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Determining the relative physical stability of anhydrous and hydrous crystal forms of GW2016

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Abstract

The slurry equilibration method used to determine the relative physical stability of polymorphs is extended to a crystal system of an anhydrate and hydrate. The method involves preparation of organic/aqueous slurries of known water activity containing mixtures of the anhydrate and hydrate forms. The slurries equilibrate to the lowest free energy form, from which the relative physical stability is determined as a function of relative humidity. The method is particularly valuable when one or more of the crystal forms is kinetically stable, since solvent-mediated transformation accelerates the conversion process. Results are provided for GW2016 anhydrate and monohydrate crystal forms, for which it was determined that interconversion occurs between 7 and 15% relative humidity.

Keywords: GW2016; Anhydrate; Hydrate; Crystal form; Relative physical stability; Crystal form selection

1. Introduction

Polymorph screening of new chemical entities is a key element of pharmaceutical development. This step preferably transpires early in development in order to establish the polymorph space of molecule and ultimately to find the optimum crystal form. Although it is not essential and may even be undesirable or impossible, the preference is to develop the thermodynamically stable crystal form over the temperature/relative humidity range to which the drug will be subjected.

Several methods exist to assess the relative physical stability of polymorphs. The solubility method is the most common approach, but only works when the crystal forms do not transform during the time course of the study (Grant and Higuchi, 1990). Another problem with this method is that some crystal forms may have solubilities too close to discriminate experimentally. Thermal methods can also be used, and are a valuable complement to solubility measurements if it is difficult to find the right solvent to use in the study (Grunenberg et al., 1996; Burger and Ramberger, 1979a,b; Sacchetti, 2000).

A third method, which is less often considered, can actually be the most definitive approach in ranking the stability of polymorphs. This method, which is termed slurry equilibration in this article, involves preparation of a suspension containing a mixture of two polymorphs in an appropriate solvent. The slurry is stirred at a set temperature and monitored for changes in crystal form by a definitive technique such as powder X-ray diffraction (pXRD). The solvent is chosen such that the compound has adequate solubility to provide a solvent-mediated transformation from the higher to the lower free energy form. In effect, the crystals of the

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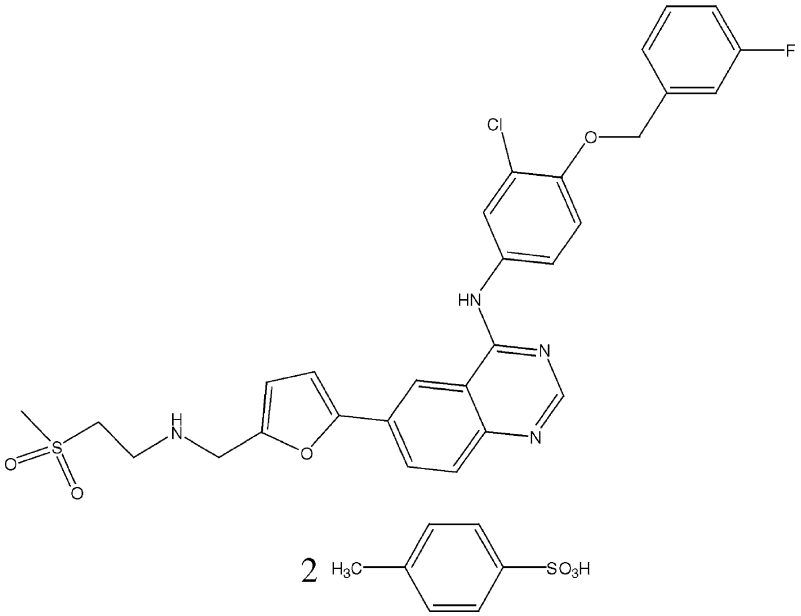

Fig. 1. Molecular structure of GW2016.

more stable form remove the nucleation barrier of the dissolved compound and the transformation becomes rate-limited by dissolution of the less stable form crystals or by crystal growth, which is generally rapid for nonviscous solvents near room temperature.

