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Process Development Challenges to Accommodate A Late-Appearing Stable Polymorph: A Case Study on the Polymorphism and Crystallization of a Fast-Track Drug Development Compound

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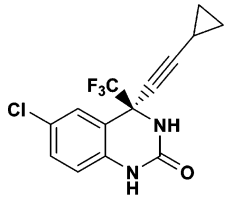
Abstract:

The case of disappearing/late-appearing stable polymorphs and their impact is well-understood by scientists in the pharmaceutical industry. This paper discusses an instance where a more stable crystal form was discovered during the development of a fast-track drug candidate. Challenges in adapting to the discovery of the new crystal form during this accelerated drug development program and approaches to develop a robust crystallization process are discussed.

Introduction

Polymorphs of a molecular material can be envisioned as minima in the energy landscape of a single component system. Metastable polymorphs would constitute local minima in the energy landscape with the thermodynamically stable form being the absolute minimum at a given temperature and pressure. The search for absolute minimum and energy differences between local minima of drug substances is the goal of material and formulation scientists in the pharmaceutical industry. While significant efforts are made by drug development groups to identify and characterize crystal forms early in development, there are many instances where new crystal forms have been discovered later in development during process scale-up. The late emergence of thermodynamically stable crystal forms is often explained by Ostwald's law of stages which states that the least stable crystal form is likely to crystallize first. Dunitz and Bernstein[1] discuss the phenomena of disappearing polymorphs in detail. They elaborate how in many instances, control of polymorphs is related more to the control of crystallization conditions. According to Dunitz and Bernstein,[1] "...once a polymorph has been obtained, it is always possible to obtain it again; it is only a matter of finding the right experimental conditions." However, consistent, controlled manufacture of less stable crystal forms can be quite challenging. The polymorph issues encountered with ritonovir (Norvir) serves as a well-documented case study on how the discovery of a more stable polymorph can have a potentially disastrous effect on the supply of an essential drug product.[2,3] In this paper, we discuss another instance where the issue of a late-appearing stable polymorph was encountered, but with no significant impact on the overall development of the drug. The challenges faced by the drug development team to adapt quickly to overcome this challenge in a fast-track drug development scenario will be discussed. The API's propensity to form solvates with a number of solvents and the impact of this on the identification and selection of a crystallization system will be peripherally addressed. Also, the use of in-process analytical tools to gain better understanding of crystallization processes will be highlighted.

Compound A is a nonnucleoside reverse transcriptase



inhibitor of human immunodeficiency virus type-1 (HIV-1). The first crystal form of compound A was discovered through sublimation experiments and unambiguously characterized through single crystal analysis as a neat (solvent-free) phase (designated as Form I). This neat form was chosen for further development. The chemical process used for the drug substance synthesis has been previously described[4,5] and is illustrated in Scheme 1. The final chemical step of the process involved a dephenethylation conducted in neat formic acid; after reaction workup the crude drug substance (Compound A) was crystallized from a toluene/heptane mixture.

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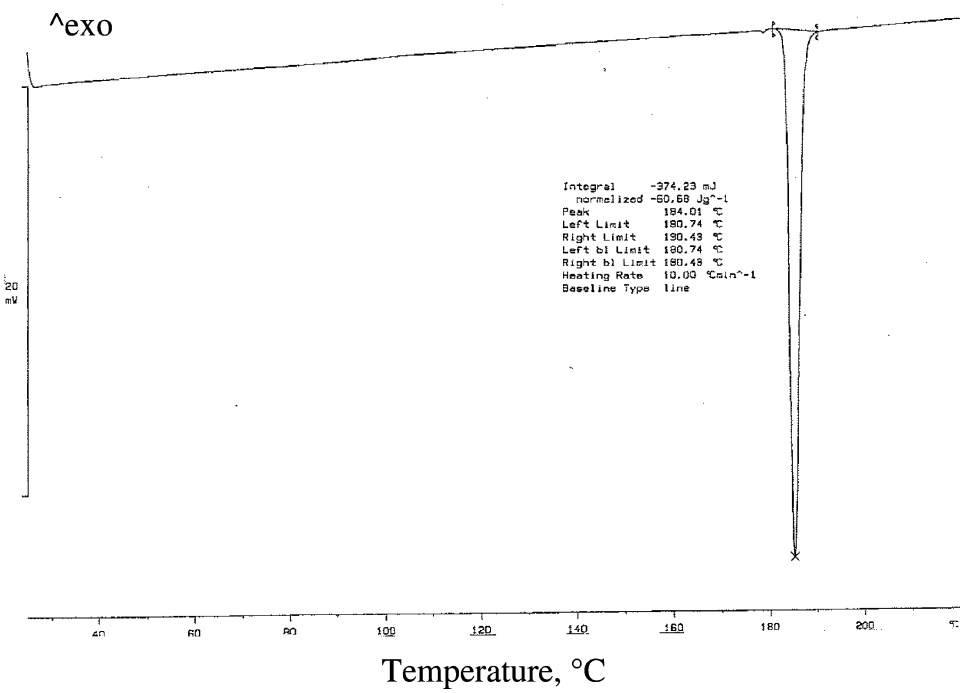
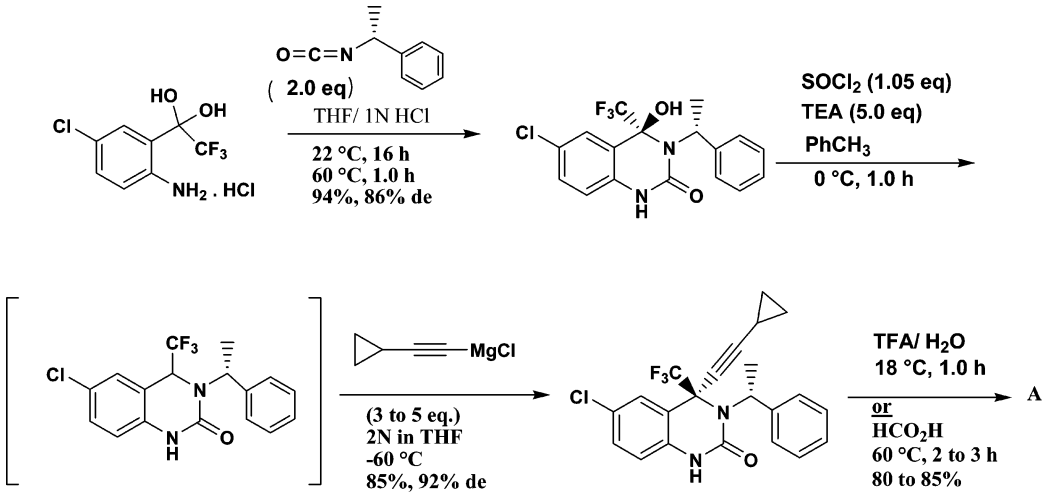


