

Development of a continuous “self-seeding” microfluidic crystallization device for active pharmaceutical ingredients

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1. Introduction

As the latest stage in the continuous production of an active pharmaceutical ingredient (API), the crystallization step further purifies the API and introduces the desired crystalline morphology combined with a suited crystal size distribution. Microfluidic devices are mainly used to investigate all possible thermodynamic and kinetic parameters involved in crystallization from solution [1]. A widely used device for crystallization screening is a droplet microfluidic system, where the material in solution is divided into droplets, separated by a counter-solvent or air inside tubing [2-5]. Droplets may vary from micrometer to millimeter scale depending on the application. Due to an excellent control of the parameters, droplet based microfluidics is widely used for nucleation studies, as well as for protein crystallization [6,7]. Yet, industrial applications of continuous flow crystallization are also reported [8-10].

Crystallization of API as any other molecule in solution occurs during a nucleation and a crystal growth phase. Recent publications bring an addition to this classical two-phase crystallization theory. Vekilov described nucleation in protein and amino acid solutions inside pre-existing metastable clusters which consist of dense liquid and are suspended in the solution [10]. Revalor et al. described two existing features of supersaturated solutions: molecular orientation and density fluctuations [11]. These density fluctuations are also reported to exist of solid-like structures like (meta)stable clusters. Gebauer et al. use in their nucleation theory pre-nucleation clusters or PNC as a term [12]. In their set-up, the nucleation of crystals from solution is preceded by a prenucleation phase, followed by nucleation of these prenuclei clusters and growth to full-size crystals [12-16]. It is stated that the formation of these PNC clusters is closely linked to phase separation phenomena, leading to the formation of solid material [12].

The API used for this study possesses two competing crystal forms: an undesired needle form and a desired cubic form, thermodynamically more stable at ambient temperature. As the solubility curves of both forms are extremely close at all temperatures, the standard batch crystallization process has issues concerning selectivity and yield. This paper describes the formation of large, stable particles or prenucleation clusters at temperatures beyond solubility that concede with the above theories. The existence of these particles is also used to benefit the fastened formation of the desired conformation in a self-seeding way, inside a continuous droplet crystallizer.

2. Experimental Methods

The active pharmaceutical ingredient was delivered by a pharmaceutical company and will be designated as ‘API’ for confidentiality reasons. As solvent, 98% pure

isopropyl acetate (IPAC) was purchased at Sigma-aldrich (Belgium) and used as such. Counter-solvent Fluorinert FC70, a perfluorinated oil, was purchased at Hampton Research, USA, and used as such.

Diluted samples were dissolved in a furnace at 85°C, right below the boiling temperature of IPAC at atmospheric pressure. Further sample treatment before loading into the droplet system may vary and will always be specified. The droplets were generated using a Nemesys syringe pump by Cetoni GmbH (Germany), using Hamilton 10 mL glass syringes (purchased at VWR international bvba, Belgium). Typical flow rates between 0.5 and 1.5 $\mu\text{l}/\text{sec}$ were used. The liquids were pushed inside 500 μm inner diameter PFA+ tubing from IDEX Health and Science GmbH, Germany. Syringes and tubings were set at temperature by means of homemade heating elements. Temperature was controlled by using pt100 thermocouples.

The formed droplets and crystals were analyzed and photographed using normal and inverted Nikon microscopes (Nikon Belgium), images from the microscopes were taken by a Zyla camera from Andor Technologies (Oxford Instruments Ltd. , UK), using NIS-Elements software (Nikon, Belgium). Macroscopic pictures were taken using normal digital cameras.

Dynamic light scattering experiments or DLS for particle size determination were performed on a Malvern zetasizer 1000HSA (Malvern INC, UK). Temperatures varied between 25 and 74°C, 10 scans were performed per measurement; the solution was prepared separately and poured into a closed rectangular quartz cell.

