Organic Process Research & Development **2009,** *13,* 1254–1261

Full Papers

Endoscopy-Based in Situ Bulk Video Imaging of Batch Crystallization Processes

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Abstract:

External bulk video imaging (eBVI) of crystallization processes has proven to be a promising technique for metastable zone determination. In this contribution the endoscopy-based in situ bulk video imaging (iBVI) method is introduced. The video data are processed using the mean gray intensity method and by a digital image processing technique which aims to detect the first crystals during nucleation. The experiments have been carried out in a small-scale calorimeter CRCv4, during which the compensation heater and infrared spectroscopy signals were monitored. It is concluded that monitoring the onset of the apparent nucleation, formation of particles with detectable size, using the mean gray intensity (MGI) trend delivers similar performance to the calorimetric and IR spectroscopy signal, whereas the crystal recognition method is the fastest, allowing detection of nucleation earlier. The endoscopy-based nucleation monitoring technique is proposed as a complementary tool to existing process analytical technologies (PAT) since it provides an in situ, low-cost, robust, probe-based method for metastable zone identification which can be easily integrated and automated with existing laboratory hardware and software.

1. Introduction

Crystallization is one of the most important unit operations in the pharmaceutical industry since it has the role of separation and purification. Furthermore, it strongly influences the downstream processing activities[1−3] and end-product properties which are mostly related to the particle size distribution and crystal shape.[4,5] With the new trend of process analytical technology (PAT)-based process design, monitoring and control, it is feasible to reproducibly ensure the product properties.[6−9] In order to guarantee constant product quality on the industrial production sites there is a growing need for the application of new, robust and low-cost sensing technologies.

The detection of the formation of fine particles due to nucleation is the most often monitored event during the operation of crystallizers. Nucleation monitoring is used for metastable zone identification experiments and crystallization process control. The term nucleation is often used in practice to indicate the formation of fine particles of detectable size and number. This usually involves the formation of critical clusters (the theoretical point of nucleation) and their growth into particles with detectable size and/or number. The goal is to detect the formation of nuclei as close as possible to the actual point of nucleation (formation of critical cluster) since this can improve the crystal size distribution of the product, eliminating the formation of unwanted fines. The application of the existing in situ sensor technologies has focused on the solid-[10−16] and

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10.1021/op900019b CCC: $40.75
Published on Web 05/11/2009

liquid-phase monitoring.[13,14,17−19] These sensors sample the solid or liquid phase in close proximity to the probe; therefore, good mixing is required in order to obtain representative measurements. These probes may have difficulties in detecting the first formed crystal because the crystal has to pass below the probe which may be influenced by the crystallization vessel size. Additionally, mounting these probes in zones with suitable flow in the case of larger reactors in an industrial environment may represent major difficulties limiting their use. In the case of systems with higher solid density, achieving the mixing necessary to suspend all particles and obtain representative sampling in the measuring zone of the probes may not be possible. Furthermore, just a few crystals may not be enough to trigger a signal which is significant compared to the background noise of the instrumentation. Also, there is the possibility that for low solid concentrations the sensor hardware and signal processing software may filter out the first particles. Alternative solutions to the in situ monitoring are the external monitoring[20−23] and flow-through cell-based solutions.[5]

Recently, the proof of concept of the external bulk video imaging (eBVI) strategy was presented,[22] and it was shown that it can be used for nucleation onset monitoring and metastable zone identification with performance similar to that of the existing focused beam reflectance measurement (FBRM) and ultraviolet−visible (UV/vis) spectroscopy.

The paper presents the in situ, probe-based implementation of the BVI approach. The probe-based implementation is based on endoscopy, often called rigid borescopy. The endoscope or borescope has found intensive application in diagnostic healthcare and engine testing. The applications to chemical process monitoring are mainly related to the determination of drop size distribution in liquid−liquid dispersions,[24−26] to the visual monitoring of heat transfer coefficient determination experiments,[27] and to the solid fraction monitoring for a circulating fluidized bed.[28] To our knowledge, endoscopy-based crystallization process monitoring has not been reported in the literature.

2. In Situ Endoscopy-Based Bulk Video Imaging

The application of endoscopy is relevant whenever the locations to be observed are difficultly accessible and in situ imaging is required. The endoscope comes as a probe-type sensor, and its parts consist of the lens system, cold light source, digital imaging system, and video acquisition hardware connected to a computer. In this work we use the endoscope to monitor the crystallization onset by detecting the gray level change in the liquid bulk properties and by detecting the first crystals. Furthermore, the endoscopy is proposed for the monitoring of mixing conditions in the vessel and particle settling events. In order to process the video data transferred to the PC, robust and sensitive image-processing techniques are proposed.