Figure 1. **Differential scanning calorimetry thermogram of Form I drug substance.**

Scheme 1. **Synthesis scheme for the preparation of Compound A**



Crystallization from toluene/heptane affords a mixed toluene/heptane solvate which undergoes a thermal conversion to compound A with a variable level of amorphous phase material. To produce drug substance with the requisite purity and prepare material of a uniform crystalline phase, the crude drug substance was recrystallized from methanol to provide a crystalline, stoichiometric methanol solvate. The isolated methanol solvate was designated as Form II. Form I was obtained by a thermal conversion of Form II by drying the wet cake of Form II at 90 °C for 4 h followed by further drying at 120 °C for 3 h. The transformation of Form II to I was monitored using differential scanning calorimetry (DSC). During the development of this drug candidate as many as 29 batches of Form I material were produced by this thermal conversion process at scales up to 150 kg. Figure 1 shows the DSC thermogram of the material obtained from the thermal conversion process where a single melting endotherm with melt onset at 181 °C corresponding to Form I was observed. During the drying of the 30th batch, a significant change in melting behavior was observed in the process control samples analyzed by DSC. Material taken from the dryer showed a new lower melting endotherm with melt onset of 174 °C (Figure 2a, b). At the end of the drying process, a material with single melting endotherm with melt onset of 174 °C was isolated (Figure 3). This material was designated as Form III. Once Form III had been generated, Form I material could no longer be produced via the thermal desolvation process except by extended heating at melt temperatures of Form III. Powder X-ray pattern of the material collected during batch 30 during the drying process shows a mixture of two forms, thus indicating a transition from one to the other (Figure 4). Scanning electron micrographs of Forms I and III show significant difference in morphology of the forms, indicative of differences between

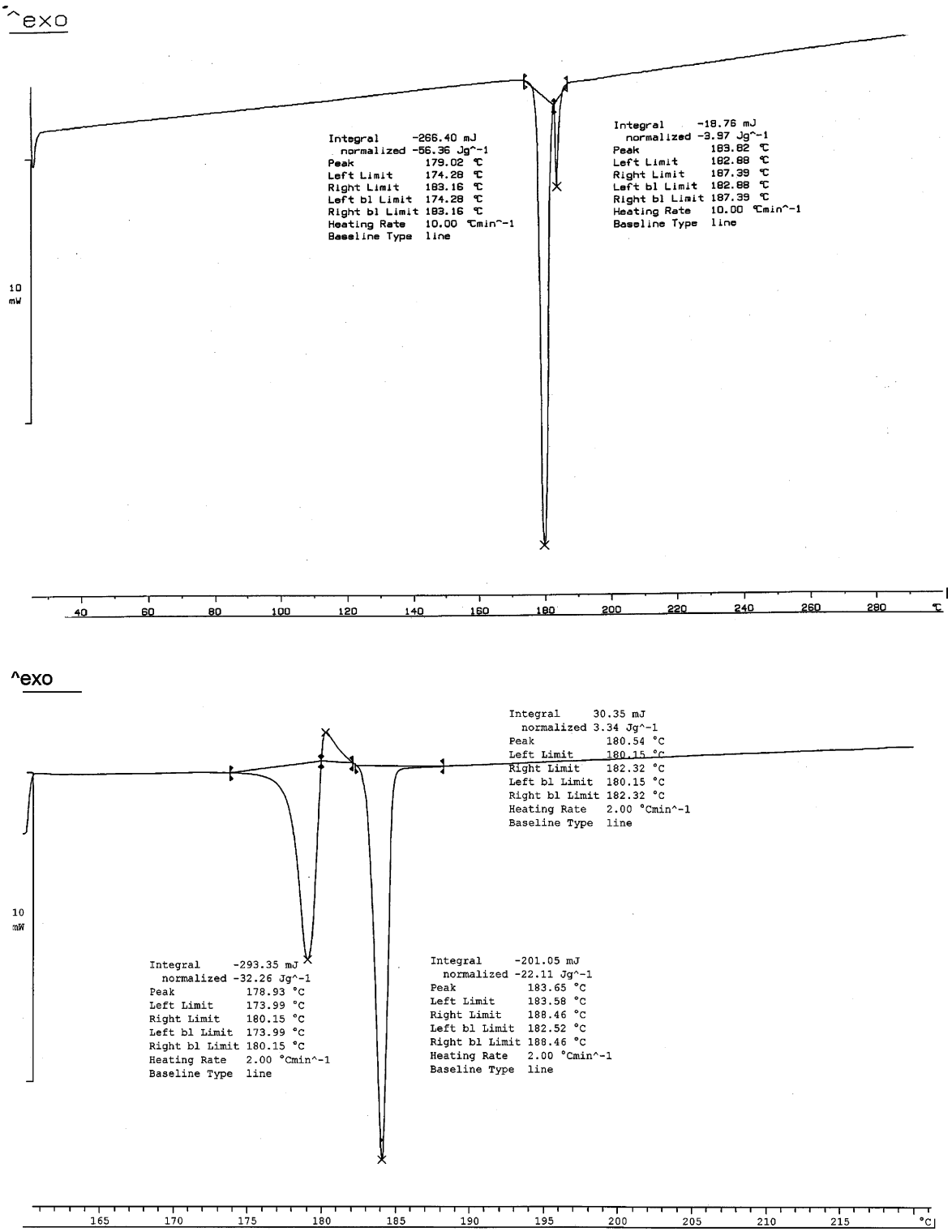


Figure 2. **Differential scanning calorimetry thermogram of the thermal conversion process. New lower melting endotherm was observed. (Top) Scanning rate 10 °C/min. (Bottom) Scanning rate 2 °C/min.**

crystal forms (Figure 5). It should be noted here that Form I was obtained from desolvation of the methanol solvate as clearly evident from the surface heterogeneities shown in Figure 5a. However, morphology of Form III crystals show fused nonsolvated crystals which may indicate a solvent-mediated transformation from methanol solvate to neat Form III. Precise mechanism of transformation to Form III and drying conditions which led to this transformation is not

