CRYSTALLISATION IN CO_2 MEDIUM : FROM PHASE EQUILIBRIA TO PRODUCTS

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Despite a solubilization in the CO_2 + solvent mixture, a CO_2-soluble compound was precipitated by a semi-continuous antisolvent process. Methylene chloride was selected as solvent because concentrated solutions provided the necessary input threshold to counterbalance the solubilization. When experiments are carried out in the one-phase region of the CO_2-DCM system, the powder shows a cotton-like aspect, and is made of elongated particles. Concentrated solutions of 40 and 100mg/ml and flow rates of 67ml/min and 3ml/min for carbon dioxide and solution, respectively, combine yields above 64% and medium size in the 80-100µm. When experiments are carried out in the two-phases region, size decreases down to 30µm and the powder is free-flowing.

INTRODUCTION

There is a growing interest in crystallisation assisted by supercritical CO_2, either due to concern for green technologies, either because of the intrinsic process performances. There are five equipments permanently settled in the laboratory, including a phase-equilibria set-up. Several compounds have been studied, and when possible, phase equilibria were investigated in order to select the process (RESS, SAS, impregnation) and/or select suitable conditions for a successful precipitation. Indeed, solubility and phase behaviour are critical parameters that contribute to yield and product quality. Solubility of the solute species in the chosen SCF is the first parameter for the process selection. A substance soluble in the SCF is micronized by the RESS process. Dealing with carbon dioxide, many drugs have been thus processed [1]. Compounds micronized by RESS have a solubility in CO_2 (at 40-60°C and 25MPa) in the $0.3 - 7\ 10^{-4}$ mole fraction range. The Supercritical Anti-Solvent (SAS, used hereby as a generic term) process is preferred for non soluble compounds. Literature provides numerous examples of precipitation of proteins, polymers, some lower molecular weight compounds, and encapsulation of drugs or proteins [1-6]. When available, solubility of these compounds is in the 10^{-7}-10^{-5} mole fraction range.

Precipitation of the CO_2 -soluble cholesterol by an antisolvent process was first investigated by Wubbolt [7], using dimethyl- or diethyl- ether as solvent. For experiments carried above the CO_2-solvent critical pressure, experimental yields were ranging from 40 to 90%, due to the high residual solubility of cholesterol in the remaining vapor phase. Recrystallization in a batch SAS process, was recently proposed from acetone solution by Liu et al. [8]. The batch mode proceeds in the L-V coexistence region for the acetone-CO_2 system, and the crystallization of the solute is carried out in the liquid phase. Needle-like or plate-like habit for cholesterol was obtained.

In the following, interrelation between phase behaviour and results of precipitation are illustrated for the case of cholesterol. Another example is given by De Gioannis [9] which considers the precipitation of griseofulvin in a batch stirred reactor.

I. SOLUBILITY AND PHASE BEHAVIOUR

Solubility of cholesterol was measured in pure methylene chloride (DCM), acetone (ACET) and anhydrous ethanol (ETOH). Cholesterol solubility in pure CO_2 and in solvent + CO_2 mixtures (up to 22% w:w in solvent) was previously determined with a one-pass flow procedure and a gravimetric determination [10]. Experimental data from a solvent content ranging from 0 (data in pure CO_2) to 100% (data in pure solvent) were then fit according to the empirical equation proposed by Wubbolts [7]. Solubility trends as a function of the solvent content are given in Fig. 1 for DCM and ACET. In ETOH, the curve is close to the curve with acetone.

Figure 1. Solubility of cholesterol in solvent+CO_2 mixtures, at 40°C and 18MPa. Lines are correlated data according to [7], symbols are experimental data. Triangle symbols for methylene chloride as solvent, circle for acetone.

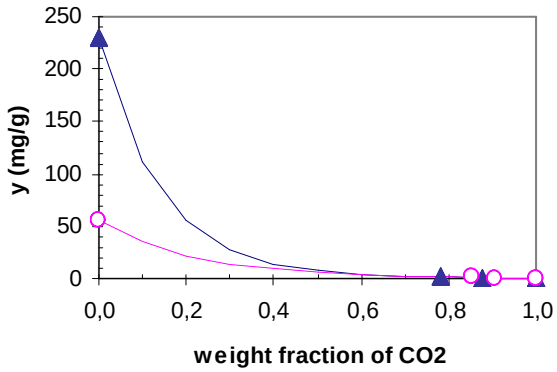
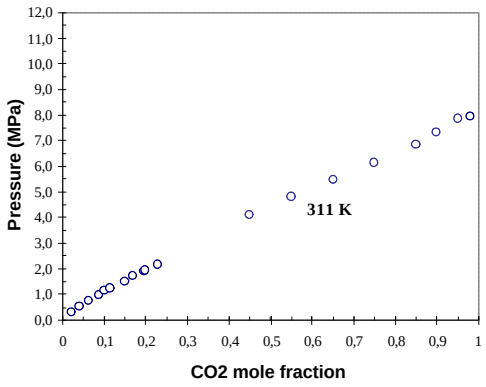