3. Results and Discussion

3.1 Solubility and crystal morphologies

The API crystallizes into two distinct morphologies, an undesired needle-like structure and the thermodynamically more stable cubic form at ambient temperature, as depicted in Figure 1. The solubility curve of the cubic crystals of the API in IPAC is shown in figure 2, blue points. It can be stated that A. upon evaporative crystallization cubic crystals are never formed, B. cubic crystals may be formed upon cooling crystallization using specific cooling conditions: slow cooling rates at 2 °C/hour to 5°C e.g.. A white deposition is observed at elevated temperatures for API mixtures with solvents shown in Figure 3. This deposition disappears at elevated temperatures, close to the boiling point of IPAC, but is stable over time. The product only starts to crystallize at temperatures below the solubility line. This indicates that the white deposition or “cloud” is a specific interaction between IPAC or any other solvent and the API. It does not change the solubility data but does influence the crystallization kinetics. In figure 2 the highest temperature at which this white “cloud” is observed is shown with triangles. Dynamic light scattering tests were performed for different solutions of API/IPAC at elevated temperatures, see figure 4. For all conditions particles of about 1 μm in size are present inside the solutions, although the temperature is clearly beyond solubility. It was observed that this stable solid product or cloud has an influence on the crystallization kinetics of the API in IPAC during cooling crystallization. To show this effect the following test was performed: a 800 mg/mL API/IPAC solution was dissolved at 85°C. A part was sucked up by a heated syringe and injected in a 500 μm wide tube at room temperature. After 1 hour, the solution in the middle of the tube did not crystallize at all, see figure 5 left. Another part was set to stabilize at 50°C for 30 minutes and injected afterward in a 500 μm wide tube at room temperature (figure 5 right). The solution started to crystallize

immediately into cubic crystals and after 6 minutes everything was fully crystalline. This indicates that the white deposition can be used as a precursor of nucleation for the cubic API crystal, or are possibly stable non-growing nuclei. Their presence leads to a very fast crystallization of the material inside the small tubing. The temperature at which the solution is put to form the white “cloud” is further denoted as (pre)nucleation temperature.

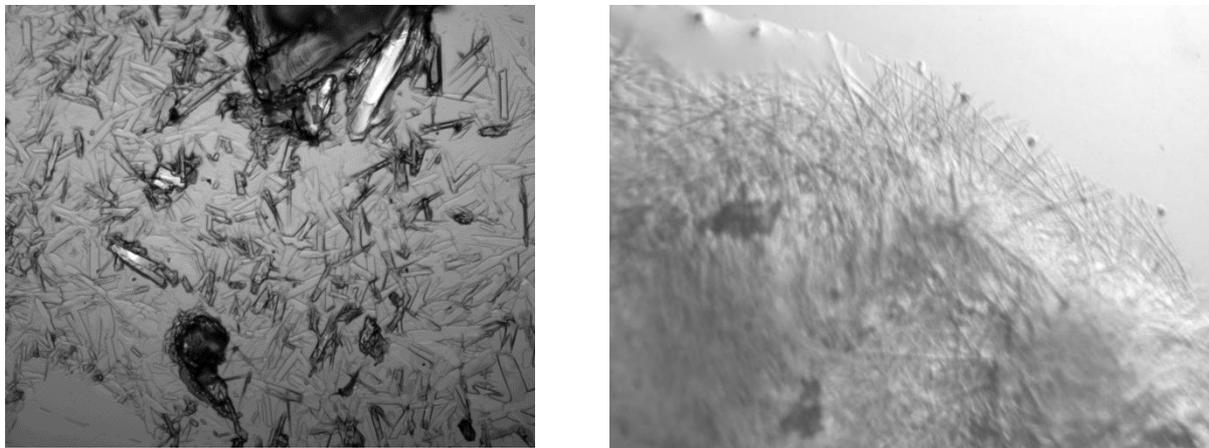


Figure 1: API after cooling crystallization resulting in cubic crystals (left) and after evaporative crystallization resulting in needle-like crystals (right). The API was dissolved in IPAC at a concentration of 0.7 g/mL and dissolved at 85°C.

