Advances in On-line Monitoring and Control of the Morphological and Polymorphic Forms of Organic Crystals Grown from Solution[†]

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Abstract

Organic crystals grown from solution are known to exhibit multiple morphology as well as habits which are of great importance to the end-use property of the product such as the bioavailability and the downstream processing such as in filtration and drying. The crystal morphology can also dictate other quality measures such as the size. Compared with the great amount of research work that has been done on the on-line measurement of other quality measures such as the size and concentration using various spectroscopy techniques, the literature on the on-line measurement and manipulation of crystal morphology is scarce. Attempts were made in the past to use laser diffraction and ultrasound spectrometry for shape monitoring. These methods have not proved to be very successful due to the difficulty in extracting detailed shape information from the signals corrupted by noise and multiple scattering. In this paper, we describe a new approach for on-line crystal morphology measurement and control which is based on the integration of on-line imaging, multi-scale image analysis and crystal morphology modelling, and present the results obtained on applying the approach to the batch crystallisation of (L)-glutamic acid. On-line imaging proved capable of capturing high fidelity crystal shapes and polymorphic transitions in real time. A multi-scale image analysis method was proposed to extract the crystals from the image background and to calculate shape descriptors which were then used for shape recognition and to derive monitoring charts showing the ratios of different polymorphs in real time as well as the relative average growth rates of facets of crystals. Calculating crystal growth rates and estimating kinetics parameters for needle-shaped crystals was also investigated. Finally, a methodology called 'camera model' for integrating on-line imaging and crystal morphology modelling was presented.

Key words: Crystal morphology, Crystal polymorph, On-line imaging, Image analysis, Morphology modeling, Morphology control, Glutamic acid

Introduction

High value-added speciality chemicals such as pharmaceuticals are often manufactured in batch crystalli-

sation processes. The shape, size and polymorphic form are properties of great importance to crystalline drug products. It is known that certain crystal morphological forms and habits have been related to difficulties in the dissolution rate, process hydrodynamics, solid-liquid separations, drug tableting, storage and handling, or in milling and grinding. The control of a product in crystallographic form is important in industrial production in that different polymorphs often exhibit different physicochemical properties

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implying variability in product performance, e.g. via a different solubility and hence bioavailability for a drug compound.

In the past, a significant effort has been devoted to the development of on-line analytical techniques for monitoring deterministic operating variables including the concentration using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR)¹⁻³, to monitor the crystallographic structure using X-ray diffraction (XRD) and Raman spectroscopy^{4,5}, and the crystal size distribution (CSD) using laser diffraction⁵, ultrasonic spectroscopy⁶ and light scattering techniques⁷⁻⁹. In contrast, the literature on monitoring crystal morphology is scarce¹⁰.

In addition to the ultimate objective of morphology control in manufacture, the on-line monitoring of crystal morphology can also help the development of crystal morphology modelling and prediction techniques. This has not yet come to the level of being able to consider the simultaneous effect of all engineering factors or operating conditions critically related to product morphology such as solvents and cooling rates or heat transfer effects¹¹, despite the significant progress and the availability of commercial software systems such as HABIT¹² and CERIUS2¹³. It is known that minor changes in supersaturation, cooling rates, reactor hydrodynamics, pH and the presence of impurities can have a significant impact on the crystal product. In addition, there are unexpected factors which can become deterministic such as wall effects on heat transfer, baffles, and impeller materials and types¹⁴. As a result, on-line measurement and real-time classification of crystal polymorphous is important because it can partially compensate the inability in modelling the morphology and shape. On the other hand, being able to measure morphology on-line in real time will no doubt provide a tool that can help the development and validation of new and more sophisticated crystal morphology prediction models.

This paper reviews the previous efforts on the on-line sensing of crystal morphology and presents recent advances in developing an enabling technique for the real-time measurement and manipulation of the morphology of growing crystals through integrating imaging, image analysis and morphology modelling.

