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THE IMPORTANCE AND CONTROL OF THE NUCLEATION PROCESS FOR THE CRYSTALLIZATION OF PHARMACEUTICAL SUBSTANCES

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Contents

Introduction

Crystallisation in pharmaceutical manufacturing is a very broad and intensively studied topic, majority amount of the APIs (Active Pharmaceutical Ingredients) produced are crystalline phases. Every year, progress in this field is described in a number of methodological and commercial publications, case studies and monographs. Recent examples include papers[1–3]. The last time this topic was mentioned in Chemicke Listy 15 years ago[4] and we have therefore decided to update it.

From a technological point of view, crystallization is a very efficient separation and purification process. In pharmaceutics, crystallization from solution is mainly used, rarely crystallization from melt. In pharmaceuticals, crystallization from solution is mainly used. From a physicochemical point of view, crystallization can be considered as a solidification process of phase transformation with subsequent formation of a crystalline phase from precursors in the liquid phase. The quality parameters monitored for the final material are: yield, chemical and phase (especially polymorphic) purity, particle size distribution and dimensional homogeneity, crystal shape (morphology), surface quality (roughness, porosity), surface energy, flow properties, mechanical properties (hardness, plasticity, elasticity), residual solvent content, and last but not least economic and ecological aspects. It is essential to note that a large number of process parameters influence the observed quality parameters (Table I) and, moreover, that not all process parameters have the same weight in the crystallization of a particular API, since each API behaves differently. The crystallization of a "tailor-made" product requires the control of all these and perhaps even more (initially unknown?) parameters for an optimal result. Adherence to the design parameters for a crystallized API affects both the control and energy consumption of the downstream processes: filtration, drying and milling, and ultimately the critical parameters of the final dosage form (bioequivalence and stability). A persistent problem of crystallization in the pharmaceutical industry is the threat of polymorphic transformations in the crystallizing

Table I
Process parameters of crystallization that affect the properties of the final product

Process parameters	
Temperature and pressure in solution	Residence time of the product in the mother liquor
Rate of cooling or evaporation of the solution	Stirring intensity of the solution
The degree of supersaturation of the solution and the rate at which it is reached	Concentration and temperature gradients
Solvent or mixture of solvents	Mechanical, ultrasonic, microwave, laser and other shocks
Water content in the solvent	pH of the solution
Presence of impurities or additives in the solution	Seeding material and its quality
Selection of antisolvent	Influence of the technology operator

medium and the formation of unwanted polymorphs (physical impurities).

The basic concepts for the crystallization process were introduced by Ostwald[5], from whom came the description of the role of supersaturation of the solution in controlling nucleation, the concept of the metastable zone and the law of states, which describes the formation of different polymorphs in a crystallizing polymorphic system. Ostwald's work was followed by Volmer[6] who developed a kinetic theory describing the relationship between solution supersaturation, interfacial tension and nucleation rate and was thus at the origin of Classical Nucleation Theory (CNT), fully described e.g. in the modern work of Karthika et al.[7].

Nucleation is the beginning of the birth of the crystalline phase from solution. It is a stochastic process and the current challenge is to understand nucleation at the molecular level. According to CNT theory, nucleation is induced by thermal fluctuations and subsequent aggregation of building material at the atomic level in supersaturated solution. The original thermal equilibrium state becomes metastable by supersaturation. The physicochemical properties of the resulting crystalline material depend on the manner in which nucleation takes place. Nucleation leads to the formation of nuclei of critical size (nuclei), whose size ranges in the order of 10^{-10} to 10^{-9} m (10–1000 molecules, so-called precursors), and their formation time can range from fractions of a second (e.g. in crystallization by antisolvent precipitation) to days (free or thermodynamically controlled crystallization). Excessive nucleations (so-called kinetically controlled process, even 10^{-13} s) are comparable in time to the vibrational frequencies of atoms and are therefore challenging to study. The structure of the nucleus and its ability to grow into a crystal has long been studied by various analytical techniques, as this knowledge is essential for controlled crystallization. The methodological advances in this field are quite evident. X-ray scattering methods (ultra-small angle, small angle, wide angle), scanning probe microscopy (SPM) and laser techniques such as FBRM (Focused Beam Reflectance Measurement) are used. These techniques rather capture the growth of crystals, i.e. the state when the crystal structure is already formed. A relatively new technique is the BlazeMetrics system, which acquires data with a single in-situ probe for simultaneous high dynamic range microscopy and real-time Raman spectroscopy measurements. It turns out that describing the nucleation process as accurately as possible and understanding its consequences leads to targeted control of crystallization, and this is the goal of a perfectly mastered technology.