In this research, the slurry equilibration method was generalized in its use beyond polymorphs to a system of anhydrous and hydrous crystal forms. In some cases, the conversion between a hydrate and anhydrate may be sufficiently rapid that the relative physical stability is readily assessed during the course of a moisture sorption study. In other cases, however, the anhydrous and hydrous crystal forms are kinetically stable, and it is not easy to determine the relative humidity at which they interconvert. Kinetic stability can be a major problem when one only has 2–3 months to decide which crystal form to select and take to the clinic. The generalized slurry equilibration method presented in this article provides a rapid means to determine the relative physical stability of anhydrous and hydrous crystal forms, which enables a better decision to be reached sooner in selecting crystal form. The method was applied specifically to GW2016 (see Fig. 1), which exists in one anhydrate and one monohydrate crystal form. The theoretical basis for this method is examined in this article, which serves to underscore the key assumptions underlying the analysis.

The influence of water activity in a crystallization medium composed of an organic/water cosolvent system was previously examined for theophylline (Zhu et al., 1996) and ampicillin (Zhu and Grant, 1996). This work was mainly directed at studying crystallization from solution, but the basic principles of examining water activity can be used for slurry equilibration to determine the relative humidity under which a hydrate becomes more thermodynamically stable than an anhydrate or lower hydrate. Seeding experiments in the study of ampicillin anhydrate and trihydrate in a methanol/water mixture demonstrated the importance of having both forms present to overcome kinetic stability of a metastable form.

2. Theory

The essence of the slurry equilibration method is that conducting the process in a liquid phase can accelerate the slow transformation kinetics of crystals surrounded by vapor, since a solvent-mediated conversion is possible (Zhu et al., 1996; Zhu and Grant, 1996). The liquid phase must then contain water at a

well-defined thermodynamic activity that can be easily varied. Cosolvents comprised of organic and aqueous components are perfect systems, since the partial vapor pressure of water—or, equivalently, RH—and hence thermodynamic activity has been measured for several mixtures containing organic solvents routinely used in pharmaceutical laboratories. Water composition can be easily varied to scan the relative humidity range from 0 to 100%.

It is important to establish the conditions under which a crystal surrounded by air/H_2O remains unchanged when submersed in an organic/H_2O liquid. Fig. 2 illustrates the process of moving a crystal from an air/H_2O vapor phase (initial state) to an air/organic/H_2O vapor phase (intermediate state) and then submersing it into an organic/H_2O liquid phase (final state). Thermodynamically, the crystal will be identical in the initial and final states if the changes in chemical potentials of the components of the crystal and the overall free energy change of the solid are zero. The total molar free energy of a solid phase (G^s) at any temperature and pressure is

$$G^s = X^s_d \mu^s_d + X^s_w \mu^s_w + X^s_o \mu^s_o \quad (1)$$

in which X represents the mole fraction, μ the chemical potential, s the solid, d the drug, w the water and o the organic. From thermodynamics, the Gibbs–Duhem equation for the solid phase relates changes in chemical potential to one another according to

$$X^s_d \, d\mu^s_d + X^s_w \, d\mu^s_w + X^s_o \, d\mu^s_o = 0 \quad (2)$$

First, Eq. (2) is applied to step 1 in Fig. 2. In the initial state (air/H_2O), $\mu^s_o = 0$, and by the assumption that the organic component does not absorb significantly into the solid phase, i.e., $X^s_o \sim 0$, μ^s_o is also small in the intermediate state (air/organic vapor/H_2O phase), implying $X^s_o \, d\mu^s_o \sim 0$, since both terms are small. The water chemical potential is identical in both the initial and intermediate states, so the second term in Eq. (2) is zero. In order for the Gibbs–Duhem relation to hold, $d\mu^s_d = 0$ (since X^s_d is not zero). In physical terms, the chemical potential of the drug does not change for step 1 because the composition of the solid remains constant; that is, there is exactly zero change in the mole fraction of water and (by assumption) only a small change in mole fraction of the organic component, leading to only a small change in mole fraction of the drug. Therefore, the total molar free energy