Previous Work

Laser diffraction techniques were investigated previously for the recognition of non-spherical particles, but only limited success has been achieved in this

area. The main difficulty has been in obtaining a single-particle pattern in mixtures due to problems associated with partial scattering of the particles¹⁵⁻¹⁷. Mougin et al.^{6, 18, 19} attempted to interpret the ultrasound attenuation spectra in order to identify dynamic changes in polymorphs during the crystallisation of an organic compound. A correct interpretation was found possible though some additional information needs to be provided which is not always readily available. Studies have also been made to produce crystals with different size and shape properties through supersaturation control using ATR-FTIR³. The modification of temperature to maintain supersaturation values during nucleation and crystal growth helps produce uniform size and shape properties, nevertheless no information on particle shape is obtained during the experiment. This practice requires concentration and supersaturation models for the compound to crystallise obtained from calibrations, and the temperature range for control may be limited.

In laboratory studies, photo-microscopy has been the most widely used method for analysing crystal morphology. Samples are taken periodically from the reactor and observed using a photo-microscope imaging system. Software systems are also often available for quantitatively analysing the images. Patience and Rawlings²⁰ reported an interesting approach for automating this process using a flow cell in a laboratory study of the crystallisation of sodium chlorate. The flow through the cell was periodically stopped to allow a photo-microscope to capture images after the crystals had settled down at the bottom of the flow cell. In this way, the crystals of the compound were lying on a habit face and showing a particular shape to be characterised by a commercial image analysis software. They also developed an automatic control system by manipulating the impurity in the feed. The system has some limitations. Firstly, the sampling period is from 20 to 30 seconds, whereas significant morphological events in crystallisation might take only a few seconds. In addition, the use of a flow cell is clearly not ideal for many industrial processes. The flow cell configuration requires a pump to circulate the solution, and this might cause crystal breakage, as discovered by Mougin¹⁸. The on-line image analysis approach reported by Patience and Rawlings employs boxed-area and aspect-ratio to describe the crystal shape along with predefined cluster regions for the classification of the two studied shapes, square and triangle. Their strategy for crystal recognition does not allow detection of any new crystal shapes existing within the reactor, and requires the assump-

tion of some shapes and calibration to predefine the corresponding classification regions for the shapes. In addition, the regions are fixed throughout the whole process, meaning that changes in morphology due to, for example, the crystal growth of each face or unexpected impurities may lead to a low amount of crystals being detected or may mislead the process control. Lasentec Inc, USA, developed a Particle Vision Measurement (PVM) probe which can be used for visualisation of the process in-situ. The PVM uses a charged coupled device camera with light sources fitted into the probe²¹. Barrett and Glennon¹⁰ used the PVM probe to visualise the crystals in the determination of the meta-stable zone of an inorganic compound. Patience²¹ made an attempt to analyse images obtained by the PVM probe in the crystallisation of sodium chlorate. He encountered difficulties in the analysis of the images using a commercial image analysis software tool. More recently, Wilkinson et al.^{22, 23} reported an approach using video imaging and image analysis for the on-line measurement of particle length and circularity in the crystallisation of drug compounds. Their approach uses a threshold method for the segmentation of the crystals from the images, and a low lens magnification for achieving a high measurement rate of the crystals.

The Framework

Fig. 1 depicts the structure of the work. It mainly

consists of three parts: (a) use of an on-line video imaging system for monitoring batch crystallisation; (b) obtaining quantitative information from images through image segmentation and image analysis to calculate shape descriptors and perform polymorph and morphology recognition; and (c) calculating crystal growth rates and estimating growth kinetics parameters.

Experiments

Experiments were carried out using a glass-jacketed reactor of 500 ml, a data interface board and a PC running WinISO process control software provided by Hazard Evaluation Laboratory Ltd. (**Fig. 2**)^{24, 25}. To control the temperature, a Julabo FP50-HD thermostatic bath was employed. A reflux condenser was used to circulate water at 6°C to minimise solvent loss at the higher reactor temperatures. Reactor stirring was performed using a pitched-blade stirrer rotating at a constant speed of 200 rpm. The temperature was measured using a platinum resistance thermometer (PT100), turbidity was measured with an in-house-built turbidimetric fibre-optic probe system. Both signals along with pH values were logged into the computer system. Observation and recording of the process were carried out in real time using on-line imaging with an instrument developed by the pharmaceutical manufacturer GlaxoSmithKline, UK. For off-line image analysis, the PVS830 described below

Fig. 1 The framework

Fig. 2 The experimental system

was employed, using a pipette to take samples from the reactor solution at several instances during the process. The samples were quickly placed and covered on the sample glass slide of the previously calibrated PVS830, and images were captured and analysed. The time from taking a sample until the off-line image analysis was from 10 to 20 seconds.