2. Nucleation process

The CNT theory was developed by studying the crystallization of simple inorganic substances from solution, metals from melts and ceramics in the solid phase. Mullin[8] divides the nucleation process as follows:

– Primary homogeneous nucleation
– Primary heterogenous nucleation
– Secondary nucleation

The initial assumption is the formation of a spherical nucleus of critical size r*, after overcoming the homogeneous nucleation barrier ΔG^*_{homo} (see[4] for more details). However, the primary homogeneous nucleation mechanism is an idealization of the process, where we assume that foreign surfaces play no role in it. However, this does not respect the reality of crystallization in practice, which is limited by crystallizer walls, stirrer, dust particles, etc. It is reported[9] that primary nucleation is only applicable up to a volume of 100 μL of solution. Therefore, heterogeneous primary nucleation is closer to reality, as it preferentially occurs on foreign surfaces (i.e. the energetically advantageous place, the so-called active centre, where nucleation is more likely to occur) and thus can occur at lower supersaturations of the solution than homogeneous nucleation. For nucleation barriers, $\Delta G^*_{homo} > \Delta G^*_{hetero}$. An example of primary heterogeneous nucleation is crystallization by an antisolvent. In this case, the rate of addition of the antisolvent to the solution can control the particle size distribution of the crystallising phase or the final product.

Secondary nucleation is a process that occurs after primary heterogeneous nucleation, where the nuclei formed bump into each other or into the walls of the crystallizer or stirrer and the resulting fragments act as initiation material for further development of the crystalline material. This process may be referred to as unintentional seeding. In contrast, intentional seeding crystallization is

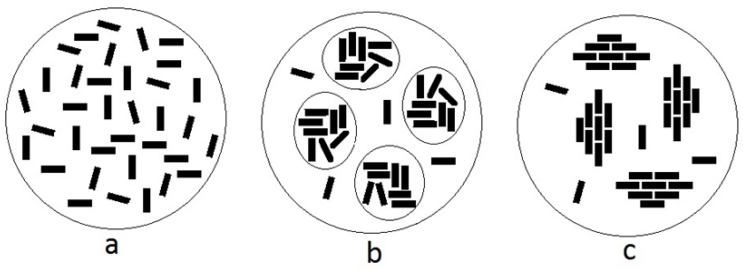

Fig. 1. **Two-step nucleation. a) Solution before nucleation; b) 1st nucleation step: formation of liquid regions ("droplets") with high supersaturation; c) 2nd nucleation step: structuring of "droplets" into internally ordered nuclei**

a process used, for example, in the targeted crystallization of a selected polymorph when the crystallized API exhibits polymorphic behavior.

2.1. Two-step nucleation model

Over time, it became clear that the CNT model was not suitable for describing nucleation for all categories of chemical compounds. The main limitations of the CNT model are that we assume an ideally spherical shape of the nucleus while describing its surface as planar with neglect of the dependence of surface tension on curvature.

Based on the study of protein crystallization, a two-step (2S) nucleation model was formulated by Vekilov[10] to explain the observed nucleation rate. According to the two-step nucleation model, solute molecules first rapidly aggregate into metastable clusters (aggregates) hundreds of nanometers in size. These metastable aggregates are the suspended regions of highly supersaturated solution or dense liquid (the term "droplets" can be used in English for these regions). In the second step, the "droplets" are reorganized into ordered structures – nuclei (Figure 1). This second phase is the controlling step of the whole process, and its speed seems to increase with the size of the molecule. Galkin et al.[11] monitored liquid-liquid separation and nucleation of a hemoglobin solution with a microscope in the differential interference contrast imaging mode. They observed the formation of a dense liquid phase at high haemoglobin concentration in which nucleation of the polymer deoxyhaemoglobin S occurred. Previous experimental studies such as dynamic and static light scattering[12], differential scanning calorimetry[13] and low-angle X-ray scattering[14,15] confirmed that two-step nucleation occurs not only for proteins but also for colloidal particles and especially for small organic molecules (pharmaceuticals). The two-step nucleation model was also confirmed by computer simulation[16] and in particular the nucleation rate was correlated with the crystallization rate and the width of the metastable zone for large volume crystallizers in industry[17]. The nucleation rate is defined as the number of nuclei formed per unit time in a unit volume.

3. Control of the nucleation process in pharmacy

Crystallisation is a two-step process where nucleation is usually followed by crystal growth. However, these two steps can also occur in parallel and which one dominates[18] depends on the supersaturation of the solution. Nucleation dominates when the supersaturation of the solution is near or greater than the upper limit of the metastable zone (high supersaturation). Crystal growth, on the other hand, dominates at low supersaturation. This is because in nucleation, the kinetics is a function of supersaturation Δc^n , where c is the concentration and n = 3–6 (in crystallization, n = 1). This means that nucleation depends on supersaturation more strongly than crystal growth.