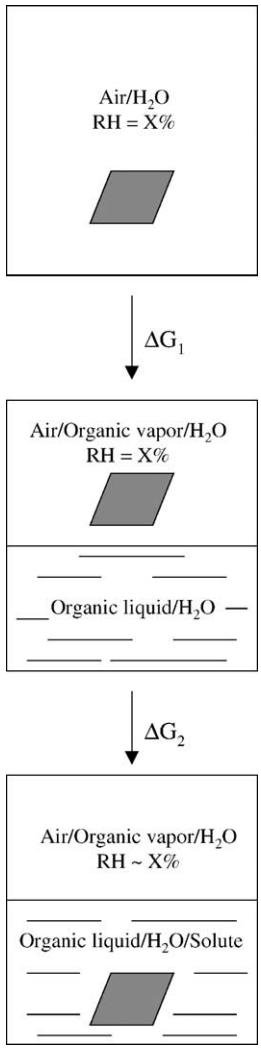

Fig. 2. Schematic illustration of the process of moving a crystal from an air/H_2O phase (initial state, top panel) to an air/organic/H_2O vapor phase (intermediate state, middle panel) to an organic/H_2O liquid phase (final state, bottom panel). The RH over the liquid matches that of the air/H_2O phase by selection of the organic/H_2O composition.

change of the solid for step 1 (ΔG^s_1) is zero, since there is no change in mole fractions and given that at constant temperature and pressure,

$$dG^s_1 = \mu^s_d \, dX^s_d + \mu^s_w \, dX^s_w + \mu^s_o \, dX^s_o \quad (3)$$

Second, Eq. (2) is applied to step 2 in Fig. 2. The key feature in step 2 pertains to the solubility of the drug in the organic/H_2O mixture. When the drug is submersed

in the liquid, it in general dissolves and interacts with the water and organic components, thereby changing their chemical potentials. Most notably, the RH of the solution in the final state would be altered from that in the intermediate and initial states as depicted in Fig. 2. However, if the drug's solubility is sufficiently small, then the water and organic chemical potentials will be only slightly affected, so they will have values similar to those in the intermediate state, since at equilibrium the chemical potentials of the components are identical in the liquid and vapor phases. The Gibbs–Duhem equation then dictates that $d\mu_d^s \sim 0$ (X_d^s is not zero) in passing from the intermediate state to the final state in Fig. 2. The same reasoning for step 1 leads to the conclusion that $\Delta G_2^s = 0$.

Thus, there is no change in chemical potentials and the overall free energy ($\Delta G^s = \Delta G_1^s + \Delta G_2^s$) of a solid exposed to air/H_2O and submersed in a cosolvent of the same relative humidity, assuming (1) small penetration of organic solvent into the solid phase and (2) small solubility of the drug in the cosolvent. It is possible then to determine the relative physical stability of anhydrates and hydrates in a liquid rather than a vapor phase.

Absorption of organic solvents into a solid phase is generally not studied in the development of a drug, but information is obtained through solubility studies in organic solvents and in residual solvent testing. Obviously, the slurry equilibration method would need to be conducted in a solvent for which there is no solvate formation.

The assumption of low solubility of the drug in the organic/H_2O vehicle is not necessary but it does simplify application of the method. If the solubility is low, the calculated RH based on literature data for organic/H_2O mixtures will be accurate. The study could be done in mixtures for which the solubility is high, but it would be necessary in such a case to measure RH if high accuracy is desired. The case of GW2016 will illustrate that the conclusion is not significantly impacted by high accuracy in the RH value.

3. Materials and methods

3.1. Materials

GW2016 anhydrate and monohydrate forms were sourced from GlaxoSmithKline (Research Triangle Park, NC) in high purity. Methanol (MeOH) was used as the cosolvent and was obtained in high purity from Burdick and Jackson (Muskegon, MI). House RO water was used.

