# SUPERCRITICAL ANTI SOLVENT CRYSTALLIZATION OF THEOPHYLLINE: EXPERIMENTAL AND THEORETICAL INVESTIGATION

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## ABSTRACT

Supercritical Anti Solvent (SAS) precipitation has been used to produce micronized crystals of Theophylline, a phosphodiesterase inhibitor and asthma medication. Dichloromethane-Ethanol mixture (1:1 volumetric ratio) was selected as the liquid solvent, considering the higher solubility of the compound in this mixture than in both pure solvents. A synthetic method was used to measure the solubility of Theophylline in a Dichloromethane-Ethanol-$CO_2$ mixture. The obtained solubility curve was very helpful to gain insight of the effect of liquid solution concentration on the crystals size. In this work experiments have been performed at 10 MPa and 309.15 K. The resulting crystals were characterized using scanning electron microscopy. We observed that the increase of the concentration, i.e. the increase of the supersaturation ratio $\beta$, leads to a reduction of the crystals size.

## INTRODUCTION

In the recent years, there has been a growing interest in the use of supercritical fluids as media for the production of particles, either because of a growing concern for "greener" technologies, either because of an improved quality of the product. Among the different processes currently under development, the Supercritical Anti Solvent (SAS) process is frequently preferred because of the relatively low solubility of many pharmaceutical compounds in $CO_2$.

SAS precipitation technique is developed to produce micrometric and sub-micrometric particles that are not attainable by conventional methods. In this process, the compressed fluid forms a solution with the liquid and further induced the precipitation of the dissolved solid. The supersaturation is induced by the change of the liquid composition upon counter-diffusions of liquid and fluid. When the compound to crystallize exhibits a much lower solubility in the liquid-fluid mixture than in the liquid, conditions of supersaturation are generated that result in the apparition of the solid phase. The most attractive characteristics of SAS precipitation are the possibility to control particle size and morphology, by changing operative conditions, and to eliminate the solvent residue without post-processing of the produced powders. Scientific literature contains information on several materials that have been processed by SAS using different apparatus and conditions [1-3]. As a general consideration, the mean particle size that can be obtained by SAS precipitation ranges from

0.1 μm to several micrometers. For what concerns the particles shape, spherical amorphous microparticles and crystals can be obtained. The precipitation process is based on quite complex mechanisms, especially when the process is operated in a continuous mode. It is the result of the interplay of fluid dynamic (jet break-up), mass transfer, nucleation kinetic and thermodynamic (high pressure ternary phase equilibria). As a consequence, ternary systems of solute/solvent/fluid have to be considered. For this reason, equilibrium data are essential to select the operative conditions as well. Numerous papers reported the solubility of solid measured in pure $CO_2$ or in $CO_2$ + solvent mixtures [4-7].

In this work Theophylline has been chosen as solute compound and Dichloromethane-Ethanol mixture (1:1 v:v) has been selected as the liquid solvent considering the larger solubility of Theophylline in this mixture than in both pure solvents. This drug is used in the asthmatic pathology since it enhances the respiratory centre increasing the respiratory volume and the vital pulmonary capacity. The aim of this work is to gain insight into the effect of liquid solution concentration on the Theophylline crystals size and morphology, obtained by SAS precipitation. The solubility curve of Theophylline in the solvent mixture has been also obtained by a synthetic method in order to help the theoretical investigation.

## I - MATERIALS AND METHODS

### Materials

Carbon dioxide was supplied from Air Liquid (99.5% purity). Theophylline (purity = 99%) was purchased by Sigma Aldrich. Dichloromethane (DCM, purity = 99.5%) and Ethanol (EtOH, purity = 99.5%) were supplied from Prolabo.

### Methods

The equipments and methods used for measuring the solubility of a solid in solvent/$CO_2$ mixtures and for the continuous SAS precipitation of organic compounds have been already detailed elsewhere [8-10].

## II - RESULT AND DISCUSSION

### Theoretical part

In order to gain insight into the effect of liquid solution concentration on the crystals size a brief description of fundamentals in crystallisation is reported in the following section.

*Crystallization process.* The formation of a solid particle results from two mechanisms: nucleation (formation of solid nuclei and apparition of an additional phase in the system) and crystal growth.

- Kinetics of nucleation

The rate of nuclei appearing, i.e. the frequency of nucleation, is the number of the nuclei formed per unit of time and per unit of volume. This frequency, $J_N$, is written in the case of homogeneous nucleation:

$$J_N = N_0 j \exp\left( -\frac{f V^2 g^3}{(kT)^3 \ln^2 b} \right) \quad (1)$$

with

j [$s^{-1}$] = frequency to which the nuclei of critical size become of supercritical size, i.e. of radius r>r*

$N_0$ [$m^{-3}$]= solubility expressed in a number of molecules per unit of volume.

