinity; for pXRD, both peak widths and positions remained the same and for DSC melt onset and enthalpy values were within the variability expected from this analysis technique (Table 2 and section 5 of the Supporting Information).

Table 2 Differential Scanning Calorimetry Data: onset melting temperature (T_{fus}) and enthalpy of fusion (ΔH_{fus}). Relative enthalpy of fusion, with respect to the most stable form, are also given (ΔH_{form}).

	T_{fus} (°C)	ΔH_{fus} (kJ/mol)	ΔH_{form} (kJ/mol)
AH-A	138.7	35.9	2.4
AH-A-ground	138.6	35.9	
AH-B	137.5	38.3*	0.0
AH-B-ground	137.5	38.3	
AH-C	138.9	34.7	3.7
AH-C-ground	139.1	34.5	

*In order to estimate the variability, five replicate measurements were performed on AH-B. The needle-like crystal morphology found for AH-B making it the most challenging material to prepare for analysis. The mean of these replicates was 38.3 kJ/mol with a standard deviation of 0.2 kJ/mol. (see section 5 of the Supporting Information)

Scanning electron microscopy (Figure 2) shows images for the three anhydrous polymorphs studied both before and after grinding. AH-A consists of angular prisms, typically less than 40 μ m in size, with some particle aggregation. AH-B consists of needles of 2-3 μ m in width and up to 120 μ m in length. AH-C consists of flakes (5-20 μ m) aggregated into larger sintered particles averaging 60 μ m in size. In each case, grinding effectively reduced the particle size. For AH-A the particle shape has been impacted with

many particles appearing more rounded. For AH-B grinding appears to have shortened the needles, but not impacted the needle width. For AH-C the aggregates have been broken to some extent, with the flake like morphology retained. Sizes are estimated from

analysis of particles within a larger collection of images, with only representative images presented here.

Figure 2 Scanning Electron Micrographs of fluconazole AH-A (a,b), AH-B (c,d) and AH-C (e,f), before (a,c,e) and after grinding (b,d,f).

3.2. Critical water activity for anhydrate to MH conversion

The critical water activity for MH *versus* AH-A and AH-B was established at 25 °C. Table 3 summarises the results of slurry experiments starting with 1:1 mixtures of the anhydrous form of interest and the MH. At a water activity (A_w) of ≤ 0.2 , the MH converts to the respective anhydrous form. Only at a A_w above 0.30 do we see full conversions to the MH for both anhydrous forms. Thus, we infer that the critical water activity for AH-A *versus* MH is in the range between 0.20 and 0.30, and for AH-B between 0.30 and 0.39. At low water activity AH-C was found to convert to AH-A within 30 min of slurry preparation and therefore it was not possible to establish the critical water activity between AH-C and the MH. The differing critical water activity values found for MH relative to AH-A and AH-B are in line with the relative stability of these polymorphs. Note that during AH-B seed preparation, a MH and AH-A (1:1 w/w) slurry, in anhydrous butan-2-one, was found to convert to AH-B, indicating AH-B as the more thermodynamically stable polymorph at room temperature.

Table 3 Solid forms identified after equilibration of anhydrous: MH mixtures in propan-2-ol/water at 25 °C.

Water activity (A_w)	1:1 AH-A:MH starting mixture	1:1 AH-B:MH starting mixture
0.00	AH-A	AH-B
0.10	AH-A	AH-B
0.20	AH-A	AH-B
0.30	MH	AH-B
0.39	MH	MH
0.49	MH	MH

3.3. Hydration of polymorphic forms

Figure 3 shows the hydration kinetics of the three anhydrous fluconazole polymorphs in the form of conversion-time plots.

Data at two relative humidities are provided, 54.5 and 75.3 %. Given that both these values are in excess of the measured critical water activity it would be expected that in all experiments the anhydrous forms would be metastable with respect to the MH.