3.2. Methods

MeOH/H_2O mixtures were prepared by volume and composition was converted to mole fraction using molecular weights and room temperature densities (0.787 g/ml for MeOH and 1.00 g/ml for H_2O). Water activity (a_w) was calculated from (Zhu et al., 1996)

$$a_w = 0.0056 + 1.398X_w - 0.647X_w^2 + 0.153X_w^3 + 0.0845X_w^4 \quad (4)$$

A 1:1 ratio (25 mg/ml anhydrate and 25 mg/ml monohydrate) of both crystal forms was added to vials and reconstituted with the MeOH/H_2O mixtures. After initial mixing, an aliquot was removed and dispensed for analysis by pXRD (model PADV, Scintag, Cupertino, CA) to ensure that peaks of both crystal forms were detectable. The pXRD analysis was conducted on the wet slurry sample—deposited onto zero background quartz plates—to minimize any potential effects of drying. The X-ray equipment was operated at 45 kV/40 mA, using a Cu Kα radiation source with a Peltier-cooled solid state Si(Li) detector. Data was collected at 0.03° digital resolution and scanned from $2\theta = 2^\circ$ to 40° at $2\theta = 1^\circ$ for 1 min. Samples were stirred and equilibrated at 25 °C in a water bath. Sufficient sample was prepared to assay approximately five later timepoints by pXRD. There was no preset schedule for the timepoints except the first one, which was set at 1 day. Later timepoints were set according to observations on the rate of transformation up to the previous timepoint. As it turned out, the transformation appeared to be essentially completed by 1 day, so the only other analysis was conducted at 7 days. As no change in the pXRD patterns was observed between 1 and 7 days, equilibrium was judged to be attained.

4. Results and discussion

The solubilities (at 25 °C) of GW2016 anhydrate and monohydrate are very close in the MeOH/H_2O mixtures and have their maximum value in pure

Table 1
Accelerated RH stability results for GW2016 anhydrate and monohydrate in MeOH/H_2O mixtures at 25 °C

Water volume (%)	Water weight (%)	Water mole fraction	RH (%)	Equilibrium form
0	0	0.00	0	Anhydrate
2	2.5	0.04	7	Anhydrate
5	6.3	0.11	15	Monohydrate
10	12	0.20	26	Monohydrate
20	24	0.36	43	Monohydrate
40	46	0.60	66	Monohydrate
60	66	0.77	80	Monohydrate
80	84	0.90	91	Monohydrate
90	92	0.95	95	Monohydrate
100	100	1.00	100	Monohydrate

MeOH, which is 2.0 mg/ml. Given the molecular weight of the free base (581 g/mol), the molar solubility is 3.4 mM, which, even if it were this high in the mixtures, would not be expected to alter the water activity significantly from the values calculated in Eq. (4) (i.e., no change in RH over the solution). (The solubilities for both forms in pure water are <0.001 mg/ml.) There was no observed decomposition (by HPLC) of the drug in the MeOH/H_2O mixtures during the 1-week period for the solubility measurement.

The results in Table 1 illustrate the crystal form conversion pattern as a function of calculated water activity/RH. The transformation rate was very rapid, as observed by pXRD, not changing from the 1-day timepoint onward. The pXRD patterns are provided in Fig. 3. The top panel shows the results for the pure crystal forms of the anhydrate and monohydrate. The middle panel illustrates that the 1:1 mixture converted to the anhydrate in the liquid phase with a water activity equivalent to 7% RH. Likewise, the bottom panel demonstrates that the monohydrate is the stable form at a water activity equivalent to 15% RH. The summary in Table 1 notes that in general, the monohydrate becomes the thermodynamically stable form somewhere between 7 and 15% RH and remains stable up through 100% RH. Thus, the monohydrate is judged to be the best crystal form to develop in terms of relative physical stability.

It is possible that the transformation was incomplete at one day, since a limit of detection of the two forms was not established by this pXRD method. It is clear, however, from the pXRD patterns that a significant amount of change occurred, particularly based on the major peaks at ~4.5° for the anhydrate and ~6.5° for the monohydrate form. In addition, no further changes were observed by pXRD from 1 to 7 days. The presence of a slowly transforming residual amount of undetected anhydrate in the monohydrate or vice versa does not impact the conclusion on relative physical stability of the forms. A complementary technique such as KF was not used, since historical batch data had always confirmed that powders with the pXRD pattern of the anhydrate form always contained at most a few tenths of a percent of water; correspondingly, powders with the pXRD pattern of the monohydrate form always contained 1.9% water, which is the theoretical value based on 1:1 stoichiometry.