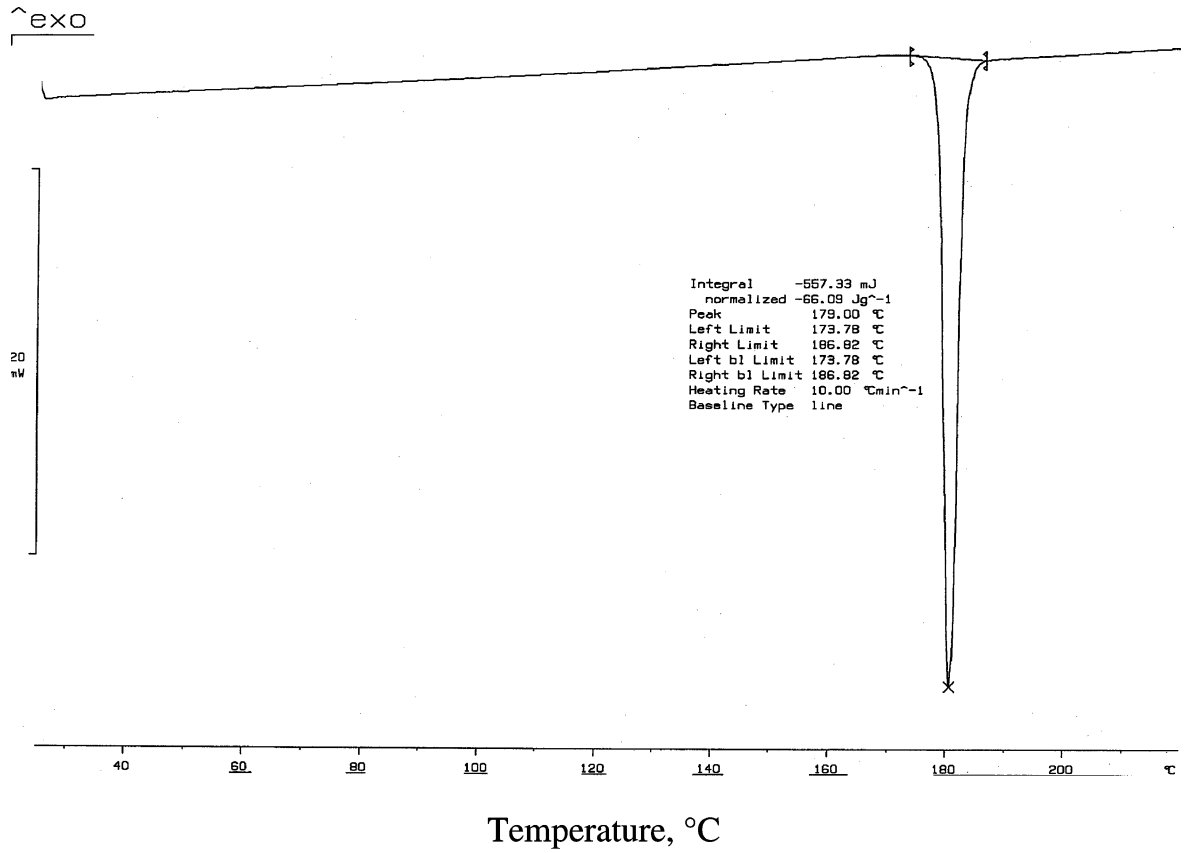


Figure 3. **Differential scanning calorimetry thermogram of Form III drug substance.**

known. This underscores the risk of isolating crystals from suboptimal, poorly controlled processes such as thermal conversion in the dryer. Once more stable Form III crystals were obtained from the thermal conversion process in the dryer and Form I could no longer be isolated, development work focused on efforts to identify a procedure to isolate Form III directly from a crystallization process.

Discussion on the Crystal Forms of Compound A. Compound A is a nonhygroscopic neutral molecule that has very low aqueous solubility (less than 10 μg/mL) and high permeability (Caco2 permeability > 150 nm/s). It can be considered as a Class II compound according to the Biopharmaceutics Classification System (BCS) with dissolution-limited bioavailability.[6] It is soluble in most organic solvents with a tendency to form solvates in many of the common solvents. During an extended polymorphism screening study several solvates including methanolate, ethanolate, and propanolate (from 2-propanol) were discovered. To date only two anhydrous crystal forms have been discovered (Forms I and III). Form I was discovered initially during development, and the discovery of Form III has been described in the previous section. After the discovery of Form III, its single-crystal structure was obtained and confirmed to be a neat form as well. Table 1 summarizes the thermodynamic properties of Forms I and III. The main diagonal represents melting behavior of forms. The upper half matrix describes the thermodynamic relationships, based on the observations outlined in the lower half matrix. Based on Burger's heat of melting rule,[7] Forms I and III are enantiotropically related with N-3 (Form III) more thermodynamically stable than N-1 (Form I) at temperatures below 120 °C. The transition temperature is inferred to be between 120 and 174 °C (T_m of lower melting Form III). At ambient conditions, far from the transition temperature, Form III is likely to be significantly more stable than Form I. This may explain the disappearance of Form I once Form III was isolated.

Table 1. **Thermodynamic interrelationships between Form I and III**

Tm (°C), ΔHm (KJ/mol)	Form I	Form III
Form I	181 °C, 19.10	Enantiotropic by BHM* Transition temperature inferred to be between 120 - 174 °C
Form III	Form I converts to Form III at 120 °C Form I converts to Form III at 40 °C/ 75% RH	174 °C, 20.80

*Burgers Heat of Melting rule: This rule states that if the polymorph with the higher melting point exhibits lower heat of fusion, the system is enantiotropic, otherwise, it is monotropic.[7]