Figure 3 then illustrates a number of key features. It is apparent that hydration rates are dependent not only on the relative humidity but also on the starting polymorphic form. For the as-crystallised, unground samples all three anhydrous forms demonstrated a kinetic barrier to hydration at 54.4 %RH (Figure 3a) with no weight gain observed over 6 months. Increasing the RH to 75.3 % (Figure 3b) accelerates the hydration kinetics which are now clearly different for each anhydrous form. For the first 16 weeks, hydration was only observed for AH-A and AH-C with the hydration rate for AH-C being markedly faster (0.0473 α /day) than that of AH-A (0.0109 α /day) (Table 4). After a 16-week induction period there is a small yet measurable gain in weight (0.0002 α /day) for AH-B with the rate increasing over time (0.0008 α /day at 25 weeks). AH-B thus experiences a higher kinetic barrier to hydration at 75.3 %RH than AH-A or AH-C.

Table 4 Maximum observed slope of conversion fraction with time (as da / day) at 54.5 and 75.3 %RH.

%RH		AH-A	AH-B	AH-C
54.5	As-cryst.	0.0000	0.0000	0.0000
	Seeded	0.0002	0.0000	0.0019
	Ground	0.0011	0.0000	0.0013
75.3	As-cryst.	0.0109	0.0008	0.0473
	Seeded	0.1023	0.0141*	0.1188
	Ground	0.1329	0.0048	0.1152

*Hydration slowed after 60 days to 0.0020 da / day. The maximum observed hydration rate is estimated from the slope of the weight gain across consecutive data points.

Figure 3 Water vapour sorption of anhydrous fluconazole polymorphs held at 54.5 (a,c,e) and 75.3 %RH (b,d,f): (a, b) as-crystallised, (c, d) including the impact of MH seed addition (5 % w/w) and (e, f) particle size reduction (ground). Values calculated from an average weight gain of 5 sample replicates held in a sealed humidity vessel. Error bars are given for all data sets but may not be discernible where the variability was low.

The impact of MH seeding on the kinetic barrier at 54.5 %RH is seen in Figure 3c. This appears to have most effect on AH-A and AH-C which now become susceptible to hydration while AH-B remains kinetically stable. Clearly the hydration rate of AH-A is significantly slower than that of AH-C. At 75.3 %RH (Figure 3d) seed addition had a dramatic effect with AH-A and AH-C now demonstrating similar hydration behaviour. For AH-B the 16-week induction period, seen without seeding, is no longer present, although the hydration rate remains relatively slow compared to AH-A and AH-C (e.g., 0.0141 α /day (initial rate) relative to 0.1188 α /day for AH-C, Table 4). The impact of particle size reduction, as revealed through the ground samples is seen in Figure 3e and f. For AH-A and AH-C the results mirror that of seeding with both forms exhibiting similar hydration behaviour. AH-B, however, continues to show much slower kinetics. An absence or decrease in hydration induction time suggesting that grinding may have removed the barrier to MH nucleation on the surface of the AH-C crystals. For AH-A hydration occurred at a faster rate than seen with seed addition, suggesting that there is an additional factor at play. AH-B continues to present a kinetic barrier to hydration at 54.5 %RH, however at 75.3 %RH hydration was observed with an induction period of approximately 20 days, after which hydration continued at a steady rate.

Very little variability was found within the weight gain of sample replicates with the exception of AH-A, AH-C (without grinding or seed addition) and AH-B (seeded) held at 75.3 %RH, where variability was seen in sample replicate weight gain. For AH-A and AH-C the variability occurred during hydration but did not impact the induction period (prior to hydration) or the time taken for hydration to complete; the variability being at a maximum at a conversion fraction of 0.5. This may simply be related to a physical difference between sample replicates, such as particle size. However, for AH-B (seeded) variability increased during the first 60 days exposure at 75.3 %RH, within this period each sample hydrated to a differing conversion fraction, after this initial period each sample then continued to hydrate at the same rate, albeit offset (see section 6 of the Supporting Information). Variability was not seen for ground AH-B, where MH nucleation was first

required on the surface of the AH-B crystals, this suggest that the variability seen in the seed data set was due to either the seed distribution or seed contact with the AH-B crystals; once MH nucleation was established within the AH-B crystals, variability in the hydration rate was no longer apparent.