Figure 2. Liquid-vapor coexistence line for the CO_2 – DCM system.



In pure CO_2, the solubility of cholesterol at 40°C is ~ 0.2-0.5 mg/g in the 10-20MPa pressure range, that can be considered as quite large for an organic compound. However, cholesterol solubility in the pure organic solvent is 100 to 400 times higher. When CO_2 + solvent mixtures are considered, the solubility decreases as the mixture is enriched in CO_2. When the CO_2 content in the mixture is higher than 80%, solubilities in the three solvents become in the same range. On a solubility basis, it seems advantageous to precipitate cholesterol from organic solutions (as in SAS) rather than from CO_2 solutions (as in RESS), due to the possibility of processing more concentrated solutions, and thus, of expecting higher production rates.

When solvent + CO_2 are involved in conditions of SAS, attention has to be paid to the Liquid-Vapor equilibria of the system. Figure 2 reports the *P,x* diagram of DCM-CO_2 [11]. Several authors [12-13] have already observed that morphology of the product is influenced by the existence of droplets, i.e. for conditions where CO_2 and solvent are in a vapor-liquid equilibrium.

II- PRECIPITATION OF CHOLESTEROL BY SAS PROCESS

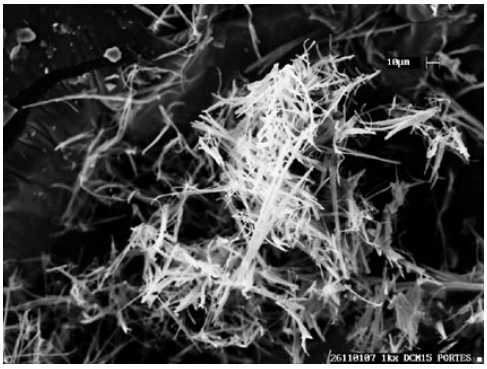
Equipment

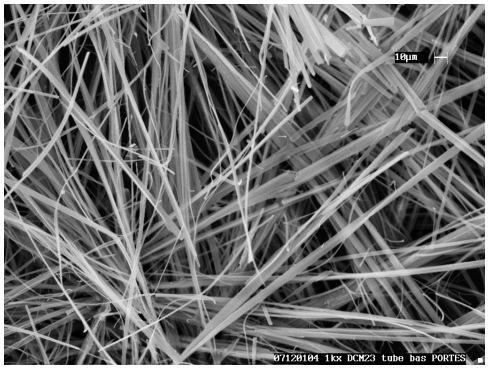
The equipment has been described elsewhere [14]. It has been updated by a vessel that is equipped with three sets of sapphire windows, allowing visual observation of the spray and of the bulk. The internal diameter and height of the vessel is 5 cm and 25cm, respectively. Nozzles were single-flow types, either disk, or swirl nozzle. We did not observe significant difference in the morphology of products with the nozzle type.
Excepted when influence of flow rates was investigated, typical flow conditions were 67 ml/min (F_{CO2}) and 3.2 ml/min (F_{sol}) for carbon dioxide and solution, respectively.

Influence of flow rates

The main difficulty in precipitation of a CO_2-soluble compounds by SAS arises from its solubilization in the CO_2+solvent mixture produced upon spraying the solution into the CO_2. Methylene chloride was chosen as solvent for dissolution. A first set of experiments were dedicated to the control of yields, with a main action on the solution and CO_2 flow rates. Temperature and pressure were 40°C and 11 MPa, respectively. The CO_2 – DCM system was thus in a one-phase region (Fig.2). The precipitation of cholesterol in these conditions gave long needle-like particles, with length varying from 60 to 300µm depending on conditions (Figure 3). Trends of size with flow rates were difficult to assess, since samples showed quite large distribution of sizes. The flow rates also impacted on thickness and width, not only on length.