The anhydrate form is kinetically stable. Samples stored in desiccators (standard vapor phase studies) as high as 25 °C/90% RH did not convert to the monohydrate at the 3-month timepoint, which is a typical timeframe in which to select crystal form. Thus, the slurry equilibration method enabled a quick determination of the relative thermodynamic stability of the crystal forms that was not possible by other means.

The generalized slurry equilibration method can be applied to drugs with larger solubility in organic/aqueous mixtures. The larger the solubility the lower the confidence will be in the calculated RH using Eq. (4). Considering an ideal chemical interaction, the presence of dissolved solute in the liquid phase will lower the chemical potential of H_2O and hence RH, by reduction in the water mole fraction. For example, in the case of GW2016, the conclusion that the monohydrate becomes thermodynamically stable above 7–15% RH would be in error; the actual RH over the solutions would be lower. In cases such

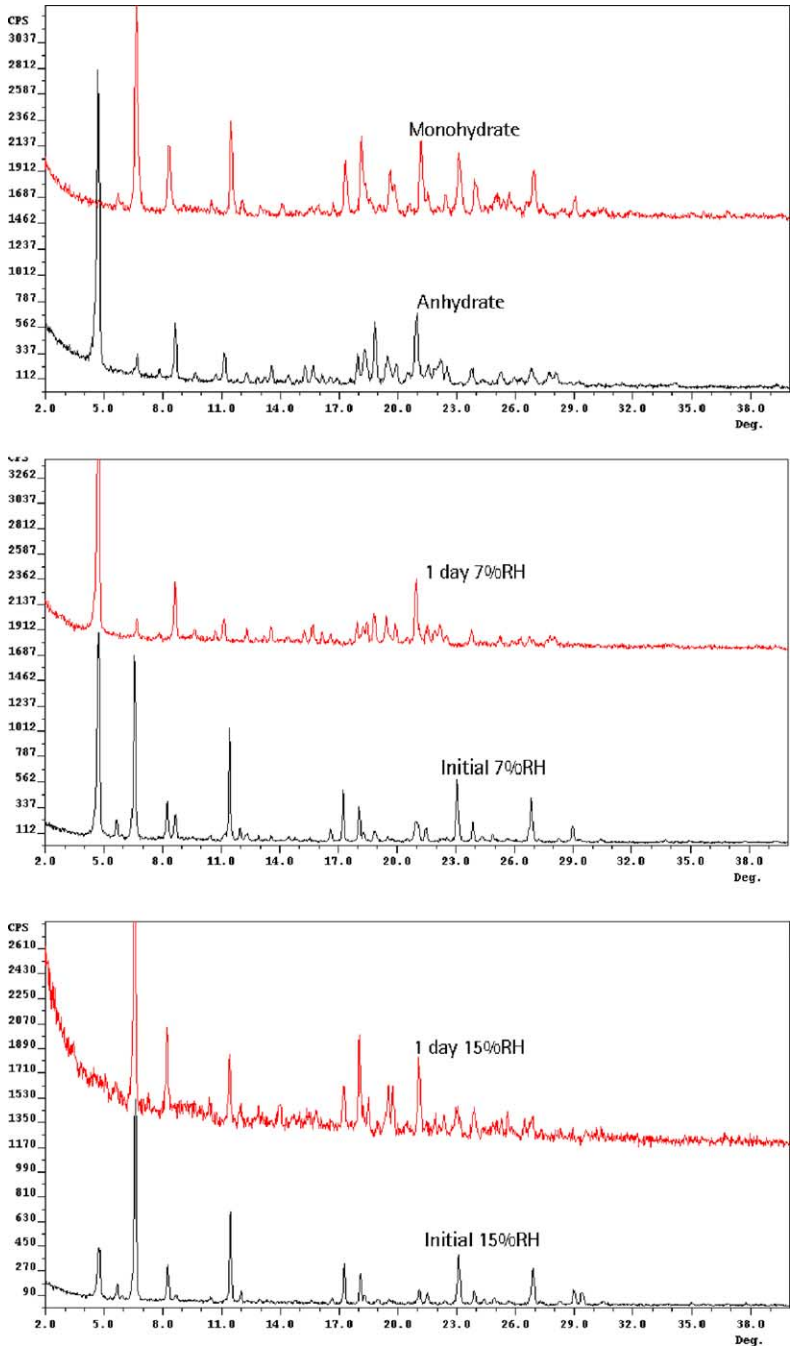

Fig. 3. The pXRD results for GW2016 anhydrate and monohydrate crystal forms. Top panel shows the pXRD patterns for the pure crystal forms. Middle panel shows the initial and 1 day results for a slurry with water activity equivalent to 7% RH. Bottom panel shows the initial and 1 day results for a slurry with water activity equivalent to 15% RH.

Table 2
Reduction in water mole fraction in a three-component MeOH/H_2O/drug system for increasing solubility of a 500 g/mol molecular weight drug

Water/MeOH system		Mole fraction of water in water/MeOH/drug system		
MeOH (%, w/w)	X_w	$S_d = 10$ mg/ml	$S_d = 100$ mg/ml	$S_d = 500$ mg/ml
0	1.000	1.000	0.996	0.965
2	0.989	0.988	0.985	0.954
5	0.971	0.971	0.967	0.936
10	0.941	0.941	0.937	0.906
20	0.877	0.876	0.873	0.841
40	0.727	0.727	0.724	0.692
60	0.542	0.542	0.539	0.511
80	0.308	0.308	0.305	0.285
100	0.000	0.000	0.000	0.000

as GW2016, this error does not affect the quality of the decision in the crystal form selection. In other cases, however, it may be necessary to measure the RH to have an accurate value.

In order to better understand the role of solubility in altering the RH, it is necessary to calculate the decrease in mole fraction of water as the drug's solubility increases. The mole fraction of water in the three-component system can be calculated from