The solid form identified at the end of the study, summarised in Table 5, is consistent with the observed weight gain. Thus, samples with a larger weight gain were found to be either fully or predominantly the MH form while those with a smaller weight gain were found to be predominantly the starting anhydrous form. Interestingly, for AH-C, the ground sample held at 54.5 %RH has a higher level of AH-A present at the end of the six months study than found for the initial sample, suggesting that conversion of AH-C to AH-A has occurred over time.

Table 5 Solid form identified after six months exposure of samples to either 54.5 or 75.3 %RH. (from powder X-ray diffraction analysis, see section 7 of the Supporting Information).

%RH		Starting Anhydrous Form		
		AH-A	AH-B	AH-C
54.5	As-cryst.	A	B	C
	Seeded	A, (MH)	B, (MH)	C, (MH), (A)*
	Ground	A, (MH)	B	C, (MH), (A)*
75.3	As-cryst.	MH, (A)*	B, (MH)*	MH
	Seeded	MH	MH, (B)	MH
	Ground	MH	MH, (B)	MH

Minor forms are given in parenthesis, *indicating the presence of only a trace of this form. A =AH-A, B=AH-B and C =AH-C.

We confirmed experimentally that all fluconazole forms convert directly to the monohydrate without any intermediates. For AH-A and AH-C, environmental powder X-ray diffraction at 90 %RH (25 °C) confirmed that the hydration of AH-A and AH-C proceeds directly to the monohydrate with no intermediate form (see section 8 of the Supplementary Information). For AH-B, pXRD patterns for partially converted AH-B confirm that the

hydration of AH-B also proceeds directly to the monohydrate with no intermediate form (see section 7 of the Supplementary Information).

3.4. Structural changes required during the hydration process

In this section the structural changes required for the hydration of the different anhydrous forms can be classified in two types: a) conformational variations and b) crystal structure variations, both are influential on hydration to a differing extent relative to the anhydrous form studied. Here we compare the conformations and the crystal packing of the three anhydrous forms with that of the MH.

Conformational variations

In order to illustrate the rotations required for conversion of each of the conformations found in the anhydrous polymorphs to that found in the MH, we have oriented all of the crystal conformations along the O13-C12 bond in Figure 4 and summarised the

torsion angle values and rotational changes required in Table 6. The six main torsion angles depicted in Figure 1 are analysed: i) τ_{Ph} which defines the orientation of the difluorophenyl group, ii) τ_{OH} which defines the orientation of the hydroxyl group, iii) τ_{t1-A} and τ_{t1-B} which define the orientation of the first $-CH_2$ -triazole group and iv) τ_{t2-A} and τ_{t2-B} which define the relative orientation of the second $-CH_2$ -triazole group. The crystal structures of the MH, AH-A and AH-C are centrosymmetric and thus have inversion symmetry. This means, that these forms contain the conformation reported in the asymmetric unit and its inverted version. For the sake of simplicity, we have only analysed one set of conformations, however the inverted versions (all torsions with opposite sign) will also be present. All crystal structures contain one molecule in the asymmetric unit ($Z'=1$) except for AH-B which contains two ($Z'=2$). Thus, two conformations (M1 and M2) are analysed for AH-B whilst only one is analysed for the rest of the forms.

Figure 4 Crystal conformations in MH, AH-A, AH-C, AH-B M1 and AH-B M2.

Table 6 Torsion values in crystal conformations of fluconazole in the MH, and AH-A, AH-B and AH-C crystal structures and the required torsional variations for the anhydrous crystal conformations to be converted to the MH crystal conformation.

