MICRONIZATION OF PHARMACEUTICALS AND FOOD INGREDIENTS USING SUPERCRITICAL FLUID TECHNIQUES

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ABSTRACT

Micronization techniques based on supercritical fluids (SCFs) are promising for the production of particles with controlled size and distribution. The interest of the pharmaceutical field in the development of SCF techniques is increasing due to the need for clean processes, reduced consumption of energy, and to their several possible applications. The food field is still far from the application of SCF micronization techniques, but there is increasing interest mainly for the processing of products with high added value.

The aim of this study is to use SCF micronization techniques for the production of particles of pharmaceuticals and food ingredients with controlled particle size and morphology, and to look at their production on semi-industrial scale. The results obtained are also used to understand the processes from the perspective of broader application within the pharmaceutical and food industries. Certain pharmaceuticals, a biopolymer and a food ingredient have been tested using supercritical antisolvent micronization (SAS) or supercritical assisted atomization (SAA) techniques.

The reproducibility of the SAS technique has been studied using physically different apparatuses and on both laboratory and semi-industrial scale. Moreover, a comparison between semi-continuous and batch mode has been performed. The behaviour of the system during the SAS process has been observed using a windowed precipitation vessel. The micronized powders have been characterized by particle size and distribution, morphology and crystallinity. Several analyses have been performed to verify if the SCF process modified the structure of the compound or caused degradation or contamination of the product. The different powder morphologies obtained have been linked to the position of the process operating point with respect to the vapour-liquid equilibrium (VLE) of the systems studied, that is, mainly to the position of the mixture critical point (MCP) of the mixture.

Spherical micro, submicro- and nanoparticles, expanded microparticles (balloons) and crystals were obtained by SAS. The obtained particles were amorphous or with different degrees of crystallinity and, in some cases, had different pseudo-polymorphic or polymorphic forms. A compound that could not be processed using SAS was micronized by SAA, and amorphous particles were obtained, stable in vials at room temperature.

The SCF micronization techniques studied proved to be effective and versatile for the production of particles for several uses. Furthermore, the findings of this study and the acquired knowledge of the proposed processes can allow a more conscious application of SCF techniques to obtain products with the desired characteristics and enable the use of their principles for broader applications.

List of abbreviations

Abbreviations and acronyms used in the text

ASES	Aerosol Solvent Extraction System
CAN-BD	Carbon dioxide Assisted Nebulization-Bubble Drying
DCM	dichloromethane
DELOS	Depressurization of an Expanded Liquid Organic Solution
DMSO	dimethyl sulfoxide
DSC	differential scanning calorimetry
EtAc	ethyl acetate
EtOH	ethanol
FT-IR	Fourier transform infrared
GAS	Gas AntiSolvent
HPLC	high performance liquid chromatography
HS-GC	headspace - gas chromatography
LS	laser scattering
m.p.	melting point
MCP	mixture critical point
MeOH	methanol
mw	molecular weight
NMP	N-methyl pyrrolidone
PCA	Precipitation by Compressed Antisolvent
PF-RESS	Pre-Filtering Rapid Expansion of Supercritical Solutions
PGSS	Particles from Gas Saturated Solution
PVA	polyvinyl alcohol
R	feed ratio solution/antisolvent
RESOLV	Rapid Expansion of a Supercritical solution into a Liquid SOLVent
RESS	Rapid Expansion of Supercritical Solutions
RESS-SC	Rapid Expansion of Supercritical Solutions-Solid Cosolvent
SAA	Supercritical Assisted Atomization
SAE	Supercritical Antisolvent Extraction
SAS	Supercritical AntiSolvent
SAS-EM	Supercritical AntiSolvent with Enhanced Mass transfer
SC-CO_2	supercritical carbon dioxide
SCF	supercritical fluid
ScMM	Supercritical Melting Micronization
SEDS	Solution Enhanced Dispersion by Supercritical fluids
SEM	Scanning Electron Microscope
SFE	Supercritical Fluid Extraction
SFED	Supercritical Fluid Expansion Depressurization
SFEE	Supercritical Fluid Extraction from Emulsions
SFF	Supercritical Fluid Fractionation
TGA	thermogravimetric analysis
VLE	vapour-liquid equilibrium
XRPD	X-ray powder diffraction

List of original publications

I R. ADAMI, E. REVERCHON, E. JÄRVENPÄÄ, R. HUOPALAHTI, *Supercritical Antisolvent micronization of nalmefene HCl on laboratory and pilot scale,* **Powder Technol, 179 (2007)**, p. 163-170. In press.

II R. ADAMI, L. SESTI OSSÉO, R. HUOPALAHTI, E. REVERCHON, *Supercritical AntiSolvent micronization of PVA by semi-continuous and batch processing,* **J Supercrit Fluids, 42 (2007)**, p. 288-298.

III E. REVERCHON, R. ADAMI, G. CAPUTO, *Production of cromolyn sodium microparticles for aerosol delivery by Supercritical Assisted Atomization*, **AAPS PharmSciTech**, accepted 18-07-2007.

IV R. ADAMI, E. JÄRVENPÄÄ, L. SESTI OSSÉO, R. HUOPALAHTI, *Influence of Supercritical AntiSolvent micronization parameters on nalmefene HCl powder characteristics*, **Adv Powder Technol**, accepted 15-12-2007.

V G. CAPUTO, R. ADAMI, E. REVERCHON, *Supercritical fluid crystallization of adipic acid using urea as habit modifier*, **Cryst Growth Design,** accepted with minor revisions 28-11-2007.

1 INTRODUCTION

The production of particles with controlled size and morphology has conventionally been a fundamental target for pharmaceutical industries. Microparticles with particle size in the range 1-5 μm to be used e.g. for inhalation delivery or aerosol devices and nanoparticles with size <0.5 μm to be used in liquid formulation are produced systematically [1]. More attention is now given also to the chemical and physical solid-state properties of the obtained particles, mainly their crystal structure, habit and the degree of crystallinity. These characteristics can have a relevant influence on the formulation, manufacturing of solid dosage form, storage stability, and properties of the final product [2,3].

The interest of food industries in nano and micro technology has not yet reached the same level, because of the limited applications of small particles as food products. However, with the continuous development of the functional foods and nutraceuticals market, more attention is currently given to products that can also deliver nutrients and bioactive compounds and, as a consequence, to the size and morphology of the particles [4,5]. Moreover, in some applications the use of particles or crystals of controlled particle size and morphology can facilitate the process of obtaining the final product. One example is the use of additives in foods or beverages to give a smooth structure to the matrix.

The use of SCF-based micronization techniques becomes of more interest due to the general tendency in every field to use clean processes to preserve the future of the planet. SCF techniques are a good solution to the environmental problems because of the limited use of, and sometimes the complete absence of, organic solvents in the process, the low energy required for carrying out the process and the absence of post-processing steps.

The interest in using SCFs in the pharmaceutical field is related to the difficulty of controlling physico-chemical properties of the particles produced using conventional manufacturing processes and to the importance of obtaining a product with the characteristics required for the final formulation into aerosols, tablets, injectable suspensions or capsules. Moreover, the international regulations for the use of solvents in pharmaceutical industries [6] require controlled use of organic solvents and the radical reduction of conventional solvents used in pharmaceutical processes.

The regulations on hygiene and good manufacturing practices [7] are also strict in the production of food-related compounds, because the human health is involved in the widest possible sense. Moreover, the processing of some compounds with a SCF may be particularly advantageous, like in the case of compounds that can degradate in presence of oxygen or during long processing time [8].

Carbon dioxide (CO_2) still remains the most used SCF, either in the micronization or extraction processes, mainly because it is non-toxic, non-flammable and cheap. It can also be re-circulated during the process or reused many times. It behaves as a lipophilic solvent and produces a non-oxidizing process environment.

The most studied SCF micronization technique is the supercritical antisolvent (SAS) process, in which supercritical-carbon dioxide (SC-CO_2) has the same role as the antisolvent in classical liquid antisolvent crystallization. Despite the many examples of applications that can be found in the literature [9-14], when a compound to be micronized is selected, it is difficult to find the relations between the operating parameters and the results. Although the process is highly complex, several published papers attempted to explain separately the role of atomization [15], mass transfer [16-18], nucleation and growth [19,20] and a significant progress has been made in attempting to isolate the effects of each of these factors using carefully designed experiments.

The choice of a satisfactory scale-up procedure also requires a deep understanding of the controlling steps of the process. Some authors proposed the scale-up of the SAS process to pilot scale. However, up to now only few examples of successful application of SAS at pilot scale to the production of micrometric particles can be found in the literature [21,22].

One of the most recent SCF micronization processes uses SC-CO_2 as co-solute, Supercritical Assisted Atomization (SAA), which interacts in the system as a pneumatic agent and improves the micronization induced by the spray in a way similar to a pneumatic gas in a spray drying process. The SAA technique [23] can be considered as the complement of the antisolvent technique, and may be used in all the cases where SAS is unsuccessful. The technique is still relatively new; therefore, not enough results are available to build up a model and the micronization mechanism has to be studied further.

The interest in the application of SCF-based micronization techniques to pharmaceutical and food-related compounds and the perception of the mechanisms involved in these processes are of interest to chemists, pharmacists and chemical engineers. The chemical engineer is fascinated by the powerful potentiality of a process as versatile and complex as micronization involving high-pressure equilibria systems. The pharmaceutical researcher can make use of the possibility of controlling the physico-chemical properties of the final compound to develop the desired formulation. The chemist finds challenges in the study of the structural modifications that may occur in an environment far from the usual micronization or reaction media. Finally, the food chemist can be surprised in discovering the value added by SCF micronization processes even to common ingredients.

2 REVIEW OF LITERATURE

Supercritical fluids (SCFs) are gases at temperature and pressure conditions above their critical point. The possibility of modulating viscosity and density and, consequently, the solvent power allows to consider them as liquid-like or gas-like, depending on the conditions and the applications. It has been known for more than a century that SCFs can dissolve non-volatile compounds [24,25]; indeed, they have been used for three decades in chromatography analysis [26]. The first industrial applications of SCFs have taken advantage of these properties as solvent for the extraction (**SFE**: Supercritical Fluid Extraction) of compounds of special value from solid and liquid matrices and for fractionating them [27-31].