For a given solute / solvent system, the rate of nucleation $J_N$ is thus a function of the temperature T and of the supersaturation ratio β (defined as the ratio of actual solution concentration C on the equilibrium concentration $C_S$) [11-12].

- Kinetics of growth

The flux corresponding to growth, $J_G$, can be expressed schematically by the following equation according to diffusion-reaction theories:

$$J_G = k(C - C_S)^n \qquad (2)$$

where k is a mass transfer coefficient and n ranges from 1 to 2.

Both rate of nucleation, $J_N$, and rate of growth, $J_G$, depends on concentration: the crystallization process is thus strongly controlled by this parameter. When concentration stands below $C_S$, the solution is unsaturated and crystallisation is therefore impossible. For concentration values between $C_S$ and C* (which is the effective concentration at which crystals appear in the solution) the system is metastable. Despite a supersaturation ratio higher than 1, the nucleation rate is too low to generate solids. However, if a crystals seed is placed in such a solution, it will grow. For concentrations higher than C*, spontaneous nucleation and growth occur competitively.

The specificity of each elaboration process lies in the arrangement of the nucleation and growth domains in space (for continuous processes) or in time (for batch processes) and in the control of residence time distribution in these domains. Supersaturation, that is the driving force of nucleation, is obtained by varying one of the operating variables of the system. In the case of crystallization by cooling, it is reduction of temperature that decreases the saturation concentration.

It is possible to show the evolution of supersaturation during the crystallization process as a function of time or space, as reported in Fig.1.

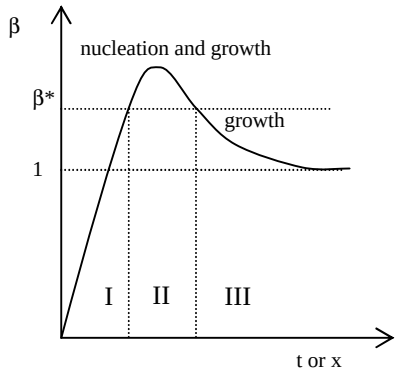


**Figure 1. Saturation ratio as a function of time or space.**

β* is supersaturation ratio large enough for nucleation to occur spontaneously. The particle size distribution (PSD) is mainly controlled by the distribution of residence times within the different zones. Residence time is the nucleation and growth area (zone II) gives the width of PSD, whereas the average size is a function of residence time in the growth zone (zone III).

Nucleation and growth of crystals from ternary system, solute-solvent-antisolvent, is governed by two mechanisms: diffusion of the antisolvent inside the organic phase and the evaporation of the organic solvent into the antisolvent phase. Antisolvent diffusion decreases the solute solubility within the organic phase, whereas the solvent evaporation increases its concentration. Therefore, high supersaturation can be achieved. Crystals size can be controlled by manipulating process variables. Crystals of small size are usually obtained when

solute are consumed mainly by nucleation, thus for concentrated solutions or large diffusion rates. On the other hand, crystals of larger average size will be formed in conditions where only few nuclei are formed and grow.

## Experimental part

In the first part of our work we studied the ternary system solute/solvents/$CO_2$ mixture (Theophylline/DCM-EtOH/$CO_2$) in order to obtain Theophylline solubility curve. We selected this kind of system, knowing that DCM helps to increase the solubility of Theophylline. For this system $CO_2$ acts as antisolvent for every $CO_2$ molar fraction. Fig. 2 shows the decreasing solubility of Theophylline when composition of the EtOH-DCM mixture increases in $CO_2$.

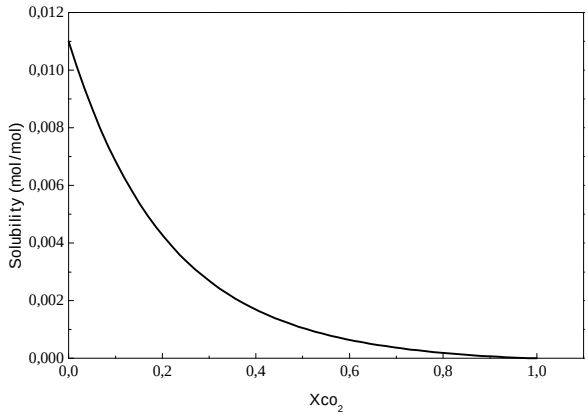


**Figure 2. Solubility curve of Theophylline at 10 MPa and at 309.15 K in $CO_2$ – DCM:EtOH (1:1 v/v) mixture.**

Crystallization from DCM-EtOH solution has been carried out at various solute concentrations of 2, 5, 10 and 25 mg/mL maintaining constant all other parameters (P=10 MPa, T=309.15 K) to study the effect of this parameter on the crystals size and morphology. The influence of several other parameters (solvent, pressure, temperature, antisolvent/solvent ratio, etc.) is under investigations. All experiments yielded to the production of a powder, excepted for the concentration at 2 mg/mL, for which no precipitation happened. The produced amount was also increasing with the concentration and a yield of ~70% was obtained from the 25 mg/mL solution.

