BATCH AND SEMI-CONTINUOUS TECHNIQUES FOR THE PRECIPITATION OF THEOPHYLLINE

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The recrystallization of a model compound, theophylline, was performed from an ethanol:methylene chloride (EtOH:DCM) solution, using carbon dioxide as antisolvent. Two variants of the antisolvent technique were used : the discontinuous mode in which CO_2 was gradually introduced into the stirred solution, and the semi-continuous mode in which the solution was co-currently introduced into the continuous flow of CO_2. This paper focuses here on the influence of pressure and temperature on the precipitate characteristics, with special emphasis of the role of phase behaviour. Conditions were thus selected to drive the representative point of the system (determined by P/T/overall composition) within or above the liquid-vapor coexistence domain. The comparison of the two processes are discussed on the particle characteristics (shape and size determined from microscopy images), crystallinity (by X-ray diffraction) and production yields.

INTRODUCTION

Supercritical fluids (SCF) are more and more employed in crystallization, especially to the micronization and the particle design, as a substitute to liquids based processes conventionally used [1,2]. One of their main interest is to obtain products with very low residual of solvents, with a reasonable consumption of organic solvents and without post washing or drying. Examples of precipitation of a given compound by both Batch (gas antisolvent) and SAS (supercritical antisolvent) processes are scarce and comparison is illustrated here with theophylline. Supercritical carbon dioxide ($SCCO_2$) have been chosen as antisolvent. Its addition decreases the solvation ability of the organic solvent and causes the solute precipitation when the saturation concentration is overset.

According to previous studies [3], a mixture of two solvents, EtOH and DCM (50:50) v/v, has been chosen to dissolve crude theophylline, since, in this case, its solubility is higher and the antisolvent effect of CO_2 is more prononced than in pure solvents. Moreover, preliminary investigations at different concentrations of theophylline have shown that concentrations around 24-25 mg/mL were a good compromise between particles size and recovery yield. Temperature and pressure are the main parameters studied in this work and their influences on the product characteristics have been analyzed in relation with the thermodynamic behaviour. Comparison of the two techniques are also discussed from the experimental results.

I- PROCEDURES AND MATERIALS

I-1. Batch process

The experimental set-up and procedure of the batch mode were described in a previous work [4]. The crystalliser consisted of a stirred vessel of 0.49 L (**Fig.1**) equipped with sapphire windows at the bottom of the vessel in order to get a visual observation of the solid formation. The CO_2, pre-cooled, was introduced into the solution through impeller of an electric motorized magnetic stirrer, whose speed was settled at 500 rpm. A flowmeter (Bronkhorst LM320) measured the CO_2 mass introduced during the experiment. The pressure was regulated by micrometering valves located, at the entrance for controlling the input flowrate, at the exit to keep constant the operating pressure. A Millipore membrane (0.22 µm pore size) placed onto a metal filter (porosity of 2 µm), was located at the bottom of the vessel to collect the particles precipitated by CO_2 addition.

The batch experiment started by the introduction of 85 mL of the solution that contained theophylline at ~ 24 mg/mL. Once the working temperature was reached, CO_2 was gradually introduced into the crystalliser. When desired pressure was obtained, the shut off valve was opened to draw down the solution. During this step, the pressure was regulated by compensation with fresh CO_2. After ~ 60 min, the precipitate was dried during 30 min by a flow of pure CO_2. Finally, the vessel was gently depressurized to atmospheric pressure and the precipitate was collected on walls and membrane.

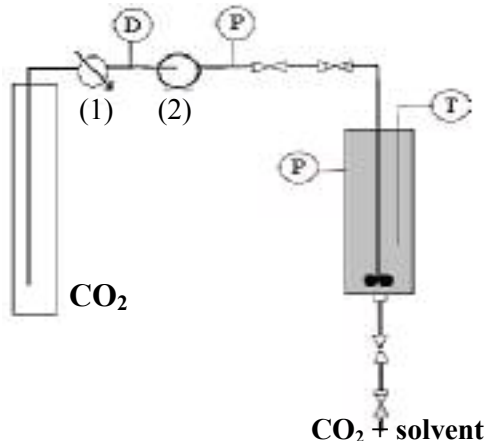

Figure 1: Batch set-up. (1) Cooling exchanger, (2) CO_2 pump.