2.1. Robust Video Data Analysis. According to both techniques the algorithm generates the interrogation window by clipping it from the current frame; it converts the image to the gray (8 bit) format, and it calculates the mean gray intensity index according to eq 1:

$$\mathrm{MGI} = \frac{\sum_{i=0}^{N} \mathrm{PGI}_i}{N} \tag{1}$$

where PGI_i is the pixel grey intensity of each pixel i and N is the total number of pixels in the picture. Such an image is a two-dimensional matrix (width × length) which contains gray intensity values ranging from 0 to 255 (0 corresponds to 100% black and 255 to 100% white). The BVI method using the average intensity value provides a robust, straightforward, real-time feasible and adjustment-free strategy to monitor the nucleation onset.[22] During the online monitoring of the crystallization process the MGI value is compared to a previously set MGI threshold value. This threshold value is selected as a function of the noise in the MGI values. In case the noise is significant, a moving average filter may be applied to the video data, and the signal is smoothed out. However, a detection delay is inherently introduced, depending on the size of the filter window. Once the threshold MGI value has been exceeded, it is concluded that the nucleation onset has started.

2.2. Interrogation Window Optimization. After the first crystallization batch has been completed, there is sufficient video information to optimize off-line the size of the interrogation window. This is required to find a compromise between the noise in the MGI trend and the influence on the mean gray value. If the interrogation window is large the particle will pass through it with high probability; thus, it can be detected.

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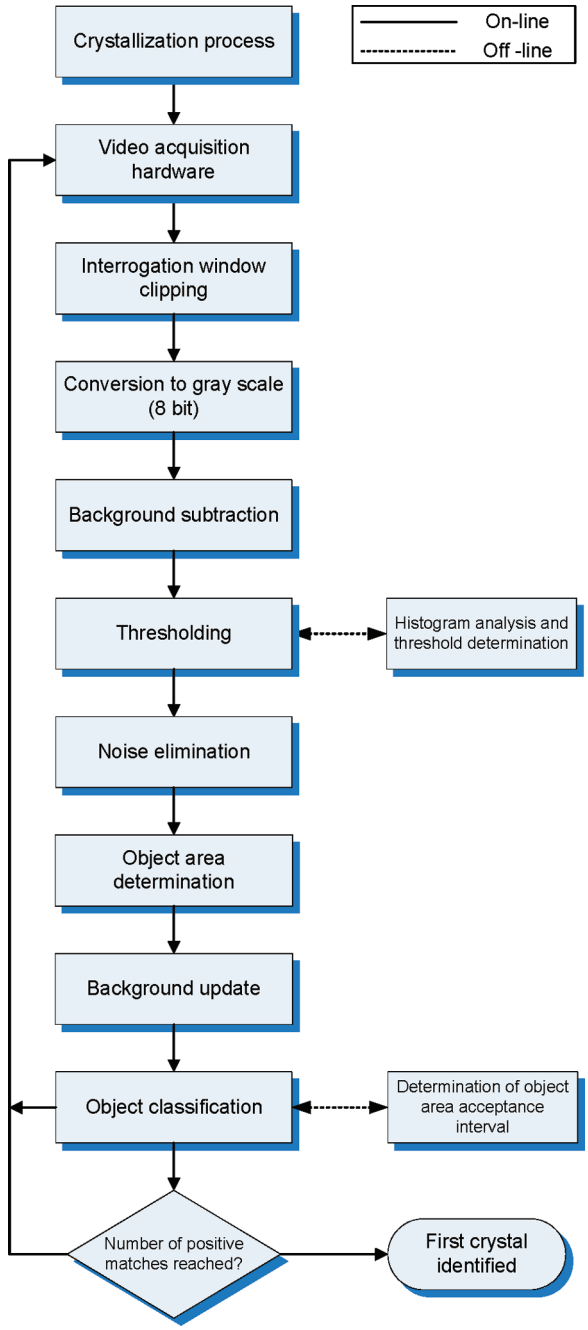

Figure 1. **Crystal recognition (CR) method.**

However, the impact on the MGI value is low since the ratio of the particle size to the interrogation window is low. In contrast to this, if the interrogation window is small, the particle passing through will cause a significant increase in the MGI value, and thus it is detected early. The optimal interrogation window size also depends on the algorithm used to detect the crystallization onset. Small windows will detect the particles faster; however, the signal will appear noisy; thus, if a threshold value of the MGI is used, this may lead to detection problems.

