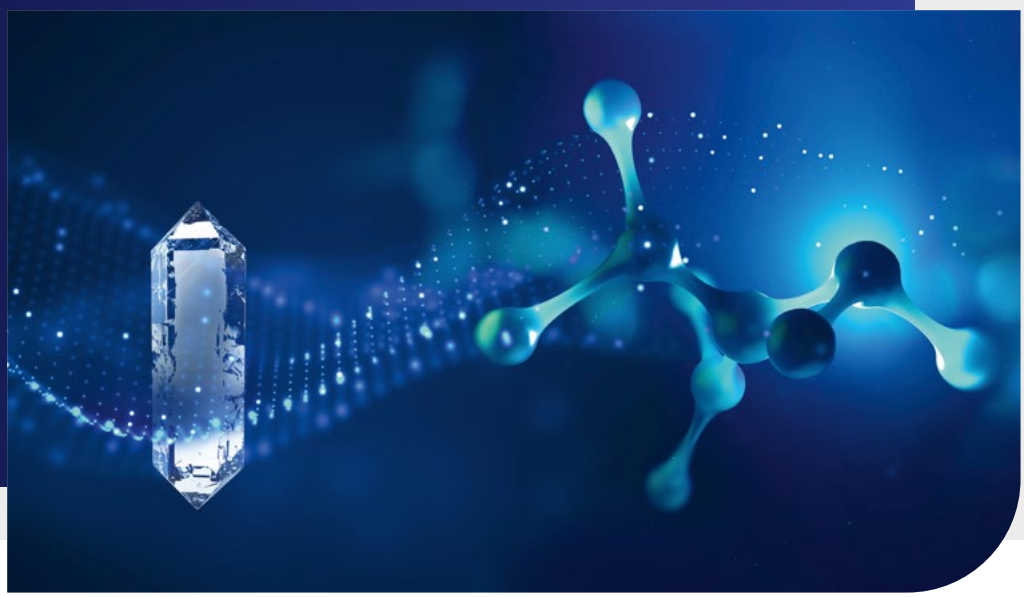


TRACKING POLYMORPH AND CRYSTAL MORPHOLOGY USING NON-INVASIVE IN SITU ANALYSIS AT SMALL SCALE

Controlling Crystallization in Pharma

In the manufacturing of solid dose formulations, crystallization is a commonly used process. A well designed and controlled crystallization process can be used to substantially improve the economical and biological performance of a drug molecule through exerting control over particle size distribution and morphology, polymorphic form, and purity. However, crystallization can be difficult to control due to the complex relationship between thermodynamic and kinetic factors and processes such as nucleation, growth and agglomeration. Therefore, in order to maximize the benefits of a crystallization, a thorough understanding is required which can be done though modeling, design of experiment stress testing and the use of process analytical technologies (PAT). By undertaking the studies to generate understanding of a crystallization process at an earlier stage with less material, it is possible to accelerate the development of drugs to market by using the ***Crystalline*** device equipped with particle view cameras and a Raman spectrometer.

Controlling Crystallization

- **Seeding Strategy** Control when and what Form of crystals appear.
- **Modelling** Using solubility, growth kinetics and particle size data, models can predict crystallization processes.
- **PAT** Inline, real-time analytics allow the monitoring of crystallization enabling feedback control strategies.

Why and how is controlling Crystallization important?

The control of an industrial crystallization is of paramount importance, especially with a molecule that has multiple polymorphs. Polymorphism is the result of different stable packing structures within a crystal lattice and can be differentiated using a range of offline techniques including X-ray diffraction, DSC and Raman spectroscopy. Different polymorphs can show completely different physical and chemical properties, and these differences can have drastic results on the therapeutic effectiveness of a molecule. These polymorphs will have a relative stability between them, one polymorph being the thermodynamic form and other a metastable form, and even trace amounts of the more stable polymorph can result in full conversion. Therefore, a crystallization process to produce a metastable polymorph requires a significant level of control and understanding at the molecular level.

The invention of process analytical techniques (PAT) has allowed for the real time in-situ monitoring of various parameters, including concentration (FT-IR, UV-Vis), cord length (FBRM), morphology (particle viewer) and solid form (Raman). By tracking one or preferably more of these parameters it is possible to take proactive measures to ensure the development of a robust crystallization procedure which gives the desired results every time. Furthermore, utilizing these technologies at the earliest stage of development can be of great benefit making it possible to gather significantly more information per experiment, reducing the number of experiments and consequently, the amount of material needed. Additionally, the detection of a process falling out of specifications (wrong polymorph or poor particle size distribution) can be mitigated through the implementation of feedback controls to salvage a batch.

