# On-chip *in-situ* observations of crystallization events under supercritical $CO_2$

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## 1. Introduction

Crystallization represents a key unit operation in separation and purification processes in the pharmaceutical industry, about 90% of active pharmaceutical ingredients (API) being obtained by this technique[1]. Most APIs are chiral and poorly soluble in water and therefore have a low bioavailability. Understanding crystallization process can help to obtain products with desired properties. The challenge for industries is to be able to produce directly highly water soluble and/or enantiopure drugs without going through several purification steps, which are costly, solvent and time consuming.

The rising demand for green technologies had promoted the interest of pharmaceutical industries for supercritical fluids. Supercritical $CO_2$ is frequently used as an anti-solvent in batch or semi-continuous mode, like the Gaseous Anti-Solvent (GAS) method, for recrystallization or co-crystallization[2].

With a few exceptions, most of the reactors have only one observation « window », thus giving only a very partial information of what is happening in the bulk. This kind of reactor might not be able to give observations of time-related events (kinetics, diffusion and induction time) and direct control of the crystal phase during precipitation processes. To address this issue, microfluidic platforms have been proved to be a great tool to investigate crystallization thanks to their optical access for *in-situ* observations.

## 2. Materials and Methods

In this present study, the API is naproxen (NPX). Naproxen is commonly commercialized in its racemic (RS) and S-enantiopure forms. The precipitation of both forms, initially dissolved in acetone, was studied by addition of $CO_2$ anti-solvent using microfluidic platform in order to access information about time-related data of precipitation in supercritical conditions.

A high-pressure microfluidic reactor was first designed and built out of silicon-Pyrex in a clean room. The design is composed of several « pools » or « wells » exhibiting a diameter of 0.5 mm and connected by a central channel, which were chemically etched in silicon (Figure 1).

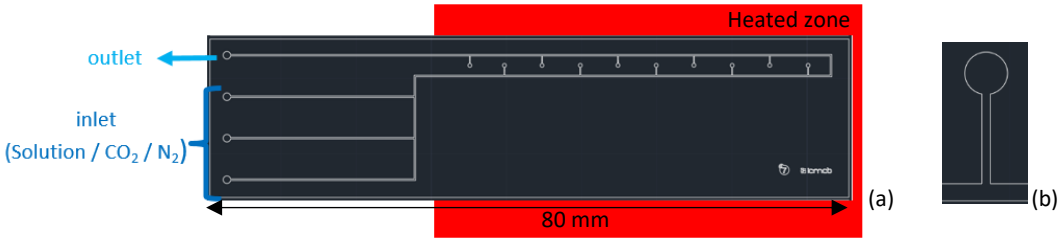


**Figure 1:** (a) Well-based design of silicon-Pyrex microchip and (b) a zoom on a well. One of the three inlets allows to add the solution of naproxen in acetone, the second one stands for the introduction of $N_2$ and the last one for $CO_2$.

The microfluidic device is heated to the desired temperature and placed under an optical Leica microscope. The solution [NPX+solvent] is introduced into microchip. Then, the excess of the solution is evacuated with a nitrogen flush from the main channel and refilled with $CO_2$. Once the $CO_2$ is added, it enters the micro-pools by diffusion and the microscope records a picture of each pool each second. A movie of NPX crystal apparition and its growth is obtained.

Furthermore, the mixing of $CO_2$ and acetone by diffusion inside the chip was simulated by numerical approaches to support the experimental results.

## 3. Results and discussion

In the literature, the precipitation of NPX is reported starting from an initial concentration of 40 mg/mL in acetone with the GAS method. Thus, in this work the experiments were conducted at this concentration at 100 bar and 40 °C. Visualization of NPX crystals and their growth as well as the modelling of the $CO_2$-acetone diffusion are shown in Figure 2.