The produced crystals have been characterized using scanning electron microscopic method. Observing SEM images reported below (Fig. 3-4), we could conclude that there are not evident changes in morphology varying the solute concentration.

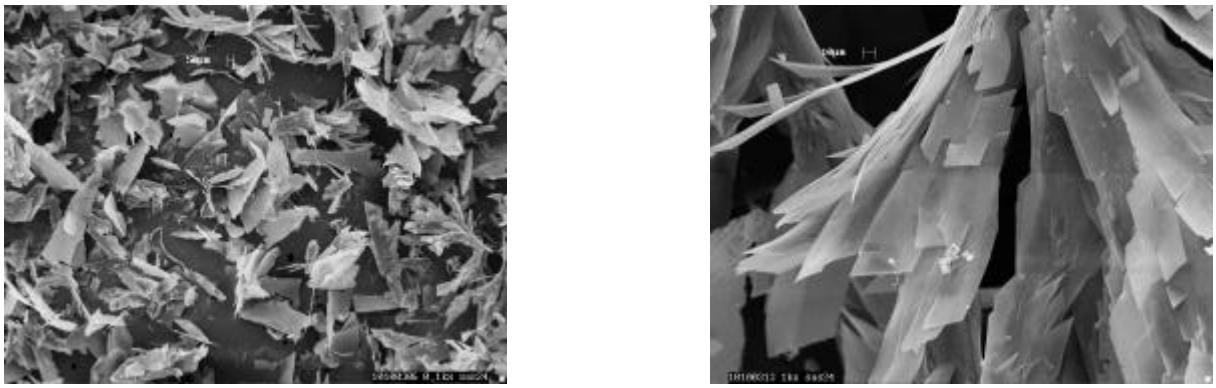

**Figure 3. SEM images of Theophylline powders generated by SAS precipitation at 309.15 K, 10 MPa, 5 mg/mL.**

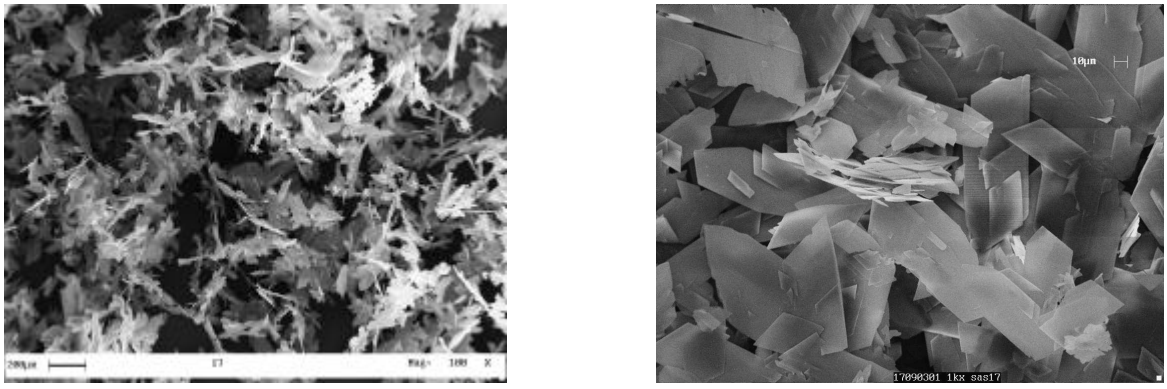

**Figure 4. SEM images of Theophylline powders generated by SAS precipitation at 309.15 K, 10 MPa, 25 mg/mL.**

However, it is possible to observe that the increase of the concentration, i.e. the increase of the supersaturation ratio β, leads to a reduction of the crystals size. The average size (the length is considered the characteristic dimension) of crystals generated by SAS precipitation at 309.15 K, 10 MPa, 5 mg/mL, is about 500 μm (Fig. 3), whereas the average size of crystals generated by SAS precipitation at 309.15 K, 10 MPa, 25 mg/mL, is about 30 μm (Fig. 4).

The solubility curve of Theophylline in mixtures of EtOH-DCM-$CO_2$ over the whole range of composition is helpful in order to gain insight of the effect of liquid solution concentration on the crystals size. Theophylline solubility curve in EtOH-DCM-$CO_2$ mixture (EtOH:DCM 1:1 v/v) and the working lines have been reported (Fig. 5a), putting in evidence $CO_2$ molar fraction range, in which the operative $CO_2$ molar fraction, of 0.97 is comprised (Fig. 5b).