The on-line imaging instrument employed in the experiments is a high-speed CCD camera system which has a maximum frequency of thirty images per second with a typical pixel resolution of 480×640 and a field of view from $140 \mu\text{m}$ to 16 mm . The camera is situated just outside the reactor wall and an imaging window is attached to the external reactor wall to avoid convexity effects on the images (**Fig. 3**). To

provide illumination, a strobe light source is used and the light is conducted using a fibre-optic guide. Camera acquisition and flashing light are synchronised to freeze the moving particles by using a camera interface box developed by scientists at the pharmaceutical manufacturer GSK^{22, 23}. The captured images are sent to a PC running VideoSavant software (IO Industries, Inc.) for acquisition, storage and management of frames.

To enhance the contrast of particles from the image background, two fibre-optic light guides can be used, both adjustable in angle and distance. The system allows the visualisation and recording in real time of every event occurring throughout the process, thus capturing the complete history of the crystallisation.

(a)

(b)

Fig. 3 (a) the on-line imaging system applied to a 5 Litre reactor; (b) images captured and the image acquisition

For an estimation of the particle size, the camera is calibrated with a glass micrometer scale (Agar Scientific Ltd.), using Mitutoyo objective lenses with Navitar Precise Eye lenses for different magnifications, allowing observations down to 5 μm .

An off-line imaging system, the PharmaVision System 830 (PVS830) from Malvern Instruments Ltd.²⁶⁾, was also used in the study. It is an automatic vision system for the analysis of the size and shape of particles. A zoom lens allows particles between 0.5 and 2000 μm to be analysed. The instrument automatically calibrates itself prior to an analysis by the measurement of both light intensity and precision grating with a known number of lines per millimetre. To perform the analysis, the sample is placed on a sample tray under a CCD video camera. A linear actuator connected to a PC moves the camera across the sample tray and the camera takes digitised video images. Particles from the images are automatically segmented by embedded computer software, thus obtaining a variety of shape parameters such as length, width, mean diameter and roundness, and supported by images of all particles for further visual understanding. The performance of the instrument is verified by reference slides which are certified and traceable to standards issued by the National Institute of Standards and Technology.

Solutions of 33.3 g of L-glutamic acid (purchased from Aldrich Chemicals) dissolved in 500 ml of fresh distilled water were prepared. The solutions were heated up to 95°C and kept constant at this temperature until everything was dissolved, then linearly cooled down to 15°C, keeping this temperature until the end of the experiment. Different linear cooling rates of 1, 0.5 and 0.25°C/min were investigated. The concentration and temperatures of the experiments were chosen to be close to those used in an industrial-scale operation. It was found that for the experiment employing a cooling rate of 0.25°C/min, the images revealed that the crystals were at first growing with a rhombic shape, an α -form (**Fig. 4 (a)** and **4 (b)**). Transformation into a β -form was observed at a temperature of 60°C, as illustrated in the real-time image of **Fig. 5 (a)** and **(b)**. For the experiment using a cooling rate of 0.5°C/min, α -form crystals were also first found and the transition to a β -form was also observed, but at a much later stage corresponding to a temperature of 30°C. No transition from α to β was observed when a cooling rate of 1°C/min was used. The results confirmed and helped explain the crystallisation mechanisms²⁴⁾.