In the last 20 years, a most challenging application has been to use SCFs to produce micronized powders of controlled particle size and distribution [10,12,14]. The different SCF-based micronization techniques were developed for several categories of compounds: pharmaceuticals [25,32-34], polymers and biopolymers [13,35], proteins [36], inorganic materials (explosives, colouring matters, catalysts, superconductors) [10] and nanocomposites [37]. The application to pharmaceutical compounds has been developed far, mainly with the view of exploring processes that could give new characteristics to the compound itself either in the formulation or the delivery systems. Up to now, very few applications to food ingredients can be found, and those that can generally concern food products with a high added value.

In this literature review, the results of the most diffused SCF-based micronization techniques are presented. Among the information that can be found in literature, the criterion applied has been to consider mainly peer reviewed publications and not to refer to materials coming from conference proceedings, because sometimes they report incomplete studies or just the starting points of ongoing research. SCF micronization techniques are briefly described, and a rich collection of results, selected for their accuracy and thoroughness of information, is presented.

2.1 SCF micronization techniques

SCF-based micronization techniques can be classified according to the role played by the SCF in the process. Indeed, SCFs have been proposed as solvents, solutes, anti-solvents and reaction media (Table 1).

Table 1. Classification of SCF-based micronization techniques according to the role of SCF in the process

CO_2 role	application	process	acronym	characteristics
solvent	compounds soluble in SC-CO_2	Rapid Expansion of Supercritical Solutions	RESS	supercritical solution sprayed into atmospheric pressure
		Rapid Expansion of a Supercritical solution into a Liquid SOLVent	RESOLV	supercritical solution sprayed into a liquid
		Rapid Expansion of a Supercritical Solution-Solid Cosolvent	RESS-SC	solid co-solvent added to SC-CO_2
antisolvent	compounds with almost zero-solubility in SC-CO_2, soluble in a solvent that has a good affinity with SC-CO_2	Gas AntiSolvent	GAS	batch operation
		Supercritical AntiSolvent	SAS	semi-continuous operation
		Supercritical AntiSolvent with Enhanced Mass transfer	SAS-EM	semi-continuous operation, vibration added to precipitation vessel
		Solution Enhanced Dispersion by Supercritical fluids	SEDS	semi-continuous operation, SC-CO_2 and solvent mixed in a tube-in-tube injector
		Aerosol Solvent Extraction System	ASES	semi-continuous operation
		Precipitation by Compressed Antisolvent	PCA	semi-continuous operation, CO_2 at subcritical conditions
		Supercritical Fluid-drying	SCF-drying	solvent used water and CO_2 modified with polar solvent
solute	compounds in which SC-CO_2 is soluble	Particles from Gas Saturated Solution	PGSS	semi-continuous operation
		Supercritical Melting Micronization	ScMM	semi-continuous operation
co-solute	compounds with almost zero-solubility in SC-CO_2, soluble in a solvent in which SC-CO_2 is soluble	Carbon dioxide Assisted Nebulization-Bubble Drying	CAN-BD	low volume mixer for $SCCO_2$-solution
		Depressurization of an Expanded Liquid Organic Solution	DELOS	compressed CO_2 used to homogenous cooling of the solution with solid particle precipitation
		Supercritical Assisted Atomization	SAA	enhanced solubilization mixing $SCCO_2$-solution

2.1.1 SCF as solvent

The Rapid Expansion of Supercritical Solutions (**RESS**) consists of the saturation of the supercritical fluid with a solid substrate. The depressurization of the resulting solution through a heated nozzle into a low-pressure vessel produces a rapid nucleation of the substrate in the form of very small particles that are collected from the gaseous stream. The morphology of the resulting solid material, crystalline or amorphous, depends on the chemical structure of the material and on the RESS parameters (temperature, pressure drop, impact distance of the jet against a surface, nozzle geometry, etc.) [10,38]. The very fast release of the solute in the gaseous medium should assure the production of very small particles. This process is particularly attractive due to the absence of organic solvents.

The authors who first proposed RESS [39,40] patented the process with respect to the possibility of producing nanoparticles, microparticles and films [41-43]. However, papers on this topic in the scientific literature confirmed this possibility only several years later [10,44]. The most important condition to be respected in the RESS technique is that the compound to be micronized has to be soluble in the SCF. This is also the most restrictive parameter for the application of this technique. Unfortunately, many pharmaceutical compounds of interest have high molecular weights and polar bonds and show a very low or negligible solubility in SC-CO_2, which is the most widely used SCF.

An interesting variation of the RESS process is the **RESOLV** (Rapid Expansion of a Supercritical solution into a Liquid SOLVent), which consists of spraying the supercritical solution into a liquid, which often contains a stabilizer [45]. When operating in this manner, it should be possible to quench particle growth in the precipitator, thus improving the RESS process performance. Moreover, by interaction among the nucleating solid particles and the compounds contained in the liquid phase, a chemical reaction step can also be added. Kropf et al. [46] patented a RESS-like process in which the supercritical solution is expanded into a gas or liquid and nanoparticles that can range between 10 and 300 nm are produced. Subsequently, Foerster et al. [47] and Kropf et al. [48] patented the generation of nanometric particles of chitosan and sterols using this process.

The potential features of RESS are very interesting from the theoretical point of view, but the results have not been particularly good in several cases. The main problem is in controlling the particle size of the precipitates. During the expansion, the particles coalesce in the supersonic free jet generated in the precipitation vessel and, therefore, needle-like particles have been obtained in many cases (Table 2). Sometimes the formation of oriented needles can be explained by the presence of electrostatic charges on the surface of the particles induced by the fast relative motion between the particles and the gas contained in the expansion vessel [49]. RESOLV configuration has been demonstrated to be more effective in producing nanoparticles, since the liquid that receives the expanding jet can suppress the particle growth. The addition of a stabilizing agent in the liquid also protects particles from agglomeration. RESOLV has the additional problem of recovering the particles from the liquid solution used to improve the process performance: in this configuration, the process is no more solventless. When the solvent used is water, the process is useful for the production of suspensions for pharmaceutical formulations [50].

In Table 2 several examples of pharmaceutical compounds micronized using RESS and SC-CO_2 as solvent are reported. It is worth noting that all the compounds have hydrophobic characteristics.

Thakur and Gupta [51-53] proposed a variation of the RESS process, which is named **RESS-SC** (Rapid Expansion of a Supercritical Solution-Solid Cosolvent), to overcome this limitation. They added to the SC-CO_2 a solid co-solvent, which can enhance the solubility of many compounds in SCFs, provides a barrier for coagulation in the expansion vessel and is easily removed by sublimation. The compound that respects all these characteristics is menthol (m.p. 32-34°C). The compound to be micronized is solubilized in the $SCCO_2$-menthol mixture and the process is then carried out as a traditional RESS. Using this process, the authors micronized griseofulvin, a highly crystalline drug with low solubility in SC-CO_2, and obtained separated particles [51] as opposed to the coalescing irregular particles obtained by RESS [54].

Chiou et al. [55] have proposed another modification of the RESS process. It consists of flowing the SC-CO_2 with the dissolved solute (meloxicam) in a filter positioned before the expansion vessel. The process is called **PF-RESS** (Pre-Filtering Rapid Expansion of Supercritical Solutions), but there is no relevant difference from the classical RESS.

The RESS process finds many applications to food ingredients because of the affinity of many natural compounds with SC-CO_2 (Table 2), and both spherical particles [54] and crystal shape particles [56,57] have been produced. Low-molecular-weight polymers such as paraffin wax materials have reasonable high solubility in SC-CO_2, while many polymers and bio-polymers have very limited or nearly zero solubility in SC-CO_2; therefore, they can not be easily processed by this technique.

Since the early publications on the RESS technique, a modelling approach to particles formation has been widely investigated [58-60] and several models for particle formation have been developed [60-62]. Recently, Weber and Thies [63] presented a simplified and generalized model accounting for the micronization and growth of particles in the RESS process, which could confirm theoretical findings from early works and reach satisfying agreement between modelling and experiments.

Table 2. Pharmaceutical compounds, bio-polymers and food ingredients micronized by SCF-based techniques. SCF used as solvent.

material	process/notes	results/observations	particle size	ref
pharmaceuticals				
2-aminobenzoic acid	RESS-SC	irregular particles, menthol used as solid co-solvent	mean 80 nm	[52]
acetylsalicilic acid	RESS	needle-like crystals	D_{90}=7-19 µm	[64]
artemisinin	RESS	crystalline particles	mean 0.5-10.6 µm	[65]
aspirin	RESS	isomeric particles with long needle-like crystals	2-5 µm	[66]
barbital	RESS	crystalline orthorhombic particles, Form II	mean 0.7-1.8 µm	[67]

material	process/notes	results/observations	particle size	ref
pharmaceuticals				
benzoic acid	RESS	isomeric particles long needle-like crystals	2-8 μm 0.2-0.3 μm	[66]
benzoic acid	RESS		0.8-1.2 μm	[59]
benzoic acid	RESS	aggregate particles	0.2-1.4 μm	[60]
carbamazepine	RESS	needle-like, prismatic, elongated prismatic crystals, Form II, Form III	0.5-2.5 μm, mean <3 μm	[68]
cholesterol	RESS		<35 μm	[59]
cholesterol	RESS	aggregate particles	100-400 μm	[60]
cholesterol	RESS	needle-like crystals rod-like crystals	1-6 μm, 1-13 μm; mean 13 μm	[69]
cholesterol	RESS		0.3-0.5 μm	[70, 71]
cyclosporine A	RESS	amorphous spherical particles	mean 4.5 μm	[72]
fluorinated tetraphenylporphyrin	RESS	coalescing spherical particles	40-80 nm	[73, 74]
fluorinated tetraphenylporphyrin	RESOLV	well separated spherical particles	23-33 nm	[74]
griseofulvin	RESS	aggregate spherical particles	mean 200±50 nm	[54]
griseofulvin	RESS-SC	irregular particles, menthol as solid co-solvent	50-250 nm	[51]
ibuprofen	RESS	capillary nozzle	mean 2 μm	[75]
ibuprofen	RESS	irregular particles, decreased degree of crystallinity	2.85-7.48 μm	[76]
ibuprofen	RESOLV	well separated irregular spherical particles	mean 40 nm	[50]
ibuprofen	RESS	irregular agglomerated crystalline particles	primary particles 100 nm	[77]
loperamide	RESS	spherical particles aggregated or not, depending from the nozzle and the use of co-solvent	0.3-0.5 μm, 1-7 μm	[78]
medroxyprogesterone acetate	RESS	irregular particles	mean 4.5 μm	[79]
meloxicam	PF-RESS	irregular shape particles	0.5-2 μm	[55]
naphtalene	RESS		1.5-3 μm	[59]
naproxen	RESOLV	well separated irregular spherical particles	mean 64 nm	[50]
phenantrene	RESS	irregular crystals	1-5 μm	[66]
phenantrene	RESSOLV	crystals, dispersion in water	0.5-1 μm	[78]
phenylbutazone	RESS	crystals, metastable form β, no polymorphic modifications	1.5-4.3 μm	[80]
phenytoin	RESS-SC	irregular particles, menthol as solid co-solvent	average 200 nm	[53]
progesterone	RESS	irregular particles	mean 4.5 μm	[79]
salicylic acid	RESS	needle-like crystals	2-5 μm	[81]
salicylic acid	RESS	needle-like crystals	2-5 μm, 1-2 μm	[66]
salicylic acid	RESS, CO_2+EtOH, CO_2	squared crystals	L/d=15.73 μm/ 4.06 μm	[82]
taxol	RESS	elongated crystals	0.3-1.7 μm	[82]
tolbutamide	RESS	crystalline orthorhombic particles, Form I, Form II, Form IV	1-8 μm	[67]