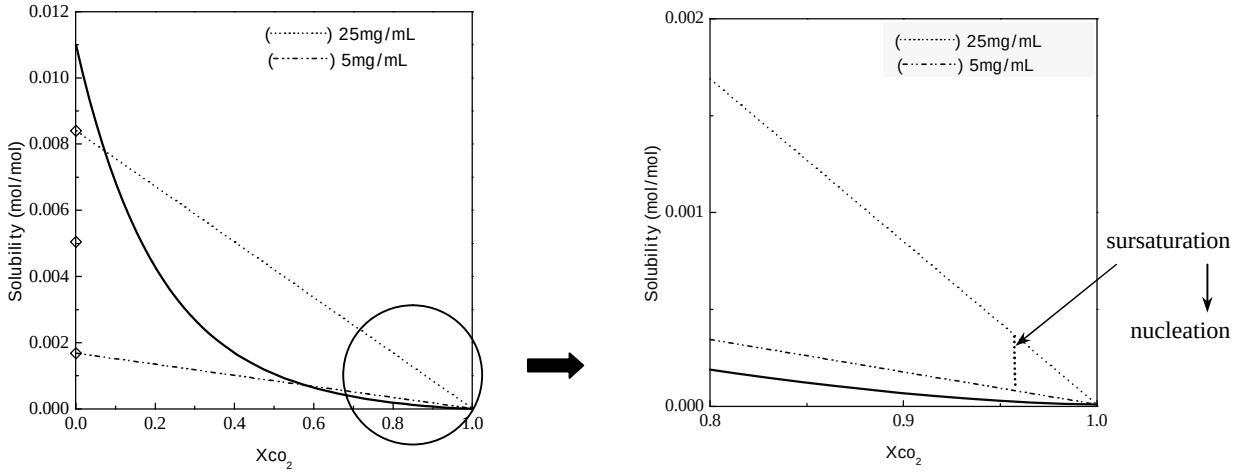


**Figure 5. a) Theophylline solubility curve in EtOH-DCM-$CO_2$ mixture at 10 MPa and at 309.15 K and working lines at 25 mg/mL and 5 mg/mL. b) Magnification of behaviour within the $CO_2$ molar fraction range of 0.8 – 1.0.**

A solution always tends to reach equilibrium because equilibrium represents a minimum of energy. For a fixed $CO_2$ concentration, if the concentration of the solute in the mixture exceeds the equilibrium value, the solution will tend to recover the equilibrium state by evacuating the solute excess as a solid phase. Starting form a given concentration of Theophylline in EtOH:DCM solvent, the addition of $CO_2$ will decrease the concentration by a dilution effect. Such decrease is represented in the Fig.5 by the straight working line.

To get conditions of nucleation, the working line should cross the saturation line, otherwise the solution is undersaturated, i.e. the actual concentration is below the equilibrium value and the system is in a stable state. For the two extreme concentrations, the difference

between the saturation curve and the associated working line is larger at a concentration of 25 mg/mL than for the diluted case (Fig.5b). As a result, supersaturation ratio is larger, and according to classical crystallization equations, nucleation is favoured so that smaller particles are expected.

Observing SEM images and analysing yield results, it is then possible to propose the following sursaturation profiles (Fig. 6). For a solute concentration of 25 mg/mL, the starting supersaturation is higher and, as consequence, the number of nuclei is greater. Less solute is available for growth. Consequently, the observed crystal size decreases with the increasing concentration.

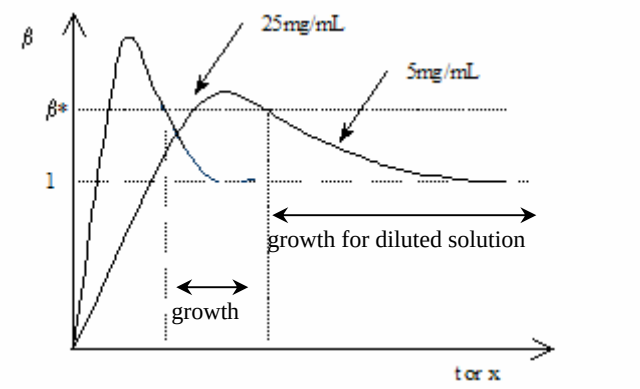


**Figure 6. Comparison between saturation ratio curves at two different concentrations.**

## CONCLUSION

In conclusion, the $CO_2$ addition to a solution of Theophylline in DCM-EtOH decreases the Theophylline solubility, providing thus the necessary conditions of successful crystallization. When processed in a continuous mode, Theophylline was recovered as flat crystals whose sizes were influenced by the initial Theophylline concentration. We showed that the observed trends – a decrease of size with an increasing concentration – were in accordance with the fundamentals of crystallization, indicating that an early burst of nuclei was a dominant mechanism for crystallization at high concentrations.

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