During the onset stage, the crystallisation experi-

(a)

(b)

Fig. 4 Observed crystals at 63°C and cooling rate 0.25°C/min in real-time (a), and in the PVS830 (b)

(a)

Parameter	Value
Area	100.00
Perim	100.00
Length	100.00
Width	100.00
Roundness	100.00
Aspect Ratio	100.00
Convexity	100.00
Extinction	100.00
Extinction Ratio	100.00
Extinction Ratio 2	100.00
Extinction Ratio 3	100.00
Extinction Ratio 4	100.00
Extinction Ratio 5	100.00
Extinction Ratio 6	100.00
Extinction Ratio 7	100.00
Extinction Ratio 8	100.00
Extinction Ratio 9	100.00
Extinction Ratio 10	100.00
Extinction Ratio 11	100.00
Extinction Ratio 12	100.00
Extinction Ratio 13	100.00
Extinction Ratio 14	100.00
Extinction Ratio 15	100.00
Extinction Ratio 16	100.00
Extinction Ratio 17	100.00
Extinction Ratio 18	100.00
Extinction Ratio 19	100.00
Extinction Ratio 20	100.00

(b)

Fig. 5 Real-time image for the cooling rate 0.25°C/min at 60°C (a) and shape dimension obtained from a sample at 60°C (b)

ments were carefully followed by the on-line imaging system. The onset point is said to occur when the first crystals start appearing in the solution, which is sometimes called the 'cloud point'. Looking at the real-time images, we were able to identify a number of appearing particles instants before the fibre-optic turbidity probe could show a pronounced reduction in light transmittance at the cloud point. The temperature at the onset points obtained by on-line imaging corresponding to the three cooling rates are summarised in **Table 1**. The results were found to be either earlier or at the same time as turbidity results²⁴.

Table 1 Crystallisation onset values obtained from on-line imaging

Cooling rate	Temperature onset	Cooling time
1°C/min	54.10°C	42 min
0.5°C/min	59.74°C	55 min
0.25°C/min	69.95°C	47 min

Image Analysis, Shape Classification and Monitoring Charts

To obtain quantitative information from the images for subsequent monitoring and control purposes, a major challenge is the availability of methods for image analysis that need to be not only tolerant of the quality of on-line images, but also accurate, fast and robust. A literature survey of the various techniques and tools led to the same conclusion as Patience²¹ had come to, i.e. that the existing methods do not work effectively for the on-line images obtained from crystallisation reactors. Images of slurries with particles suspended in a solution are much more complex than images of purely solid particles, and the major challenges lie in the fact that the slurries in a stirred reactor are in continuous motion, and that the variation of the distance between the camera lens and the particles captured in a snapshot makes the edges of some particles more vague than others. In addition, the light effect and temporal changes of hydrodynamics within the reactor may lead to varied intensity in the background.

As a result, and as part of the research, a new multi-scale approach based on the Canny²⁷ and wavelet functions^{28, 29} was developed which proved to be very promising in extracting the crystals from the image

backgrounds^{25, 28, 30}. The image segmentation method involves several steps: edge detection over two scales (so called multi-scale), mathematical morphological closing and opening to remove noises and small objects, to ultimately obtain the segmented crystal objects. **Fig. 6** shows an example of analysing a poor-quality image (to choose a low-quality image to demonstrate the capability of the method). **Fig. 7** shows the analysis results for images obtained using the Malvern Instruments PVS830 and the PVM system of Lasentec. The processing time for a typical image with a resolution of 640×480 obtained by the GSK system is between seven and ten seconds using a PC of 2 GHz. No significant difference in processing time was observed when analysing images from the PVS830 with a resolution of 756×548.

Following image segmentation, the next step is to classify the shapes and extract morphological data³¹. The shape can be defined by the object features that maintain invariance under *similarity transformations*, the transformations including rotation, translation and size scaling^{32, 33}.

There are two major groups of methods for the use of descriptors to represent a shape. The first group includes techniques that provide a detailed representation and preserve information of the object boundary and thus can enable reconstruction of the original shape from the descriptor values. These types of descriptors usually consider a shape as being a periodic mathematical function which can be represented, for example, as an expansion or as a high-order polynomial. The second group includes global shape descriptors which can also be used to characterise the region boundary, albeit not preserving details of the original shape for reconstruction. These types of descriptors include, for example, critical distances, ratios or shape factors. For the latter, we use the software of a PVS830 imaging system from Malvern Instruments Ltd. to make the calculations, which give the key shape descriptors, while for the former, we use Fourier descriptors.