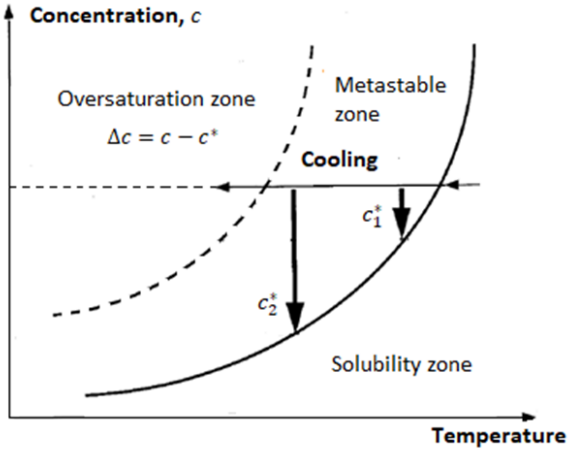

Fig. 2. **Plot of solution concentration *versus* temperature.** The supersaturation of a solution, is defined as the difference (Δc) between the current concentration (c) and the equilibrium concentration (c^*). The difference $c - c_1^*$ represents a small supersaturation (formation of a "thermodynamic" crystal), the difference $c - c_2^*$ represents a large supersaturation (formation of a "kinetic" crystal)

A process dominated by the nucleation step (kinetically controlled crystallization) is usually applied where finer particles need to be prepared. The material obtained in the dominant crystal growth step is called thermodynamically controlled crystallization. Crystals obtained by thermodynamically controlled crystallisation have a narrow particle size distribution, lower surface area, good bulk density and dry well.

The basic driving force of crystallization is the supersaturation of the solution, which is defined as the difference (Δc) between the current concentration at time (c) and the equilibrium concentration (c^*) (Figure 2), and arises in the following ways:

- by cooling,
- by the addition of an antisolvent,
- by evaporation of the solvent,
- by reaction crystallization,
- a combination of two (or three) of the above methods.

However, once supersaturation is reached, uncontrolled nucleation (primary heterogeneous) can occur and thus suppress crystal growth. This then results in small particles often coalescing into agglomerates that trap parent alkalis with residual solvent and impurities in the resulting material. This reduces the effective surface area of the crystals, which reduces the filtration rate and increases the drying time (downstream processing). Last but not least, the material obtained may be amorphous or oily phase (oiling out).

A very promising technique for controlled crystallization is the use of modified surfaces as heteronucleants. Heteronucleants force the crystallization process to proceed in unconventional ways in order to optimize the technology and the resulting product properties. E.g., Eral et

al.[19] studied biocompatible alginate hydrogels as heteronucleants for controlled crystallization and simultaneously as carriers for hydrophobic and hydrophilic APIs.

3.1. Seeded crystallization

The best known and most commonly used method of controlling the crystallization process is deliberate secondary nucleation or seeded crystallization. It is the process of deliberately introducing crystals – loops (*de facto* nuclei) of the produced API into the crystallization solution. An alternative method is to introduce a certain amount of energy (ultrasound) into the solution (sonocrystallization method). In both cases, the aim is to control the nucleation process from the outset and prevent it from running uncontrollably.

For seeded crystallization, it is essential to know the parameters of the seeding material (particle size distribution, polymorphic form, surface energy), the technique of used (powder or suspension of seeding material) and the course of crystallisation after seeded crystallization (type and length of cooling temperature ramp)[20]. The temperature of the solution at which the seeding material is introduced should also be taken into account. Seeded crystallization near the solubility curve is performed at low supersaturation (up to about 1/3 of the width of the metastable zone, see Figure 2, here expressed as $\Delta c = c - c_1^*$). In this region, little secondary nucleation occurs, crystal growth is slow and large crystals result[21], see Figure 3 left. Seeded crystallization near the supersaturation curve is performed at high supersaturation, expressed as $\Delta c = c - c_2^*$ (Figure 2), crystal growth is rapid and results in small crystals (Figure 3 right).

The amount of seeding material introduced into the crystallized solution can vary greatly and again depends on the reason for seeded crystallization and can be divided as follows:

- pinch, this amount is added mainly to avoid unwanted phases (e.g. oil phase) and uncontrolled nucleation. It is mainly used in the laboratory and is not effective when transferred to scale-up production.
- small amount (< 1% of product weight), nucleation is more controlled than in the previous case, but usually the nucleation step dominates over the crystal growth, bimodal (two-peaked) particle size distribution occurs.
- a large amount (5–10% of the product weight), this amount already guarantees crystal growth in most cases, bimodal distribution and secondary nucleation are prevented.
- massive amounts (> 10% of the product weight), this amount will reliably guarantee crystal growth.

Although a product produced in a previous batch can be used as seeding material, it is very likely that the batches will differ in particle size distribution and in specific surface area (Fig. 4, Table II).