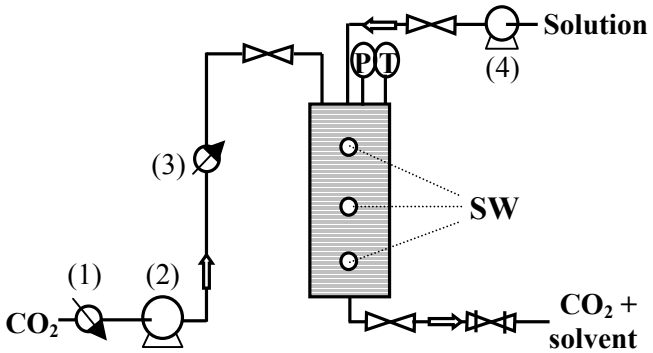

Figure 2: Semi-continuous set-up. (1) Cooling exchanger, (2) CO_2 pump, (3) heating exchanger, (4) solution pump. SW: sapphire windows.

I-2. Semi-continuous process

The apparatus is presented schematically in **Fig.2** and was described in prior studies [3,5,6]. The precipitator was a high pressure stainless steel vessel equipped with sapphire windows positioned at three different levels, to get a visual observation of the spray and of the precipitation. The solution that contained theophylline, and the CO_2 were flowing co-currently by two separated inlet ports, and were continuously discharged from the vessel bottom. The solution was sprayed into the vessel through a swirl nozzle (Lechler) with an exit diameter of 100 µm. A micrometering valve at the exit line regulated the pressure inside the vessel. Downstream the valve, an home-made cyclonic separator allowed for recovering the

liquid phase. As for the batch set-up, the precipitated particles were retained by a membrane plus a porous filter.

The semi-continuous process started by pressurization of the reactor chamber with $SCCO_2$ at the desired pressure. The system was allowed to stabilize at the working pressure, temperature and flow rate. About 40 mL of the solution was then sprayed into the precipitator through the nozzle. At the end of the spraying time, a washing step with pure CO_2 flow was carried out to eliminate the residual content of organic solvent, during ~ 30 min. The precipitate was collected onto walls and membrane after slow depressurisation of the vessel.

I-3. Materials, analysis and characterization

Carbon dioxide (purity of 99.5%) was purchased by Air Liquid. Organic solvents, absolute ethanol and methylene chloride (99.5% min), were obtained from Prolabo. Theophylline (purity of 99%) was supplied by Sigma Aldrich and used without further purification. The crude theophylline SEM pictures show particles with a rod morphology, and with sizes in the 200 μm – 1 mm range in length for 40 to 100 μm of thickness.

The characteristics of the produced particles were determined by Scanning Electron Microscopy (SEM, Leica S440) after gold-palladium coating. Their sizes were manually estimated by analysis of SEM pictures with a software (Scion Image). The particles crystallinity was determined by X-ray diffraction (XRD, Inel XRG 3000). The production yield was calculated as the ratio between the collected amount of precipitate over the processed amount, i.e. the solution concentration x its volume.

II- RECRYSTALLIZATION OF THEOPHYLLINE

II-1. By batch process

The operating temperature was varied between 298.15 and 315.15 K, at a final pressure (P_f) of 6.5 or 10.0 MPa. The different conditions and results obtained are summarized in **Table 1.** In a batch mode, the solution undergoes continuously an evolution of its composition as CO_2 is introduced, and can go trough a phase transition if the pressure/composition path crosses the L-V coexistence curve. During the experiments, the precipitation pressure (P_p) was determined by visual observation through the sapphire windows. Although qualitative, we observed a significant decrease of about 1.5 MPa in P_p, as decreased from 315.15 K to 298.15 K. The precipitation was thus obtained at an higher pressure when temperature increased, but the corresponding composition was almost insensitive (67-71% CO_2 mole fraction) to the temperature variation. This result was quite surprising because an increase of temperature generally enhanced the solubility of a compound and so, an higher quantity of antisolvent might be necessary to generate the sursaturation of the system (so the precipitation). The one-to-two phases border curves were previously determined at 309.15 and 333.15 K for the EtOH-DCM-CO_2 system [3]. At 10 MPa and whatever the temperature, the final pressure and the CO_2 mole fraction drove the system in a monophasic region. At 309.15 K (the unique temperature that fits the one-to-two phase data), it is interesting to note that precipitation occurred as the system was still in a two-phase domain, i.e. the precipitation took place into the liquid phase. Due to the closeness of the one-to-two phase curves at 309.15 and 333.15 K, it is anticipated that precipitation also occurred in the liquid phase at 303.15 and 298.15 K, also this has to be confirmed by phase equilibria investigations. It has to be also noted that temperature had not a significant influence on the final amount of CO_2 introduced, except at 315.15 K and 6.5 MPa where the CO_2 mole fraction (74.4%) was lower

than at 303.15 and 298.15 K (81.9 and 80.8%, respectively). For yields, no trends as function of pressure or temperature were observed, and values stood in the range of 60-75%, excepted at 315.15 K and 10.0 MPa where a lower value of 46% was obtained.