Process Analytical Technologies (PAT) in the Crystalline

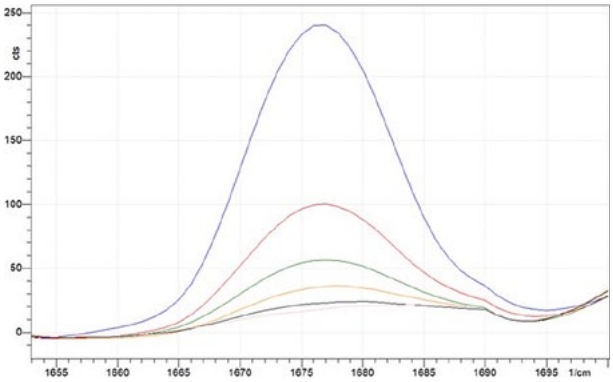
- Particle viewer – In situ microscopy looks into the vessels showing particle size and morphology
- Raman Spectrometry – Measuring the spectra of solids allows molecular characterization to track polymorphism

Using the ***Crystalline*** instrument, it is possible to generate information about the solid form, as well particle size and morphology. By making use of the integrated particle viewer and Raman spectroscopy, one may accelerate the development process of the drug development at an earlier stage and respectively using less material.

Proof of Principle: Mefenamic acid

Mefenamic acid is a nonsteroidal anti-inflammatory used in the treatment of mild pain. It has three polymorphs with Form I being the thermodynamically most stable form. Using Raman spectroscopy it is possible to track the rapid conversion of mefenamic acid Form II to Form I by monitoring the spectral region 1660- 1690 cm^{-1}.

Figure 1. Raman spectrum transformation for mefenamic acid captured using the Tornado HyperFlux integrated with the Crystalline instrument



50 mg of mefenamic acid Form II was suspended in 5 ml ethanol (EtOH) and stirred (700 rpm) using an overhead hook impeller at 25 °C in the ***Crystalline*** instrument. The ***Crystalline*** was interfaced to a Tornado HyperFlux™ PRO Plus Raman spectrometer equipped with a Hudson™ Probe adapted to the ***Crystalline*** sampling port (785 nm, total collection time of 4.8 seconds per spectrum).

Over the course of 10 mins, the C=O stretching at 1676 cm^{-1} rapidly decreases in intensity until it is no longer detectable signifying the complete conversion to the thermodynamic Form I (Figure 1). This experiment highlights how using the ***Crystalline*** instrument coupled with the Tornado Raman spectrometer, it is possible to track the fast polymorph conversions in real time.

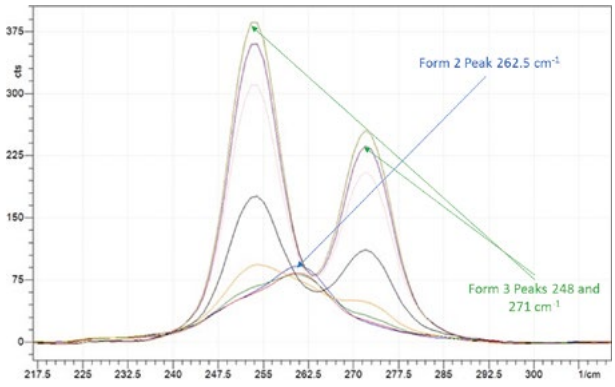
Case study: Carbamazepine Polymorphic Transformation

Carbamazapine is an anticonvulsant used in the treatment of epilepsy and is commonly marketed as a solid dosage form. It is also one of the benchmark compounds in crystallization research as there are several known polymorphs. There are 4 known anhydrous polymorphs and a dihydrate form, with Form 3 being the thermodynamically most stable polymorph and Forms 1, 2 and 4 being metastable but readily obtainable through various crystallization procedures. It is possible to differentiate between Forms 2 and 3 using Raman spectroscopy as well as optical microscopy, as the two polymorphs show different morphologies: Form 2 needles and Form 3 blocks, respectively.

This experiment was conducted using the ***Crystalline*** instrument interfaced to a Tornado HyperFlux™ PRO Plus Raman spectrometer equipped with a Hudson™ probe (785 nm, total collection time of 15 seconds per spectrum). Carbamazepine (199 mg) was suspended in isopropanol (5 ml). The sample was then heated to 70 °C and held with stirring (700 rpm) until a clear solution was obtained. The solution was then crash cooled to 20 °C at 20 °C/min to induce crystallization and held at that temperature with constant stirring for several hours. After several minutes large amounts of fine needle-shaped crystals could be seen using the particle view camera of the ***Crystalline*** instrument. By making use of in-situ Raman spectroscopy on the ***Crystalline*** instrument, a peak was observed at 262.5 cm^{-1} indicating the formation of the kinetic metastable Form 2. After ~ 100 mins a few block-shaped crystals of Form 3 appeared, together with the emergence of peaks at 248 and 271 cm^{-1} in the Raman spectra. Over the course of the next 130 mins the amount of Form 3 increased as the metastable Form 2 converted.