Torsion (°)						
	τ_{Ph}	τ_{OH}	τ_{t1-A}	τ_{t1-B}	τ_{t2-A}	τ_{t2-B}
Torsion definition	2-1-12-13	1-12-13-34	13-12-14-17	12-14-17-18	13-12-24-27	12-24-27-28
MHcrys	173.7	157.8	63.4	101.1	65.5	102.1
AH-Acrys	185.1	43.4	74.6	101.2	73.0	99.7
AH-B M1crys	190.8	55.3	59.9	79.3	-55.5	-91.8
AH-B M2crys	187.7	48.4	60.5	76.9	-64.9	-83.9
AH-Ccrys	178.7	54.3	58.2	73.1	55.8	89.6
Torsional Variation (°)						
	$\Delta\tau_{Ph}$	$\Delta\tau_{OH}$	$\Delta\tau_{t1-A}$	$\Delta\tau_{t1-B}$	$\Delta\tau_{t2-A}$	$\Delta\tau_{t2-B}$
AH-Acrys to MHcrys	-11.4	114.4	-11.2	-0.1	-7.5	2.4
AH-B M1crys to MHcrys	-17.1	102.5	3.5	21.8	121.0	-166.1
AH-B M2crys to MHcrys	-14.0	109.4	2.9	24.2	130.4	-174.3
AH-Ccrys to MHcrys	-5.0	103.5	5.2	28.0	9.7	12.5

Analysis of the conformational data in Figure 4 and Table 6 revealed that the crystal conformations found in AH-A and AH-C are very similar to the MH crystal conformation. Only the acyclic hydroxyl group requires a conformational change, whilst the remaining torsions require only minor adjustments ($\Delta\tau < 30^\circ$). Rotation of the hydroxyl group should be facile since it would not be significantly hindered sterically, only a proton will change its orientation significantly. By contrast, both crystal conformations (M1 and M2) in AH-B require a conformational change in the $-OH$ group and significant changes in two torsions defining the

relative orientation of the second $-CH_2$ -triazole group. The conformational changes required to go from the AH-B crystal conformation to the MH crystal conformation are major and result in major shape changes in the fluconazole conformation (Figure 4).

To explore this further the conformer and adjustment energies for all crystal conformations in the gas-phase were calculated. (see section 9 of the Supplementary Information.) Upon geometry optimisation of all different crystal conformations, we identified three distinct conformers C1, C2 and C3 (Table 7).

Table 7 Conformer energies in the gas-phase and water and their correspondence with the various forms of fluconazole. Energies computed at the B97-d/6-31+G level of theory.**

Form Conformation	Gas Conformer	ΔE_{gas} (kJ/mol)	ΔE_{water} (kJ/mol)	$\Delta \tau_{\text{OH}}$ (°)	$\Delta \tau_{\text{t2-A}}$ (°)	$\Delta \tau_{\text{t2-B}}$ (°)
AH-A AH-C	C1	0.0	4.8	67.5	62.8	93.7
AH-B _{M1} AH-B _{M2}	C2	3.3	1.8	57.6	-70.7	-99.5
MH	C3	4.7	0.0	183.0	62.6	95.8

The three conformers have similar stabilities, all being within 5 kJ/mol of the most stable conformer. Interestingly, the order of stabilities changes with the solvation environment. For example, the MH conformer, C3, is the most stable in water but the least stable in the gas-phase. By contrast, AH-A conformer, C1, is the most stable in the gas-phase but the least stable in water whilst C2 has intermediate stability in both environments. C1 and C3 only differ in the orientation of the hydroxyl group whilst C2 differs from C3 in the orientation of the hydroxyl group and the orientation of the second $-\text{CH}_2\text{-triazole}$ group. C2 differs from C1 in the orientation of the second $-\text{CH}_2\text{-triazole}$ group. All these conformers require some level of conformational adjustments in the crystal structures of the different forms, those vary from just 4 kJ/mol to almost 10 kJ/mol, which is typical in molecular crystals.³⁹

Finally, we calculated the energy barriers for converting the conformer in AH-B (C2) into conformer AH-A/C (C1) via rotation of $\tau_{\text{t2-B}}$ first and $\tau_{\text{t2-A}}$ second and for converting conformer AH-A/C (C1) into conformer MH (C3) via rotation of τ_{OH} . The conformational energy pathway (Figure 5) reveals that the conformation found in AH-B needs to cross three energy barriers of 29, 14 and 11 kJ/mol (21, 24 and 18 kJ/mol in water) and rotate three bonds by a cumulative total of 450° to convert to the monohydrate conformer.