material	process/notes	results/observations	particle size	ref
bio-polymers				
HYAFF 11	RESS	regular microspheres	<10 μm	[83]
PDLLA	RESS	irregular sized particles	10-20 μm	[84]
PGA	RESS	rectangles, oval particles needles	10-20 μm length 40 μm	[84]
PLLA	RESS, CO_2, CO_2+acetone	microparticles and microspheres	4-25 μm	[84]
PLLA	RESS, CO_2+HCFC	dendrites microparticles agglomerates microspheres	>100 μm 1-2 μm 10-20 μm 10-50 μm	[85]
PLLA	RESS, CO_2+THF	nanoparticles dispersed in micro and agglomerated particles	30-100 nm	[86]
PMMA	RESS, CO_2+EtOH	separated spherical particles	1.5-1.8 μm	[87]
PS-β-(PMMA-co-PGMA)	RESS, CO_2+EtOH, MeOH, propanol, toluene	separated spherical particles	1.5-2 μm	[87]
food ingredients				
β-sitosterol	RESS	tween80 and SDS used to avoid growth and agglomeration	mean 200±50 nm	[54]
caffeine	RESS	needle-like crystals	length 1-25 μm	[57]
cocoa butter	RESS	Form V crystals	0.3-1.5 mm	[56]
stigmasterol	RESS	amorphous spherical particles whiskers	0.05-0.2 μm d/L=0.2 μm/ 2-3 μm	[88]

d/L = diameter/length
D_{90} = equivalent volume diameter below which is 90% particle diameter
EtOH = ethanol
HCFC = chlorodifluoromethane
HYAFF = hyaluronan total benzyl ester
L/d = length/diameter
MeOH = methanol
PDLLA = poly D,L-lactide
PGA = polyglycolic acid
PGMA = poly glycidylmethacrylate
PLLA = poly L-lactide
PMMA = polymethyl methacrylate
SDS = sodium dodecyl sulphate
THF = tetrahydrofuran

2.1.2 SCF as antisolvent

Supercritical antisolvent precipitation has been proposed using various acronyms, but the process is substantially the same in all the cases (Table 1). A liquid solution contains the solute to be micronized. At the process conditions, the supercritical fluid should be completely miscible with the liquid solvent, whereas the solute should be insoluble in the SCF. As a rule, SC-CO_2 has been used. Therefore, contacting the liquid solution with the SCF induces the formation of a solution and produces supersaturation and precipitation of the solute. The formation of the liquid mixture is very fast due to the enhanced mass transfer rates that characterize the supercritical fluids and, as a result, micro- and nano-particles could be produced. This process has been used by several authors using different

process arrangements. The most significant differences are, however, related to whether the process operates in batch or in semi-continuous mode [9]. In batch operation (**GAS**: Gas AntiSolvent) the precipitation vessel is loaded with a given quantity of the liquid solution and the supercritical antisolvent is added until the final pressure is obtained. In semi-continuous operation (**SAS**: Supercritical AntiSolvent) the liquid solution and the supercritical anti-solvent are continuously delivered to the precipitation vessel in co-current or counter-current mode. An important role is also played by the liquid solution injection device [89-91]. The injector is designed to produce liquid jet break-up and the formation of small droplets to produce a large mass transfer surface between the liquid and the gaseous phase. Several injector configurations have been proposed and patented in the literature [92-97]. A new kind of injector has been recently proposed [98-100]. It consists in the tube-in-tube injector already proposed by other authors [22,94], but in this case SC-CO_2 flows in the inner tube and the liquid solution in the outer annulus, whereas in the traditional way it is the opposite. Spiral slots, inclined at a 45° angle, are added onto the annulus to improve the mixing of SC-CO_2 that is forced to contact on the thin swirl film and the solution.

A high-pressure vapour-liquid equilibrium (VLE) and mass transfer between the liquid and the SCF also play a relevant role in SAS. Particularly, VLEs of the ternary system solute-solvent- antisolvent and the position of the operating point in SAS processing with respect to these VLEs, can be decisive for the success of the process [22]. When the supercritical antisolvent micronization process is performed properly, it is possible to obtain nanoparticles (diameter <200 nm), submicro- (diameter 0.2-1 μm) and microparticles (diameter 1-5 μm), hollow micrometric particles (balloons, diameter >10 μm) and crystals by varying the operating conditions. The formation of a single supercritical phase is the key step for the successful production of nanoparticles [101]. The formation of very large crystals observed in some cases [9] may be connected to SAS precipitation from a liquid-rich phase due to a modification of the shape and the extent of the miscibility gap. When two phases are present, different morphologies that can be related to the precipitation from a liquid rich phase (crystals) and an SCF-rich phase (amorphous particles) can be observed simultaneously. The relative quantities of precipitates correlate to the partition factor of the solute between the two phases. It is interesting to note that in some cases semi-crystalline and crystalline particles can be obtained, in my opinion, because the crystallization rate of the compounds is high and the crystallization time is shorter than the characteristic time of the SAS process. Another kind of micrometric particles, called balloons, have been produced by SAS at homogenous subcritical conditions [102]. Balloons are particles with the size of 10-180 μm and with hollow or porous interiors. They can find application in the micro-encapsulation or coating processes. The washing step with pure supercritical

antisolvent at the end of the precipitation process is also fundamental to avoid the condensation of the liquid phase that otherwise rains on the precipitate modifying its characteristics.

When the injection of the liquid solution is properly performed, the limits of the SAS process are in the difficulty of predicting VLE modifications induced by the presence of solute on the binary liquid-SCF system. Very complex phase-behaviours can be produced. In the simplest case the shift of the mixture critical point (MCP) towards higher pressures can be observed. In the case of a large increase of the MCP pressure, very large pressures will be required to obtain a single-phase system and the successful production of nanoparticles [103].

The supercritical antisolvent technique has found many applications in the pharmaceutical field, mainly because of the possibility of producing a powder with controlled particle size and distribution using a non-expensive process, solvents already in use in the pharmaceutical protocols and a non-polluting antisolvent. A large selection is reported in Table 3. In addition to pharmaceuticals, proteins are also shown in Table 3 mainly because of their various applications as drugs for treatment of life-threatening and chronic diseases [36]. Different solvents that have affinity with SC-CO_2 can be used, which increases the possibility of applying the technique to several compounds with different characteristics. The chosen solvent also has an influence on the final product, and some solvents are more successful than others, mainly due to their interaction with the solid compound. Due to their optimal interaction with SC-CO_2, the most used solvents are dimethyl sulfoxide (DMSO) and N-methyl pyrrolidone (NMP). Other commonly used solvents are methanol (MeOH), acetone, toluene and ethyl acetate (EtAc) (see Table 3). Ethanol (EtOH) is less used mainly because, due to its chemical structure and the possibility to easily form hydrogen bridges, it interacts strongly with the solid compound dissolved in it and modifies the VLE solvent-antisolvent-solute in an unpredictable way. Water cannot be used as solvent because of its relatively low solubility in SC-CO_2. Consequently, it is difficult to micronize hydrophilic compounds by SAS. Moreover, adding even a small quantity of water into a solvent used in the SAS technique relevantly changes the VLE solvent-antisolvent. For example, the addition of small amounts of water into a DMSO solution produces an enlargement of the area of operation in the VLE diagram. The two-phase region is enlarged, the MCP is moved to higher pressures and, consequently, the region of gas miscibility below the MCP is enlarged [104].

To micronize also water-soluble compounds, some authors have tried to modify the SC-CO_2 characteristics by adding a polar solvent, mainly EtOH, and decreasing the hydrophobicity

of the SCF [105-107], but the results are, as a rule, mainly primary particles in the range 100-500 nm, agglomerated in large aggregates of several tenths of microns. Particles were produced only in few cases [108], and up to 19 wt% of EtOH in CO_2 was used as modifier. At such compositions the mixture solvent-antisolvent-cosolvent is not in supercritical conditions, but in the conditions of an expanded liquid [109].

Because of their limited solubility in SC-CO_2, supercritical antisolvent micronization techniques have found many applications in polymers and bio-polymers [13]. The major results obtained in the micronization of bio-polymers and bio-compatible polymers are reported in Table 3. These bio-polymers can be used in pharmaceutical applications as drug carriers, or in food processing to stabilize some labile compounds used in nutraceutical and functional foods production [110].

The limit in the application of this technique to food ingredients is that chlorinated solvents (e.g. dichloromethane) and other solvents classified as Class 3 in the Federal Register of the International Conference of Harmonization [111] (e.g. DMSO) are used to dissolve the untreated compound. They are not accepted by the European Directive concerning the use of additive and other substances in food [7,112-114] and the purity of food additives [115]. However, successful micronizations have been performed using EtOH and spherical connected amorphous particles have been obtained in the case of soy lecithin phospholipids [116,117] and orthorhombic crystals in the case of ginkgo ginkgolides [118].