2.3. Video Data Analysis for Crystal Recognition. The goal of this approach is to recognize the first detectable crystal based on the video stream acquired from an external imaging device or from an in situ endoscope. The proposed crystal recognition (CR) method is presented in Figure 1 and is based on digital image processing techniques. For this method the size of the interrogation window should be as large as possible since it increases the particle detection probability. The interrogation window is clipped from the current frame and converted to an 8 bit gray-scale format. The background subtraction is carried out so that from the current figure the previous one is subtracted. This way the background image is always updated, and it contains the illumination changes which may occur during the operation. Image thresholding is performed to convert the gray scale image to a black and white or binary format.

The conversion threshold is the design parameter of the algorithm and is determined off-line on the basis of the histogram information. After thresholding, some of the noise left in the image can be removed using morphological image processing transformation that is specific for binary images.[29] Depending on the application, several operations can be evaluated such as removing isolated foreground pixels (the *clean* operation) or making a certain pixel a foreground pixel if at least five pixels among the neighboring 8 pixels are foreground; otherwise the pixel is considered as background pixel (the *majority* operation). The size of the objects obtained after noise reduction is given by the area of the object. In case the size of an object falls within a certain specification, it is concluded that the object is a crystal. This interval is another design variable which is application specific. The other objects which do not meet this criterion are discarded. Small objects may occur due to imperfections during the noise removing step or may be true crystals. The Gaussian blurring was also tested for noise removal; however, since it is a low pass filter it tends to remove the small crystal patterns. Instead, image morphology-based techniques are preferred for noise removal.

Large objects are likely to occur due to the air bubbles which are occasionally entrained during mixing. It is considered that the nucleation onset has occurred when the objects are identified within a certain time window (e.g., three matching objects in a second or 25 frames). This last step is an additional measure to filter false nucleation onset events.

Since the collected data is abundant, the CR algorithm should have conservative settings in order to obtain a robust monitoring tool with good performance. For illustration, during our experiments, using an interrogation window of 300 × 326 with 25 frames/s and expected object size between 10 and 30 pixels, 2445000 pixels are collected every second. For the design of the CR method the Matlab Image Processing Toolbox[30] was used.

The proposed image processing algorithms were applied to recognize the first detectable crystal for the external and in situ video imaging acquisition. The experimental setup of the external video monitoring is presented in a previous work,[22] and results are compared to the FBRM and UV/vis spectroscopy signals.

In order to show the performance of endoscopy-based in situ (iBVI) method, it is compared to the calorimetric signal and IR spectra. Within the field of chemical reaction modeling

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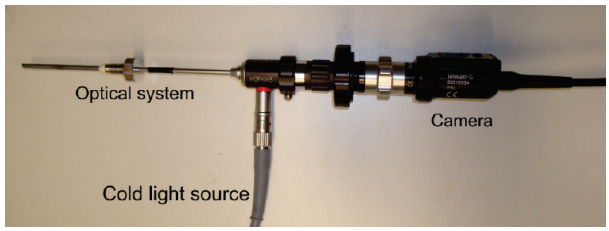

Figure 2. **The Karl−Storz endoscope.**

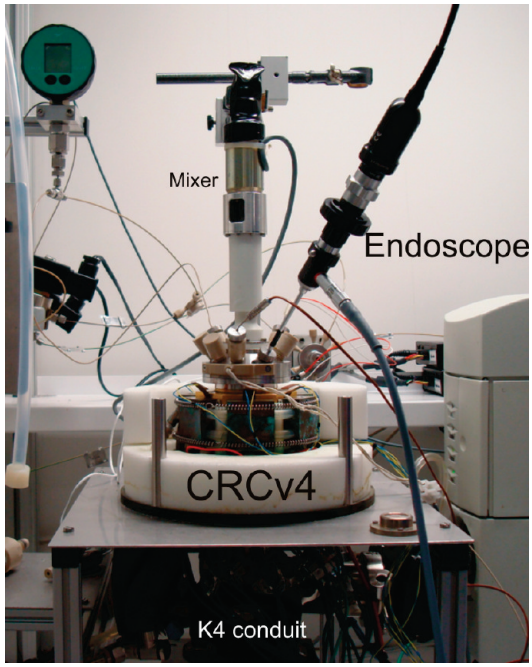

Figure 3. **The experimental setup with the CRCv4 unit.**

and simulation, reaction calorimetry is employed for the determination of the kinetics-related parameters and reaction enthalpy. The calorimetric signal used for comparison is the power output of the compensation heater.