Figure 5 Conformational energy pathway for conformer interconversions. Calculations were performed at the B97-d/6-31+G** level of theory in the gas-phase (orange line) and using an SMD solvation model for water (black dashed line). In contrast, the conformers in AH-A/C only require the crossing of one energy barrier of 11 kJ/mol (18 kJ/mol in water) and rotate $\sim 100^\circ$ in the acyclic hydroxyl group. Thus, the conformational change required for the hydration process is significantly more dramatic for AH-B than AH-A/C.

Crystal Structure Variations

Looking at the hydrogen bonding interactions found in the various structures. AH-A forms discrete dimers and AH-B and AH-C chain motifs between fluconazole molecules (Figure 6 b-d). Those

hydrogen bond interactions were calculated to be stronger for the hydrogen bond chains (60 kJ/mol per HB) than for the hydrogen bond dimers (50 kJ/mol per HB). All fluconazole-fluconazole hydrogen bonds are disrupted by water in the monohydrate (Figure 6a). In the MH form, water interacts with fluconazole via three hydrogen bonds, a water environment commonly seen in organic hydrates.⁵⁰

Figure 6 Hydrogen bonding network found in fluconazole (a) MH, (b) AH-A, (c) AH-B and (d) AH-C.

Deriving crystal structure similarities for these forms was challenging; because the hydration occurs with changes in composition, expansion of cells and conformational changes, classic tools for structure similarity analysis (i.e. COMPACT algorithm)⁵¹ do fail to reveal detailed similarities and thus an expert analysis was done visually by overlaying molecular fragments with Materials Mercury.⁵²

The similarity between the anhydrous and monohydrate structures can be considered by comparing the position and orientation of the molecules within the structures. A molecule would be described to have positional and orientational similarity if the molecules in the anhydrous and MH structures were found to be at the same site and the functional groups were aligned. When assessing

positional similarity, the impact of the water molecules on the spacing of the fluconazole molecules was considered. The hydrogen bonded dimers found in the AH-A structure form a 2D layer parallel to the {111} planes. The molecules within this plane have both positional and orientational similarity with the MH (110) plane. The presence of water molecules in the MH structure increases the molecular distances between the fluconazole molecules within the dimer (Figure 7a and b). Looking three dimensionally, the molecules within the adjacent plane retain orientational similarity however they are positionally offset, therefore a translation of the plane would be required to match the MH structure. A high level of similarity can also be found by comparing the MH and AH-C structures. Here a common 2D layer consisting of parallel chains is found in the (200) plane of AH-C compared

to the (0-11) plane of the MH. Since the water in the MH structure sits offset from this plane, there is very little positional difference between the fluconazole molecules in the AH-C and MH structures (Figure 7e and f). There is also a degree of 3D similarity through the {002} planes, all molecules are found to have positional similarity and half of the molecules have orientational similarity.

Finally considering AH-B (Figure 7c) there is a degree of positional similarity with the MH structure (Figure 7d) found comparing the (200) plane of AH-B to the (100) plane for the MH. The similarity is restricted within four localised fluconazole molecules and does not extend to adjacent molecules in the (200) plane, these four molecules are found to repeat within this plane, each repeat being offset by approximately one fluconazole molecule.

Figure 7 Similarity found between anhydrous and MH structures. (a) AH-A (111) and MH (110) (b) AH-B (200) and MH (100) and (c) AH-C (200) and MH (0-11). Repeat distances and angles are measured from the central carbon (C12) using Mercury.⁵³ For AH-B vs MH the matching molecules are highlighted in red.

To understand the susceptibility of the crystal surface to hydration this packing similarity assessment can be expanded to consider external crystal faces. The predicted growth morphologies

for the various anhydrous forms are presented in Figure 8 and show reasonable agreement with those observed experimentally (see SEM images, Figure 2). For AH-C similarity with the MH

was found to be present on the dominant morphological faces, {002}. In AH-C the {002} faces represent 60 % of the total facet area in the calculated morphology (Figure 8c). Given that the form was crystallised from a polar solvent, we expect the triazole functionality to dominate the chemistry of the surface. On this surface, conversion of the hydrogen bonding network of AH-C to that of the MH may be readily achieved via some surface expansion accompanied with the incorporation of the water.