	Conditions			*CO_2 mole fraction*		*Product*		
Run	T (K)	Pf (MPa)	Pp (Mpa)	At Pp*(%)	At Pf (%)	Yield (%)	Size: length	x width (µm)
Batch 1	315,15	10,0	6,6	71	84,4	46	40 to 105	15 to 30
Batch 2	309,15	10,0	5,7	69	84,2	76	20 to 100	5 to 20
Batch 3		6,5	5,8	70	74,4	55	30 to 130	10 to 35
Batch 4	303,15	10,0	5,5	67	85,3	62	25 to 95	8 to 15
Batch 5		6,5	5,2	70	81,9	67	30 to 75	7 to 20
Batch 6	298,15	10,0	5,2	70	85,3	68	40 to 130	10 to 60
Batch 7		6,5	5,0	62	80,8	58	25 to 70	10 to 20

Table 1: Results obtained with the batch process (CO_2 introduction speed at about 0.5 MPa/min)

A SEM picture of particles collected during batch 7, is given in **Fig.3**. The particles morphology was similar whatever the conditions and consisted of hexagonal flake pile-up. An accurate determination of particles sizes was not easy because of their high degree of aggregation but, roughly speaking, the particles size was in the range of 5x20 to 60x130 µm. Temperature and pressure did not seem to affect notably the morphology or size, but acted more on the agglomeration of theophylline particles. At all temperatures, many plate-like particles aggregated without apparent order, but at 303.15 and 298.15 K, some of them seemed to be extended outward from a common center formed structures with a more spheroid shape (as detailed in Fig.3).

II-2. By semi-continuous process

Different conditions of temperature and pressure were investigated to determine their influence on particles morphology, size and yield : at 333.15 K from 16.0 to 8.5 MPa, and at 309.15 K from 16.0 to 6.5 MPa (**Table 2**). Most of results has been already discussed [3] so we briefly summarize the observed behaviour. A corresponding SEM picture is given in **Fig. 3**. At 333.15 K, the yield was quite insensitive to pressure variation and values were in the 80-90% range, but it was less at 309.15 K with values included between 65 and 85%. Concerning the particles size at 333.15 K, an increase of pressure had not a considerable effect except at 10.5 MPa where a minimum was observed. At 309.15 K, the particles size decreased from 6.5 to 7.5 MPa and then increased notably up to 16.0 MPa.

The behaviour was enlightened via observation of the mixing and phase regime as function of the operating conditions. When pressure/composition drove the system within the L-V coexistence domain, the solution flow broke up into droplets, whereas a simpler mixing occurred when the system was driven in a monophasic domain. In our case, the pressure corresponding to the transition between droplets and mixing regime, was observed between 9.5 and 10.5 MPa at 333.15 K, and between 7.5 and 8.5 MPa at 309.15 K. When droplets were formed, the crystallization took place in the liquid phase, and the nucleation and growth were spatially confined into them. As a results, particles were smaller at conditions in the vicinity of L-V coexistence curve. Temperature was found to influence notably the particles size, and also emphasized the trend as function of pressure.

	Conditions		*Regime*	*CO_2 mole fraction*	*Product*		
Run	T (K)	Pf (MPa)		At Pf (%)	Yield (%)	Size: length x	width (µm)
SAS 1	333,15	16,0	Mixing	96,8	87	20 to 50	10 to 40
SAS 2		10,5	Mixing	96,9	85	5 to 30	2 to 10
SAS 3		9,5	Droplets	96,8	91	10 to 50	5 to 25
SAS 4		8,5	Droplets	96,7	90	10 to 70	10 to 30
SAS 5	309,15	16,0	Mixing	97,2	65	100 to 300	30 to 100
SAS 6		10,0	Mixing	96,4	77	40 to 95	10 to 40
SAS 7		7,5	Droplets	96,6	82	4 to 15	1 to 5
SAS 8		6,5	Droplets	96,6	69	10 to 60	5 to 30

Table 2: Results obtained with the semi-continuous process (CO_2 and solution flow rates: 67 & 3 mL/min)

The particles obtained at 10 and 16 MPa were smaller at 333.15 K than at 309.15 K. In both case, the system is in the mixing regime, but at 333.15 K and 10 MPa it was closer to the phase transition zone.

