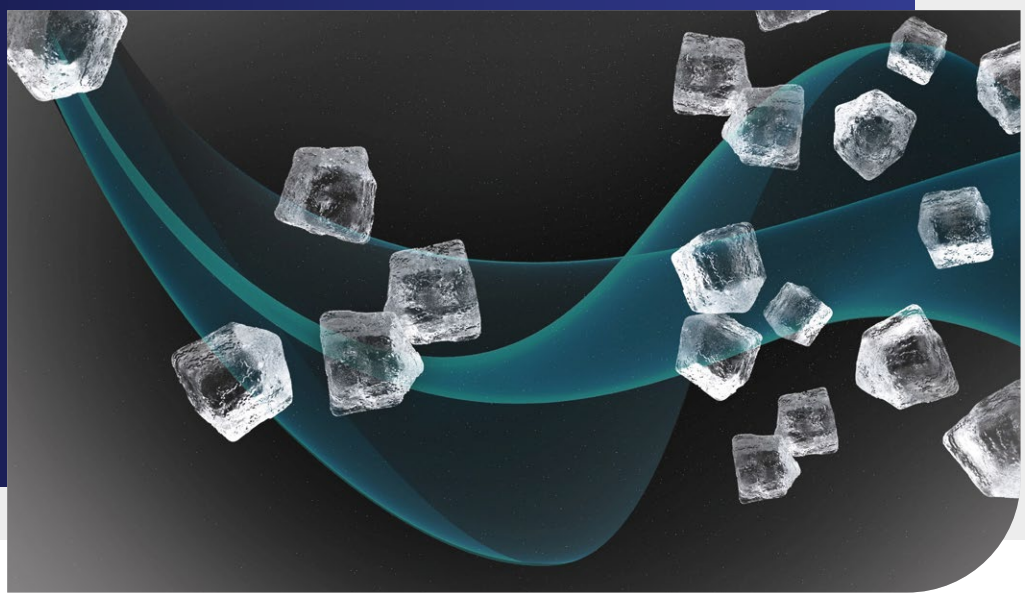


MEASURING SECONDARY NUCLEATION THROUGH A SINGLE CRYSTAL SEEDING APPROACH

What is crystallization?

Crystallization is a common process in the pharma industry in the manufacturing of solid form doses. It is a highly complex process consisting of kinetic, thermodynamic and chemical factors that compete against each other making crystallization difficult to control, therefore understanding the steps involved in crystallization is paramount. Crystallization can be separated into two processes nucleation and crystal growth. Nucleation is important to control, predict and understand as it plays a significant role in the polymorphic form, particle size distribution and downstream particle properties.

Why introduce seeding protocol?

- Control crystallization process
- Control of polymorphic form
- Influence particle size distribution

Benefits of this approach

- Easy to incorporate into crystallization workflow
- Rapid screening platform
- Greater understanding of the crystallization process

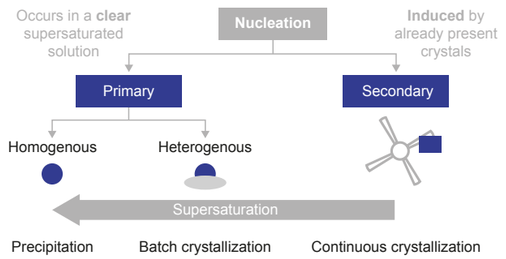
Benefits of the Crystalline

- Easy to use
- Disposable reactors
- Non-invasive analytics
- Overhead stirring
- Seeding and antisolvent integration
- Small volume imaging and Raman

Types of nucleation

Crystal nucleation is the formation of new crystalline entities from a supersaturated liquid phase and, plays a large role in the final crystal size distribution and polymorphism of a compound. Nucleation can be categorized as primary or secondary. Primary crystal nucleation can be either homogeneous which occurs in a clear solution in the absence of crystalline material of its own kind or heterogeneous which occurs in the presence of impurities or foreign entities. Secondary nucleation occurs as a result of the presence of crystals of the same compound in a supersaturated suspension and is typically seen after seeds are added (Figure 1). Secondary nucleation influences the particle size distribution of the final product and therefore affects downstream processing and particulate product quality.