An extensive review on shape description techniques was made by Zhang and Lu³⁴. They concluded that Fourier descriptors based on spectral transforms provide robust performance, accuracy, compact features and low computation complexity, as well as being the most promising method to overcome noise. Fourier descriptors have been used by several researchers for the contour analysis of particles^{35, 36}. This technique is popular due to its invariance under two-dimensional transformations, i.e. rotation translation and scaling, and its ability to describe a region

Fig. 6 The segmentation method applied on a sample in-situ image with highly irregular pixel intensity. (a) Original image, (b) edges detected at the first scale, (c) edges detected at the second scale, (d) edges of first and second scales, (e) morphological closing on image (d), (f) region-filling on image (e), (g) morphological opening on image (f), (h) segmented particles after removing those with less than 200 pixels from image (g), (i) segmented particles with the original grey-scale intensity superimposed

boundary in detail, preserving shape information for reconstruction. The calculated Fourier shape descriptors were then used by a shape classification algorithm, the adaptive resonance theory (ART2)^{37, 38} for classification purposes. ART2 is a neural network that adopts a learning mechanism that is both unsupervised and recursive. Since it is unsupervised learning, unlike back propagation neural networks that require training using pairs of predefined shape clusters and descriptors³⁶, ART2 automatically determines the number of clusters and the assignment of data pat-

terns in a way that patterns in a cluster are more similar than those in a different cluster. The recursive learning feature is a mechanism that can continuously update the knowledge with new data available, without corrupting the existing knowledge already learned using previous data and without the need to make up the new data with previous data for re-training or re-learning, also very important for on-line use (in some literature it is called incremental learning). Being recursive is extremely useful for on-line use since new data are continuously made available.

Fig. 7 Examples of analysis results for image obtained using PVS830 of Malvern Instruments, (a) and (b); and using PVM of Lasentec, (c) and (d). **Fig. 7** (a) is by courtesy of Lasentec¹⁰⁾

The methodology for image segmentation, descriptor calculation and shape recognition was applied to the on-line raw images obtained during cooling crystallisation of (L)-glutamic acid. The number and volume of the crystals captured in the frame over a fixed time interval were used to estimate the number and volume fractions between the two polymorphs, alpha and beta.

For estimation of the ratios between the prismatic and needle shapes, each time point in **Fig. 8** and **9** represents 60 images taken in the last 10 minutes. **Fig. 8** shows the relative percentage of the number of the two polymorphic forms, alpha and beta, plotted against time, from crystallisation onset throughout the polymorphic transition. The trends illustrate that the number of crystals with prismatic alpha shape became a minority at around 30 minutes from crystallisation onset. This type of chart is very useful to process engineers and operators to obtain the quantitative information about the polymorphic transitions inside the reactor. Due to the natural progress from onset through growth of the crystals, images of the first instants during onset contain only a few crystals

Fig. 8 2-D crystal polymorphic transition charts in number percentage for the polymorphs alpha and beta. Each point represents the previous ten minutes. (Onset at $t=0$)

Fig. 9 2-D polymorphic transition for the polymorphs alpha and beta. Each point represents the previous ten minutes. (Onset at $t=0$)

compared to images of subsequent stages. As a result, clearer and less fluctuating shape trends are obtained as the time progresses and the amount of crystals detected increases. Nevertheless, the broad features of the polymorphic transformation are still evident through detecting the changes in crystal shape.

Due to the differences in particle size, a volume fraction can provide a better idea of the amount of compounds in solid state with a particular shape. The crystal shape patterns in volume fraction based on the last ten minutes is shown in **Fig. 9**. It is worth noting that in the volume fraction plot, the crossing-over is observed earlier than in the number fraction

plots, meaning that the amount of (L)-glutamic acid beta-form becomes predominant in the solid state at the crossing-over in **Fig. 9**, albeit at the same time-point the amount of crystals with prismatic shape, alpha-form, still constitute the majority.