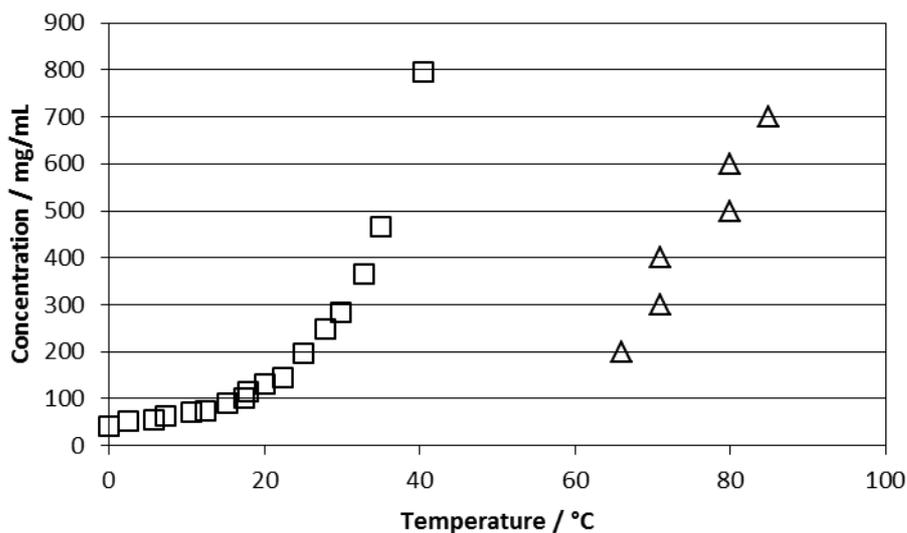


Figure 2: Solubility line of API/IPAC in squares, highest temperature of visible occurrence of prenucleation clusters (triangles) for different API/IPAC concentrations.

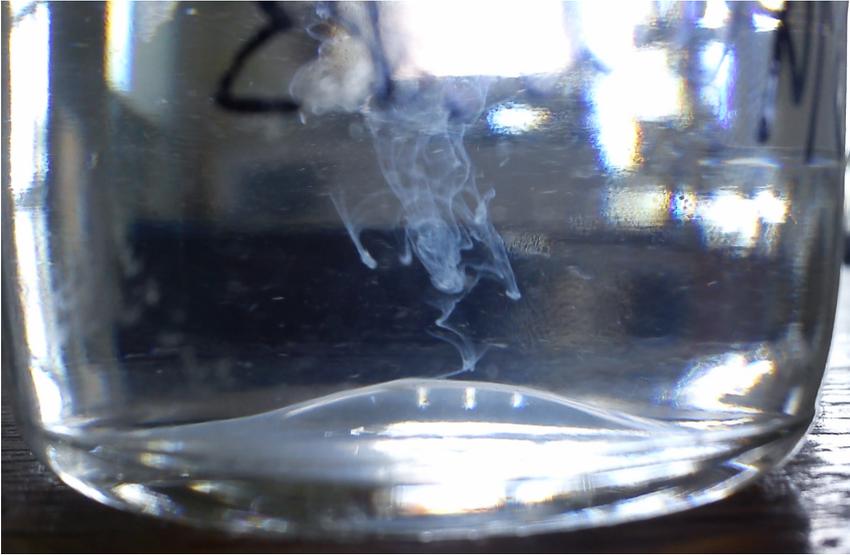


Figure 3: Appearance of the white deposition inside a API/methyl isobutyl ketone 100mg/mL solution at 20°C.