$$X_w = \frac{\Omega_w/M_w}{\Omega_w/M_w + \Omega_o/M_o + S_d/1000\rho_s M_d} \quad (5)$$

in which Ω_w is the weight fraction of water, Ω_o the weight fraction of organic (Ω's are for the three-component system), S_d the drug solubility (in mg/ml), ρ_s the density of the solution (g/ml), M the molecular weight and all subscripts are the same as defined previously. Further, the weight fractions are calculated from

$$\Omega_w = \frac{1 - S_d/1000\rho_s}{1 + \Omega_w/\Omega_o} \quad (6)$$

and

$$\Omega_o = 1 - \Omega_w - \frac{S_d}{1000\rho_s} \quad (7)$$

Note that the weight ratio Ω_w/Ω_o in Eq. (6) is a constant for changes in drug solubility and can be calculated from the weight fractions of the organic/H_2O mixture. The solution density is in general not measured, but will be estimated for the purpose of this calculation by the MeOH/H_2O density, which is tabulated at 20 °C (Perry et al., 1984).

Water mole fraction results are given in Table 2 for a drug with molecular weight 500 g/mol. Table 2 illustrates that not until the drug's solubility reaches 500 mg/ml, does one observe significant changes in the mole fraction of water. It is not possible to calculate the RH of the three-component system, but the small changes in mole fraction of water indicate that it would not change significantly until the drug's solubility exceeded 100 mg/ml. Note that the smaller the molecular weight of the drug, the bigger the impact on water mole fraction. Eqs. (5)–(7) can be used for the specific parameters associated with any individual case of organic solvent and drug to provide the estimate for water mole fraction changes.

5. Conclusions

The generalized slurry equilibration method was successfully applied to an anhydrate/monohydrate crystal system for GW2016. It provided a rapid conversion process (1 day) that determined the relative physical stability of the crystal forms as a function of RH, which was not possible to assess by standard means (i.e., storing powder in vials in desiccators) due to kinetic stability. The method is accurate when the solubility of a drug is low in the organic/aqueous medium. The method can be used for higher solubility compounds, but, based on theoretical considerations, the RH at which interconversion of the crystal forms transpires would be less accurate and would need to be measured if high accuracy is required.

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