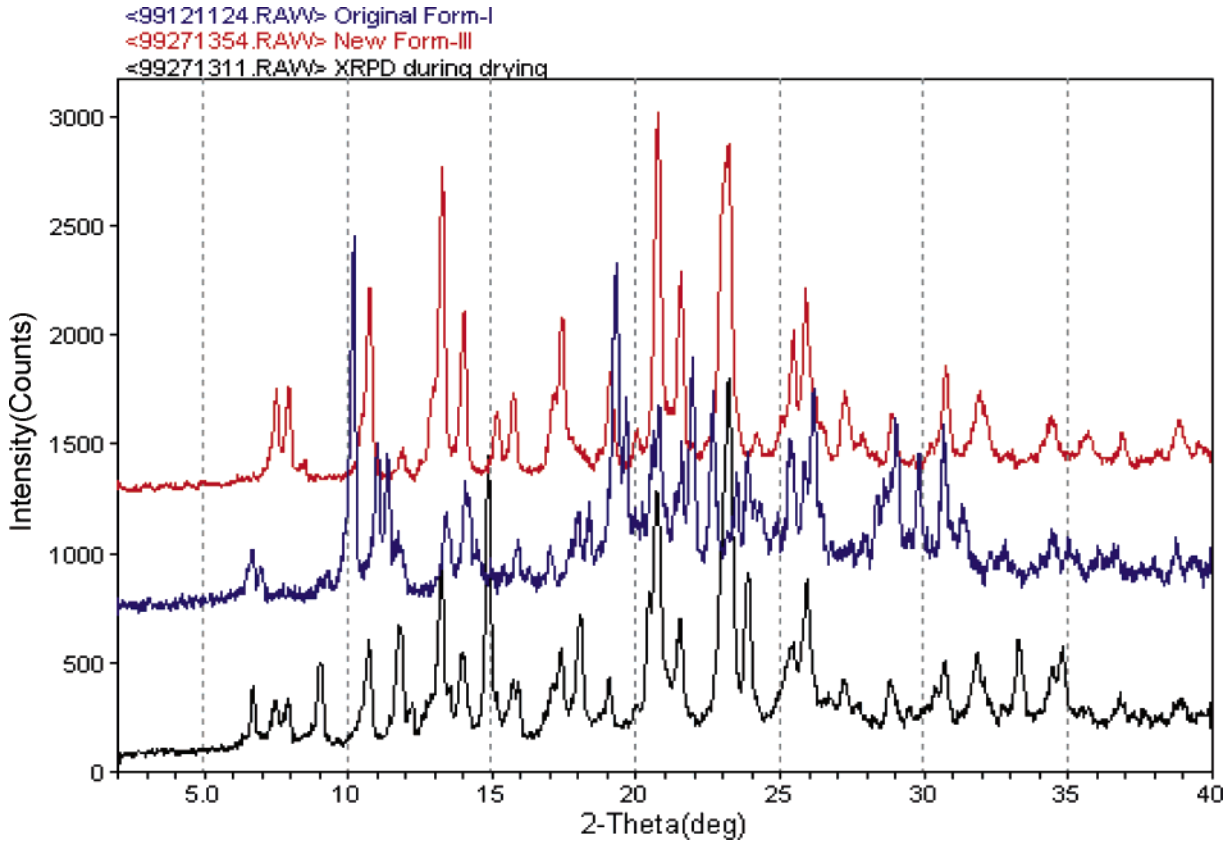


Figure 4. **Powder X-ray diffraction pattern of material obtained during the drying process (Top) Form III. (Middle) Form I. (Bottom) Material obtained during the drying process. A mixture of Form I and III was observed by PXRD analysis.**

Table 2. **Structural parameters for Forms I and III**

parameters	Form I	Form III
crystal system	monoclinic	tetragonal
space group	$P2_1$	$P4_122$
crystal habit	acicular	rhomboid
a	16.810 Å	11.593 Å
b	9.080 Å	11.593 Å
c	18.541 Å	67.066 Å
β	92.52°	90°
V	2827.3 Å^3	9013 Å^3
Z	8	24
Z′[a]	4	3
density	1.479 g/cm^3	1.391 g/cm^3
lattice energy[b]	418.4 kcal/mol	449.6 kcal/mol

[a] Z' = number of molecules per asymmetric unit. [b] Universal Force Field.

The crystal structures for Forms I and III have been determined using single-crystal X-ray crystallography. The structures are very different in terms of molecular packing. Table 2 summarizes the structural parameters. The hydrogen bonding in Form I is made up of two sets of dimers along the *a*-axis. The dimers are held together through a $\pi-\pi$ stacking along the *c*-axis and N−H- - -Cl−C interactions along the *b*-axis (Figure 6). Form III is also held together by dimers but through an infinite chain along the *c*- and *b*-axes, whereas van der Waals interactions are along the *a*-axis (Figure 7). Table 3 summarizes the intermolecular distances in each form. It is clear from this table that Form I contains more and stronger intermolecular interactions than Form III. Furthermore, Form III contains relatively large channels (~3.5 Å in diameter) along the *c*-axis, which might explain the large difference in densities between the two forms (Figure 8). Form I exhibits a higher density, contradicting the density rule[7] that states, "If one modification of a molecular crystal has a lower density than the other, it may be assumed to be less stable at absolute zero". Exceptions to this density rule have been reported in the literature in about 10% of the molecular crystals exhibiting polymorphism.[8] Burger and Ramberger explain that in instances where directed forces such as hydrogen bonds are present it may not be possible to conclude a correlation between density and potential energy.[7] The lattice energy calculations using the universal force field confirm that Form I has a more stable crystal packing and hence a higher density.

Direct Crystallization Process of Form III. Since Compound A exhibited tendencies to form solvates in many organic solvents, the search for an acceptable solvent for direct crystallization of anhydrous form III resulted in few leads. After careful screening, *n*-propanol was chosen as a

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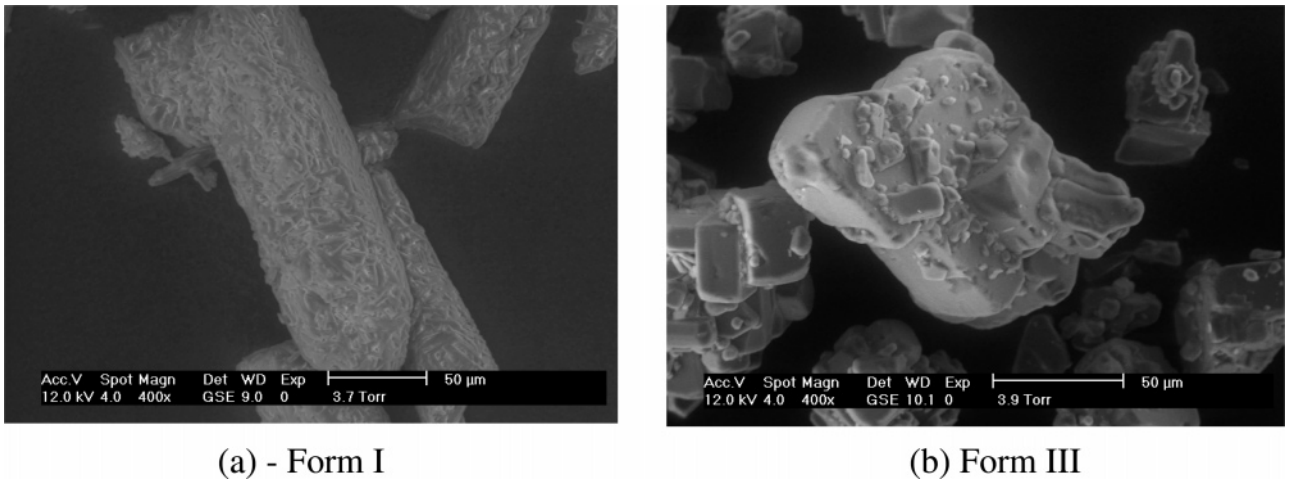