Chattopadhyay et al. [119,120] proposed a batch supercritical antisolvent (GAS) micronization process enhanced with the addition of a vibrating surface in the precipitation vessel, with the aim to reduce the size of the final particles. The technique was named Supercritical AntiSolvent with Enhanced Mass transfer (**SAS-EM**). They produced griseofulvin (antifungal, antibiotic) particles as small as 130 nm and lysozyme (enzyme) particles of about 190 nm.

From the Table 3 it can be noted that the control of particle size is not an easy task. For the majority of compounds, only a specific typology of particles (e.g. nanoparticles, microparticles, crystals and balloons) was obtained for each one, showing that it is still not well known how to modulate the operating conditions to get different typologies of particles for the same compound. Although many chemical compounds with different characteristics have been processed by supercritical antisolvent techniques, indications cannot be found. The way to proceed in the case of new compounds is still to study the system of interest, following the results obtained with similar systems.

Some authors have studied SAS process mechanisms to give an interpretation of the results [15-18]. The process is very complex and not easy to be modelled, because it depends on the

interactions among thermodynamics, mass transfer, jet hydrodynamics and nucleation kinetics. Werling and Debenedetti [16,17] proposed a mathematical model based on the mass transfer between solvent and antisolvent in a single droplet of solvent considering the influence of density and diffusivity at subcritical [16] and supercritical conditions [17]. Rantakylä et al. [19] studied the influence of the initial droplet size on the particle size of the final formed solid product. Perez et al. [121-123] in the case of polymers, at conditions of complete miscibility between the organic solvent and CO_2, proposed that droplets are the result of the formation of a liquid-liquid phase split. Jarmer et al. [20] proposed a nucleation and growth rate model for PLLA (poly L-lactide) related to the characteristics of the suspension density and suggested the importance of the role of secondary nucleation.

Only few authors considered the influence of the thermodynamics and phase behaviour of the system on particle morphology and size [22,103,124,125] and, due to the difficulties in the construction of ternary diagrams, in many cases some authors considered the corresponding binary diagrams solvent-CO_2 and neglected the influence of the solute in the system [126]. However, such approximation cannot be applied when the presence of the solute largely modifies the VLEs [22,103,125].

The influence of fluid dynamics on particle morphology is also a relevant aspect in the SAS process, because the atomization produces large surfaces between the liquid and the fluid phase. Some authors have studied the fluidodynamics of solutions at the exit of the injector in the supercritical medium [15,127,128], while others have studied the characterization of the jet break-up and atomization in supercritical conditions [91,129,130].

Elvassore et al. [131] performed a theoretical investigation of mass transfer at high pressure for ternary systems and developed a mathematical model for mass transfer between a droplet of a polymeric organic solution and a compressed gas antisolvent under conditions of miscibility [18]. Martín and Cocero [132] tried to combine the description of all the physical phenomena relevant for the SAS process into a single model. Phase equilibrium, mass transfer, jet hydrodynamics and crystallization kinetics were considered. The phase equilibrium data and solid-fluid interfacial tension parameters were used for the calculations. The results of the model were compared to experimental results of ascorbic acid, but the model proved to be descriptive rather than predictive. Furthermore, the experimental system chosen for the comparison does not represent a reliable example of micronization.

Table 3. Pharmaceutical compounds, bio-polymers and food ingredients micronized by SCF-based techniques. SCF used as antisolvent.

material	solvent	process/notes	results/observations	particle size	ref
pharmaceuticals					
5-fluorouracil	EtOH/DCM	SEDS	spherical particles	<0.5 μm	[133]
α-chymotrypsin	HCl-acidified water (pH2.04)	PCA, CO_2+EtOH	nanometric irregular particles interconnected	<100 nm	[107]
amoxicillin	NMP	SAS	amorphous spherical particles	mean 0.2-12 μm	[134, 135]
amoxicillin	NMP	SAS pilot	amorphous spherical particles	mode 0.2-0.8 μm	[21]
amoxicillin	DMSO, EtOH/DMSO	SAS	amorphous spherical particles	0.2-1.6 μm	[136]
ampicillin	NMP	SAS	aggregated and separated amorphous spherical particles	mean 0.26 μm	[137]
ampicillin	EtOH	SAS	aggregated and separated amorphous spherical particles	mean 1.26 μm	[137]
antibody 4D5Fab fragment	water	SEDS, CO_2+EtOH	activity of processed antibody 45%		[105]
antibody D1.3Fv fragment	water	SEDS, CO_2+EtOH	activity of processed antibody 3%		[105]
ascorbic acid	acetone	PCA	small irregular crystals elongated crystals	1-4 μm mean 30 μm	[138]
atenolol	MeOH	ASES	spherical aggregated particles	mean 42.74±20.62 μm	[139]
atenolol	EtOH	ASES	spherical aggregated particles	mean 20.84±11.34 μm	[139]
atenolol	iso-propanol	ASES	spherical aggregated particles	mean 16.83±10.60 μm	[139]
β-lactamase	water	SEDS, CO_2+EtOH			[92]
baicalin	MeOH	SEDS-PA	rod-like particles twisted fiber-like particles fibrous net-like particles	L/w=10-30 μm /0.8-2.2 μm L/w=4-8 μm /0.2-0.6 μm	[140]
beclomethasone-17,21-dipropionate	acetone	GAS	irregular particles with a more defined crystalline structure	mean 1.8 μm, MMAD 7.9 μm	[141]
budesonide	DCM	ASES	same crystallinity of untreated	<5 μm	[142]
budesonide	DCM	PCA	spherical particles	mean 1-2 μm	[143]
budesonide	MeOH	SEDS	plate-like particles	5–30 μm	[144]
budesonide	acetone	SEDS	smooth irregular spherical particles	1–3 μm	[144]
budesonide	DCM	ASES	crystalline particles	D_{50}=6-8 μm	[145]
budesonide	acetone	SEDS	irregular crystalline particles	mean 2-3 μm	[146]
carbamazapine, indomethacin, ketoprofen, paracetamol, theophyline	EtOH-DCM, MeOH/CO_2	SEDS	microspheres	<1 μm	[147]

material	solvent	process/notes	results/observations	particle size	ref
pharmaceuticals					
carbamazepine	MeOH	SEDS	crystals, polymorphic forms α, β, γ	several tenth μm	[148]
carbamazepine	DCM	SEDS	crystals, polymorphic forms α, β, γ	several tenth μm	[148]
carbamazepine	acetone	GAS	needle-like particles	mode 31 μm	[149]
carbamazepine	acetone	GAS	needle-like particles, decrease of % of Form III, modification in Form I	L/d=161.86 μm/ 40.35 μm	[150]
carbamazepine	DCM	GAS	needle-like particles, decrease of % of Form III, modification in Form I, partial amorphisation	L/d=173.36 μm/ 38.65 μm	[150]
carbamazepine	EtAc	GAS	needle-like particles, decrease of % of Form III, modification in Form I	L/d=97.46 μm/3 7.27 μm	[150]
catalase	EtOH:water 90:10	ASES		mean 1 μm	[151]
cefonicid	DMSO	SAS	spherical submicroparticles and empty shells	from 0.2 μm to >50 μm	[103, 125]
cefoperazone	DMSO	SAS	submicrometric particles, micropetric particles, large crystals	mode 0.25-0.5 μm	[125]
cefuroxime	DMSO	SAS	submicrometric particles wrinkled microparticles balloons	0.1-0.9 μm 1-3 μm 5-20 μm	[125]
chlorpropamide	acetone	SAS	columnar habit crystals	several tenth μm	[152]
chlorpropamide	EtAc	SAS	platy crystals	several tenth μm	[152]
cholesterol	acetone	GAS	needle-like and flatted tabular crystals	>50 μm	[153]
cholesterol	DCM	SAS	plate-like crystals long crystals	1-15 μm; 10-50 μm, 10-500 μm	[69]
cilostazol	DCM, glacial acetic acid	SAS	irregular interconnected crystals	mean 0.90-4.52 μm	[154]
copper indomethacin	DMF	ASES	porous spherical particles, bipyramidal crystals, spheres made of small crystals	<5 μm or 10-50 μm	[155]
copper indomethacin	NMP	ASES	large spheres irregular particles	mean 20 μm 2-5 μm	[155]
copper indomethacin	DMSO	ASES	agglomerates of irregular platelets	around 200 μm	[155]
copper indomethacin	DMF	GAS	rhombic or bipyramidal particles depending on the expansion rate	2–100 μm	[155]
copper indomethacin	NMP	GAS	clusters of needle-like crystals	50-100 μm	[155]
copper indomethacin	DMSO	GAS	clusters of needle-like crystals	50-100 μm	[155]
copper indomethacin	DMF	GAS	slow expansion: rhombic crystals fast expansion: crystals	mean 50 μm mean 20 μm	[155]
cromolyn sodium	MeOH	ASES	amorphous particles	0.1-20 μm	[156]
dexamethasone phosphate	MeOH	SAS-EM	coalescing spherical nanoparticles	150-200 nm	[157]
ephedrine	EtOH	SEDS	long needle-like, short rod-like crystals	0.5-1.5 μm	[99]