3. Materials and Experimental Equipment

The experimental work has been carried out using potash alum hydrate. The experiments were carried out by dissolving 11.67 g in 30 mL of deionized water, which corresponds to a saturated solution at 52 °C.[11] The solution was kept at 60 °C for 50 min to ensure entire dissolution, after which the cooling was started with a 0.2 °C/min rate. The stirrer speed was set to 200 rpm to minimize air entrainment in the liquid. The endoscope (Figure 2) is a rigid borescope with 3.8 mm diameter, model 84384, 30 mm focal depth and 70° direction of view, type Telecam C 20212034. The illumination is provided by a cold light projector model 81472, and the video data are acquired and processed using a Telecam DX 202320/20 unit. The acquisition rate is 25 frames/s, and the video signal is compressed using a Pinnacle TV capture card with hardware encoding capabilities.

The experiments were carried out in the combined reaction calorimeter (CRCv4), Figure 3, a small-scale reaction calorimeter that combines the principle of power compensation and heat balance.[31]

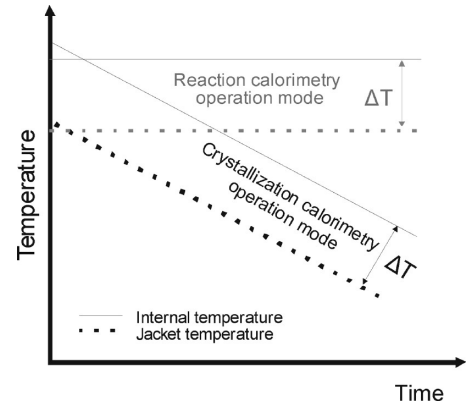

Figure 4. **Reaction and crystallization calorimetry operation modes of the CRCv4 unit.**

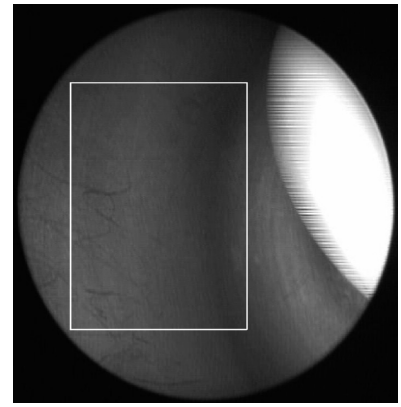

Figure 5. **Endoscopy image showing the interrogation window; the gray surface is the reactor wall while the white area is the mixer.**

The Hastelloy reactor vessel has a working volume of 25−45 mL; it uses a metal block as an intermediate thermostat, and it is able to withstand pressure up to 30 bar. The temperature working range of CRCv4 is between −20 and 200 °C. The isothermal conditions are maintained using the power compensation principle. Peltier elements are coupled to an online feedback control to compensate for the change of the overall heat transfer through the metal block during the measurement, making time-consuming calibration unnecessary. The liquid is stirred using a magnetically driven impeller with a four-blade Teflon mixer. As the CRCv4 is designed for isothermal operation, in order to accommodate the operation of crystallization processes the temperature control strategy has been modified as described in the next section.

3.1. Crystallization Calorimeter Operation. The most important feature of the CRCv4 calorimetric vessel is that, in spite of highly exothermic reactions, is able to ensure isothermal conditions.[32] Based on the implemented power compensation method the liquid temperature is controlled by an electrical

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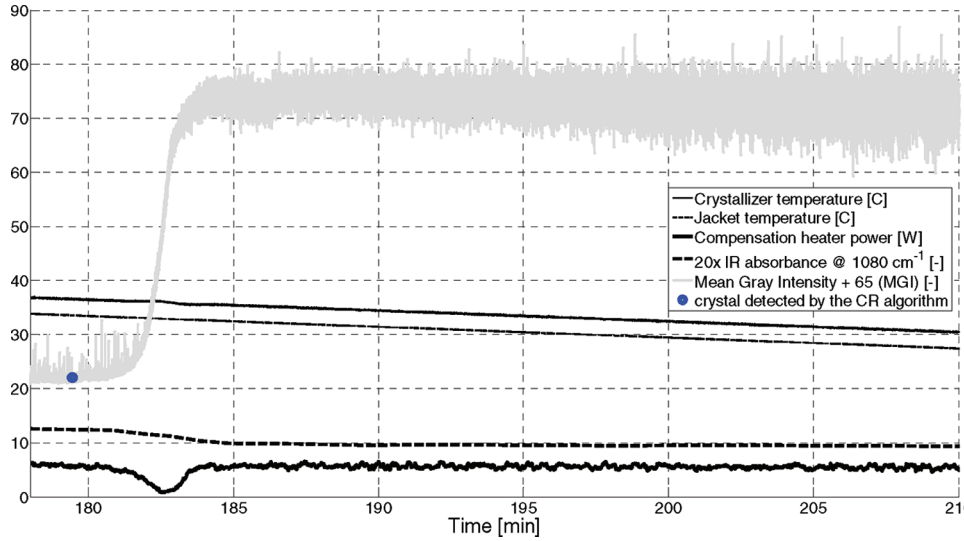

