Crystallization is a common step used during the synthesis of organic compounds to isolate
and purify the desired product. In recent years the importance of crystallization in the pharma-
ceutical and high value chemical industries has increased due to the following factors1:
1. A requirement to reduce impurities to extremely low levels that may not have historically
   been detectable.
2. A desire to tightly control physical crystal attributes for:
   a. Better formulation and improved reproducibility and bioavailability.
   b. Improved downstream processing – filtration, drying, milling.
3. The need to crystallize more complicated molecules of increasing molecular weight.
4. Increasing requirements to duplicate crystal attributes across multiple scales during
   development and manufacture to meet regulatory requirements.

These demands mean process chemists now spend more time developing better crystalliza-
tion processes for (a) intermediate synthesis steps, where impurity rejection and efficient
downstream processing are critical; and for (b) the final synthesis step, where the crystalliza-
tion process will produce the final active ingredient under strict regulatory guidelines and with
the desired bioavailability.

In recent years the Process Analytical Technology (PAT) framework2 has provided guidance
on the use of novel measurement technologies, often inline or at-line, to design, analyze,
and control pharmaceutical processes. Critical process parameters (CPPs) and critical quality
attributes (CQAs) for crystallization processes, such as supersaturation, crystal size and
impurity profile, can be measured inline or at-line during the development and manufacture of
an active ingredient.

<table>
<thead>
<tr>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
</tbody>
</table>
The challenge remains that the successful implementation of PAT technologies often requires a level of expertise that necessitates the creation of specialist groups which can only focus on a small number of high priority projects at one time.

For crystallization processes, an opportunity exists to deploy simpler technologies that can be readily adopted by scientists without any specific PAT expertise. An example of this type of technology is real-time microscopy\(^3\), which provides scientists with high quality images and videos of crystals and crystal structures inline as process conditions are changing.

1 The Importance of Crystal Size, Shape, and Structure

Crystal size, shape, and structure are critical quality attributes that require control during scale-up and manufacturing. For intermediate synthesis steps the role size, shape, and structure play in the development of successful processes may not be clear at first, since the crystal product will simply be dissolved before the initiation of the next reaction steps. However, crystal products generated as part of an intermediate crystallization steps must be isolated, and these isolation properties are directly related to the size, shape, and structure of the product. In Figure 1, four real-time microscopy images are shown for different crystal products. The ease with which each of these products could be isolated using a filtration step can be imagined.

![Figure 1: Four real-time microscopy images of different crystal products with differing crystal size, shape, and structure](image)

The crystals in Figure 1 (a) will likely filter quickly and consistently. The large "boulders" will leave plenty of space for the filtrate to pass between them as they pack on the filter.

Flat platelet crystals like those shown in (b) can be some of the most difficult to filter. Platelets tend to stack on top of each other creating a layer of crystals that the filtrate cannot pass through. This leads to long and potentially variable filtration times, depending on how the crystals are discharged from the crystallizer.
The small crystals shown in (c) will plug the gaps left by the larger crystals, making it difficult for the filtrate to pass through the bed of crystals. The presence of a significant population of fine (small) crystals often causes extended filtration times and can be particularly difficult to isolate and move to the next process step.

The dendritic crystal shown in (d) is quite common in organic processes that rely on seed crystals to initiate the processes. Imperfections on the crystal surface lead to crystal growth from these areas and long crystal branches growing from a seed core. It is difficult to predict how something like this will filter, but it is likely to break apart resulting in variable filtration times.

2 Factors Affecting Crystal Size, Shape, and Structure

In Figure 2, a process flow diagram illustrates some of the options available to scientists who wish to develop a crystallization process that results in the desired product and process performance. Critical process parameters need to be designed and controlled so as to ensure crystallization mechanisms, such as nucleation growth or agglomeration occur predictably. If this can be achieved then crystal quality attributes, such as crystal size and the impurity profile, can be optimized carefully.