Figure 1. Crystal nucleation scheme



Measuring secondary nucleation

The ***Crystalline*** offers the ability to quantify secondary nucleation utilizing the in situ visual monitoring, particle counter and the transmissivity measurements to identify the secondary nucleation threshold within the metastable zone width (MSZW). This approach follows the single nucleus mechanism (Figure 2) and uses a systematic single crystal seeding method in a small batch-wise crystallization process to follow the number density of crystals during the experiment, which determines the rate of secondary nucleation rate and provides the identification of supersaturation limits at which secondary nuclei can be formed and detected.

Figure 2. Visualization of nucleation

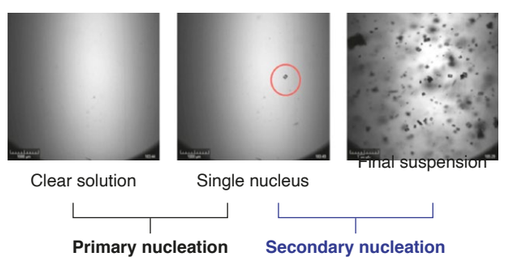
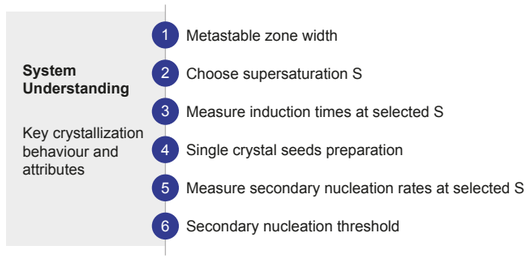
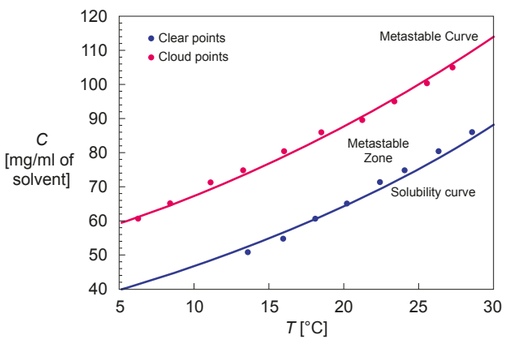


Figure 3. Experimental workflow



The described workflow (Figure 3) allows for a rational discrimination between primary and secondary nucleation events. It consists of 6 stages and starts the generation of the solubility and metastable curved using the transmissivity data collected using the ***Crystalline***, from these curves it is possible to determine the MSZW (stage 1) in order to define the crystallization window (Figure 4). Once the MSZW has been determined, several supersaturations need to be selected. The supersaturations selected must be sufficiently close to the solubility curve to avoid unwanted spontaneous primary nucleation, so secondary nucleation can be measured. Spontaneous primary nucleation can be monitored through induction time measurements. The next step is to generate single crystals and characterize the size of the crystals and to calibrate the camera. In order to measure the secondary nucleation, the camera needs to be calibrated using polystyrene microspheres to calculate the suspension density (Np) from the number of particles on the screen (N). The secondary nucleation then can be collected at a range of supersaturations and crystal sizes to determine the secondary nucleation threshold. This threshold can be used in the industrial crystallization design process to either avoid or enhance secondary nucleation, considering that fluid dynamics in the experiments and under industrial conditions might be substantially different, possibly influencing this secondary nucleation threshold.

Figure 4. Solubility and metastable curves



Case study

Isonicotinamide is an isomer of nicotinamide and it is widely used for co-crystallization. In this study Isonicotinamide is used in ethanol and the objective is to accurately measure secondary nucleation rates while clearly distinguishing secondary and primary nucleation processes. The approach consists in seeding a supersaturated solution with a known amount of well-characterized crystals under conditions at which primary nucleation does not occur. Using ***Crystalline***, on a 2.5-5 ml scale, it is possible to add a single seeded crystal to a clear, supersaturated and agitated solution at constant temperature whilst the number of crystals subsequently formed is monitored.

The seeded experiment shows a suspension density increase 6 minutes after the single seed crystal was added. In the unseeded experiment the suspension density raised only after 75 minutes, (Figure 5) showing that spontaneous primary nucleation in the unseeded experiment is detected much later compared to secondary nucleation induced by the seed crystal in the seeded experiment. The suspension in the seeded experiment is therefore the result of secondary nucleation initiated by the seed crystal. Furthermore, the observed secondary nucleation rate was dependent on the seed crystal size as well, indicating that secondary nucleation is faster using larger single seed crystals (Figure 6).