material	solvent	process/notes	results/observations	particle size	ref
pharmaceuticals					
fluconazole	DCM	SAS	needle-like crystals, anhydrate Form I, Form II	several hundred μm	[158]
fluconazole	EtOH	SAS	needle-like crystals, anhydrate Form II	several hundred μm	[158]
fluconazole	acetone	SAS	needle-like crystals, anhydrate Form I	several hundred μm	[158]
flunisolide	DCM	ASES	different polymorphic form than untreated	<5 μm	[142]
flunisolide anhydrous	MeOH	SEDS	polymorphic particles Form IV, Form II, Form IV+ Form III	several tenth μm	[144]
flunisolide anhydrous	acetone	SEDS	polymorphic particles Form III, Form I+ Form III	several tenth μm	[144]
fluticasone-17-propionate	DCM	ASES	spherical amorphous particles, ribbons particles with different polymorphic form	<5 μm	[142]
glycine	water	SCF-drying, EtOH+CO_2 (0-19 wt%)	α-glycine, β-glycine and γ-glycine, cocoon-like, urchin-like, irregular crystals	>10 μm	[159]
griseofulvin	DCM	SAS-EM (GAS)	needle-like and nanometric particles	volume-average 310 nm	[119]
griseofulvin	THF	SAS-EM (GAS)	needle-like and nanometric particles	volume-average 310 nm	[119]
griseofulvin	acetone	GAS	tetragonal crystals long needle crystals bipyramidal crystals	L/w=12-1000 μm /3-200 μm L/w=50-200 μm /10-300 μm 40-2000 μm	[160]
hydrocortisone	acetone	SEDS	network of needles	several hundred μm	[161]
hydrocortisone	MeOH	SEDS	flake-like particles, mixture of flakes and needles	several tenth μm	[161]
hydrocortisone	MeOH	SAS-EM	needle-like particles irregular spherical particles low degree of crystallinity	<180 nm 1-13 μm	[162]
hydrocortisone acetate	DMF	ASES	crystals	mean 5 μm	[163]
insulin	DMSO, DMF	ASES/GAS	spherical aggregated particles	D_{90}<4 μm, D_{10}<1 μm	[164]
insulin	DMSO	SAS	irregular coalescing particles	1-5 μm	[165]
insulin	water	SEDS, CO_2+EtOH	spherical particles, more or less aggregated	50-500 nm	[106]
insulin	EtAc	GAS	discrete spherical particles, very narrow PSD	0.3-0.7 μm	[166]
insulin	EtOH	GAS	discrete spherical particles, very narrow PSD	0.05-0.03 μm	[166]
insulin	DMSO, MeOH	ASES	primary spherical particles aggregate	primary particles 0.2 μm	[167]
insulin	DMSO	GAS	discrete spherical particles, very narrow PSD	1.4-1.8 μm	[166, 167]

material	solvent	process/notes	results/observations	particle size	ref
pharmaceuticals					
insulin	MeOH	GAS	discrete spherical particles, very narrow PSD	0.2-0.7 μm	[166, 167]
insulin	HFP	PCA	coalescing spherical nanoparticles	mean 50 nm	[168]
insulin	EtOH-water	ASES		mean 1 μm	[151]
ipratropium bromide	DMF	ASES	regular elliptical particles	aerodynamic diameter 0.6-3 μm	[169]
lactose	water	SEDS, CO_2+MeOH	particle morphology depending on the nozzle	3-10.5 μm	[170]
lactose	water	SEDS, CO_2+MeOH/ EtOH	crystals, monohydrate changes the hydratation	mean 100 μm	[171]
lobenzarit disodium	water	ASES, CO_2+EtOH		0.2-0.6 μm	[172]
lysozyme	DMSO	SAS	irregular coalescing particles	1-5 μm	[165]
lysozyme	water	SEDS, CO_2+EtOH	spherical particles, more or less aggregated	500 nm	[106]
lysozyme	DMSO	GAS	discrete spherical particles, very narrow PSD	0.05-0.2 μm	[166, 167]
lysozyme	DMSO	GAS, CO_2+DMF (30%v/v)	discrete spherical particles, very narrow PSD	mean 0.1 μm	[166, 167]
lysozyme	DMSO	GAS, CO_2+EtOH (30%v/v)	discrete monodisperse spherical particles	0.02-0.04 μm	[166, 167]
lysozyme	DMSO	GAS, CO_2+acetic acid(8%v/v)	discrete spherical particles, very narrow PSD	0.05-0.25 μm	[166, 167]
lysozyme	DMSO	GAS	discrete monodisperse spherical particles	0.05-0.07 μm	[166, 167]
lysozyme	DMSO	GAS	discrete monodisperse spherical particles	0.01-0.05 μm	[166, 167]
lysozyme	DMSO	GAS	amorphous spherical particles, more or less agglomerated	200-300 nm, mean 180 nm	[173]
lysozyme	water	SCF-drying, EtOH+CO_2 (0-19%wt/wt)	agglomerated nanoparticles microparticles microspheres	200-300 nm 1-50 μm 1-15 μm	[108]
lysozyme	DMSO, water	SEDS	irregular agglomerated particles	primary particles 1-5 μm	[174, 175]
lysozyme	DMSO	SAS-EM (GAS)	irregular separated particles or aggregated	mean 190 nm	[120]
lysozyme	DMSO	ASES	aggregated nanospheres	<100 nm	[176]
methylprednisolone	THF	ASES, CO_2 or ethane	crystalline particles	mean 5 μm	[163]
myglobin	DMSO	ASES	spherical agglomerated particles	0.03-0.4 μm	[167]
myglobin	DMSO	GAS	spherical polydisperse particles	0.03-0.4 μm	[166, 167]
myglobin	MeOH	GAS	spherical particles broad PSD	0.05-0.3 μm	[166, 167]

material	solvent	process/notes	results/observations	particle size	ref
pharmaceuticals					
nicotinic acid	absol EtOH	SEDS		0.4-0.75 µm	[177]
nicotinic acid	MeOH	SEDS	crystalline smooth tabular particles	1-5 µm	[178]
nimesulide	CHF, DCM	SAS	needle and thin rods shaped crystals Form I		[179]
nimesulide	acetone	SAS	needle and thin rods shaped crystals, meta-stable Form II		[179]
n-trimethylchitosan	DMSO-water	PCA	coalescing particles and spherical microparticles	1-10 µm	[104]
oxeglitizar	EtOH+CHF, EtOH	SAS	needle-like crystals, polymorphic form A	>50 µm	[180]
oxeglitizar	THF, DCM	SAS	needle-like crystals, polymorphic form A, traces form B	>50 µm	[180]
oxeglitizar	EtOH/THF (50:50), EtOH	SAS	needle-like crystals, polymorphic form A and form B	>50 µm	[180]
paracetamol (acetaminophen)	EtOH	SEDS	irregular prismatic crystals	10–15 µm	[181]
paracetamol (acetaminophen)	EtOH	PCA	irregular crystals small and elongated	mean 2 µm mean 30 µm	[138]
paracetamol (acetaminophen)	EtOH	SEDS	irregular crystals	3-20 µm	[182]
paracetamol (acetaminophen)	acetone	PCA	irregular squared particles	average 2 µm	[183]
paracetamol (acetaminophen)	acetone	GAS	irregular particles	90-250 µm	[183, 184]
p-HBA	MeOH	ASES	rhomboidal platelets	length 3-10 µm	[176]
p-HBA	acetone	ASES	needle-like crystals	length>30 µm	[176]
p-HBA	EtAc	ASES	rod-like crystals	length>30 µm	[176]
phenantrene	toluene	GAS	platelet-shape particles formed by agglomerated crystals	160-540 µm	[185]
phenantrene	toluene	GAS	mono and bimodal PSD	mean 21-210 µm	[186, 187]
prednisolone acetate	acetone	ASES		mean 1 µm	[188]
prendisolone	DCM	ASES	decrease of degree of crystallinity	<5 µm	[142]
RhDNase	water	SEDS, CO_2+EtOH	spherical separated particles	50-500 nm	[106, 189]
rifampicin	DMSO	SAS	amorphous particles, coalescent nanometric spherical separated micrometric	mean 0.4–1 µm 2.5–5 µm	[190]
rIgG	water	SEDS, CO_2+EtOH	decrease of biological activity 50%		[191]
salbutamol	MeOH + acetone	SEDS		0.5 µm	[177]
salbutamol sulphate	DMSO	SAS	crystalline rod-like particles star-like particles	L/d=1–3 µm/ 0.2–0.35 µm	[192]
salbutamol sulphate	MeOH	SAS	needle-like crystals	D_{50}=9.03-35.33 µm	[193]

material	solvent	process/notes	results/observations	particle size	ref
pharmaceuticals					
salmeterol xinafoate	MeOH, acetone	SEDS, CO_2+MeOH		1-10 μm	[92]
salmeterol xinafoate	MeOH, acetone	ASES		1-10 μm	[177]
salmeterol xinafoate	MeOH	SEDS	needle-like particles		[177]
salmeterol xinafoate	acetone	SEDS	platelet-like particles		[177]
salmeterol xinafoate	EtOH	SEDS	whilst habit crystals	3-17 μm	[194]
salmeterol xinafoate	acetone	SEDS	blade-like habit crystals, Form I, Form II	3-17 μm	[194]
salmeterol xinafoate	MeOH	SEDS	platelet-like crystals	98% mean 0.5-10 μm	[195]
sulfamethizole	acetone, DMF	SAS-EM	tubular habit platy habit	140-220 μm 29-56 μm	[196]
sulfathiazole	acetone	SEDS	Form I crystals amorphous spherical particles	>10 μm <2 μm	[197]
sulfathiazole	MeOH	SEDS	Form I (fused lumps), Form III (flat elongated hexagons), Form IV (prismatic solids), and their mixtures	>20 μm	[197]
sulfathiazole	acetone	SAS	prismatic crystals	>750 μm	[152]
sulfathiazole	MeOH	SAS	needle-like, tabular crystal habit	>750 μm	[152]
terbutaline sulphate	EtOH, MeOH, water	SEDS	amorphous, crystals, form A, form B, monohydrate	3-10 μm	[198]
terbutaline sulphate	EtOH/DMF	ASES	agglomerate and separate spherical particles	200-300 nm	[199]
tetracycline	NMP	SAS	needle-like particles irregular amorphous particles	0.6-0.8 μm 150 nm	[134]
tetracycline	THF	SAS-EM (GAS)	very fine particles, narrow PSD	mean 125 nm	[200]
theophylline	EtOH/DCM	SAS	lamellar crystals and rosette crystals	L/d=5-300 μm/ 1-100 μm	[201]
triamcinolone acetonide	DCM	ASES	decrease of degree of crystallinity	<5 μm	[142]
trypsin	DMSO	SAS	irregular coalescing particles	1-5 μm	[165]
bio-polymers					
ALAFF	DMSO	GAS		0.8 μm	[202]
β-PLLA-co-PDLLA-co-PGA 62.5:12.5:25	DCM+TFE	ASES	spherical separated particles, semi-crystalline	D_{50}<10 μm	[203]
dextran	DMSO	PCA	spherical particles, mono and bimodal PSD	several nm-tenth μm	[121]
dextran	DMSO	SAS	spherical particles	mean 125-150 nm	[204]
ethylcellulose	DCM+DMSO	SAS	spherical coalescing particles	mean 5 μm	[205]
HPMA	DMSO	SAS	spherical particles	100-200 nm	[204]
HPMC, PVP	EtOH-DCM, MeOH/CO_2	SEDS	microspheres	<1 μm	[147]
HYAFF 11	DMSO	GAS		mean 0.6 μm	[202]
HYAFF 11	DMSO	GAS	separated spherical particles	0.4 μm	[83]