Figure 3. Cholesterol produced by SAS. Solvent: CH_2Cl_2, C_{chol} : 40mg/ml. P / T : 11MPa / 40°C; F_{CO2} = 67ml/min. F_{sol}: 1.8ml/min (left) – 6.2 ml/min (right).





Experimental yields varied between 25 and 69%, when the initial concentration of cholesterol in DCM was 40 mg/ml (Figure 4). Variations of yields with flow rates (and concentration as described in next section) were analyzed by mass balance calculations based on solubility behaviour of cholesterol in CO_2+DCM system (Figure 5). The non-zero solubilization of the component in the mixture – that is specific to cholesterol compared to

other components processed by SAS- requires the use of a high input of solute to counterbalance its output, and appropriate combinations of flow rates.

Figure 4. Influence of CO_2 and solution flow rates on the yield of cholesterol

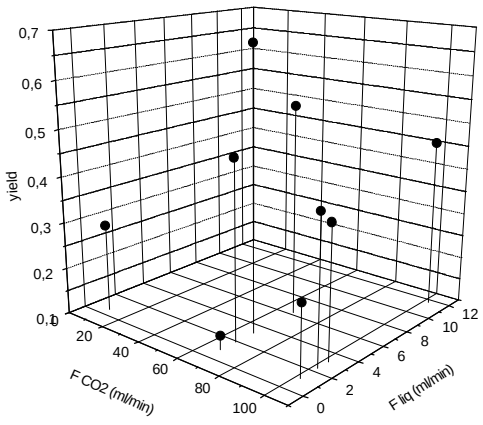
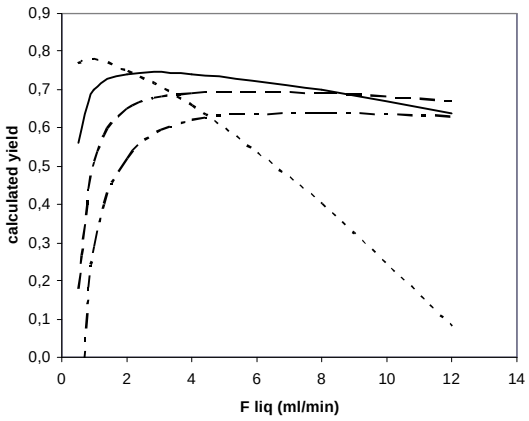


Figure 5. A mass-balance calculation for evaluating yields for various flow rates. F_{CO2} : (---) 10ml/min, (-) 33ml/min, (– – ·) 67 ml/min, (– · –) 102ml/min.



Influence of solvent

Methylene chloride was a suitable solvent, since the high solubility of cholesterol in DCM provided the necessary input threshold. Two experiments were performed using Ethanol or Acetone instead of DCM. Because of the lower solubility of cholesterol in pure solvent (18.6 – 18.8 – 105 mg/ml at 20°C in EtOH – ACET – DCM respectively) and similar range of solubility in the CO_2+solvent, the feed did not overcome the output and very low yields were obtained, 4% and 4.6% respectively.
The morphology of produced cholesterol was dependent of the solvent of dissolution. With DCM, elongated and needles-like particles were produced, for conditions in the one-phase region of the system. With ethanol, particles showed a plate-like morphology, whereas in acetone, a needle-type morphology was obtained.

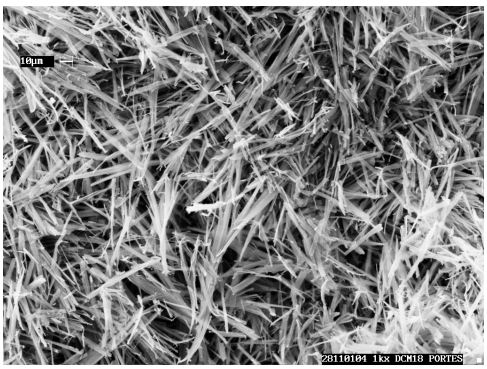
Influence of concentration of cholesterol in the methylene chloride solution

Various concentrations were investigated, because this parameter is acting on sursaturation and consequently on size and yields. With solutions of 20mg/ml – 40mg/ml- 100mg/ml, yields were 5% - 69 – 84%, with length of particles decreasing from 200µm – 100µm and 80µm. The main differences in particles came from the width, with more particles of larger width as concentration increased. The low yield obtained with solution of 20mg/ml is due to the insufficient input of cholesterol and the lower sursaturation ratio generated with diluted solutions.