For AH-A the similarity to the MH structure was found within the {111} faces. However, these faces represent only 6% of the total predicted crystal morphology (Figure 8a) and they were not observed in the experimental morphology (Figure 9) (see section 2 of the Supporting Information for full indexing of AH-A and a new crystal structure determination at 296K). The localised similarity found between AH-B and the MH is predicted to be within an internal plane. Breakage of the AH-A and AH-B crystals during grinding would be consistent with exposure of the {111} planes of AH-A and {200} planes of AH-B to the surface.

Figure 8 Predicted growth morphologies of (a) AH-A, (b) AH-B and (c) AH-C.

Figure 9 Face indexing of AH-A

4. DISCUSSION

We have performed some experimental hydration studies on three anhydrous polymorphs of fluconazole (AH-A, AH-B and AH-C) at 54.5 and 75.3 %RH. Despite the RH value being above the critical water activities for all polymorphs, hydration was not observed at 54.5 %RH unless the system was activated by seed addition or grinding. At 75.3 %RH, hydration was observed most significantly for AH-C, then AH-A and only marginally for AH-B. The sigmoidal shape of the hydration isotherms reveals an initial induction period, possibly governed by the required nucleation of the MH, followed by a growth period;⁵⁴ this was particularly evident for AH-A and AH-C (Figure 3). Hydration slows towards the end; it can be presumed that this is because hydrating zones start to overlap.

Seeding or grinding of the samples considerably accelerated the hydration process for all forms. At 75.3 %RH the kinetics of hydration for AH-A closely mirrors that of AH-C, with AH-B becoming considerably more susceptible to hydration. Most interestingly, both the seeding and the grinding seem to eliminate the

induction period required prior to hydration of AH-A and AH-B. Thus, both crystal size reduction or the presence of MH crystals allow for a much more facile nucleation of the hydrate on the surface of the anhydrous crystals. Additionally, the enhanced slopes of the hydration curves, observed with seeding and grinding, suggests different propagation rates once nucleation has occurred, although again AH-B behaves differently with its rate remaining relatively low.

The accelerating impact of crystal size reduction underlines the importance of crystal surfaces in the nucleation process; higher surface areas offering greater probability of a nucleation event. The precise mechanism of such an event is however, more difficult to visualise. One potential route might be condensation of water on the surfaces facilitating a solution mediated pathway. However, this seems less likely since the anhydrous forms of fluconazole were found to be non-hygroscopic (see section 5.5 of the Supporting Information). For AH-A and AH-B, even estimating a low surface area, the water content at 75.3 %RH is not sufficient to form a monolayer coverage of water on the crystal surface. This leads us to explore more fully potential solid-state processes which might be possible.

To aid the discussion of the impact of crystal structure on the hydration kinetics of the different polymorphs, we have summarised the results of our kinetic studies, structural analysis and energy calculations in Table 8.

Clearly, at a basic level the hydration rates are concomitant with the level of similarity between the anhydrous and hydrated forms. The conformational and packing similarity found between the AH-C and MH structures is consistent with AH-C demonstrating the greater ease of hydration. AH-A has 2D similarity with the MH, this does not extend in three dimensions without movement of adjacent molecular layers. Interestingly striations are seen within the crystals following hydration of AH-A, (Figure 10b) suggesting a degree of shear in the crystal during hydration. The direction of these striations is consistent with movement aligned to the {111} planes. The conformational and relatively more modest packing similarity found between AH-A and the MH is in line with the intermediate hydration behaviour of AH-A.