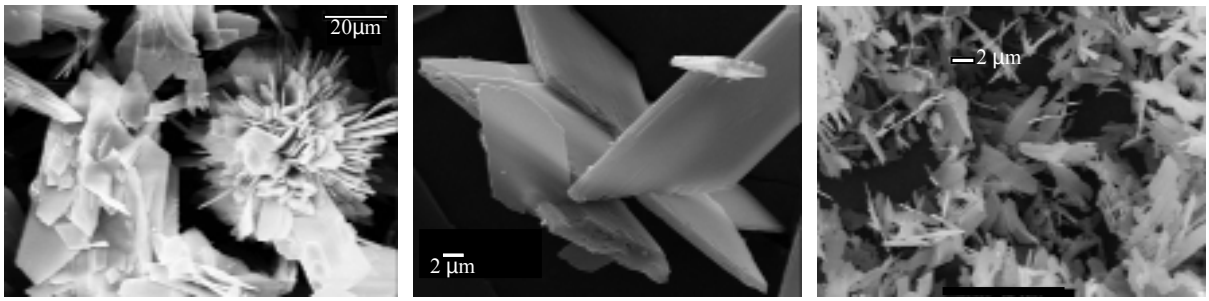

Figure 3 : SEM images of some theophylline powders : Batch 7, SAS 1 and SAS 7, respectively.

III- COMPARISON OF THE TWO TECHNIQUES AND CONCLUSION

In both supercritical techniques, the micronization of theophylline by recrystallization was successfull. The particles showed the same morphology whatever the process, but the size ranges were generally higher in the batch process, excluded to the SAS 5 (309.15 K and 16.0 MPa) where the obtained particles were relatively longer. Theophylline was appeared to be quite insensitive to the experimental conditions when precipitation was performed in the batch mode. The powder consisted in agglomerated particles, dispersed in size within the range 5 x 20 to 60 x 130 µm. When precipitation was performed in the semi-continuous mode, the particles sizes could be manipulated with the operating conditions, specially in regards of the phase behaviour. The smaller particles of theophylline were thus obtained at 7.5 MPa for 309.15 K, corresponding to conditions close to the phase transition zone. In terms of yield, the semi-continuous mode provided the recrystallization of almost 82% of the initial amount (in conditions of smallest size, i.e. SAS 7), whereas quite similar yield were obtained with the batch process but of a powder of larger particles.

Concerning the crystallinity, three X-ray diffraction patterns are given in **Fig.4**. The produced powders exhibited different patterns than those of crude theophylline because new peaks were detected after the CO_2 treatment. The new peaks might sign a new crystalline phase, although extra-investigations are required to accredit this assumption. X-ray analysis are currently on going to get a clearer picture over all the sample produced.

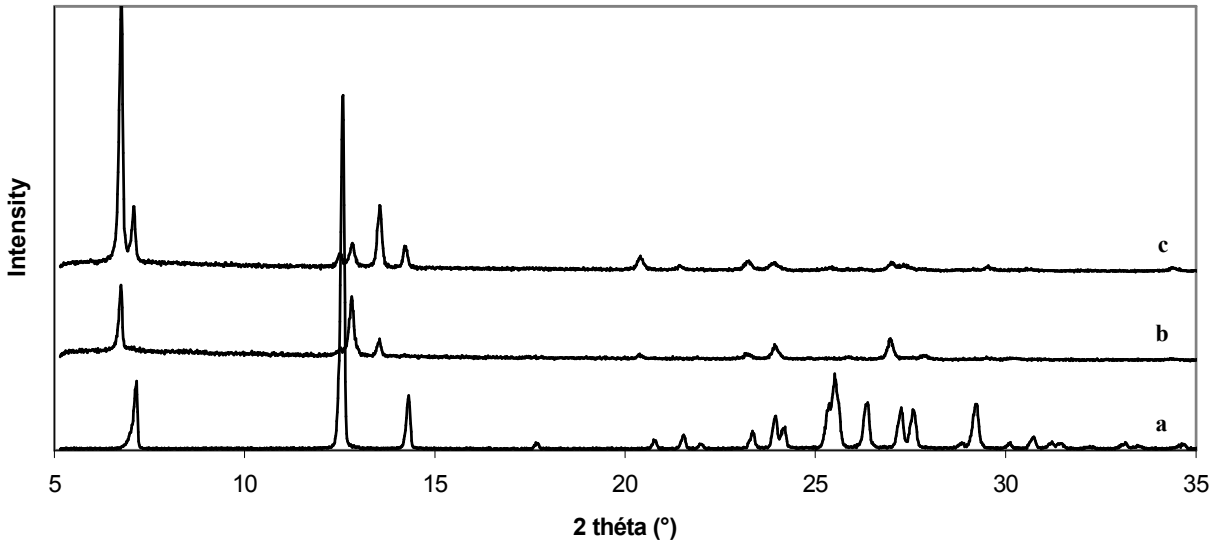

Figure 4: X-ray diffraction patterns. a) Crude theophylline, b) Batch 2, c) SAS 6.

To conclude, the semi-continuous process seems to be the best process to combine small theophylline particles, low degree of aggregation and satisfactory yield, providing the experimental conditions were settled near the critical region of the solvent-CO_2 system. X-ray patterns seemed also to differ according to wether the CO_2 treatment was done by batch or semi-continuous.

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