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# USE OF ISOTHERMAL MICROCALORIMETRY IN PHARMACEUTICAL PREFORMULATION STUDIES
## Part I. Monitoring crystalline phase transitions

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## Abstract

Solid-state transformation kinetics of two crystal forms of a synthetic tetrapeptide was monitored by isothermal microcalorimetry and complementary solid-state analytical techniques. Form B, the crystal form obtained from the synthetic process transformed to Form D upon exposure to higher relative humidity conditions (>60% RH). The transformation occurred rapidly at higher temperature, and relative humidities. An intermediate phase of very low crystallinity (amorphous-like phase) was observed when transformation was slow. Form D was observed to be physically stable at higher relative humidities and thus the preferred crystal form for development. Exposure of Form B to high relative humidity was the only process through which Form D was obtained. Optimal conditions for transformation of Form B to Form D were determined by microcalorimetric experiments and were used for larger scale processing of Form B to Form D.

**Keywords:** amorphous, crystallization, microcalorimetry, polymorphism, solid-state transformation

## Introduction

Pharmaceutical compounds commonly exist in various solid forms such as crystalline polymorphic forms, solvates or amorphous forms. Preformulation characterization of an active pharmaceutical ingredient (API) involves screening for various crystal forms, characterizing their properties and establishing thermodynamic stability of various solid forms leading to the selection of an optimal form for formulation development. Though most solid forms are discovered during initial screening, it is not uncommon to discover more stable forms (polymorphs or solvates) during chemical process scale up or sometimes even in marketed formulations. Different crystalline forms (polymorphs or solvates) differ in their crystal packing leading to differences in lattice energy and entropy. These differences result in significant differences in physical properties such as stability, solubility, dissolution rate, and perhaps color [1]. Hence, it is important to carefully select a crystalline form with desirable properties and stability such that crystalline transformations do not occur during the life of a drug product. Since the physical form of the drug can have profound impact

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on in-vitro and in-vivo performance of a drug product, regulatory agencies increasingly stress the importance of use of appropriate analytical methods for detection of various solid forms of an active substance and their control during different stages of drug development [2]. Various analytical techniques such as calorimetry, powder X-ray diffraction, Raman and NMR spectroscopy are available for characterization of solid forms. Of the many techniques available, isothermal heat conduction microcalorimetry is being most widely used to probe a variety of solid-state reactions and has become an established tool in preformulation research [3, 4]. The instrument consists of a small closed reaction vessel in contact with a large heat sink such that the reaction vessel is at a constant temperature. Heat flow sensors located between the reaction vessel and heat sink measure heat generated due to a physical or chemical reaction [3, 4]. The thermal power $P=\mathrm{d}Q/\mathrm{d}t$ due to the reaction is recorded and monitored as a function of time [3, 4].

The case study presented here involves a development candidate, which is a synthetic tetrapeptide with very high water solubility. Initial preformulation screening of the compound resulted in identification of three crystalline forms designated as A, B and C. Forms A and C were metastable and solvated forms. Further it was difficult to remove solvent of crystallization from these two forms therefore they were not considered for further development. Form B was extremely hygroscopic and during routine stability analysis, Form B was found to be transforming to a new solid form under high humidity conditions, which was designated as Form D. Form D had adequate physicochemical properties for product development but was obtained only through transformation of Form B on exposure to high humidity conditions at temperatures >40°C. This report details the utility of IMC in monitoring the conversion process and selection of optimal conditions for large scale batch production of Form D from Form B. For complete understanding of the nature of conversion and themodynamic stability of the forms, complementary techniques of powder X-ray diffractometry (XRD), thermal analysis and water vapor sorption analysis were utilized.

## Materials and methods

Antisolvent precipitation was used for obtaining Form B, which was used as received. Form D was obtained by exposing Form B to 40°C/80% RH for 2 weeks.

### *Thermal analysis*

A Seiko DSC 220 differential scanning calorimeter was used for thermal analysis. Samples in covered aluminum pans were scanned from 20 to 220°C at 5°C $\mathrm{min}^{-1}$ under a nitrogen purge of 50 mL $\mathrm{min}^{-1}$.

### *Powder X-ray Diffractometry*

Powder diffraction patterns were obtained by exposing approximately 60 mg of sample to $\mathrm{CuK}_{\alpha}$ radiation (40 mA×45 kV) using a Bruker D5000 powder X-ray diffractometer. Samples were size reduced gently prior to analysis to eliminate particle size effects on

peak intensities. Diffraction patterns were obtained between an angular range of 3–40°2θ, with a step size of 0.04°2θ and counts were accumulated for 1 s at each step.

*Moisture sorption isotherms*

Moisture sorption isotherms were obtained by exposing approximately 15 mg of the sample to various humidity conditions in a DVS 100 moisture balance.