The lack of conformational similarity and only localised packing similarity between the AH-B and MH structures provides a plausible explanation for the kinetic stability of AH-B to hydration. During hydration the molecular conformation, packing and hydrogen bonding pattern of the anhydrous form will need to change to match that of the hydrate. Thus, it seems reasonable that a closer structural similarity with the MH, would facilitate an easier hydration process. The fact that AH-B has the smaller particle size (Figure 2) yet demonstrates the greater kinetic barrier to hydration, shows that the hydration rate cannot be purely related to surface area and particle size; molecular conformation and packing can also be influential.

Looking at the overall thermodynamic drive, we observe a correlation between the ease of hydration and the forms relative order of stability (Table 8). Thus, the least stable form, AH-C, hydrates faster than the intermediate form, AH-A, which also hydrates faster than the stable form, AH-B (ΔH_{form} in Table 8).

Considering other energetic factors which may be controlling the kinetics of hydration: a) the highest energy barrier required for conformational conversion and b) the absolute value of the attachment energy for the softest crystal plane (lowest attachment energies). The first value relates to the maximum energy barrier required to convert the conformation of the anhydrous form to the conformation of the hydrate. This conformational energy barrier is just ~ 11 kJ/mol for AH-A and C but 29 kJ/mol for AH-B (Table 8). The second value relates to the quantification of the softest

plane in the crystal structure. Attachment energies have been shown to correlate with the kinetics of solvate formation for theophylline.⁵⁵ Softer planes may allow an easier breakage of crystals or diffusion of solvent into the crystal structure to produce the hydration process. We note that microscopic images of our samples, after the hydration process reveal only small changes in the morphologies upon hydration. The morphologies remain very well defined, (Figure 10). Examination of the hydrated crystals, between crossed polarising filters, confirmed that the hydration process has completed in a single-crystal to single-crystal manner.

Figure 10 Photomicrographs taken to compare the physical appearance before and after hydration at 75 %RH. (a) AH-A and (b) MH formed from hydration of AH-A. (c) AH-B and (d) MH formed from hydration of AH-B when seeded with MH. (e) AH-C and (f) MH formed from hydration of AH-C.

Thus, we expect that polymorphs with softer planes would have faster kinetics of hydration since diffusion of the water into the structure would be more facile. These values are reported in Table 8. AH-C has the softest plane (smallest $-E_{att}$ value) followed by AH-B and AH-A. In this case, the values do not correlate with the kinetics because conformational changes are not accounted for in the attachment energies alone. We then calculate the overall kinetic energy barrier as the sum of the absolute value of the attachment energy plus the conformational energy barrier. This overall value is calculated to be 43, 56 and 31 kJ/mol for AH-A, B and C respectively and correlates very well with our observed kinetics of hydration of our original crystals. We notice, however, that the conformational changes required may be much more energetically costly within the crystal lattice, especially for those required for the AH-B form, where significant geometrical changes are required. Our gas-phase (or water) calculations combined with the attachment energies, however, are a convenient approximation. On grinding the crystals, the diffusion control of hydration becomes less dominant and the kinetics become controlled by the conformational energy barrier. Thus, on grinding both the AH-A and AH-C show almost identical behaviour.

5. CONCLUSIONS

We have studied the hydration kinetics of three anhydrous forms of fluconazole: AH-A, AH-B and AH-C. AH-B was found to demonstrate the greater kinetic barrier to hydration than AH-A or AH-C. These kinetic studies, combined with a crystal packing, molecular conformational similarity analysis and energy calculations have revealed some important conclusions. Firstly, experiments showed that the hydration rates of ground and seeded (with MH) samples are significantly increased relative to the as-crystallised materials. In the case of grinding considerable impact on the average size of the anhydrous crystals is evident while the crystallinity of the forms is maintained (neither pXRD nor DSC analysis show evidence of detectable amorphisation). These observations are accompanied by changes in the hydration profiles (the shape of the conversion-time curves) and suggest that three processes may be controlling the kinetics of hydration: a) diffusion of water inside the crystal lattice, b) nucleation of the monohydrate at crystal surfaces and c) growth of the monohydrate until full conversion is attained. The first two processes may be clearly influenced by grinding and seeding whilst growth may be more related to water diffusion and the similarity between the anhydrous forms and the MH.