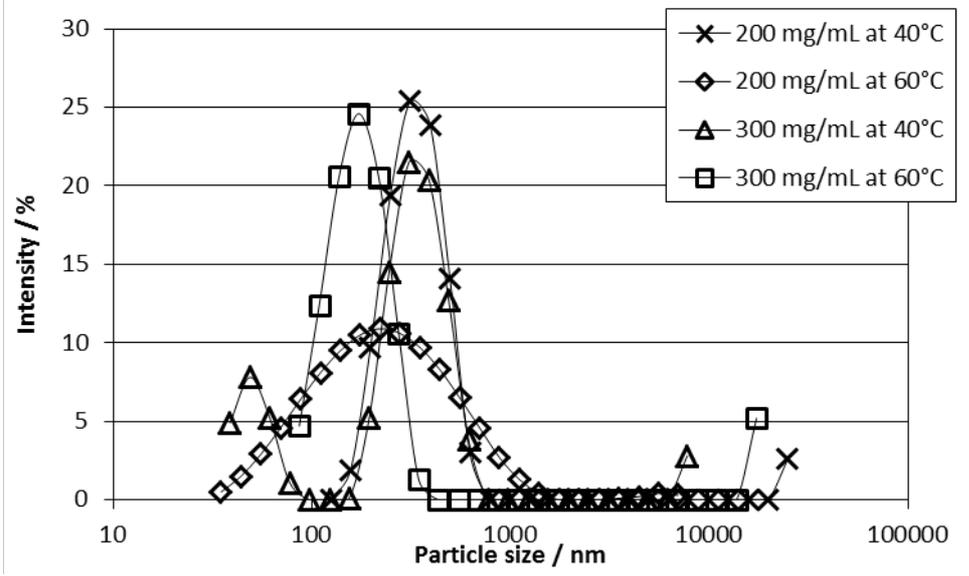


Figure 4: Dynamic Light Scattering measurements on API/IPAC solutions of 200 and 300 mg/mL at 40 and 60 °C; after 30 minutes stabilization at temperature.