*Isothermal microcalorimetry*

A Thermometric® Thermal Activity Monitor (TAM) was used for isothermal microcalorimetric experiments. Solid-state transformation was characterized by exposing the two polymorphs to various relative humidity conditions at various temperatures. Approximately 100 mg of the material was weighed into a 3 mL glass ampoule. A microhygrostat containing saturated solutions of various salts was placed in the ampoule to generate the required relative humidity above the material. Saturated salt solutions of NaBr (65% RH), NaCl (75% RH), KCl (80% RH) and pure distilled water (100% RH) were used in the microhygrostats. Washed sea sand prepared similar to the sample was used as a reference. Microcalorimetric power output in μW was obtained as a function of time. Post TAM analysis of the material was performed by XRD.

## Results and discussion

Initial analysis of the two forms, B and D indicated them to be anhydrous. For selection of the most stable form for further development it was necessary to determine the relative stability (thermodynamic and kinetic) of the two forms. The stability determination was performed by physico-chemical characterization of solids using a number of analytical tools as described in the methods section. The two forms were crystalline and differentiated by their X-ray powder diffraction patterns as shown in Fig. 1. The most intense peaks of Form B and Form D were at 5 and 15.2°2θ, respectively. As shown in Fig. 1, the 15.2°2θ peak of Form D partially overlapped with Form B and hence whole patterns were used for monitoring qualitative changes of Form B to Form D. XRD patterns were obtained at periodic intervals of a sample of Form B stored at 25°C and 2.4, 6, 40, 60 and 80% RH over a three-week period. A gradual disappearance of Form B to a phase of low crystallinity (predominantly amorphous) was observed over week 1 in the sample at 80% RH. Further monitoring resulted in crystallization of Form D during second week of storage. Samples stored at other humidities did not reveal any changes over three weeks. Storage of Form D at the same conditions showed no phase changes over three weeks at all humidity conditions. Moreover, extended storage of Form D at 25°C/80% RH for 6 months did not result in any solid-state changes. Thermal analysis by DSC revealed Form B to melt at 217°C and Form D to melt at 224°C. Both of the forms decomposed during melting. The overlap of the melt and decomposition events made it difficult to use heat of fusion rule to obtain the relative thermodynamic stability of the two forms [5].

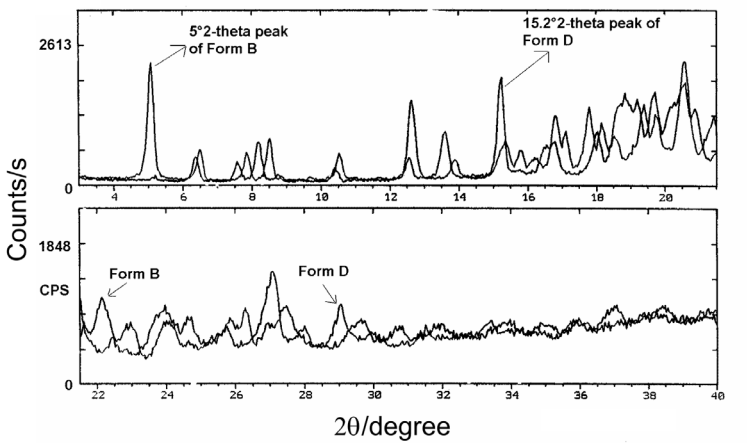


**Fig. 1** XRD patterns of Forms B and D

The dynamic water vapor sorption isotherms at 25°C of the two forms are shown in Fig. 2. As is evident from the sorption isotherms, Form B was very hygroscopic and sorbed up to 40% by mass of water at high %RH conditions. The significant amount of water sorption by a crystalline material was suggestive of possible incorporation of water molecules into lattice channels [6, 7]. Analysis by XRD of Form B after dynamic sorption analysis revealed collapse to a low crystalline phase [6, 7]. The dynamic nature of sorption analysis, where sample exposure to a %RH condition is time constrained, did not allow for complete transformation to Form D. In contrast, Form D sorbed up to 5% of water by mass at high %RH conditions and no solid-state changes were evident in post vapor sorption material.

The results described above were suggestive of being the thermodynamically stable form relative to Form B. Additionally Form D was kinetically stable over an extended period of time at high %RH and temperature conditions (such as 40°C/75% RH). The low hygroscopicity and physical stability of Form D at all

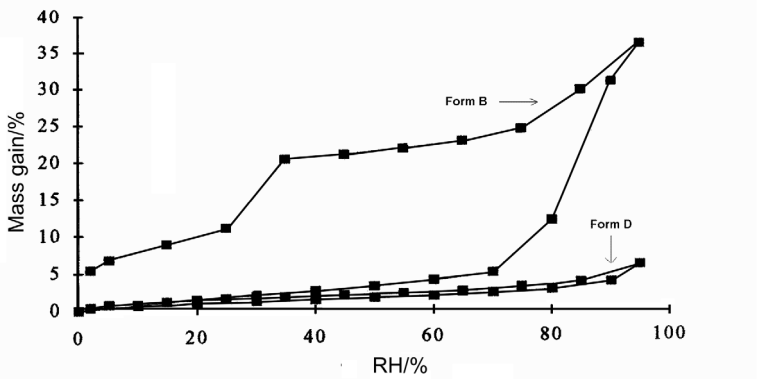


**Fig. 2** Moisture sorption isotherms

conditions of study of were desirable both from physico-chemical and processing perspectives. Hence, it was selected as the form suitable for solid dosage form development.