A comparison of the crystal packing and molecular conformations between the anhydrous forms and the MH revealed that AH-C has the closest degree of similarity to the MH followed by AH-A and AH-B. This correlates well with the observed relative hydration kinetics. Calculation of conformational similarities and energy barriers revealed that, when compared to AH-A and AH-C, the AH-B conformation has to cross many more and higher energy barriers to convert to the MH conformation. This is consistent with the fact that AH-B shows the slowest hydration rate. The attachment energy values for the different planes in the various forms suggest which anhydrous form will be easier to break upon grinding and indicate the ease of water diffusion into the lattice. It was found that the combination of conformational and packing differences provide an overall kinetic energy barrier to hydration of AH-A, AH-B and AH-C. The values of this energy barrier correlate very well with the observed hydration kinetics.

In summary the hydration behaviour of AH-A, AH-B and AH-C is aligned with their conformational and packing similarities relative to the MH structure. Surface similarity may be required for nucleation whereas a higher level of packing similarity allows for growth of the hydrate. Where there is little surface similarity or structural similarity, as with AH-B, there is a greater level of kinetic stability.

ASSOCIATED CONTENT

Supporting Information

A new crystal structure for AH-A at 296K including crystallographic information and crystal indexing (CCDC Deposition Number 1951951). Form identification by pXRD for crystallised solid forms. Determination of the critical water activity (slurry method). Physicochemical properties for each polymorph including, TGA, DSC scanning electron microscopy images (where not included in manuscript). Hygroscopicity of anhydrous polymorphs. Hydration of anhydrous forms; sample weight taken during a six months exposure at 54.5 and 75.3 %RH with form identification for final samples. Environmental pXRD on AH-A and AH-C at 90 %RH and 25 °C. Adjustment and conformer energies for the monohydrate and the three polymorphs studied.

The Supporting Information is available free of charge on the ACS Publications website.

Table 8 Summary of hydration kinetics, structural similarity and energetics analysis for AH-A, AH-B and AH-C in regard to the monohydrate.

		Anhydrous Forms		
		AH-A	AH-B	AH-C
Experimental Hydration Kinetics	Hydration Rate (α /day) 75% RH, as cryst.	0.0109	0.0008	0.0473
	Hydration Rate (α /day) 75% RH, ground.	0.1329	0.0048	0.1152
Structural Similarity	Conformational Similarity	Similar	Very Different	Similar
	Required Conformational Rotations	OH by $\sim 100^\circ$	triazole by $\sim 170^\circ$ OCH-CH ₂ by $\sim 125^\circ$ OH by $\sim 100^\circ$	OH by $\sim 100^\circ$
	Packing Similarity	2D similarity, dimers	Localised	3D similarity
	Surface Similarity	None	None	Dominant face
Energetics Thermodynamics	ΔH_{form} (kJ/mol) (T=138-139 °C)	2.4	0.0	3.6
Energetics Kinetics	Conformational Energy Barrier (kJ/mol)	10.7	29.0	10.7
	-E _{att} Softest hkl plane (kJ/mol)	32.9*	27.0*	19.9*
	Overall Energy Barrier (kJ/mol) [§]	42.6	56.0	30.6

* Those being the {001}, {020} and {002} for AH-A, AH-B and AH-C respectively. [§]This is calculated as the sum of the conformational energy barrier and the -E_{att} of the softest plane.

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Author Contributions

PAB has designed and executed most experiments as well as major contributions to the writing of the manuscript. KRB and MC have contributed crystallography and SEM imaging expertise respectively. AJCC has contributed with computational expertise, project design discussions and manuscript writing. RD and RJD have contributed with work design discussions and manuscript revision..

Notes

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The Impact of Crystal Structure and Molecular Conformation on the Hydration Kinetics of Fluconazole

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SYNOPSIS TOC

Three polymorphs of fluconazole exhibit significantly different kinetic behaviour towards hydration. Here we demonstrate that conformational variations, seeding and particle size reduction all impact the hydration kinetics of the fluconazole polymorphs. Larger conformational changes seem to impact the hydration kinetics most significantly.

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