Figure 5. Nucleation rate of seeded (green) and unseeded (red) crystallization

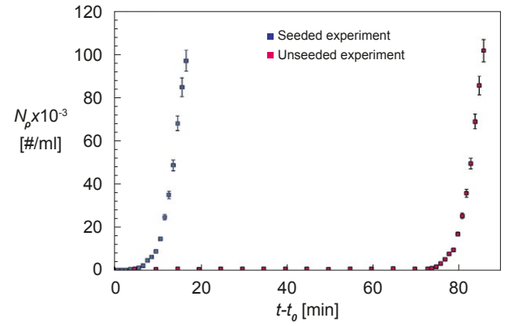
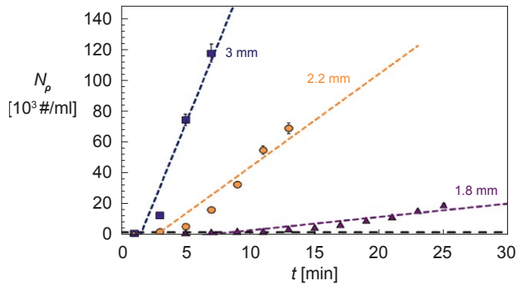
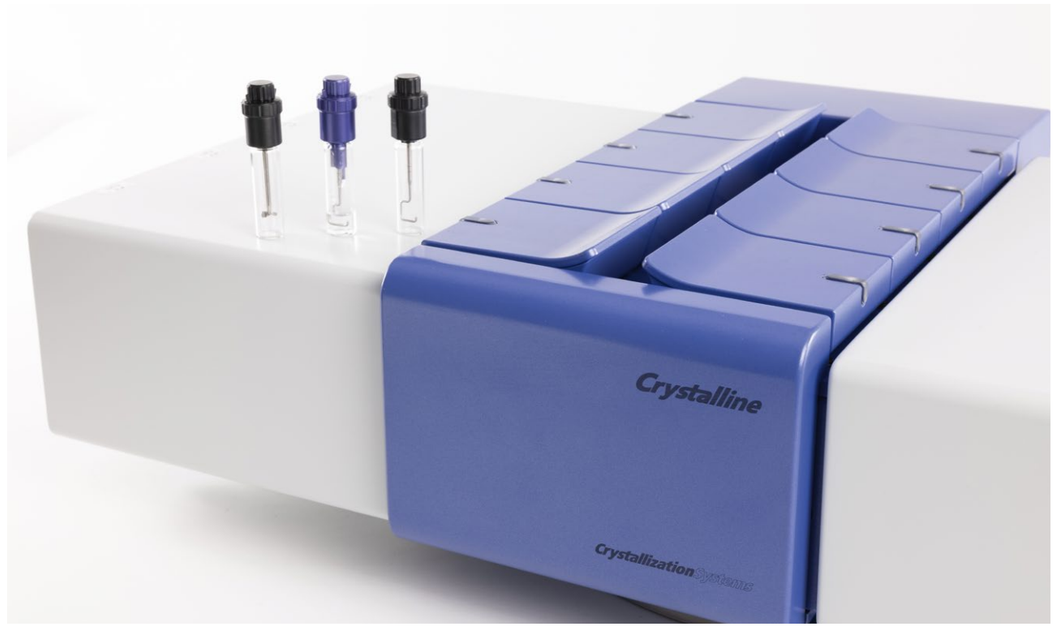


Figure 6. Secondary nucleation rate with seed crystal size





Conclusions

A novel and reproducible single crystal seeding approach in batchwise cooling crystallization has been developed allowing the study of secondary nucleation using ***Crystalline***. Upon seeding a well characterized single parent crystal in an accurately controlled supersaturated solution, the suspension density starts increasing after a delay time allowing the determination of the secondary nucleation rate. The developed method enables a systematic study of secondary nucleation kinetics, which improves the control on this crucial step of crystallization. This novel seeding procedure can be incorporated in crystallization workflow procedures allowing rapid development of industrial crystallization processes.

Specifications Crystalline	
Reactors	8
Reactor Type	8 ml vials
Working Volume (ml)	2.5-5 ml
Temperatures profiles	8
Temperature range (°C)	-25 to 145
Temperature accuracy (°C)	0.1
Heating/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

References:
Briuglia. M, Sefcik. J, ter Horst. J; Measuring Secondary Nucleation through Single Crystal Seeding; Cryst. Growth Des. 2019, 19, 1, 421–429

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