Figure 5. **Scanning electron micrographs of Form I and III material. (a) Form I. (b) Form III.**

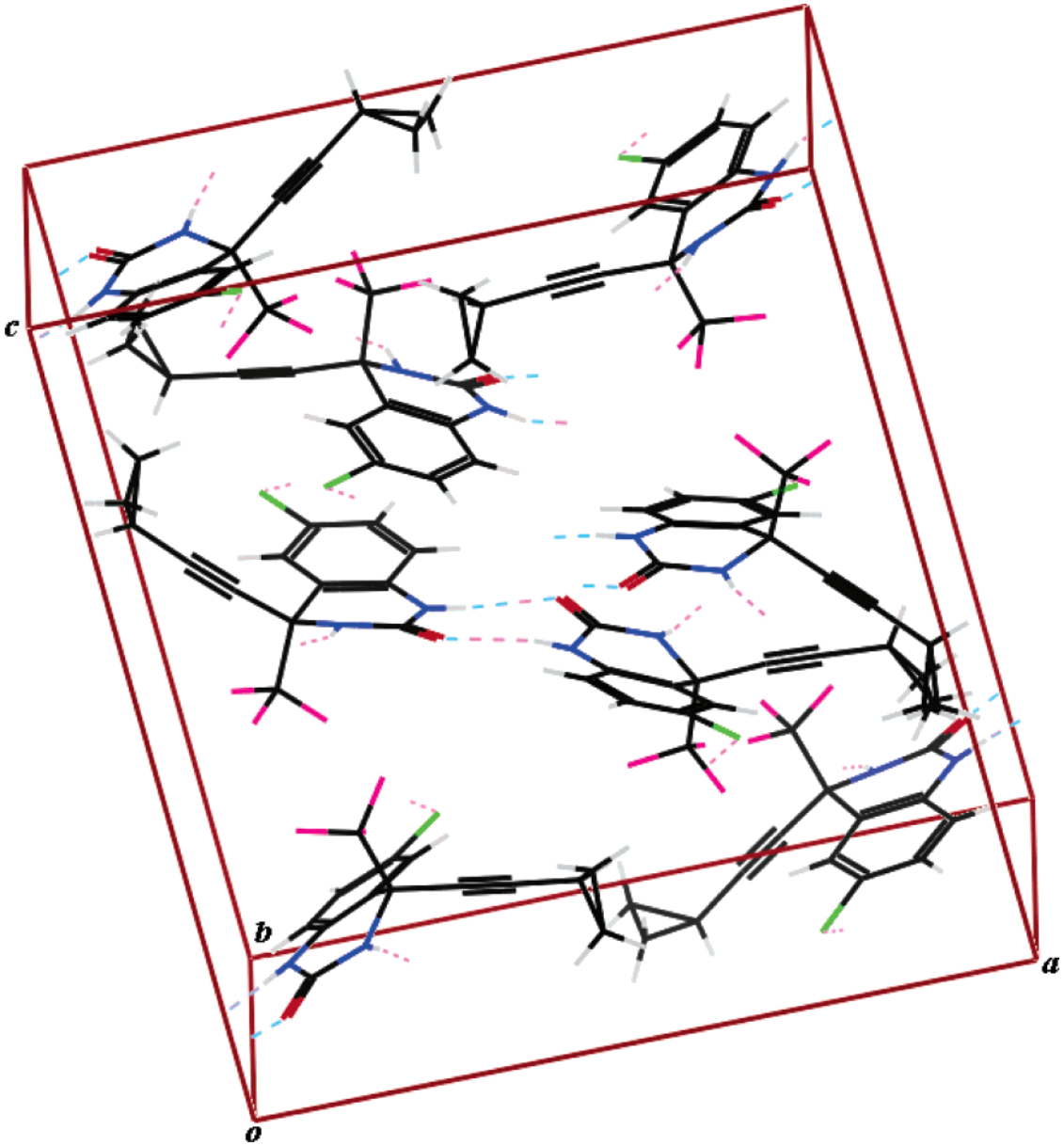

Figure 6. **Form I unit cell.**

solvent for crystallization process development. Due to the high solubility of Compound A in *n*-propanol, use of an antisolvent (water) was required to achieve the required yield target and to provide a slurry with acceptable flow properties.

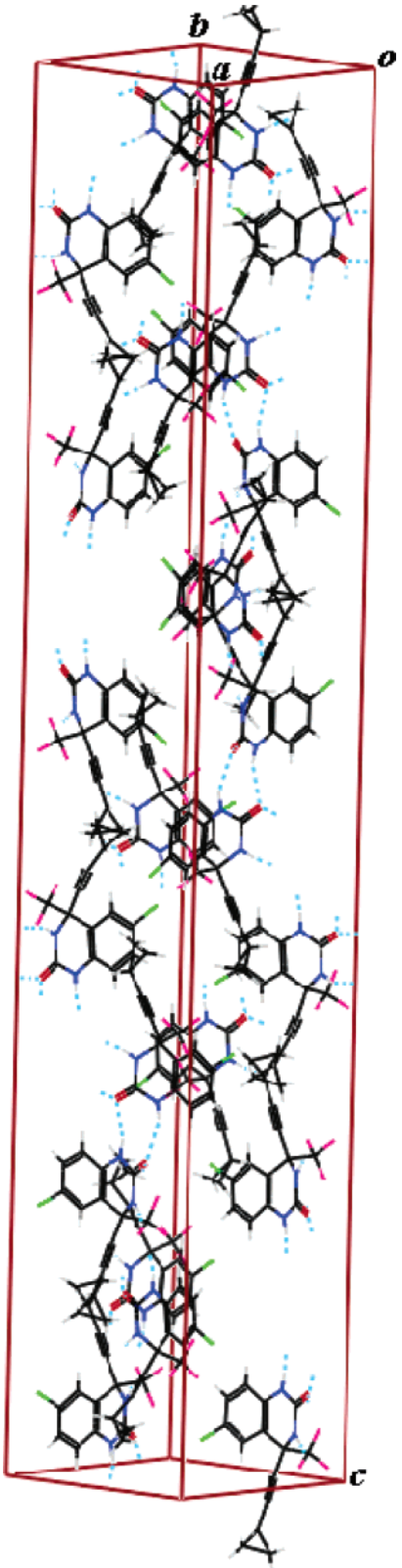

Figure 7. **Form III unit cell.**

The *n*-propanol/water crystallization system was developed and scaled up to 1-L scale to demonstrate process feasibility. The crystallization protocol involved the use of Form III seeds (with a loading of up to ~5% by weight) to ensure growth on existing crystals of Form III and to avoid formation of undesired crystal forms. However, during one