The dynamic change in crystal shape can also be depicted in **Fig. 10**, which illustrates the values of shape roundness of alpha- and beta-form crystals as crystallisation in the reactor progresses. Each point represents a single crystal, and its corresponding roundness value can be read from the vertical axis. As expected, alpha-form crystals have higher values of roundness, while beta-form crystals have low roundness values, and the crystals are clearly separated into two clusters due to the difference in round-

Fig. 10 Evolution of roundness values for the crystal shapes detected as alpha and beta forms

ness values, with the upper cluster representing alpha-form crystals and the lower cluster corresponding to beta. The plot reveals that at the very early stages of crystallisation, many more crystals have high roundness values than low roundness values, a reflection of the fact that there are more alpha- than beta-form crystals. As time progresses (accompanied with reactor cooling), more crystals have low roundness values (beta-form), and few have high roundness values (alpha-form), indicating the transition from alpha- to beta-form.

Fig. 11 (a) and (b) show the evolution of length, width and equivalent diameter of the crystals for alpha- and beta-forms, respectively. For both cases, a clear relationship is observed between the patterns of crystal length and width. Nevertheless, differences can be observed. For instance, the beta-form crystals show a low difference in crystal width from the first to the last minutes, whereas the crystal length values rise from around 110 μm with more pronounced fluctuations to values of nearly 250 μm during the period of phase transition. In contrast, for the alpha-form crystals, although there are also differences in the crystal length and width patterns, they present more similarities compared with the needle-shaped crystal patterns. This reflects the actual differences in growth rate for the different crystallographic faces. In the beta-form crystals, the [101] crystallographic face clearly has a predominant growth rate compared with the other faces of the crystals, therefore leading to the characteristic needle shape. The crystal habit of the alpha-form, on the other hand, maintains the prismatic ratio as a result of similar growth rates of the crystallographic faces.

Fig. 11 Evolution of morphological parameters for the crystal shapes detected as (a) beta and (b) alpha forms

In contrast to other on-line techniques for measuring the crystal size that can only provide a value similar to the equivalent diameter, the present monitoring charts obtained from image analysis show the trends of changes in crystal size in more detail. For instance, in our studies, the image analysis results make evident that oscillations in crystal size in needle-shaped crystals mainly correspond to crystal length with minor changes in crystal width, whereas the morphological dimensions of the prismatic crystals oscillate with similar trends.

Crystal Growth Rates and Kinetics

The information obtained from imaging and image analysis was also used to estimate the crystal growth rates²⁵⁾. This was conducted for cooling ($0.1^{\circ}\text{C}/\text{min}$) crystallisation of LGA which produced needle-shaped β LGA. As an example, **Fig. 12** shows the mean values of length along with supersaturation, temperature and turbidity. Each point is the average of the previous 60 seconds containing 300 images. **Table 2** gives growth rate values estimated for both the length and width, and the literature result for the same chemical.

Comparing the estimated growth rate values with the literature, the growth rates of different faces of beta-form crystals of glutamic acid have been investigated previously by Kitamura and Ishizu³⁹⁾ using the single crystal method. The literature reported that the growth rate value at the relative supersaturation of 0.5 is lower compared with the values obtained here in the same direction, the crystal length, in the supersaturation range from 0.47 to 0.51. This difference is consistent with the difference in temperature. Clearly, higher growth rates are obtained at higher temperatures. Other factors within the crystalliser vessel are also associated such as the existing hydro-

dynamics or collisions that promote crystal surface dislocations which enhance the growth, compared with the conditions of measurement in a single crystal. Although it is expected that at higher temperatures the values of growth rate will also be higher, it is observed in **Table 2** that, for length, the growth rate tends to increase under the (simultaneous) effect of supersaturation increase.

Kitamura and Ishizu³⁹⁾ also reported that for the width, the growth rate was too small to be measured. Here, the measurements of crystal width involve a combination of the faces and the estimated growth rates for the width are 4 to 6 times smaller than those for the crystal length.