Table II
Effect of the seeding material on the final crystalline product. Parameters evaluated: particle size distribution – PSDD90, where D90 corresponds to 90% of particles smaller than the mentioned value; specific surface area – SSA and yield

Exp.	PSD_{D90} [μm]	SSA [m^2/g]	Yield [%]
1	42	1,7	90
2	88	1,0	82
3	102	0,75	70

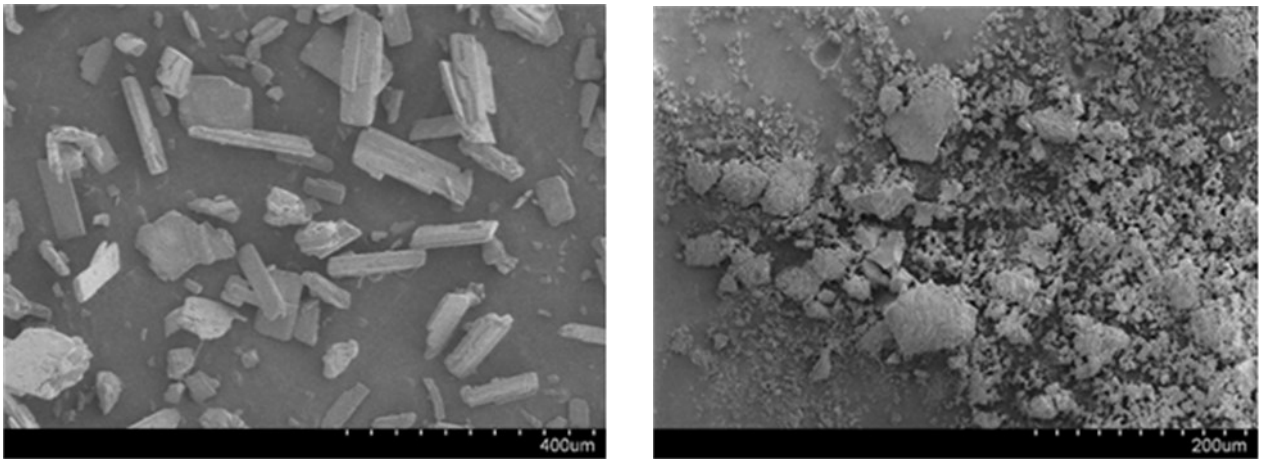

Fig. 3. **Left: SEM image of the material called "thermodynamic crystal", obtained at relative supersaturation $\Delta c = 2$. Right: SEM image of a material called "kinetic crystal" obtained at relative supersaturation $\Delta c = 20$**

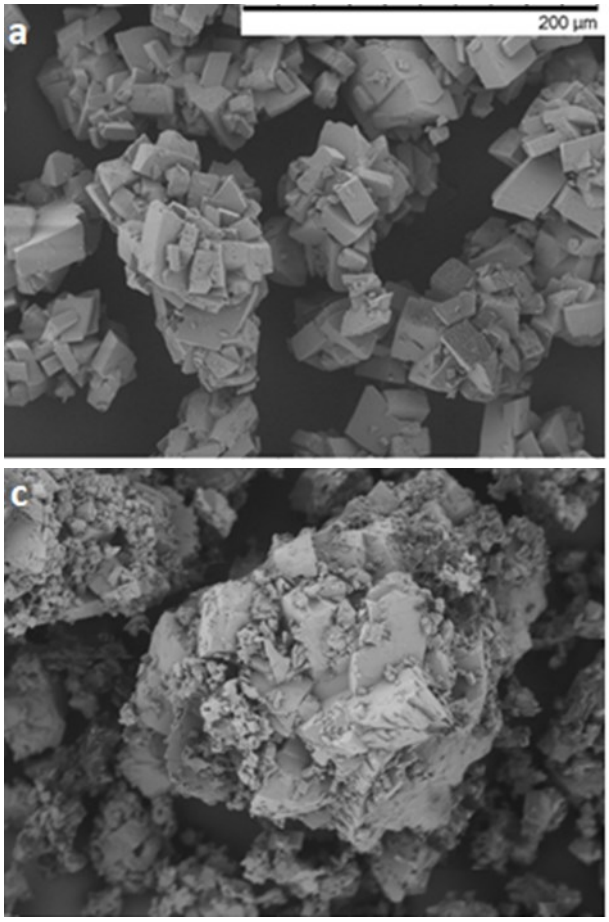

Fig. 4. **SEM images of crystalline APIs monitoring the effect of the grafting material on the final crystal.** a) API grafted with sieved material, parameter D90 = 42 µm; b) API grafted with the material in a), parameter D90 = 88 µm; c) API grafted with the material in b), parameter D90 = 102 µm

If the product exhibits polymorphic behaviour, a polymorphic purity analysis is required. In addition, the tendency of some solutions not to crystallize and form an oily phase must be taken into account.