material	solvent	process/notes	results/observations	particle size	ref
bio-polymers					
HYAFF 11	DMSO	SAS	agglomerated spheres	15-20 µm	[83]
HYAFF 11	DMSO	SAS	submicronic particles		[206]
HYAFF 11 p75	DMSO	GAS		mean 0.8 µm	[202]
HYAFF 11p75	DMSO	SAS	submicronic particles		[206]
HYAFF 11p80	DMSO	SAS	submicronic particles		[206]
HYAFF 302	DMSO	SAS	submicronic particles		[206]
HYAFF 7	DMSO	GAS		mean 1 µm	[202]
inulin	DMSO	SAS	spherical particles connected by humidity bridges	<1 µm	[204]
PCL	acetone:DCM :isopropanol	SEDS	mixture of separated and agglomerated particles	25-85 µm	[207, 208]
PDLLA	acetone+EtAc	SEDS, CO_2+N_2	spherical nanoporous particles	9-10 µm	[207, 208]
PDLLA-co-PGA (50:50)	DCM+TFE	ASES	irregular agglomerated particles, amorphous	D_{50}<120 µm	[203]
PDLLG	EtAc:acetone: isopropanol	SEDS	spherical separated particles	6-10 µm, 15-57 µm	[207, 208]
PHBV	DCM	SAS	irregular or spherical particles, aggregated or separated, disomogeneity of mw	mean 3-9 µm	[209]
PLLA	DCM	ASES	spherical particles	1-10 µm, 10-30 µm	[210]
PLLA	DCM	GAS	spherical and irregular particles	0.5-3 µm	[129]
PLLA	DCM	PCA	spherical and elliptical particles	<3 µm	[211]
PLLA	DCM	ASES	ovoidal microspheres with a smooth to slightly rough surface	mean 10-12 µm	[212]
PLLA	DCM	ASES	spherical separated particles and particles with cracks and holes	mean 6-50 µm	[213]
PLLA	DCM	ASES	spherical semicrystalline particles	D_{10}=1.76 µm, D_{90}=6.78 µm	[214]
PLLA	DCM:acetone :isopropanol	SEDS	mixture of spherical particles and irregular particles	7-10 µm	[207, 208]
PLLA	DCM	SAS	spherical particles	1-2 µm, mean 1.4 µm	[204]
PLLA	CHF	PCA	spherical agglomerate particles	mean 1.6 µm	[127]
PLLA	DCM	SAS	spherical particles or fibres	1-5 µm	[215]
PLLA	DCM	ASES	discrete microspheres	<2 µm	[176]
PLLA	DCM	SAS	fibres and/or microspheres	mean 1–3 µm	[130]
PLLA	DCM	PCA	spherical particles	mean 193±19 µm	[96]
PLLA	DCM	PCA	connected nanoparticles spherical wrinkling microparticles	0.1-2 µm 5-50 µm	[122]
PLLA	DCM	CTAR	elliptical regular particles	1-3 µm	[97]
PLLA	EtOH/DCM	SEDS	spherical particles	1.1-3.6 µm	[133]
PLLA	DCM, THF, 1-4 dioxane	ASES	spherical particles	0.6-2.3 µm	[216]
PLLA	DCM	SAS	coalescing particles	3–15 µm	[19]

material	solvent	process/notes	results/observations	particle size	ref
bio-polymers					
PMMA	acetone	GAS	fractionation of different molecular weights		[217]
PVA-PLLA	DCM	ASES	spherical semicrystalline particles	D_{10}=1.21 µm, D_{90}=7.29 µm	[214]
food ingredients					
albumin	water	SEDS, CO_2+EtOH		50-500 nm	[106]
β-carotene	DCM	GAS	irregular particles	<1 µm	[218]
β-carotene	EtAc	GAS	platelet-like crystals	2-10 µm	[218]
β-carotene	DCM	SEDS-PA	irregular particles, increase of purity in the particles	0.4-2.9 µm	[100]
bixin	DCM	SEDS-PA	needle-like particles	d/L=0.3-1.1 µm/ 2-16 µm	[98]
ginkgo ginkgolides	EtOH	SAS	orthorhombic crystals	L/d=10-200 µm/ 3-50 µm	[118]
lycopene	DCM	SAS	needle-like particles	10-80 µm	[219]
maltose	water	SEDS, CO_2+EtOH	free flowing white powder		[170]
soy lecithin	EtOH	SAS	partly coalescing spherical particles	1-10 µm, 5-45 µm	[116]
soy lecithin	EtOH	SAS	partly coalescing spherical particles	10–40 µm	[117]
sucrose	water	SEDS, CO_2+EtOH	free flowing white powder		[170]
tartaric acid	acetone	PCA	plate-like crystals	10-20 µm	[220]
tartaric acid	MeOH+EtOH	PCA	plate-like, needle-like crystals	25-45 µm	[220]
tartaric acid	EtOH	PCA	irregular crystals	35-60 µm	[220]
trehalose	water	SEDS, CO_2+EtOH	free flowing white powder		[170]

ALAFF = benzyl ester of alginic acid
CHF = chloroform
d/L = diameter/length
D_{10} = equivalent volume diameter below which is 10% particle diameter
D_{90} = equivalent volume diameter below which is 90% particle diameter
DCM = dichloromethane
DMF = dimethylformamide
DMSO = dimethyl sulfoxide
EtOH = ethanol
EtAc = ethyl acetate
Fab = fragment antigen binding
Fv = fragment variable
HFP=1,1,1,3,3,3-hexafluoro-2-propanol
HPMA = hydroxpropylmethacrylic acid
HPMC = hydroxypropyl methylcellulose
HYAFF = hyaluronan total benzyl ester
HYAFFp = hyaluronan partial benzyl ester
L/d = length/diameter
L/w = length/width
MeOH = methanol
MMAD = mass median aerodynamic diameter
NMP = N-methyl pyrrolidone
PCL = polycaprolactone
PDLLG = poly D,L-lactide-co-glycolide
PDLLA = poly D,L-lactide
PGA = polyglycolic acid
PHBV = polyhydroxybutyrate-valerate
PLLA = poly L-lactide
PSD = particle size distribution
PVP = polyvinyl pyrrolidone
PVA = polyvinyl alcohol
TFE = 2,2,2-trifluoroethanol
THF = tetrahydrofuran

2.1.3 SCF as solute

In the **PGSS** (Particles from Gas Saturated Solution) process, a compressible gas (i.e. CO_2) is dissolved under pressure in a melted or liquefied substance acting as a solute in the process. Polymers are the best candidates for this kind of process because the capacity of SC-CO_2 to reduce the glass transition temperature helps the melting [221]. High

temperatures are often required to liquefy other compounds to be micronized. This gas-saturated solution is later expanded with consequent supersaturation and fine particles precipitation [222]. This method shows some advantages and some disadvantages over other techniques based on SCF. When compared with RESS, the consumption of CO_2 is lower by three orders of magnitude for PGSS. When compared with SAS, no organic solvents are needed for PGSS. In the case of polymers, the knowledge of the pressure-temperature behaviour gives information about the pressure needed to melt the substance to be micronized and form a liquid phase at a given temperature, and allows to calculate its composition [44]. Some examples of applications of PGSS are reported in Table 4.

The high temperatures often required may lead to some chemical changes of the compounds during processing by PGSS [223,224]. Hence, this technique is not suitable for pharmaceutical compounds. Polymers and bio-polymers can be easily treated by PGSS, but the results obtained are mainly aggregated materials [225]. Not many applications of this technique to food ingredients are found in literature. Münüklü and Janksen [226] proposed the PGSS process with the name Supercritical Melting Micronization process (**ScMM**) for the micronization of edible fats. Solid microparticles and hollow microparticles of rapeseed 70 were produced using a continuous operated pilot plant. Rapeseed 70 is hardened rapeseed oil with a melting point of 70°C, used in the food industry as an emulsifier e.g. in margarine production. Therefore, it is important to have a powder with the morphology and size of micro particles. Moreover, hollow particles of edible fats are suitable for encapsulation.

Theoretical investigation of PGSS is at the beginning, and few papers have been published in this area to date. An exhaustive description of the PGSS process demands the investigation of many different mechanisms and phenomena, including supercritical fluid thermodynamics, jet-spray hydrodynamics, droplet fluid dynamics, crystallization kinetics, bubble formation and droplet coalescence. Elvassore et al. [227] have investigated PGSS thermodynamics. Li et al. [228] have considered the nozzle hydrodynamics in the PGSS process. They also developed a general model based on the atomization and the crystallization mechanisms that considers both the melt crystallization and the gas-solution crystallization. Strumendo et al. [229] investigated the behaviour of an isolated gas-saturated solution droplet in a gaseous environment and developed a mathematical model that describes the transport phenomena within the droplet and in the surrounding atmosphere.

Table 4. Pharmaceutical compounds, bio-polymers and food ingredients micronized by SCF-based techniques. SCF used as solute.

material	process/notes	results/observations	particle size	ref
pharmaceuticals				
cyclosporine A	PGSS	amorphous spherical particles	mean 4.5 μm	[72]
nifedipine	PGSS	irregular porous particles	mean 15-30 μm	[223,224]
bio-polymers				
catalase	PGSS	porous macroparticles		[225]
galactosidase	PGSS	porous macroparticles		[225]
PLA	PGSS	porous macroparticles		[225]
PLGA	PGSS	porous macroparticles		[225]
food ingredients				
rapeseed 70	ScMM	empty balloons, form α, 84% crystallinity	8-90 μm	[226]

PLA = poly-lactide PLGA = poly D,L-lactide-co-glycolide

2.1.4 SCF as co-solute

The techniques that use CO_2 as co-solute have something in common with the micronization by spray-drying: the SCF and the solution are intimately mixed and then sprayed in a drying atmosphere. These SCF-based micronization techniques are very interesting because they can be applied to water-soluble compounds difficult to handle with the other SCF techniques without excluding the possibility of using other organic solvents. The relatively low temperatures used allow to treat thermolable compounds as well.