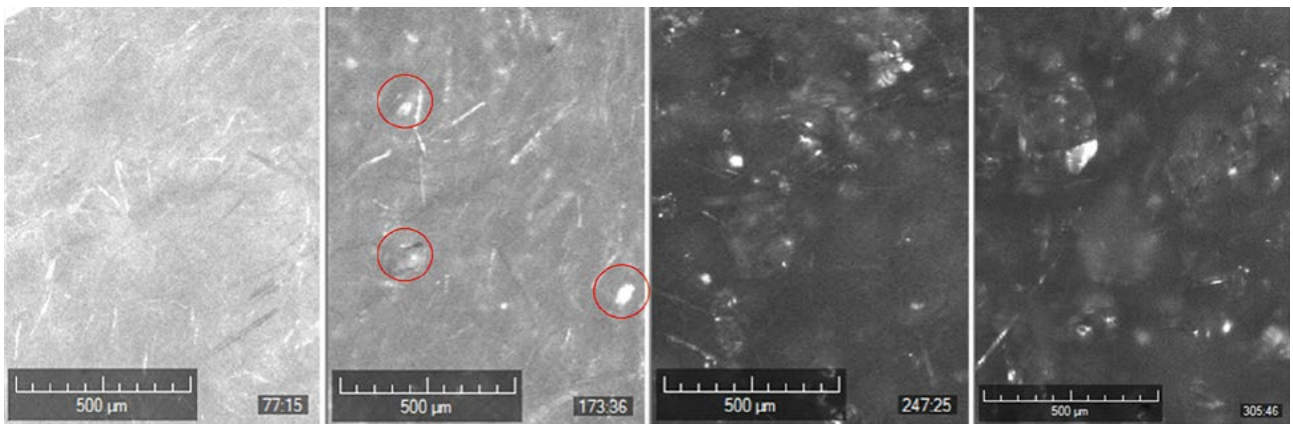
This can be seen in the increase in the number of block shaped crystals on the particle viewer and the decrease in needle-like crystals. Furthermore, the peak at 262.5 cm^{-1} associated with Form 2 decreased in intensity until it was no longer visible at the end of the experiment, and the peaks of Form 3 grew in intensity showing that the thermodynamic Form 3 was now the majority.

Figure 3. Raman spectrum transformation for carbamazepine captured using the Tornado HyperFlux integrated with the Crystalline



References
F. Tian; J.A. Zeitler; C.J. Strachan; D.J. Saville; K.C. Gordon; T. Rades (2006). Characterizing the conversion kinetics of carbamazepine polymorphs to the dihydrate in aqueous suspension using Raman spectroscopy. J. Pharm. Biomed. Anal., 40(2), 271–280.
Ouyang, J., Chen, J., Rosbottom, I., Chen, W., Guo, M., & Heng, J. Y. Y. (2021). Supersaturation and solvent dependent nucleation of carbamazepine polymorphs during rapid cooling crystallization. CrystEngComm, 2021,23, 813-823

Figure 2. Morphology transformation of carbamazepine Form 2 (needles) to Form 3 (Blocks) captured using the Crystalline instrument



Nucleating Form 2 → *Converts to Form 3*

Crystalline Specifications	
Reactors	8
Reactor type	8 ml vials
Working volume (ml)	2.5 - 5 ml
Temperature profiles	8
Temperature range °C	-25 - 145
Temperature accuracy °C	0.1
Heating and Cooling rate (°C/min)	0 - 20
Stirring modes	Overhead or stirrer bar
Stirring rate (rpm)	0-1250
Turbidity (%)	Every reactor
Chiller necessary	Yes
In-line analytics	4-8 particle view imaging cameras and/or Raman probes
Particle size and shape analysis	Yes – with particle view imaging cameras
Extra functions	Reflux, antisolvent, seeding, evaporation and pH monitoring integration
Data export	CrystalClear, Word Report, XML

Benefits of the Crystalline

- More data with less material
- Kick start crystallisation development earlier
- Non-invasive in line turbidity, Raman spectroscopy and particle viewer analysis

Tornado Specification for HyperFlux™ PRO Plus Raman Spectrometer and Hudson™ Probe	
Laser Wavelength (nm)	785 nm
Probe Types	Immersion and Non-Contact
Probe Materials	Stainless Steel, Hastelloy, Titanium, other materials possible
Range (cm^{-1})	200 to 3300
Laser Power (mW)	5 to 495
Multiplexing Capacity	4 channels or 8 channels
Operating Range (ambient) (°C)	0 to 35
Chemometric Support	Embedded SIMCA and PEAXACT engines
Data export	.spc, .csv

Conclusion

We have demonstrated that by utilizing the ***Crystalline*** device coupled with a Tornado Raman spectrometer, it is possible to monitor and track polymorphism and morphology at low working volumes. Typically, the smallest commercial vessel where Raman spectroscopy and in-solution microscopy can be used as PAT tools is 100 mL, but by using the experimental setup described in this document it is possible to obtain the same data while working at scales as low as 2.5 ml. By working at these reduced volumes, the required material required for crystallization development can be significantly reduced. Solid form selection and research can be undertaken earlier in API development, increasing the speed they can reach manufacturing and become available to the patient.

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