In order to do this effectively, scientists need to obtain process understanding for their crystallization process. As stated previously, such process understanding can be obtained using PAT tools; however, many of these require a significant learning curve and expertise to implement successfully.

**Figure 2:** Flow diagram illustrating how input properties and process parameters influences crystallization mechanisms, and the subsequent impact on process and product performance
3 Real-Time Microscopy and Relative Backscatter Index

With real-time microscopy, it is possible for any scientists to begin understanding the key mechanisms that take place in a crystallization process, and how critical process parameters influence these mechanisms. Viewing videos and images of crystals directly inline as they change provides unambiguous process understanding without any data analysis, or complex interpretation required.

Many recent examples can be found in the literature where this technique is applied to understand and optimize crystallization processes in particular to optimize cooling rates⁴, control polymorphism⁵, identify and address phase oiling and phase separation⁶. To further automate and enhance the development of process understanding from images and videos, image analysis routines such as Relative Backscatter Index (RBI)³ have been developed. Relative Backscatter Index is a measure of the overall reflectivity of the crystal system at a given point in time, and is similar to a turbidity measurement in that it can be used to follow crystallization progression using a simple univariate trend.

The combination of real-time microscopy, RBI, and recorded process parameters for a crystallization processes become powerful PAT techniques that any scientist can use to understand a crystallization process. In Figure 3, a simple example is shown where the RBI is used to measure the dissolution and nucleation temperature for an organic compound. With this information scientists can easily determine the solubility curve for their system and optimize the nucleation temperature, which has been shown to correlate directly to process consistency and purity⁷.

Figure 3: Combining real-time microscopy with RBI and temperature to develop measure the dissolution and nucleation temperature for an organic compound
4 The Influence of Cooling Rate on Crystal Size and Shape

Supersaturation is the driving force for crystallization processes and directly influences crystal nucleation and growth rates (Figure 4). This relationship is explained in great detail by Nyvlt et al.8, but for the purposes of this paper, it should be clear that the choice of cooling rate in a crystallization process has a dramatic effect on the prevailing level of supersaturation and the final crystal size distribution.

In Figure 5, two crystallization processes are compared, each with a different cooling rate. The RBI trends in each case indicate the difference between the process growth kinetics, endpoint, and batch time.

![Figure 5: Two crystallization processes (A) and (B) with a slow and fast cooling rate respectively, result in crystals of different size and shape](image)

Figure 4: Supersaturation influences crystal nucleation and growth rates, which ultimately dictates the crystal size distribution.
The slower cooling rate, Process A, results in noticeably larger crystals, as shown in the real-time microscopy images, whereas the faster cooling rate results in smaller crystals which are thinner. This example illustrates a classic example where the effective design of a crystallization process requires a trade-off to be made. In this case, a balance must be drawn between a faster crystallization time, which may save time in the plant, versus a smaller, thinner crystal, which may pose challenges during filtration and drying. Scientists can use the information provided by real-time microscopy and the RBI trend to develop the process understanding they need to make informed decisions.

5 Identifying Polymorphs During Crystallization Development

Strict understanding and control of the polymorphic behavior of an active ingredient are vital at all stages of development. For intermediate synthesis steps, the isolation properties of a crystalline product can depend heavily on the polymorphic form, and consistently producing the correct polymorph demonstrates good understanding and control of the process.

In some cases, new polymorphic forms can appear during development and fast identification is critical. In other cases where the presence of more than one polymorph is known, and an in-process transition is required to produce the desired process, it is extremely useful to apply a PAT methodology to ensure that the transition from the less stable to more stable polymorph occurs consistently across all scales and operating conditions.

In Figure 6, a polymorphic transformation is studied. Both the RBI and real-time microscopy images provide detailed and unambiguous process understanding. When the initiator is added nucleation occurs immediately, as indicated by the rapid increase in the RBI signal, corresponding to the formation of crystals. While the temperature remains constant at 50 ºC, the RBI reaches a steady state, but soon increases again rapidly – indicating a second nucleation event has occurred. Checking the real-time microscopy images confirms the presence of a second crystal morphology has nucleated, which can be verified as a different polymorphic form using offline XRD analysis. Over time, the transition from the less stable form to the more stable form occurs, until the RBI trend again reaches a steady state, and the real-time microscopy images show that only needle shape crystals persist.