Table 3. **Intermolecular distances**

interaction type	heterogeneous atom distances (Å)	
	Form I	Form III
H-bonding	2.827	2.857
"Dimers"	2.742	2.857
	2.834	2.874
	2.791	2.898
		2.826
		2.771
−Cl- - -H−N−	3.384	4.084
	3.347	
	3.409	
	3.412	
$\pi-\pi$ staking	3.47	none
	3.56	

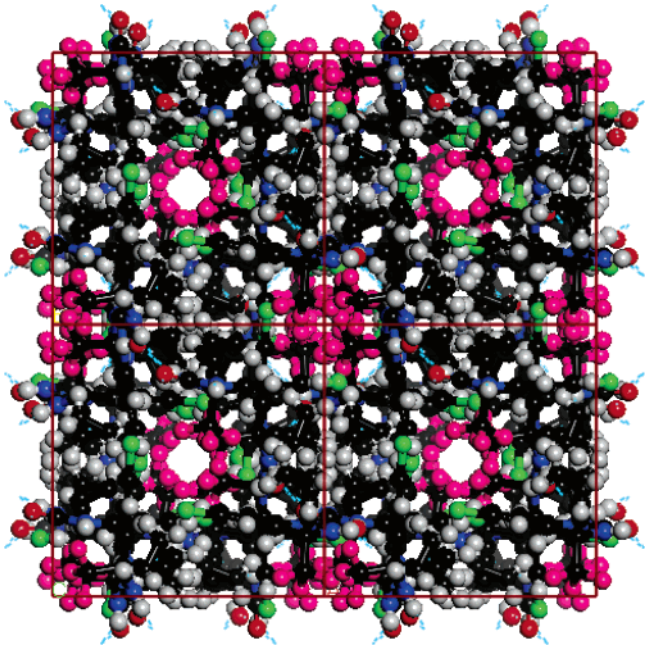

Figure 8. **Form III channel along *c*-axis.**

of the experimental batches, a new crystal form with needlelike morphology was observed. Analysis by PXRD indicated the presence of a new phase different from that of Form III. Further characterization of the material confirmed the material to be *n*-propanol solvate. Formation of solvate despite substantial amount of Form III seeds present was investigated as this is not desirable for isolating neat Form III crystals. Efforts were also undertaken to study the phase behavior of this solvate in *n*-propanol/water solvent systems to develop crystallization protocols for isolating neat Form III directly from crystallization (next section). This was solved using in-process monitoring using in situ optical microscopy (Particle Vision Measurement probe, Lasentec Inc.). With the in situ microcopy, it was clearly evident that during the water addition, a quasi-emulsion phase was formed with the hydrophobic layer enriched with drug substance (*n*-propanol phase) and the aqueous phase containing small amounts of the drug substance. Unlike stable emulsions, the quasi-emulsion phase observed is not thermodynamically stable with easy separation of hydrophobic/hydrophilic

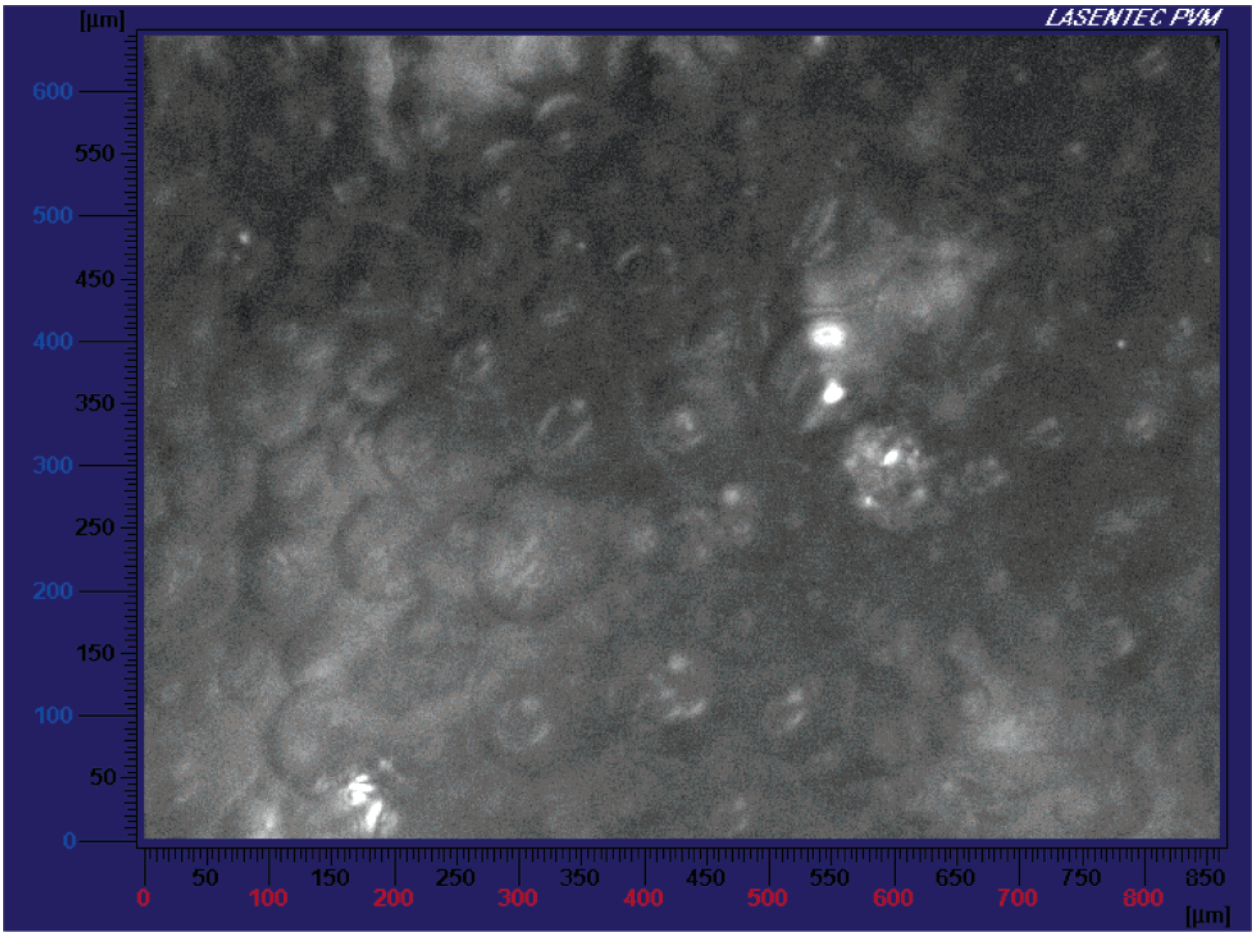

Figure 9. **In situ optical micrograph during the crystallization process. A quasi-emulsion phase was observed.**