Figure 6. **Comparison among the mean gray intensity (MGI), crystal detection method (CR), IR spectroscopy, and power compensation signal trends; the two parallel lines show the liquid bulk and jacket temperature.**

compensation heater which ensures good control dynamics. Another control loop, which is independent from the previous one, controls the jacket temperature. As soon as heat is generated in the liquid bulk, the heat transferred by the compensation heater is reduced and later on increased to preserve the isothermal conditions.

For crystallization purposes which imply decreasing temperature trends, the calorimeter operation is modified so that the advantages offered by the power compensation technique are preserved, Figure 4. Although the internal and jacket temperatures change in time during the crystallization process, by ensuring a constant temperature driving force between these, the operation principle of the power compensation technique is maintained. According to this operation strategy the heat of crystallization is calculated similarly to the heat of reaction.[31] The temperature set points for the two control loops are prescribed independently.

The Fourier transform infrared - attenuated total reflectance FTIR-ATR spectroscopy measurements were carried out using a ReactIR 4000 system and the ReactIR 3.03 software package, both from Mettler Toledo. The FT-IR spectrometer is connected via a K4 conduit to an ATR-IR crystal directly built into the bottom of the reactor vessel.

The crystallization unit and all the peripheries are controlled by LabVIEW, v6.1, from National Instruments.

4. Results and Discussion

A snapshot from the video data collected during the experiments is presented in Figure 5, where the white box is the interrogation window. Any subsequent data processing is carried out on the clipped image section.

The comparison among the video signal processed by both nucleation detection techniques, the MGI and CR, the calorimetric signal and IR spectroscopy signal are presented in Figure 6. Note that the magnitudes of the signals have been adjusted for plotting purposes. These results show that the MGI index-based bulk video imaging is able to detect the nucleation onset with performance comparable to that of the other PAT techniques. However, the crystal detection-based technique

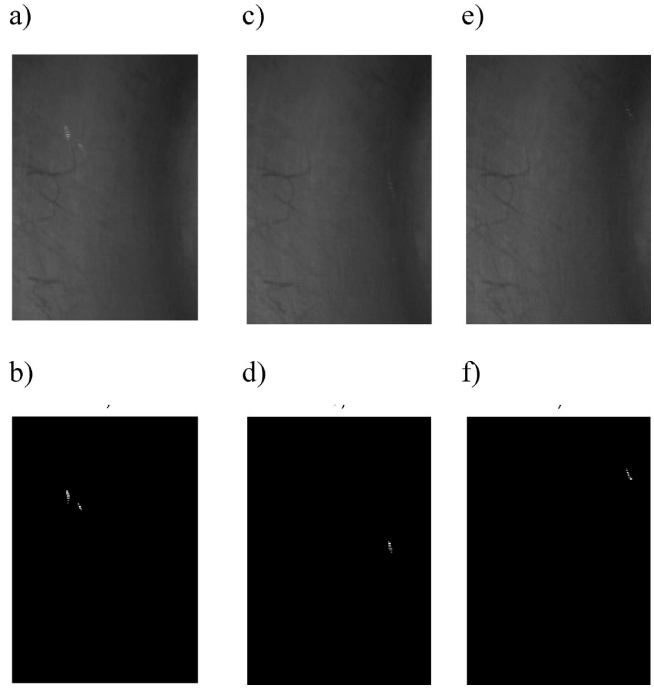

Figure 7. **Captured original images (a, c, e) and the results of the crystal detection method (b, d, f) around 179.4 min.**

provides the fastest means for nucleation detection. Enough change in the bulk concentration must happen for the ATR-IR to detect nucleation, which can result in a delay in the nucleation detection. The delay is larger, the smaller the size of the particles, since the formation of a very large number of small particles can generate a cloud formation, which will be detected easily by the change in the MGI, but may lead to small concentration variation. The IR signal shown in Figure 6 indicates a change of about 30% in the absorbance value at the wavelength of 1080 cm^{-1} due to nucleation and subsequent growth; however, there is also a counteracting effect caused by the crystallization of the potash alum as dodecahydrate. This leads to the incorporation of a significant amount of water in the solid phase (about 0.85 g of water for every gram of anhydrous potash alum crystallized) during the crystallization.