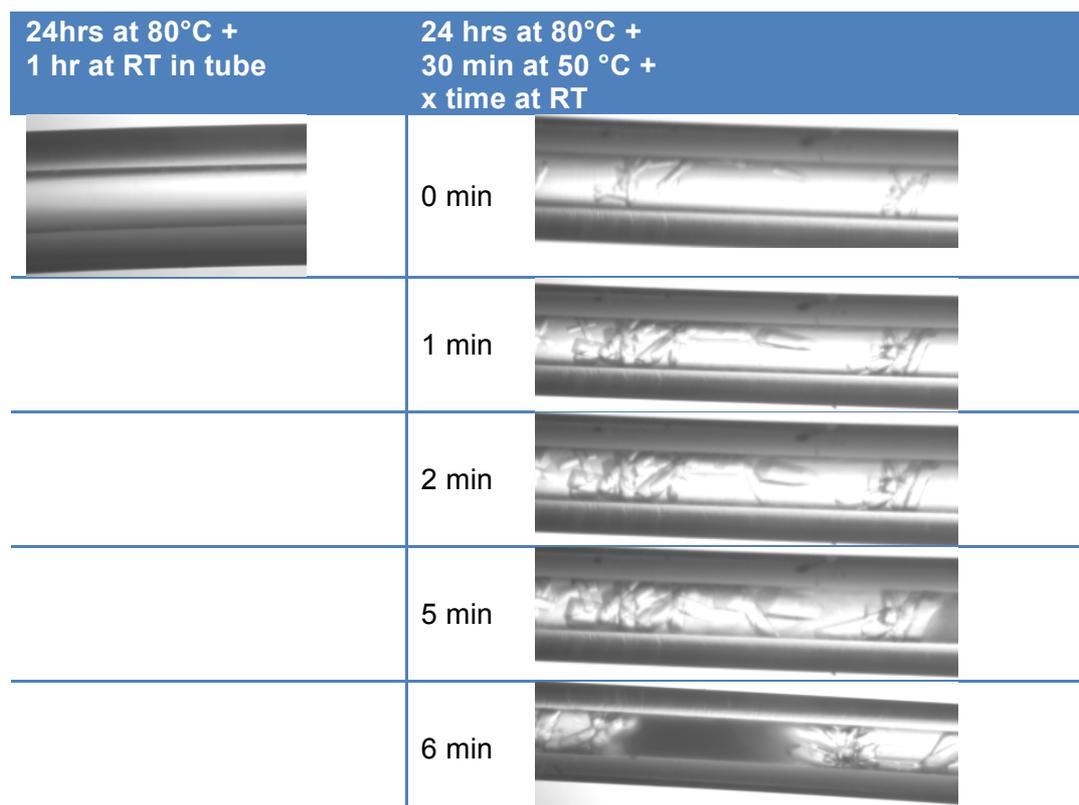


Figure 5: Influence of the (pre)nucleation at 50°C on the crystallization at room temperature inside a tube of a 800 mg/mL API/IPAC solution.

3.2 Droplet formation and crystallization scenarios

For obvious reasons, temperature control is of main importance during cooling crystallization. An in-house setup was therefore constructed, see figure 6. This setup permits the control temperature over several points where temperature drops may occur and alter the crystallization behavior of the API. All crystallization scenarios deal therefore with several temperature zones of importance for the process: dissolution temperature, prenucleation temperature (inside furnace and temperature of the heated syringes), temperature of the tube before the T-junction and the temperature of the droplet collector. At a flow of 1 μ l/sec for each line some concentrations already crystallize inside the tube before the droplet generation and therefore crystals are already seen that block the flow of material through the tubing, see figure 7. The temperature before the T-junction is set in such way that further nucleation before droplet generation may occur, without blocking the channel. For crystal growth the droplets are collected and studied in a static way under a microscope.

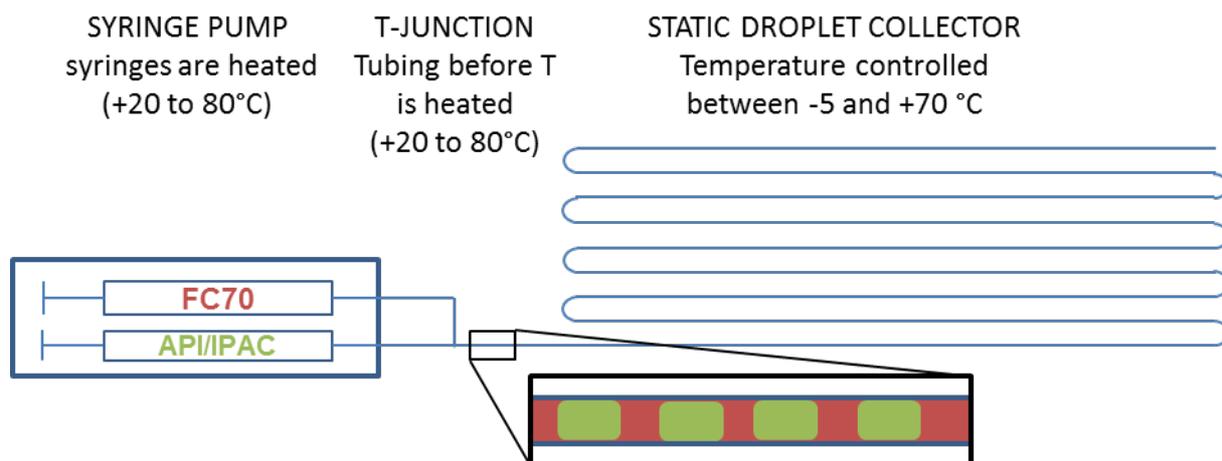


Figure 6: Schematic illustration of the droplet generation and storage in the microfluidic setup

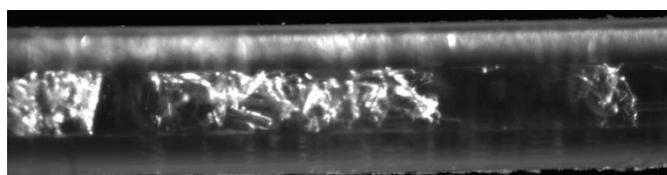


Figure 7: Flow blocking cubic crystals before the T-junction at 20 °C for a 600 mg/mL API/IPAC solution, pre-nucleated at 45 °C.