Supercritical Assisted Atomization (**SAA**) is a recent process [23,230] in which the SCF acts as an atomizing medium. Atomization, also assisted by an inert gas, is generally used in the spray drying of solutions. The innovative aspect of the SAA process is the solubilization of SC-CO_2 in the liquid solution formed by the solvent and the (solid) solute, and the subsequent atomization of the gas-solid-liquid mixture using a thin wall nozzle. Indeed, gases are released slowly from the liquid phase and their contribution to a two-step atomization is not relevant [231]. Gases at supercritical conditions are released from the liquid phase by a faster process [230]; therefore, SC-CO_2 can improve the atomization process, contributing to a two-step atomization. The operating conditions that are characteristic of the SAA process are the formation of a single, expanded liquid, phase in the mixing device. When SAA is properly conducted, two atomization processes take place: the first one is the production of primary droplets at the exit of the nozzle by pneumatic atomization and the second one is the destruction of these droplets by the fast release of CO_2 from the internal of the droplet (decompressive atomization). Depending on the process temperatures and the chemical characteristics of the solid solute, amorphous or

crystalline particles have been produced [232]. The SAA process has been successfully performed using water, organic solvents with relatively low boiling points (EtOH, MeOH, acetone) and mixtures of them. Solvents with high boiling points, typically used e.g. in SAS micronization (i.e. DMSO, NMP, toluene) cannot be used because of the difficulty of removing them during precipitation. The limit of this process is that the smallest particles produced depend on the dimensions of the smallest secondary droplets generated (one droplet-one particle process). The dimensions are connected to the classical parameters that control droplet dimensions during atomization: surface tension, viscosity and quantity of SCF dissolved in the liquid. The scale-up of the process has been performed, based on the geometrical similitude, on a semi-continuous plant with a precipitator and a saturator volume that is five fold the laboratory scale (14 dm^3 and 0.15 dm^3 respectively) [233].

CAN-BD (CAN-Bubble Drying) has similar features as SAA [234], that is, a two-phase mixture of CO_2 and the formation of a liquid solution [235]. The key principle is different from SAA, because the CO_2 is not solubilized in the solution but contacts the liquid in a near zero volume tee (<1 μL) with a short residence time.

Li et al. [236,237] used a process called Supercritical Fluid Expansion Depressurization (**SFED**) to produce microparticles of pharmaceutical compounds. The apparatus and the process are identical to the SAA technique [230], the micronized products and the operating conditions used have also been reproposed. No additional contribution can be found.

In another process, called Depressurization of an Expanded Liquid Organic Solution (**DELOS**) [238], a compressed gas, CO_2 not necessarily at supercritical conditions, is used for the production of micro- and submicro crystalline particles from an organic solution. The compressed CO_2 is solubilized, at a given pressure and temperature, in the organic solution of the solute to be crystallized. It has the role to produce, by Joule-Thomson effect, a homogeneous sub-cooling of the solution with supersaturation and solid particles precipitation [239]. Up to know, the process has been successfully applied to inorganic colorants [238,239] and some preliminary experiments on fatty acids and some drugs have been reported in some conference proceedings [240].

These micronization techniques are relatively new, but many applications of SAA and CAN-BD on pharmaceutical compounds can already be found (Table 5). In all cases, spherical particles are produced with different degrees of crystallinity, mainly because of the operating temperature used. An interesting application can be found in those compounds that can be used as carriers or excipients in foods due to their high solubility in water or their particular conformations. Examples of this are cyclodextrins [241], lactose [242] and bio-compatible or natural polymers [243,244] that have been successful

micronized as spherical amorphous micrometric particles (Table 5). No modeling studies on these techniques are present in literature yet. These processes seem to be very promising, and in the future, when more data are available and the mechanism of particle formation is clearer, the translation into mathematical models will be performed.

Table 5. Pharmaceutical compounds, bio-polymers and food ingredients micronized by SCF-based techniques. SCF used as co-solute.

material	solvent	process/notes	results/observations	particle size	ref
pharmaceuticals					
α-cyclodextrin	water	SAA	crystalline spherical particles	95% 0.1-5 μm	[241]
α-lactose	water	CAN-BD	spherical aggregated particles	mean 1 μm	[242]
amphotericin B	EtOH	CAN-BD	irregular particles	mean 1 μm	[245]
ampicillin	buffered water (pH7)	SAA	amorphous spherical particles	mean 0.8-5.6 μm	[246]
budesonide	EtOH	CAN-BD	spherical particles	mean 1 μm	[245]
carbamazepine	MeOH	SAA	micronic needle-like particles	several tenth μm	[230]
cromolyn sodium	water	CAN-BD	spherical irregular coalescing particles	number-average 0.58 μm, volume-average 0.40 μm	[242]
dexamethasone	acetone	SAA	amorphous spherical particles	<3 μm	[230]
DL-alanine		CAN-BD		0.3-0.5 μm	[234]
erythromycin	MeOH, EtOH	SAA	spherical particles	<3 μm, 88% particles volume 1-3 μm	[247]
erythromycin	acetone	SAA	spherical particles and coalescing particles	<3 μm	[247]
erythromycin	EtOH	SFED	spherical particles and coalescing particles	<3 μm	[236]
griseofulvin	acetone	SAA	spherical semicrystalline particles	mean 0.5-2.5 μm	[248]
HMR1031	MeOH	SAA	amorphous spherical particles	MMAD 1.6-4 μm	[249]
hydroxypropyl-β-cyclodextrin	water	SAA	amorphous spherical particles	95% 0.1-5 μm	[241]
LDH	water	CAN-BD	85% activity lost		[250]
lysozyme	water	CAN-BD	aggregates, divided when sucrose and tween80 used as additive, amorphous powder	1–3 μm	[250]
naproxen	95%EtOH: 5% water	CAN-BD	irregular coalescing particles	primary particles 0.91 μm, agglomerates 0.5-5 μm	[245]
rifampicin	MeOH	SAA	separated deflated spherical particles	<3.2 μm, mean 0.7-1.8 μm	[251]
rifampicin	MeOH	SAA pilot	separated deflated spherical particles	mode 0.3-0.4 μm	[233]

material	solvent	process/notes	results/observations	particle size	ref
pharmaceuticals					
salbutamol sulphate	water	CAN-BD	spherical particles	number-average 0.70 µm, volume-average 0.95 µm	[242]
terbutaline	water	SAA	very regular and homogeneous particles	>99% 1-4 µm	[252]
tetracycline	water	SAA	separated spherical particles	<2.4 µm, mean 0.5-1.3 µm	[251]
tetracycline	water/ EtOH	SFED	approximately spherical particles	1-3 µm, mean 1.3-1.5 µm	[237]
triclabenzadol	MeOH	SAA	irregular crystals	<2 µm	[230]
bio-polymers					
chitosan	5%acetic acid aqueous solution	SAA	spherical particles with decreased degree of crystallinity	99% 0.1-1.5 µm	[243]
PLLA	DCM	SAA	spherical particles	1.5-3.5 µm	[244]
PMMA	acetone	SAA	spherical particles, decrease of the Tg	0.05-1.6 µm	[244]
food ingredients					
palmitic acid	EtOH	CAN-BD	leaf shaped coalescing particles	0.43-0.65 µm	[245]

DCM = dichloromethane MeOH = methanol Tg = glass transition temperature
EtOH = ethanol MMAD = mass median aerodynamic diameter

2.2 Polymorphic modifications induced by SCF

In the last five years, one development in the application of SCF-based micronization techniques has been the production of particles not only with controlled particle size and distribution, but also with controlled crystal habit or polymorphic form.

A solid compound can exist in several forms, either as crystalline or amorphous. Gross structural modifications such as polymorphism and solvate formation are notably common for certain groups of drugs. Polymorphs are crystalline phases containing the same molecules or ions but having different conformations and/or packing arrangements in the solid-state. Solvates are also crystalline phases and are formed when solvent molecules are present in the crystal lattice, leading to molecular adducts with the host molecules. If the solvent is water, the molecular adducts are termed hydrates [253]. The crystal habit is the external shape of a crystal. An identical compound can have many crystal habits due to the different growth rate of each crystal surface. The habit does not reflect the internal structure of a crystal. A crystal that has an identical internal space lattice can show different crystal habits. If a pharmaceutical compound that has only one polymorphic form undergoes a habit modification, its bioavailability may be changed [2,3].

Polymorphic forms can have remarkably different physical properties in pharmaceutical compounds. These can include solubilization rate and melting point, which result in different stability and bioavailability of drug products. If a mixture of polymorphs occurs in a pharmaceutical formulation, the quantitative control of crystallization is required to ensure a fixed proportion of forms. In addition, solid-state recrystallization phenomena, which may have conversion times between seconds and years, have to be suppressed to maintain integrity during product shelf-life. It should be remembered, however, that processing techniques such as milling, drying and compression can also introduce polymorphic modifications and transformation [253]. The effect of a solvent or solvent mixture on the formation of stable and unstable polymorphs is well known [254,255].

In some food applications, the polymorphic form might be important when specific characteristics are required. An example is cocoa butter, which exists as six different polymorphic forms identified with roman numerals in order of their different melting point. Different polymorphs give different consistencies to the final product (chocolate). Form V is the polymorph preferred to the others, because it gives the chocolate a better structure and taste. The other forms feel too sticky or thick in the mouth. Form VI is associated with fat bloom, a condition where the cocoa butter separates out during storage. Although Form V is the best tasting polymorph of cocoa butter, Form VI is the most stable [256]. Food scientists in the chocolate industry seek to develop techniques that lead cocoa butter to solidify in the desirable Form V polymorph.

Several studies have showed that the dramatic change in solid morphology after processing by SCF is not only related to particle size and distribution, particle shape and porosity, but sometimes the crystal pattern is modified as well. SC-CO_2 can be a suitable solvent for producing crystallographically pure phases when mixtures of polymorphs of a particular pharmaceutical compound are involved, thus avoiding the use of organic solvents. Using a SC-CO_2 treatment, a new polymorph of deoxycholic acid was formed [257]. Deoxycholic acid crystals were stored in a pressure vessel with SC-CO_2, after which new X-ray diffraction peaks not found in the bulk deoxycholic acid crystal were observed. The crystals obtained were found to be a metastable form of deoxycholic acid. Equimolar powder mixtures of the two carbamazepine polymorphs (Form I, Form III) were submitted to treatment with SC-CO_2 at 350 bar and 55°C either under a dynamic flow or under static conditions [258]. There was an enrichment of the mixture in terms of Form III, essentially due to a conversion of Form I via solubilization in SC-CO_2 followed by re-crystallisation of the less soluble polymorph.