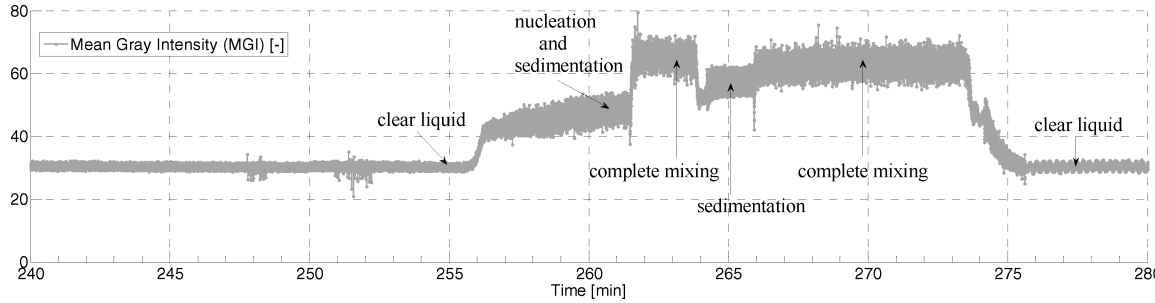

Figure 8. **Mean gray intensity trend calculated on the basis of the endoscopy video data and the process states shown in Figure 9.**

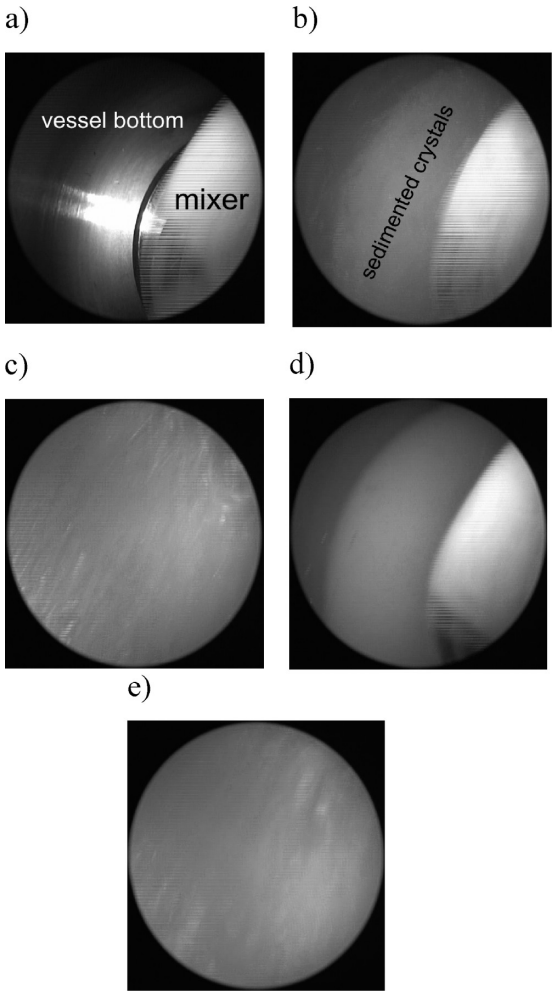

Figure 9. **Crystallization process conditions at several time instances captured by the endoscope; (a) clean solution at 255 min, (b) nucleation and sedimentation at 261 min, (c) complete mixing at 263 min, (d) sedimentation at 265 min, (e) complete mixing at 270 min.**

Therefore, the actual detected decrease in the solution concentration is significantly smaller compared to cases when solid forms without incorporating solvent molecules in the crystal structure. Additionally, the counteracting effect of the temperature change on the absorbance has to be taken into account, too. With robust chemometrics approaches, which take the temperature effect into account, the ATR-IR can be made more sensitive.[13]

The crystal recognition algorithm presented in this article was applied to the acquired video data. The nucleation onset was identified on the basis of the information provided by