3.2. Sonocrystallization

As already mentioned, another way to control nucleation is by controlled introduction of ultrasonic energy into the supersaturated solution, or sonocrystallization. Sonocrystallisation uses ultrasonic energy at frequencies greater than 20 kHz and a phenomenon called cavitation[22]. Cavitation is defined as the formation, growth and collapse of bubbles (voids) in the sonicated liquid. In crystallisation experiments, ultrasonic devices with a frequency of 20–100 kHz are used (at higher frequencies, a so-called cavitation barrier could occur). The cavitation barrier is a phenomenon in which the compression and decompression cycle caused by ultrasonic waves is so short that molecules in the liquid cannot be separated and form bubbles, thus preventing the cavitation effect[23]. The application of ultrasound has a positive effect on the nucleation rate, the width of the metastable zone, and prevents the formation of oily and amorphous phases. By appropriately adjusting the input parameters of the incoming ultrasound (power, wave amplitude), the desired particle size distribution and surface properties of the crystals can be prepared. It is also possible to use sonocrystallisation for so-called sonomilling – i.e. particle size reduction without their isolation. In doing so, an ultrasonic generator is used to introduce energy of 10–100 W (optimally in the range of 20–80 W) into the crystallized system. Ultrasonic waves can be introduced into the system either continuously or pulsed. By varying the intensity of the introduced waves and using a cooling temperature ramp, different types of crystalline material can be prepared (Figure 5). In Tab. III three identical crystallization experiments are shown, differing only in the intensity of the introduced waves. From Fig. 5 and Table III, the influence of the intensity of the waves on the design of the final crystalline material is evident.

4. Monitoring of the nucleation and crystallisation proces

Advances in in-line measurement techniques make it possible to monitor and control the progress of the crystallization proces. These techniques are called Process Analytical Technologies (PAT) and are recommended by regu-

Table III
Effect of ultrasound intensity on the resulting particle size distribution – PSDD90, where D90 corresponds to the 90% of particles that are smaller than this value

Exp.	PSD_{D90} [µm]	Ultrasound intensity [%]
1	102	20
2	74	40
3	33	60

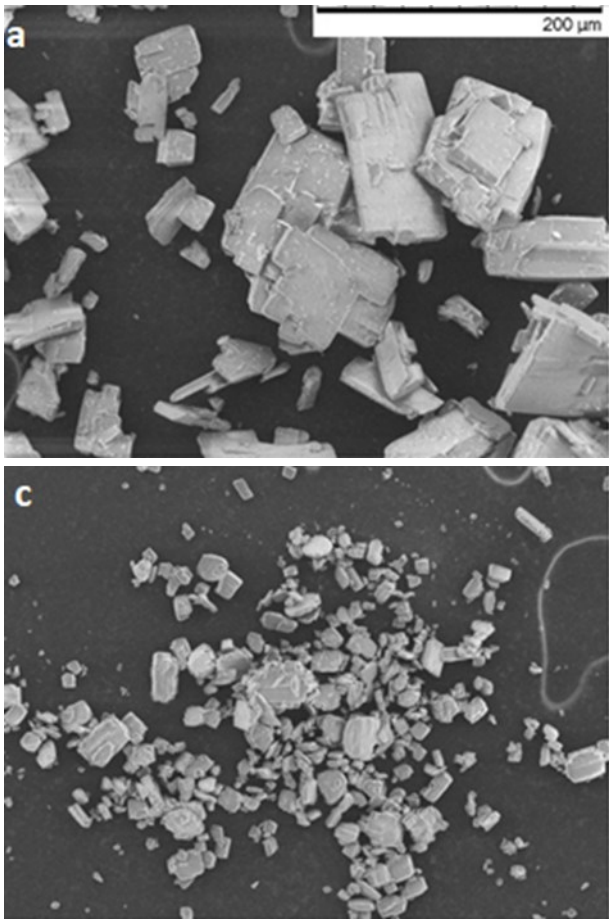

Fig. 5. **SEM images of crystalline API obtained by sonocrystallisation under the same conditions, only the amplitude of ultrasonic waves differed.** a) 20 % of ultrasonic wave intensity, parameter D90 = 102 µm; b) 40 % of ultrasonic wave intensity, parameter D90 = 74 µm; c) 60 % of ultrasonic wave intensity, parameter D90 = 33 µm. The intensity can be adjusted on the ultrasound probe (intensity is proportional to amplitude), the terminology in the literature is not clear (see ref.[24])

latory authorities (e.g. FDA) in the pharmaceutical industry. Some of the most commonly used in-line instrumentation include Mettler-Toledo's FBRM laser probe and, most recently, the Blaze system from BlazeMetrics company. These techniques, with a sensitivity of approximately 0.5 µm, detect the formation of a new phase in the crystallization system (however, this is not nucleus detection). Their main advantage is that they are sensitive to dynamic changes in the system, be it primary heterogeneous nucleation, secondary nucleation or polymorphic transformation in suspension.