The capability to measure crystal growth rates could, in principle, be used in the estimation of para-

Fig. 12 Evaluation of crystal length along with supersaturation, temperature and turbidity. Each point represents the average of previous 60 seconds

Table 2 Estimated growth rate values for length and width of needle-shaped crystals

Temperature average, $T (^{\circ}\text{C})$	Relative supersaturation $\sigma = S - 1$	Length growth rate $R_L \times 10^8 \text{ (m/s)}$	Width growth rate $R_W \times 10^9 \text{ (m/s)}$
68.34	0.471	2.440	5.575
68.18	0.478	2.561	5.838
68.01	0.485	3.136	5.852
67.84	0.492	2.837	6.044
67.68	0.502	2.997	5.526
67.51	0.512	2.995	5.019
25	0.5	1.3* (literature ³⁹⁾)	—

meters associated to the kinetics of growth, in this case image analysis providing the possibility of estimating the kinetics in different morphological directions of the crystals. Here, although the conditions are of cooling temperature, it is tempting to try to fit the growth rates obtained from imaging data to a kinetic model. Due to the low relationship found between width growth rates and supersaturation it was considered that for the case of width, it would not lead to a sensible estimation. Therefore, only the estimation of kinetic parameters associated with the crystal length was attempted.

To estimate the parameters, that is the growth kinetic constant k and the order n , a semi-empirical kinetic model was used

$$R = k\sigma^n$$

which can be re-written in logarithmic form to estimate the parameters from a linear regression (**Fig. 13**)

$$\ln R = n \ln \sigma + \ln k$$

Strictly speaking, the value of k is dependent on temperature following the Arrhenius equation⁴⁰. Here, although the variation of temperature is relatively low for the studied points, the estimation was made above all to investigate the potential of using imaging in the

Fig. 13 Data plot for the estimation of kinetic parameters, k and n , of growth in crystal length ($r^2=0.95$). The arrow indicates an outlier

estimation of growth kinetics, with the possibility of using the technique in future research in isothermal experiments. **Table 3** summarises the obtained values.

Looking at the obtained value of the exponent, the estimation suggests that the growth in length is close to a dislocation controlled BCF mechanism. However, one needs to bear in mind that the semi-empirical kinetic model used is in fact describing the growth in the direction of a (100) face, which for this particular morphology is non-existent. The growth in the crystal length is mainly driven by the {101} family of faces, being aware of the existence of an angle between the growth rate in length and the growth rate of the (101) face. Due to the rotation of the crystals in the three-dimensional space in the solution, it is difficult to provide a precise estimation of the growth rates for the face (101), $R_{(101)}$, which would be given by the equation

$$R_{(101)} = R_L \cos 36^\circ \quad (6.3)$$

Nevertheless, for the crystal morphology of the beta-form crystals, it is reasonable to assume that the growth rate in length is very close to the growth rate of the faces {101}.

The present results are encouraging. They clearly show that in the current state, the technique can provide a very close approximation of the growth rates and kinetics associated to some particular crystal faces. And they also show that if the rotation of the crystal in the solution can be quantified, the results provided by the imaging technique can lead to estimation of the same parameters of crystal growth of facets with high accuracy.

The Camera Model: Integration of Imaging and Crystal Morphology Modelling

The integration of crystal morphological modelling with in-situ shape measurement using on-line microscopy provides a potentially useful instrument for crystallisation study, for example, for validation of morphology prediction models, for constructing 3-D shapes from on-line 2-D images, as well as for classification of the polymorphic and morphological forms.

Table 3 Estimated kinetic parameters of growth in length for needle-shaped beta-form crystals

Temperature range (°C)	Relative supersaturation, σ	Kinetic growth constant, $k \times 10^7$ (m/s)	Kinetic order n
67.51 < T < 68.34	0.471 < σ < 0.512	1.761	2.61

An approach for the integration called *camera model* was proposed by Li et al^{41, 42}. It involves a means of generating a library of 2-D images through rotating a 3-D crystal in the multidimensional space, and a method for matching the 2-D images in the library to those 2-D images obtained on-line.