This case study demonstrates the ease with which scientists can obtain extremely useful process understanding using a PAT, which is simple to implement and requires very limited data analysis expertise.
Figure 6: Monitoring the nucleation of two polymorphs, and the subsequent transition to the more stable form.

6 Recognizing and Addressing Oiling Out (Phase Separation)

Oiling out can occur in crystallization processes where impurity levels are high and/or supersaturation is generated at a very fast rate (typically through anti-solvent crystallization or a salting out with an acid-base reaction). Oiling out occurs when the system is driven to a point in the phase diagram where a liquid-liquid phase split becomes possible, and a product-rich oil phase is formed in the solvent matrix. Crystallization will subsequently occur but the product is more prone to contain residual impurities and have a variable crystal size distribution. Processes that oil out and then crystallize can also be very difficult to isolate and leave a residue on crystallization equipment that is very difficult to clean, particularly at larger scales.

Recognizing when oiling out occurs is important so that changes can be made to the process early in development, minimizing the chance of a poorly performing process reaching larger scales. Visual observation of oiling out, by looking through the glass walls of a crystallizer, is often impossible because the solution just looks turbid, much like it might appear if it were to crystallize normally.
In Figure 7, a crystallization process is shown where a fast cooling rate results in an oiling out event. The RBI identifies an initial "nucleation" event – but the real-time microscopy images reveal that oil droplets have appeared, rather than crystals (a). Over time, the oil phase disappears and crystals nucleate and grow, while real-time microscopy images reveal that crystals are actually growing out of the oil droplets as well (b). Subsequently, the oil phase disappears completely and only crystals remain, which then go on to grow until the end of the process (c).

This phenomenon has been shown in the literature, and methods to prevent oiling out range from adjusting starting concentration\(^9\), to adjusting the seed size and loading\(^10\).

7 Conclusions

Crystallization is a unique unit operation that offers scientists a method to isolate and purify products in a single step. The pressure to deliver optimized crystal products with the desired attributes is increasing and scientists need to obtain better process understanding to support their optimization efforts. Many PAT tools suitable for supporting the development of crystallization processes are extremely valuable, but can be complicated enough so as to limit their adoption outside dedicated and specialist groups. Real-time microscopy combined with simple image analysis, in this case Relative Backscatter Index (RBI), offers an opportunity for every scientist to begin utilizing PAT to design, analyze, and control crystallization processes as part of the PAT framework.

In this paper, examples focus on process optimization and demonstrate the ease with which relevant data can be obtained and analyzed, without any special expertise needed. This results in crystallization processes that will deliver the required critical quality attributes consistently at every scale required.
Appendix: ParticleView with PVM Technology

ParticleView V19 with PVM® technology is a probe-based instrument that visualizes particles and particle mechanisms in real time. High resolution images are continuously captured without the need for sampling or manual offline analysis. A process trend, sensitive to changes in particle size and concentration, is automatically combined with the most relevant images providing scientists with comprehensive process understanding.

www.mt.com/ParticleView

How does ParticleView work?
ParticleView uses a high resolution camera and internal illumination source to obtain images even in dark and concentrated suspensions or emulsions. With no calibration needed and easy data interpretation, ParticleView quickly provides critical knowledge of crystal, particle, and droplet behavior.

What is RBI?
ParticleView V19 with iC PVM™ uses information from every image that is collected to calculate an innovative process analytical trend called Relative Backscatter Index (RBI). RBI is a measure of the overall reflectivity of a particle system and indicates how particle size, shape, and concentration is changing over time.

RBI is used to understand how changing process parameters affect process performance and combined with high resolution images provides comprehensive process understanding.
References

1. Powder Technology, 150, pp 133 – 143
3. www.mt.com/ParticleView