4.1. Focused Beam Reflectance Measurement (FBRM technique)

It is a laser probe that uses technology to measure light reflected on particles from a light beam focused into the medium being measured. When the light beam emitted by the laser hits the crystal, the sensor records and analyzes the reflected signal. The FBRM method uses a unique discriminating circuit to evaluate the duration of the bounce-back interval as the beam moves from one end of the particle to the opposite end. This time interval is multiplied by the scanning speed and the result is the length. The length measured by this method is called the chord length. It is equal to the length of the straight line between any two points on the edge of the particle or agglomerate. The system is capable of measuring tens of thousands of chord lengths per second, which provides a powerful source of data for the evaluation of their distribution (the values measured during one second are sorted by chord length linearly into 1 400 classes).

The system provides a real-time indication of the size and concentration of solid particles at a selected point in the process stream. Particles in the size range from 0,5 µm to 2,5 mm are measured and are not assumed to be spherical in shape. The method provides an indication of the size distribution and number of particles in the selected size category at two-second intervals and allows monitoring of the number of particles in specific grain size regions (fine, coarse, etc.).

The advantages of this technique include simple operation and very easy maintenance or calibration of the instrument. However, the method is limited by the fact that it does not display the shape of the particles. However, this is an attribute and a limitation, given that shape is important and especially for non-spherical particles if changes in shape and size occur over time. The laser probe gives high precision results and is mainly used to dynamically quantify the effect of process variables such as: reaction rate, temperature and cooling rate, antisolvent addition rate, mixing rate, etc.[26,27]. The results of the laser probe measurements are presented in graphical form as weighted or unweighted particle size distributions, where the weighted distribution emphasizes larger particles (crystal growth) and the unweighted distribution emphasizes small particles (nucleation process). The probe is also able to detect polymorphic transition of API in suspension, which

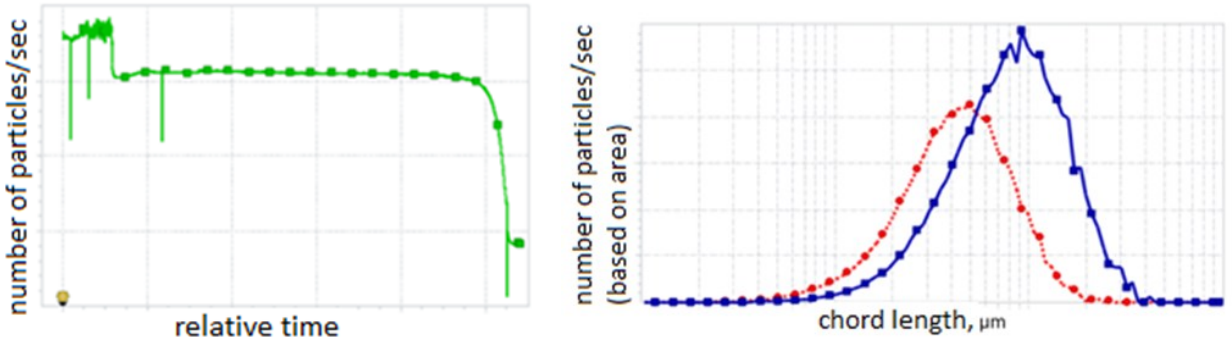

Fig. 6. **FBRM record. Sharp decrease in the total number of particles in the crystallizing suspension over time (left), shift in the distribution of particle lengths towards larger particles (right).**

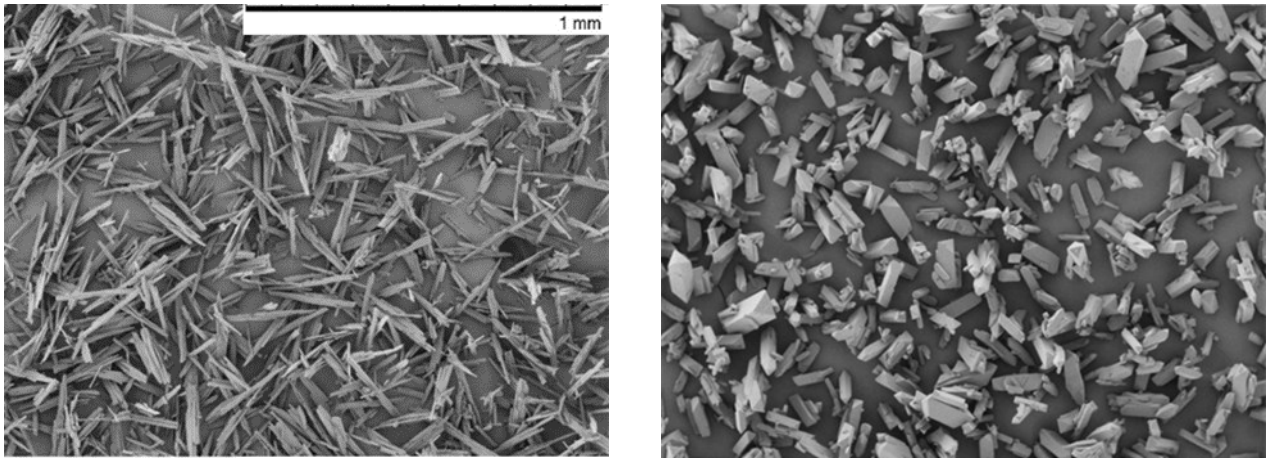

Fig. 7. **SEM recording. Dimorphic transformation of one crystal modification (left) to another (right).** Electron microscope images confirm the laser probe recording, see Fig. 6 right

is usually accompanied by a step change in particle size, Figures 6 and 7.