3.3 Crystallization scenarios using pre-nucleation or “self-seeding” conditions

Crystallization scenarios involve several steps between the dissolution and finally the crystal growth conditions. In an exemplary case, a 400 mg/mL concentration API in IPAC was used. A long dissolution step to prevent any residual particles was performed for one night at 80 °C. Afterwards, the solution was lowered in temperature for one hour to induce the formation of pre-nuclei at 45 °C. This mix was injected at temperature into the 500 μm tubing, which was placed before and after the T-junction at 19 °C. The present nuclei did not block the flow of material through the tubing for several minutes, enabling full continuous flow. The flow was stopped and the collected droplets were investigated. After 10 minutes, in 50 % of the droplets perfect cubic crystals were found where typically one crystal fills one droplet, as shown in figure 8A. The other droplets remained empty. This crystallization ends around 20 minutes. After 50 minutes, the remaining empty droplets start to be filled with needle-type crystals, see figure 8B. It is clear to see that the needle-like structure has a tendency to come out of the droplet. They have therefore an affinity with the fluorinated oil. However, the formation of both crystalline structures is separated in time due to the formation of the pre-nuclei during an isothermal annealing step at temperatures beyond the solubility line of the API.

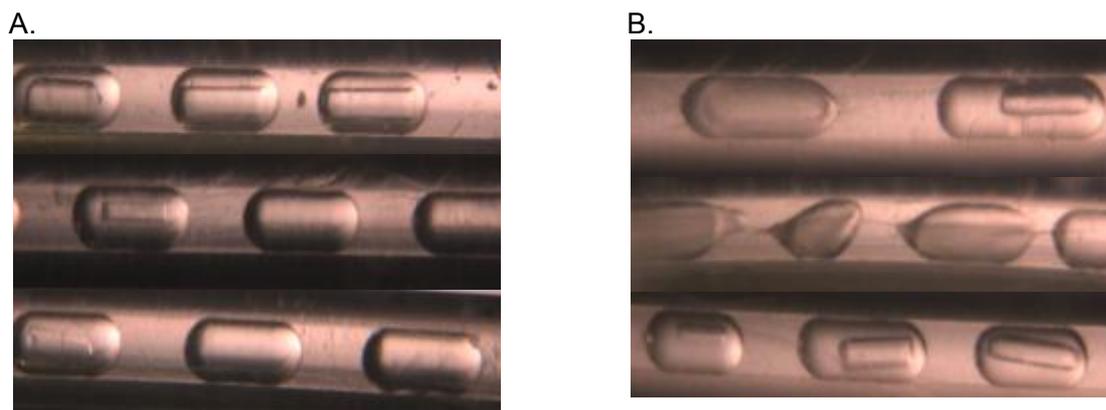


Figure 8: A. cubic crystal formation after 20 minutes of a 400 mg/mL solution of API/IPAC using a specific crystallization scenario; **B.** the filling of the empty droplets with needle-like crystals.

4. Conclusions and future work

The API used in this study has two competing crystalline structures upon crystallization. The separation of both crystal types is enabled through the existence of a specific interaction between the API and the solvent isopropyl acetate, as it forms a stable intermediate at temperatures beyond the solubility line. These particles can be regarded as nuclei of the cubic type as they induce the crystallization of cubic crystals in a shorter timescale than the appearance of needle-like crystals.

More concentrations and nucleation and crystal growth conditions are tested to look for the optimal results in crystals type and size. Also a scaling-up approach is under development.

5. Acknowledgements

We acknowledge kindly the Walloon region and the FNRS for funding, Noemi Casadevall Gras for the work on solubility, Michel Vandenbranden for the DLS experiments and Edith Norrant for the project management.

6. References

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