phases. The presence of the highly hydrophobic drug substance in high concentrations (30−50% w/v) resulted in the phase separation of the otherwise fully miscible *n*-propanol−water mixtures resulting in the formation of the quasi-emulsion phase described above (Figure 9).

Phase Behavior of Compound A in *n*-Propanol/Water System. To investigate the formation of the quasi-emulsion phase and to understand the formation of an *n*-propanol solvate, detailed experiments were carried out in 20-mL laboratory-scale equipment. An excess of Form III drug substance was slurried in *n*-propanol/water mixtures at varying concentrations and temperatures. Evidence for phase separation of the organic and aqueous phase was visually observed and noted. The corresponding solid phase was analyzed by PXRD to determine the subsequent material crystalline phase. Figure 10 shows the phase behavior information of the drug substance in the solvent system chosen for crystallization. It is clear that at high *n*-propanol concentrations and at lower temperatures, *n*-propanol solvate is the stable form. At predominantly *n*-propanol-rich conditions (>1:1 *n*-propanol/water ratio), at temperatures above 35 °C, a clear liquid phase corresponding to complete dissolution of drug substance was observed. As the water composition is increased, the quasi-emulsion region was observed. The composition range over which the quasi-

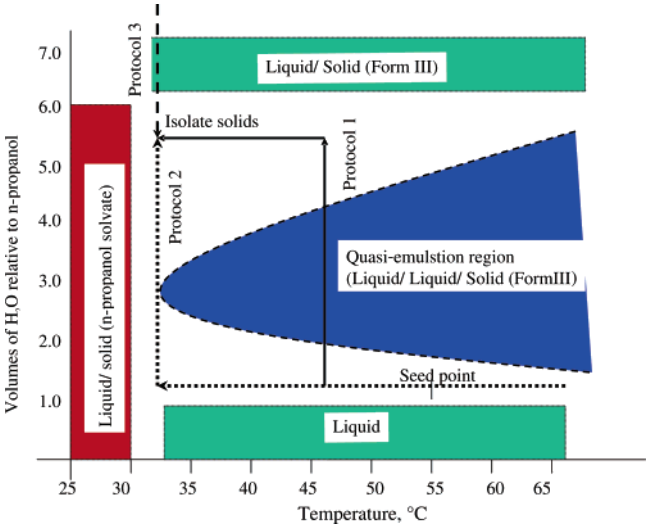

Figure 10. **Phase diagram of the crystal forms in the crystallization solvent system.**

emulsion phase was observed was found to increase at higher temperatures. This could be attributed to the fact that at higher temperatures more drug substance is soluble in *n*-propanol rendering the solution more hydrophobic. At sufficiently high water compositions, the *n*-propanol solvate was observed to desolvate to the anhydrous Form III.

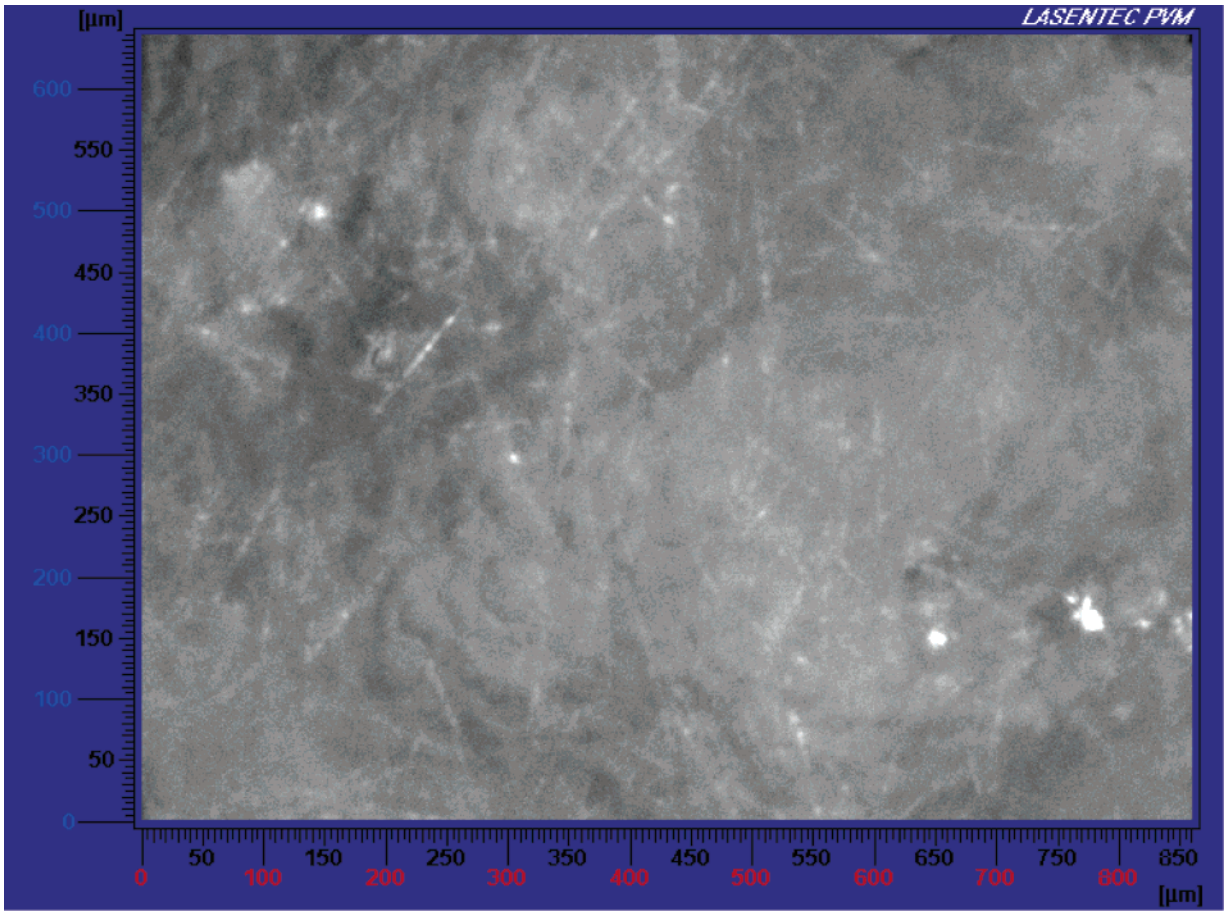

Figure 11. **In situ optical micrograph during the crystallization process. Needlelike crystals (*n*-propanol solvate) were observed.**