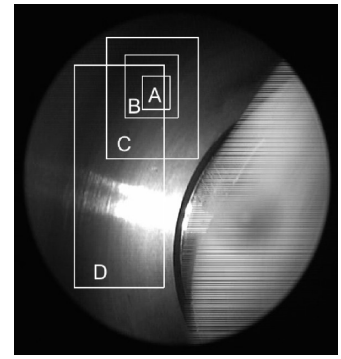

Figure 10. **Interrogation window position for the MGI calculation.**

images a, c, and e of Figure 7. The results presented in images b, d, and f of Figure 7 show that crystal recognition can be successfully performed on video data acquired by endoscopy. However, note that the size of the crystals cannot be measured accurately with this approach because crystals leave traces rather than a sharp image with the video equipment used in the endoscope system. For quantitative analysis of the information to be carried out, the illumination needs to be changed to "freeze" the particles in motion. Such technologies require pulsed light sources, e.g. pulsed laser and high speed video cameras available in various instruments, but for higher costs. These particular features of the CR method based on endoscopy prevent the exact evaluation of the minimum detectable size of particles, as the detected "trace" of the particles may depend on various experimental and system-dependent factors, such as the particle velocity (thus mixing conditions) as well as reflective index differences between the particle and solvent.

The nucleation temperature identified by the video signal was determined to be 36.2 °C, which is significantly lower than the value of approximately 48 °C estimated on the basis of data reported by Barett and Glenon[11] (0.15 °C/min cooling ramp and 1 L solution volume) for the same initial concentration. The broader metastable zone width can be attributed to the different materials of vessels, agitator, and probes as well as to the different mixing conditions in the various vessel sizes, as concluded, for example, by Yi and Myerson.[33] The authors provide a comparison of nucleation temperatures for cooling crystallization using different vessel volumes. They concluded that the metastable zone width difference can be as large as 14 °C when performing the cooling experiments in different vessels using 40 and 300 mL solution volumes.

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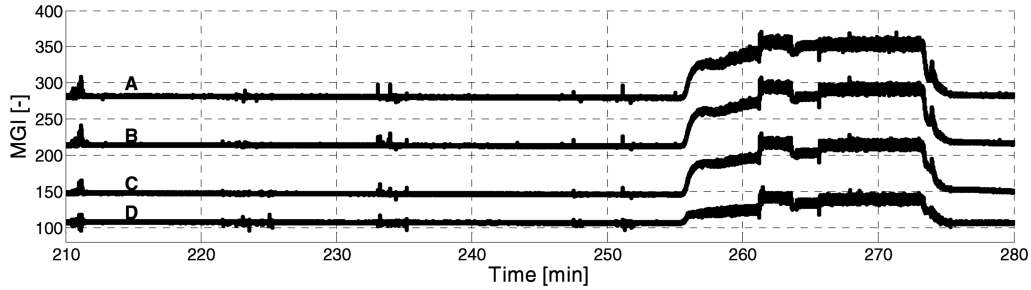

Figure 11. **MGI trends for several interrogation windows: (A) 2234 pixels (shifted 220 units), (B) 8134 pixels (shifted 160 units), (C) 27027 pixels (shifted 80 units), (D) 48580 pixels.**

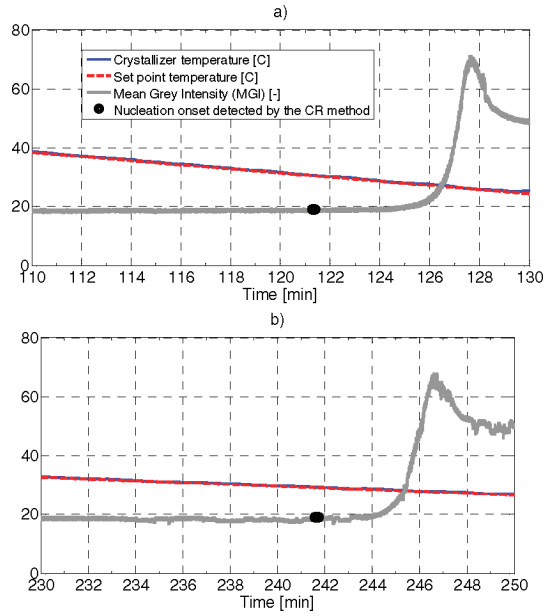

Figure 12. **Comparison of nucleation detection capability of the MGI and CR methods based on the caffeine experiments: (a) cooling rate of 0.7 °C/min, (b) cooling rate of 0.3 °C /min.**

A second set of experiments was performed to evaluate the capabilities of the bulk video imaging method to detect various process states, such as sedimentation and complete mixing. In these experiments the mixing was influenced by changing the stirrer speed. The results are shown in Figure 8.