Influence of pressure, i.e below or above the critical point of the CO_2-DCM mixture

Experiments were carried out at 40°C, varying the pressure from 11.2MPa to 8.5MPa and 7.1MPa. Initial concentration of cholesterol was100mg/ml, with flow rates of 67 and 3 ml/min for F_{CO2} and F_{sol}, respectively. Based on visual observation during the experiments, droplets were only visible for the 7.1MPa experiment. The pressure of 8.5MPa might be close to the critical pressure of the system (absence of phase behaviour data at 40°C), but no droplets were detected visually for these conditions. Lowering the pressure allowed the recovery of a more free-flowing powder, compared to the cotton-like powder produced in experiments at 11.2MPa. The particles showed also a reduction of size, with a typical length decreasing from 80 to 30µm (Figure 6). On reverse, the yield was decreasing, from 84% to 74% and to 57%, as the pressure decreased. For conditions below the critical pressure (as 7.1MPa), the maximum concentration of CO_2 in the droplets is determined by the equilibrium composition of the liquid phase, that is lower than composition given by the ratio of the flow rates. Conversely, the DCM content in the droplets is higher, and less cholesterol precipitates.

Figure 6. Cholesterol produced at 40°C, 11.2MPa (left) and 40°C, 7.1MPa (right) with F_{CO2} = 67ml/min, F_{sol} = 3.4 ml/min (left) and 2.6ml/min (right); C_{chol} = 100mg/ml.



CONCLUSION

A CO_2-soluble compound (usually micronized by rapid expansion of CO_2-solution) was precipitated by a semi-continuous antisolvent technique, providing the process is carefully controlled to limit losses due to solubilization in the CO_2+solvent mixture. Compared to ethanol and acetone, methylene chloride was the only solvent that provided the necessary input of cholesterol. For conditions above the critical pressure of the mixture, cholesterol precipitated as elongated particles. Conditions of 67ml/min and 3 ml/min for CO_2 and solution flows, respectively, together with concentration of 40 or 100mg/ml were suitable to combine yields above 64% and size below 100µm. Settling conditions below critical pressure, i.e. generating droplets instead of mixing flows, seems to lead to smaller particle sizes, in the 30µm range. Compared to parallel experiments in RESS, such sizes are still three or ten times larger than sizes obtained by RESS. Reduction of size by SAS is currently investigated by playing with temperature, besides encapsulation experiments.

Acknowledgements

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REFERENCES

[1] JUNG, J., PERRUT, M., J. Supercrit. Fluids, 20, **2001**, 179
[2] TSERVISTAS M., LEVY M., LO-YIM M., O'KENNEDY R., YORK P., HUMPHREY G., HOARE M., Biotechnol. Bioeng., 72, **2001**, 12
[3] ELVASSORE N., BERTUCCO A., CALICETI P., Ind. Eng. Chem. Res. 40, **2001**, 795
[4] TAKI S., BADENS E., CHARBIT G., J. Supercrit. Fluids, 21, **2001**, 61
[5] MONEGHINI M., KIKIC I., VOINOVICH D., PERISSUTTI B., FILIPOVIC-GRCIC J., Int. J. Pharm., 222, **2001**, 129
[6] REHMAN M.,SHEKUNOV B.,YORK P.,COLTHORPE P.,J.Pharm.Sci. 90 **2001** 1570
[7] WUBBOLTS, F., Supercritical crystallization: volatile components as (Anti-)solvents, Ph'D thesis, Technische Universiteit Delft, The Nethelands, **2000**
[8] LIU, Z., WANG, J., SONG, L., YANG, G., HAN, B., J. Supercrit. Fluids, 24, **2002**,1
[9] DE GIOANNIS B., JESTIN P., SUBRA P., 6th Int. Symposium on SCF, Versailles **2003**
[10] DOMINGO, C., VEGA, A., FANOVICH, A., ELVIRA, C., SUBRA, P., submitted
[11] VEGA GONZALEZ, A., TUFEU, R., SUBRA, P., J. Chem Eng. Data, 47, **2002**, 492
[12] WUBBOLTS F., BRUINSMA O., van ROSMALEN G., J. Crystal Growth 198/199, **1999**, 767
[13] REVERCHON E., DELLA PORTA G., PALLADO P.,Powder Technol.114, **2001**,17
[14] SUBRA, P., JESTIN, P., Ind. Eng. Chem. Res., 39, **2000**, 4178