The first step involves generating a 2-D library by rotating the 3-D crystals, which can be explained using **Fig. 14**. If the camera is placed at a point on the **X** axis, as shown in **Fig. 14**, the rotation around **X** axis will not result in different 2-D shapes, but only change the orientations of the same 2-D shape. As a result, the rotation around the **X** axis can be ignored. Since the crystal is a faceted object, when rotating around an axis, either **Y** or **Z**, from one angle to another at a very small step, for example, around the **Y** axis, the 2-D shapes corresponding to the two positions may only change in a very minor way. **Fig. 15** shows some 2-D shapes when rotating a crystal at different angles, i.e. 1°, 2°, 3°, 4°, 5°, 6°, 7°, 8°, 9°, 10°, and 11° around the **Y** axis, from which it is clear that even when rotating the crystal 11° from the initial position, the 2-D shape does not change very much. This means that the number of 2-D shapes with a significant difference to each other is limited. Here, the words “significant difference” though are vague, and can be defined by the users. For instance, it can be defined as that as long as the number of edges in the projected 2-D images changes (increases or decreases), then it is considered a new 2-D image with a significant difference from its last image. It is worth pointing out that the minimum degree at which the crystal needs to rotate is dependent on the complexity of the 3-D crystal and the initial position of rotation.

Two methods of rotation can be used. One approach is to use a constant rotation angle to continuously rotate the crystal. In this case, the value of the angle must be small enough so that the similarity distance between two 2-D shapes corresponding to any two neighbouring orientations is smaller than a predefined threshold value. An alternative approach of rotation is to use variant rotation angles. In this approach, when rotating the crystal around an axis from one position to the next, the angle between the two positions is chosen so that the similarity distance of the two 2-D shapes corresponding to the two positions is larger than a threshold value sd_1 and smaller than another threshold value sd_2 ($sd_1 < sd_2$). The advantage of this rotation method is that it can reduce the size of the 2-D image library generated.

Fig. 16 shows some examples of 2-D images gen-

Fig. 14 Crystal rotation method

Fig. 15 2D shapes obtained through rotating a crystal at different angles

Fig. 16 Some image examples in the generated image library

erated in a library. **Fig. 17** shows an example of on-line images which has undergone the segmentation procedure. **Table 4** shows the identification of crystals in **Fig. 17** by matching the 2-D crystals with those in the 2-D library generated by the camera model.

Fig. 17 Segmented on-line crystal images

Final Remarks and Future Work

In the past, pharmaceutical manufacturers have been hesitant to introduce new equipment, instruments and control techniques due to regulatory uncertainties which could lead to longer approval times and high costs⁴³. As part of the FDA initiative to encourage innovation and efficiency of manufacturing process design and control and quality assurance⁴⁴, the Process Analytical Technology (PAT) framework⁴⁵ is an important move decided on jointly by the regulatory authority and the industry, which could potentially lead to a revolutionary change in the manufacture of pharmaceuticals that requires all materials properties including concentration, size, shape, morphology and surface properties of feedstocks, intermediates and products at nano-, micro- and macro-scales, to be measured on-line or at-line. In addition, the measurements will not only be used for display purposes, but will also be analysed using advanced sensor and PAT data mining techniques to gain a fundamental understanding of the process and product behaviour

Table 4 The image matching results for crystals of **Fig. 17**

Online Images	Retrieved Reference	Similarity Distance	Polymorph
BetaM042182	1.40	β
	BetaM112182	1.44	β
	BetaM112358	1.48	β
	BetaM280330	0.88	β
	BetaM104030	0.89	β
	BetaM284330	0.96	β
	AlphaM176054	0.87	α
	AlphaM356126	0.88	α
	AlphaM176234	0.88	α
	BetaM022228	0.46	β
	BetaM022048	0.51	β
	BetaM320134	0.52	β
	AlphaM356018	0.65	α
	AlphaM354198	0.68	α
	AlphaM004016	0.68	α

(a) Schematic diagram

(b) The original pilot plant

Fig. 18 A 200 litre pilot plant donated by AstraZeneca is being installed which will be used in the scale-up study.

needed to achieve product quality control and assurance.

The results presented in this paper demonstrate that the integration of advanced on-line imaging sensors and multi-scale image analysis techniques provides great potential for implementing real-time techniques to manipulate the crystal growth and control the morphology of crystals. The work also can potentially lead to full integration of morphology modelling, measurement, control and optimisation techniques. The work reported in this paper was obtained with a laboratory-scale reactor. In the future, it is planned to extend the study to an industrial-scale 200-litre reactor (**Fig. 18**) donated by AstraZeneca which is being installed in the pilot plant of the Institute of Particle Science and Engineering, at the University of Leeds.

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