Development of Direct Crystallization Process. On the basis of the phase behavior information, several crystallization protocols were devised and evaluated for direct isolation of the desired Form III. As shown in Figure 10, in Protocol 1, the temperature of seeding was set at 45 °C and 5% seeds of neat Form III crystals were added as a slurry in a *n*-propanol/water mixture. While some liquid-phase separation and quasi-emulsion formation was still observed, the effect was not as pronounced as in earlier cases. In Protocol 2, the seeding temperature was further lowered to approximately 30 °C. In this case, while no significant emulsion formation was observed, *n*-propanol solvate crystals were observed during crystallization as evidenced by in-process microscopy (Figure 11). As noted previously the *n*-propanol solvate crystals were converted to Form III upon further water addition. In both Protocols 1 and 2, the crystallization slurries were held at 30 °C for 8 h before filtration to ensure complete conversion of any undesired *n*-propanol solvate. Protocol 2 was successfully scaled up to 300-kg scale (1000 L) in the manufacturing facility. As an alternative to both Protocols 1 and 2, a new reverse-addition process (Protocol 3) was developed. In this process, drug substance dissolved in *n* -propanol was slowly added to the aqueous phase containing seeds of Form III. This modified scheme incorporates all the elements of a robust process where Form III is the stable solid phase at all times during the crystallization process. As noted in Figure 12, some agglomerate crystals of Form III were observed, but this issue was easily overcome by use of intense mixing during crystallization and by sonication of the slurry.

Effect of Crystal Form on Dosage Form Development. Compound A was formulated as 50-mg strength tablets using Form I. Analysis of tablets stored under accelerated storage conditions (40 °C/75% RH) indicated complete conversion to Form III in dosage form. The formulation was modified to accommodate the new crystalline form and was found to be equivalent to Form I through a relative bioavailability study in humans. While polymorphs of drug substances may result in differences in bioavailability, in this instance there was no difference in the in vivo performance.

Summary

Knowledge of stability of the crystal forms and their interrelationships is critical for developing robust crystallization processes. While it may be tempting to use expeditious processes such as thermal conversion during initial drug development, they may have much greater overall negative impact on development timelines. Use of on-line analytical tools such as in-process microscopy is critical for developing knowledge on the crystallization behavior. In this paper, the case of a late-appearing, thermodynamically stable polymorph during a fast-paced drug development program and challenges encountered are discussed. We hope to have provided another convincing example for pharmaceutical

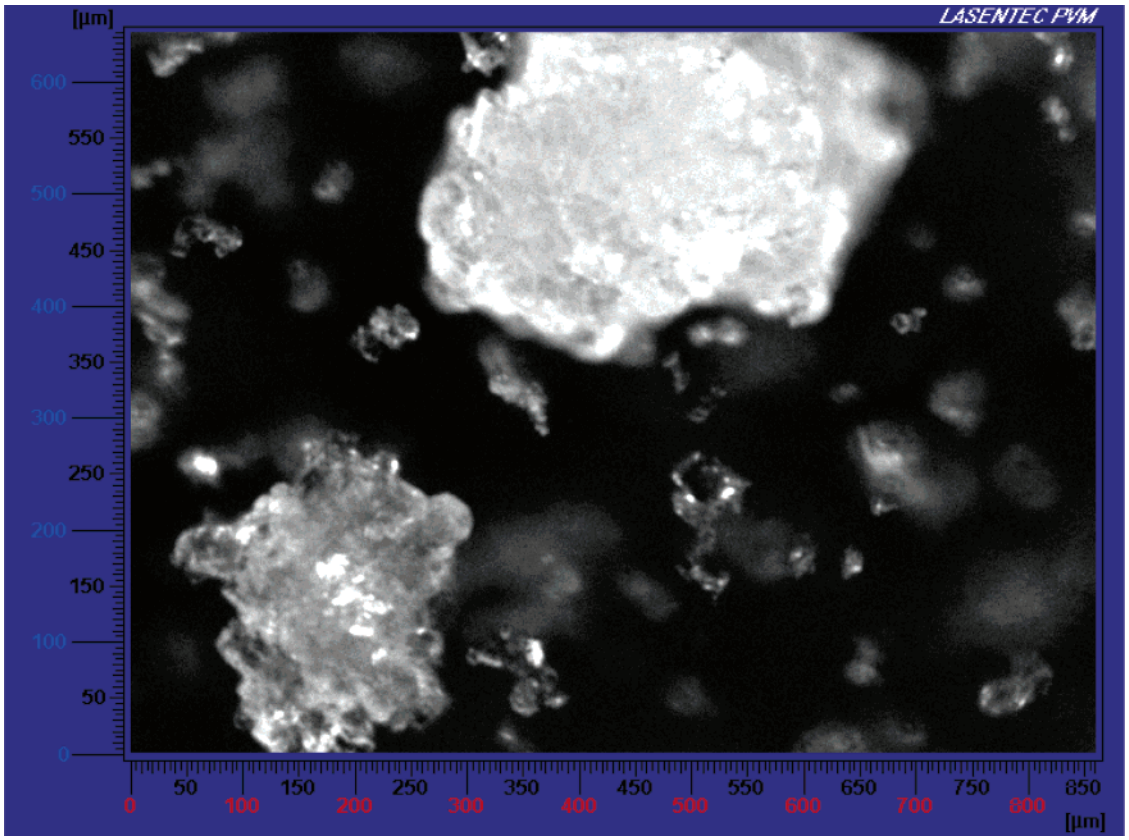

Figure 12. **In situ optical micrograph during direct crystallization process (Protocol 3).**

scientists on the need for early and thorough investigation of crystal forms.

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