4.2. System Blaze

Another laser-based technique is the Blaze system from BlazeMetrics companybegining, which collects real-time data[24]. The probe combines several techniques (similar to Mettler Toledo's Particle Track system, for example) to provide complete control over the crystallization process, without disrupting the hydrodynamics and mixing of the crystallizer, saving time and reducing costs. Compared to previously manufactured probes (FBRM, PVM, etc.), the Blaze probe is a step up precisely because of the combination of different techniques in one device. As with the FBRM, the Blaze probe monitors the particle size distribution, using the parameters D10, D50, D90, which means that 10%; 50% and 90% of the particles are smaller than the selected size). Unlike FBRM, Blaze obtains size data from image analysis. The probe obtains high resolution images of the entire process that are comparable to images from an optical or electron microscope (Figure 8). The beginning of crystallization can also be detected using the turbidity of the solution. Turbidity characterizes the attenuation of the intensity of the primary beam due to scattering as it passes through the dispersion system. The phase transformation can be detected by Raman spectroscopy, which uses a coherent laser. Because it collects data in close proximity to the probe and from a larger area, it has a much better signal compared to a standard Raman probe.

The most common type of crystallisation is cooling crystallization. The ideal result is then a correct crystalline form, unimodal (single peak) particle size distribution and high yield. However, if, for example, too fast cooling is chosen, the material crystallizes uncontrollably and particles may stick to the probe slide. In order to obtain up-to-date and unbiased process data, the probe must be cleaned. The system is often cooled to temperatures close to 0 °C to achieve the maximum possible yield. Turbidity measure-

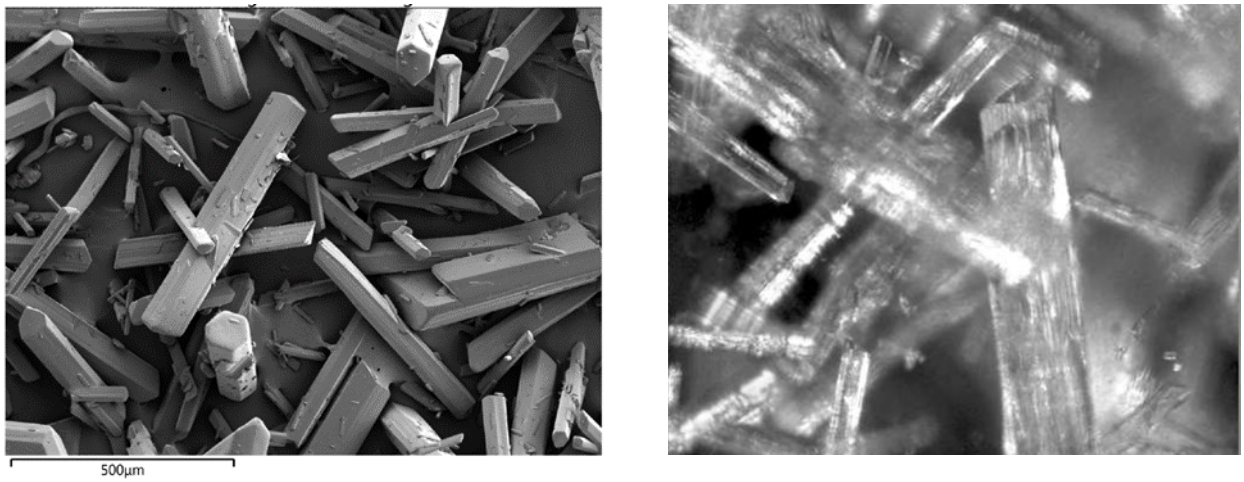

Fig. 8. **Comparison of SEM image (left) and microscopic image of the same API obtained with the Blaze system (right)**