Attempts were made to crystallize Form D from various aqueous organic solvent systems. Due to the high solubility of the API in aqueous systems, the crystallization process involved antisolvent precipitation from a solvent system containing water. The crystallization process always resulted in precipitation of crystalline Form B. The precipitation of Form B instead of Form D is explained by Ostwald's step rule, which states that the form whose free energy is closest to the free energy in solution is obtained initially [8]. It is common to obtain metastable forms during crystallization, which transform to the most stable form eventually. Very high water solubility limited only by viscosity at high concentrations of the tetrapeptide in water is a probable reason for failure to obtain Form D by crystallization. Thus, in practice Form D could be obtained only through a solid-state transformation by exposing Form B to high humidity (≥80% RH) at a range of temperatures.

As stated earlier, solid-state transformation of Form B to Form D was found to proceed through an intermediate low crystalline phase as observed by qualitative XRD studies. The transformation is schematically shown below.

$$\text{Form B} \xrightarrow{>60\%\text{RH}} \text{Phase of low crystallinity} \rightarrow \text{Form D}$$

Such solid-state transformations have been proposed to proceed through four-steps [9]. The initial step consists of molecular loosening followed by formation of an intermediate solid solution. The solid solution leads to nucleation of the new phase with subsequent growth to macroscopic crystals. It is clear that for a transformation to occur the initial step of molecular loosening is essential which appears to be facilitated here by humidity and temperature [10]. The intermediate state is a phase of low crystallinity, which being thermodynamically unstable results in crystallization of Form D.

After establishing that Form D is the desirable form for dosage form development, it was necessary to establish optimal conditions of temperature and humidity for rapid and reliable large scale preparation of Form D from Form B. The optimal conditions were defined by analyzing the transformation kinetics of Form B to Form D by isothermal microcalorimetry.

Initially, the transformation was monitored by exposure to 80% RH at 40°C. Additionally the effect of methanol vapors on transformation kinetics of Form B to Form D was assessed. The power–time curves associated with exposure of Form B to water and methanol vapors are shown in Fig. 3. As a control, pure Form D was also exposed to 40°C/80% RH. All samples were analyzed by powder XRD after the IMC run to determine the solid-state post-exposure. The power–time curve of Form B exposed to water vapor showed an exotherm signifying a reaction. Post–exposure analysis by XRD revealed the formation of Form D and the exotherm in the power–time curve was attributed to transformation of Form B to Form D. The power–time curve of Form B exposed to methanol reached the baseline without any other event indicative of a reaction process. The lack of any solid-state change in the presence of meth-

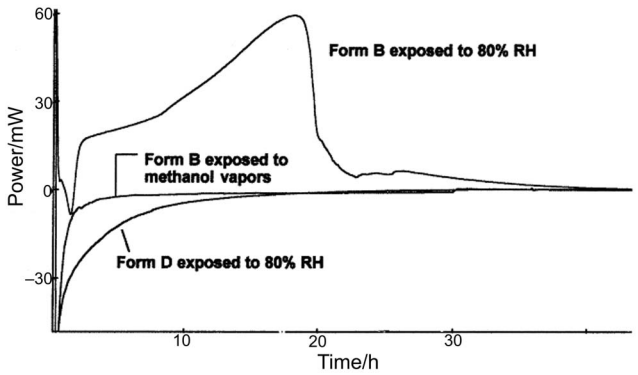


**Fig. 3** IMC curves of Form B and Form D exposed to 40°C/80% RH and vapors of methanol

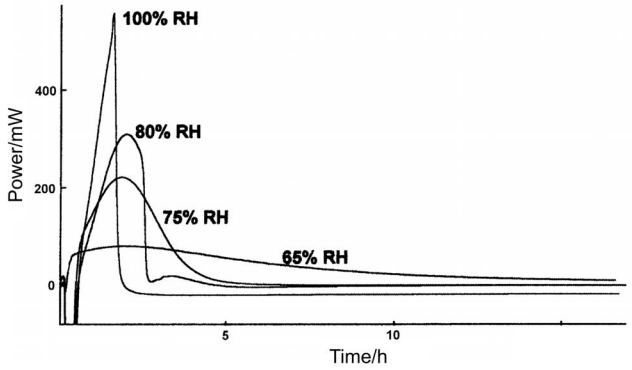


**Fig. 4** Effect of relative humidity on rate of transformation of Form B to Form D at 60°C

anol was confirmed by XRD post-exposure. Similarly, the power time curve of Form D, exposed to water vapor reached the baseline indicating lack of a reaction. XRD analysis post-exposure confirmed no solid-state changes. The results were in accordance with results mentioned previously and confirmed that IMC was of utility in monitoring kinetics of solid-state transformation.