The video data corresponding to the particular process states recorded by the endoscope during the experiments are presented in Figure 9.

These results show that using endoscopy-based bulk video imaging is feasible for monitoring several process states, and its application may be extended beyond the metastable zone determination. To analyze the sensitivity of the MGI index to the interrogation window size, its size was modified, and the MGI trends were recalculated. These interrogation windows are presented in Figure 10, and it was concluded that the detection capability of the BVI method is not influenced significantly, as shown in Figure 11. Note that the trends have been shifted for plotting purposes.

The crystal recognition method was also tested on two external bulk video monitoring experiments of a caffeine in water system[22] (cooling rates of 0.7 °C/min and 0.3 °C/min). It was found that the nucleation onset of caffeine crystallization can be detected earlier as compared to the monitoring of MGI trends, Figure 12. The corresponding processed digital images are presented in Figure 13.

Figure 13. **Crystal recognition results, corresponding to around 121 min for the caffeine experiment using 0.7 °C/min cooling rate.**

The monitoring techniques presented in this work are not suited for particle size determination for the following reasons: (1) the image resolution is low compared to the crystal size, (2) the optical system of the endoscope introduces image distortion, and (3) with the video hardware, moving objects appear as traces. The difficulty of the crystal recognition may be increased by the presence of air bubbles entrained during mixing, as illustrated in Figure 14. Nevertheless, by proper tuning of the image processing algorithm, nucleation onset detection is still feasible.

The endoscopy-based monitoring system is an excellent tool for low cost, automated, metastable zone identification experiments. Furthermore, the measurements are not biased by mixing conditions, e.g., stirring rate, and it is a promising tool to be used in the context of adaptive supersaturation[34] or direct nucleation control.[35]

5. Conclusions

In this article we have presented the endoscopy-based in situ bulk video monitoring of crystallization processes. The video data was processed using the mean gray intensity method and by using a digital image processing technique which aims to detect the first crystals during nucleation. It is concluded that the nucleation monitoring using the MGI trend delivers similar performance to the calorimetric and IR spectroscopy signal, while the crystal recognition method is the fastest.

(34) Woo, X. Y.; Nagy, Z. K.; Tan, R. B. H.; Braatz, R. D. Adaptive Concentration Control of Cooling and Antisolvent Crystallization with Laser Backscattering Measurement. *Cryst. Growth Des.* **2009**, *9* (1), 182.

(35) Abu Bakar, M. R.; Nagy, Z. K.; Saleemi, A. N.; Rielly, C. D. The Impact of Direct Nucleation Control on Crystal Size Distribution in Pharmaceutical Crystallization Processes. *Cryst. Growth Des.* **2009**, *9* (3), 1378.

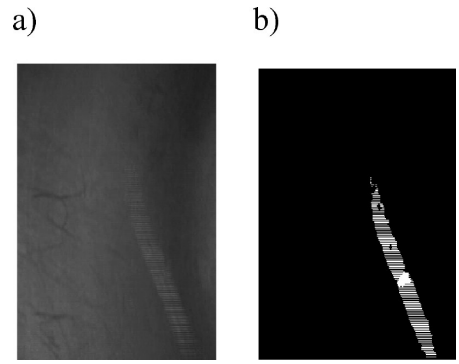

Figure 14. **Trace of an air bubble recorded by the endoscopy-based monitoring system: (a) original figure, (b) after digital image processing using the CR method.**

The endoscopy-based nucleation monitoring offers an in situ, low-cost, robust, probe-based technology for metastable zone identification for crystallization processes. Besides the advantages listed above the nucleation detection capability is similar to the existing PAT monitoring tools, and it is proposed as complementary technique. Since the endoscopy-based nucleation detection can be easily automated and integrated with existing laboratory hardware and software, it is expected that due to its low cost and simplicity the small- and medium-sized businesses would benefit from its application. The results presented in this article encourage the application of bulk video imaging to large-scale crystallization processes.

LIST OF SYMBOLS

PGI	pixel gray intensity
MGI	mean gray intensity
N	number of pixels

Acknowledgment

We are grateful for discussions on the experimental work to the calorimetry group members: Julien Billeter, Sebastien Cap, Tamas Godany, Dr. Gilles Richner and Dr. Bobby Neuhold. Z.K.N acknowledges the financial support provided by the Engineering and Physical Sciences Research Council (EPSRC), U.K. (Grant EP/E022294/1).

Received for review January 28, 2009.

OP900019B