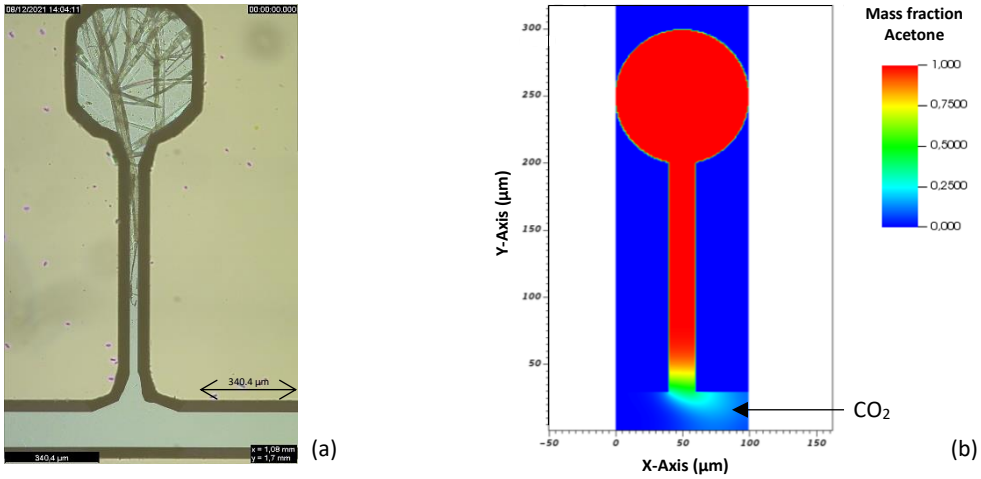


**Figure 2**: (a) On-chip precipitation of RS-NPX recorded by the optical microscope, (b) modelling of the $CO_2$-acetone diffusion.

Experimentally speaking, nucleation and growth of NPX start in the small channel connecting the main channel to the pool where they end. This is in correlation with the modelling of the $CO_2$-acetone diffusion: at their interface a local supersaturation is induced, the anti-solvent effect of $CO_2$ tends to precipitate NPX with a consequent growth of needle-like crystal.

At 40 mg/mL, precipitation of RS-NPX is slightly faster than the S-enantiopure, 4 min against 6.5 min respectively (Figure 3). This is in accordance with observations done in a sapphire reactor. Moreover, for RS-NPX, precipitation at different level of concentration were studied. The higher the concentration, the faster the precipitation occurs as in classical crystallization.

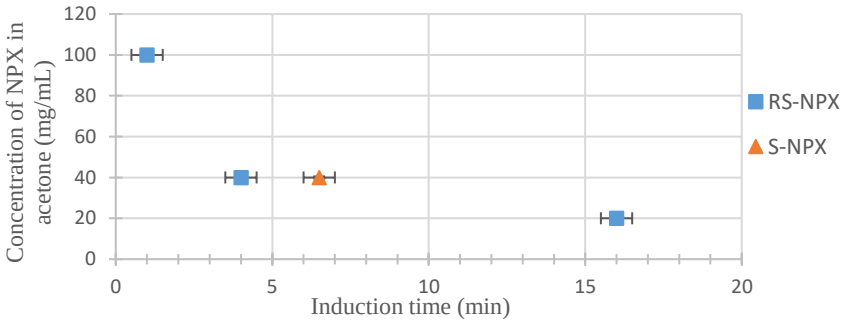


**Figure 3:** Induction time of racemic and S-enantiopure naproxen at 100 bar and 40°C.

From these data, knowledge on the growth kinetics under supercritical $CO_2$ can be acquired. *In-situ* chemical mapping of the concentration profile inside the micro-pools with RAMAN spectroscopy is in progress.

## 4. Conclusions

The implementation of high-pressure microfluidic platform offers *in-situ* and real time observation of crystallization phenomena in supercritical conditions. Relevant information on the behavior of racemic and enantiopure compounds precipitation can be obtained thanks to image analysis. These first results can be an opening perspective of enantioseparation in supercritical conditions.

## References

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