Further analysis focused on the influence of humidity and temperature on the rate of transformation of Form B to Form D. Analysis was performed at 60°C and at various %RH conditions and the power–time curves are shown in Fig. 4. At all %RH conditions, exotherms in the power–time curves indicated solid-state transformation of Form B. It was also apparent that the time for transformation to be complete was shorter at higher %RH conditions. Additional analysis on the effect of crystallinity of Form B on the rate of transformation was assessed. Form B was milled to obtain material of low crystallinity and the transformation was assessed at 60°C and 80% RH. The power–time curves of the transformation are shown in Fig. 5. As shown in the scheme above, the reaction proceeds through an intermediate state of low

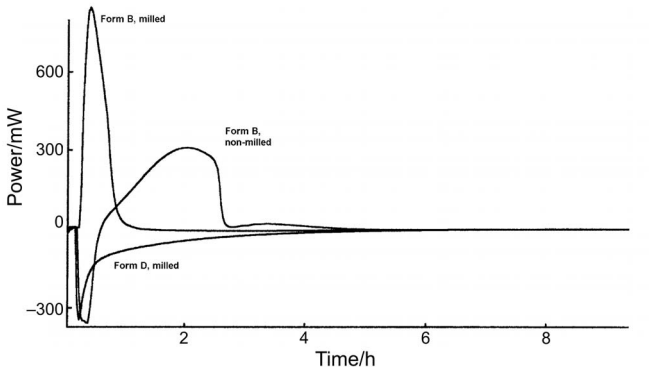


**Fig. 5** Effect of crystallinity of Form B on rate of transformation to Form D at 60°C/80% RH

crystallinity. Hence any reduction in crystallinity of Form B should eliminate the initial step and decrease the time taken for conversion of Form B to Form D. As hypothesized, the time taken for low crystalline Form B to convert to Form D was much less than that observed with highly crystalline Form B. The faster rate of transformation of low crystalline Form B provided further evidence in support of formation of an intermediate phase of low crystallinity. The transformation appered to follow the four-step process for a solid-state reaction [6].

Based on IMC experiments, optimal conditions for large-scale manufacture of Form D from Form B were defined. The transformation rate was higher at 60°C/80% RH (<3 h) than at 40°C/80% RH. Additional vapor sorption studies revealed that mass of Form B did not decrease the rate of transformation to Form D at 60°C/80% RH. Hence, the large scale production of 30 kg of Form D was performed successfully by exposing Form B to 80% RH at 60°C in a double cone jacketed blender.

## Conclusions

An unusual case study is presented where the desired solid form could be obtained only via a solid-state transformation aided by higher relative humidity. Using IMC and other complementary techniques the stability of Form D over Form B was established. IMC experiments under various conditions resulted in choice of optimal temperature and humidity conditions for large scale manufacture of Form D from Form B.

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## References

1 D. J. W. Grant, Theory and origin of polymorphism, in H. G. Brittain (Ed.), Polymorphism in Pharmaceutical Solids, Vol. 95, Marcel Dekker, New York 1999, pp. 1–33.
2 S. Byrn, R. Pfeiffer, M. Ganey, C. Hoiberg and G. Poochikian, Pharm. Res., 12 (1995) 945.
3 M. Angberg, C. Nystrom and S. Castensson, Int. J. Pharm., 81 (1992) 153.
4 S. Lindenbaum, Calorimetry in pharmaceutical research and development in J. Swarbrick and J. C. Boylan (Eds), Encyclopedia of Pharmaceutical Technology, Vol. 2, Marcel Dekker Inc., New York 1990, pp. 233–250.
5 A. Burger and R. Ramburger, Microchim. Acta [Wein], II (1979) 259.
6 J. S. G. Cox, G. D. Wodaard and W. C. McCrone, J. Pharm. Sci., 60 (1971) 1458.
7 E. B. Vadas, P. Toma and G. Zografi, Pharm. Res., 8 (1991) 148.
8 J. W. Mullin, Crystallization, 3rd Ed. Butterworth Heinemann, London, UK 1993, pp. 172–201.
9 I. C. Paul and D. Y. Curtin, Acc. Chem. Res., 6 (1973) 217.
10 S. R. Vippagunta, H. G. Brittain and D. J. W. Grant, Adv. Drug Del. Rev., 48 (2001) 3.