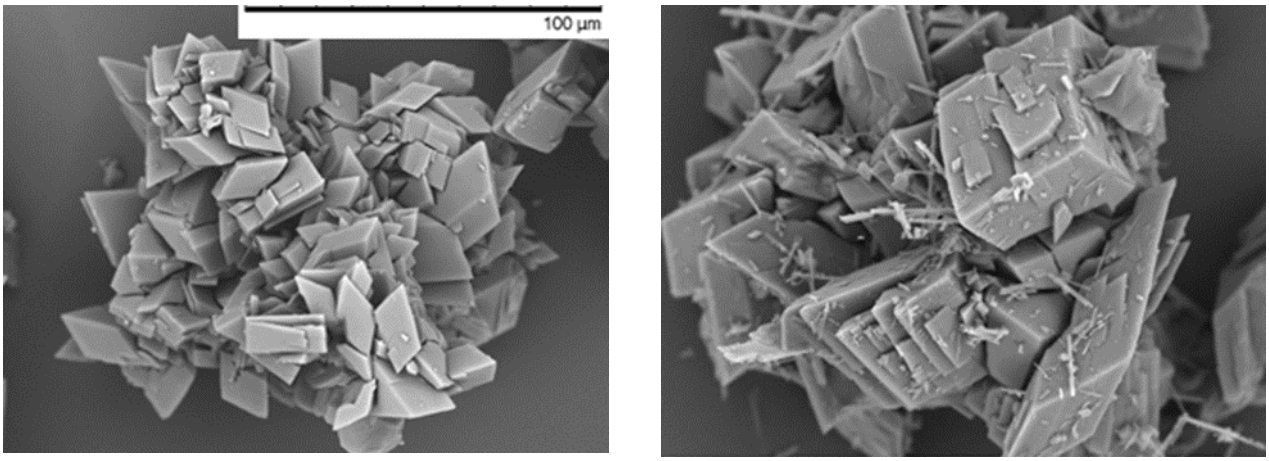

Fig. 9. **Comparison of SEM images of crystalline API obtained by grafting material with different specific surface area – SSA** (polymorphic form 1, SSA = 2 m^2/g – left; mixture of polymorphic forms 1 and 2, SSA < 1 m^2/g – right)

ments will indicate whether further cooling makes sense, whether crystal growth or secondary nucleation is occurring. If the turbidity of the solution is constant as the temperature decreases, it is unnecessary to continue cooling, thus shortening the length of the process. If the turbidity increases even at constant temperature and the system cannot be cooled further (e.g. for technological or polymorphic reasons), an optimum ratio between mixing time and yield is sought. The longer the suspension is stirred, the greater the chance of the crystals breaking against each other or, due to impact, against the crystallizer walls. All changes are immediately reflected in the particle size distribution. Compared to previously obtained distributions and combined with microscopic images, it is possible to immediately evaluate how the process will proceed. Similarly, the formation of aggregates can be seen. The probe also provides very valuable information during Ostwald rippening. Comparison of the crystalline materials after each cycle will show whether Ostwald ripening has a significant effect on the distribution and how many cycles need to be done.

The situation is similar for seeding crystallization. Here there is already some control over the crystallization by adding seeding material with defined properties. But is the selected seeding material suitable enough and added in sufficient quantities or is it added unnecessarily too much? A small amount of seeding material will cause secondary nucleation, which will result in bimodal distribution. A similar result is achieved with seeding material of the small specific surface area, in this case the same polymorph as the seeding material is not guaranteed to be produced (Figure 9). Sometimes a different polymorph can be identified by microscopy alone on the basis of the different particle morphology. However, identification by Raman spectroscopy is far more reliable, and if spectra (standards) of pure polymorphs are available, it is obvious which poly-

morph has crystallized[25].

It is also very similar for crystallization with an antisolvent. By measuring the turbidity it is possible to know when the system is fully desupersaturated and what minimum amount of antisolvent must be added. Slow addition of antisolvent produces large, well-developed crystals. If the rate is chosen appropriately, the entire particle distribution will shift to the right towards larger sizes and remain unimodal.

The probe can also be used to compare materials before filtration and after drying. During filtration and drying, agglomeration, crystal caking, surface defects in solvates or complete transformation to another polymorphic form may occur.

The development of the crystallization process and its optimisation with Blaze system becomes faster, more efficient and easier, as the number of experiments can be reduced and there is no need to sequentially take samples and wait for their analysis.

List of abbreviations

API	Active Pharmaceutical Ingredient
CNT	Classical Nucleation Theory
FDA	Food and Drug Administration
FBRM	Focused Beam Reflectance Measurement
PAT	Process Analytical Technologies
PVM	Particle Vision and Measurement
SEM	Scanning Electron Microscopy
SPM	Scanning Probe Microscopy
SSA	Specific Surface Area
2S	two step

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Abstract

The text is a contemporary continuation of an earlier publication, Kratochvíl B.: Chem. Listy *101*, 3 (2007). It describes mainly the nucleation process (two-step nucleation of active substances in pharmacy) and crystallization control processes (seeded crystallization and sonocrystallization). The focus of the work is the description of the nucleation process monitoring by modern analytical technologies, i.e., Focused Beam Reflectance Measurement (FBRM) and the BlazeMetrics system. Both methods presently provide the best available information for a deeper understanding of the nucleation process mechanism in crystallizing active substances. The work is documented by high quality and original photographic attachments of the crystallizing material.

● Gabriel R., Bártová A., Šahnić D., Kratochvíl B.: Chem. Listy *116*, 737–745 (2022).
● https://doi.org/10.54779/chl20220737