The RESS technique has been the most successful SCF technique for producing different polymorphic forms of a compound, mainly because the use of different operating conditions allows to modulate the density and viscosity of the SCF and reproduce some characteristics of organic solvents. This characteristic can also stabilize one specific polymorphic form. Examples can be found in Table 2 [56,67,68].

Using supercritical antisolvent techniques some examples of production of microparticles with different polymorphic forms can be found, as reported in Table 3. In some cases, they are obtained by varying the operating conditions, mainly the injection flow rate and the operating temperatures, which have a great influence on the growth of the nuclei during the crystallization [148,158,159,194,197]. In many other cases several polymorphic forms are found by varying the solvent used [144,148,150,158,197,198]. The most stable polymorphic form is often obtained, but in some cases it is also possible to obtain the same polymorphic form of the untreated material. For example, salmeterol xinafoate Form I and Form II processed by SEDS retained a high polymorphic purity and distinctly different physical and surface properties. Form I is, however, the most stable [259]. The most frequent crystallographic changes observed in particles obtained by supercritical antisolvent micronization processes are related to the crystal habit of the compound. Indeed, the modulating properties of SC-CO_2 and the mixture solvent-antisolvent characteristics may create an environment particularly favourable to some morphologies [155,160,176,177,194,196,220].

In some works, additives are used as habit modifiers during SCF crystallization. Chloropropamide was crystallized using the RESS process in the presence of urea as a polymorph conversion additive [260]. Jarmer et al. [261] proposed the use of poly-sebacic anhydride as growth inhibitor of griseofulvin using the SAS process. Griseofulvin crystal habit changed from acicular to bipyramidal. Caputo and Reverchon [262] proposed the use of urea as habit modifier of sulfathiazole precipitated by SAS from an acetone solution. In the presence of urea the sulfathiazole habit changed from a plate shape to spherical semicrystalline particles with a very narrow particle size distribution and with a mean particle size ranging between 0.5 and 1.0 μm depending on the sulfathiazole concentration.

The examples reported in Table 3 suggest that, among the other SCF micronization techniques, the supercritical antisolvent technique is a promising process for the production of different polymorphs of a compound.

2.3 Novel developments

This section focuses on specific applications of SCF-based micronization techniques, which differ from the micronization of neat particles, and seem to be very promising. The micronization techniques already proposed can be used more or less successfully to produce particles formed by more compounds, generally an active principle and a biocompatible polymer, at the same time with controlled particle size and distribution. This is the case of encapsulation and co-precipitation. Furthermore, the principles of the same techniques can be applied to purposes different from micronization. In SAE (Supercritical Antisolvent Extraction), SC-CO_2 is used as antisolvent and the aim is to fractionate different materials; in SFEE (Supercritical Fluid Extraction from Emulsions) SC-CO_2 is again used as antisolvent, but for drying particles already produced in the emulsion step.

2.3.1 Supercritical Antisolvent Extraction (SAE)

The Supercritical Antisolvent Extraction (**SAE**) is a challenging combination of supercritical fluid extraction (SFE) technique and supercritical antisolvent micronization (SAS) technique. The SAE process is conceptually very similar to SAS, but the scope of the process is the recovery of one or more solid compounds from a liquid mixture. It consists of the continuous flow of SC-CO_2 and of the liquid mixture in a pressurized precipitation vessel. If the process conditions have been properly selected, the liquid is rapidly extracted by the supercritical fluid and recovered in a separation vessel, while the solid precipitates at the bottom of the precipitation vessel, where it is collected. The liquid solution is sprayed in the precipitation vessel to produce a very large liquid surface, due to the formation of small liquid droplets. In this way, the rate of solubilization of the liquid phase in the supercritical medium is strongly enhanced. The process is performed at operating conditions at which the liquid solvent is completely soluble in SC-CO_2 and the compound to be recovered is not soluble in SC-CO_2. The knowledge of solubility data on the liquid solvents and of the solids in SC-CO_2 is important for the proper selection of process temperature and pressure. The process performances are controlled by the thermodynamics of the system and by the mass transfer mechanism. The advantage of the use of a supercritical fluid is the enhanced mass transfer in the separation of the undesired liquid compounds, and the selective precipitation of the product of interest. A limitation of this process is the possible formation of a ternary mixture liquid/solid/SC-CO_2. Indeed, the presence of the liquid can induce an increase of the solubility of the solid compounds in SC-CO_2. In this case, part of the solid is retained in the fluid phase, removed by SC-CO_2, and lost in the liquid recovered in the separation vessel [31].

Until now, SAE has been used in a limited number of processes, but it has a large potential for future applications, especially in the separation of high added value substances, such as antioxidants, often combined with oils and fats soluble in SC-CO_2. The compound to be selectively recovered can be naturally present in the liquid mixture, as in the case of lecithin in soybean oil. The phospholipids are insoluble, and the seed oil is soluble in SC-CO_2. The oil solubilized in SC-CO_2 is extracted and recovered in a separator operated at low pressure, and lecithin precipitates as a solid powder [263]. In the same way, Andersson et al. [264] used SAE to separate digalactosyldiacylglycerol, used in the preparation of liposomes or in oil-in-water emulsions, from oat oil. The oil was sprayed in a vessel and SC-CO_2 removed the other components of the oil except digalactosyldiacylglycerol, which precipitated with a purity of 97%.

In other cases, the compound of interest is solubilized in a liquid solvent as the result of a previous process or as pre-treatment. Mukhopadhyay and Singh [265] dissolved crude lecithin in hexane, because the oil contained in the raw material is more soluble in hexane than the pure lecithin. During the antisolvent extraction the dissolution of CO_2 in the solution causes a large partial molar volume reduction of hexane and a reduction of its solvent power for lecithin. Pure lecithin is then selectively precipitated, and the oil is removed with the hexane by the SC-CO_2.

Propolis is usually dissolved in EtOH or EtOH/water mixtures to remove insoluble material such as waxes and detritus from the hive, thus obtaining the solution called propolis tincture. It contains a high concentration of flavonoids, which are used in a wide range of cosmetic and health food preparations for their antimicrobial properties. Catchpole et al. [266] developed a combined supercritical antisolvent/extraction process for the fractionation of propolis tincture to obtain flavonoids and essential oil fractions by extraction, and to remove high molecular mass components by antisolvent precipitation. Flavonoids are practically insoluble in pure CO_2, but sufficiently soluble in CO_2+EtOH to enable their separation from high molecular mass and/or more polar components. In the first step of the process, supercritical CO_2 is used both as anti-solvent to precipitate high molecular weight components, and as a solvent to extract the EtOH and soluble components of the propolis. This extract is then fractionated in two separation steps to create a concentrated flavonoids fraction as the primary product, and an essential oil/EtOH fraction as a secondary product.

An improvement of the SAE process could be its combination with SFE in a two-step process developed using the same apparatus. First, the compounds of interest are extracted from a matrix using SC-CO_2 modified with a polar liquid solvent, then the mixture extract-

CO_2-solvent is delivered to another vessel and, changing the operating conditions, the solid is precipitated by the SAS technique. A similar approach was used by Aro et al. [267] in performing the fractionation of oat lipids using the semi-industrial plant described in this thesis work, and coupling SFE and SAS techniques in separate steps. In the first step, SC-CO_2 is used to extract the undesired triacylglycerols and moisture from the matrix of groated oat or oat flakes, and in the second step, SC-CO_2 with EtOH as modifier is used to extract the polar lipids from the treated matrix. The solution EtOH-polar oat lipids is separated in a low pressure vessel and concentrated to 1/50 of the original volume. Finally, the antisolvent separation is performed and the polar lipids are precipitated and collected, while EtOH is removed by SC-CO_2.

2.3.2 Supercritical Fluid Extraction from Emulsions (SFEE)

Supercritical Fluid Extraction from Emulsions (**SFEE**) is a novel process proposed by Chattopadhyay et al. [268] to obtain particles of nanometric and micrometric size with very narrow particle size distribution. The process consists of producing an oil-in-water, or multiple emulsions, in which the organic solvent contains the compound to be micronized. The precipitation of the compound takes place in the emulsion droplets, well separated from each other by the presence of water. SC-CO_2 is then used to extract the organic phase form the emulsions, and a water suspension in only one step is obtained, which could be directly used in some specific formulations. Water can be subsequently removed by conventional drying processes. The inventors suggest the use of high-speed centrifugation followed by decantation, by which dried particles with very narrow particle size distribution are obtained. In this process, the role of SC-CO_2 is to extract the solvent from the emulsion without solubilizing the compound itself.

The size of the particles depends on the size of the emulsion droplets; therefore, depending on the efficiency of the emulsification step, nano or microparticles with a very narrow size distribution can be obtained. Using this technique, cholesterol acetate, griseofulvin and megestrol acetate were micronized in the form of water suspensions with particle diameters ranging between 0.1-1 μm, measured in the suspension as processed [269]. Organic solvents such as EtAc, toluene or DCM and dispersing agent (surfactants) as PVA, pluronics, lecithins, Span 80 or Tween 80 have been used in the emulsion process. After the drying step, the particles obtained are prismatic-like and crystalline in morphology. The process has recently been applied to produce dry-encapsulated formulations [270].

2.3.3 Co-precipitation, encapsulation, formulation

A new trend in the use of SCF-based micronization techniques is the design of composite particles with several purposes [13,34,36,89,110,271]. These include the preparation of sustained-release drugs by incorporating the active compound, mainly pharmaceutical or nutraceutical, in a slow-dissolving (bio-degradable or bio-erasable) matrix, the stabilization of fragile molecules (mainly bio-molecules) in the solid form, and the bio-availability enhancement of poorly soluble compounds by incorporating the active compound in a fast-dissolving hydrophilic excipient [110]. Different kinds of formulations are developed according to the final purpose. Polymer/drug co-formulations and inclusion complexes, such as drug/cyclodextrin complexes, can be used either for extended and delayed release or to enhance poorly water-soluble compounds' solubility.

The main method of obtaining controlled release is to incorporate the biologically active agents within biopolymers or bio-compatible polymers, basically in the form of microcapsules and microspheres. Microcapsules are microparticles formed by an active agent core wrapped by a polymeric shell (core-and-shell system). The release rate of the drug is then determined by the drug diffusion through the polymeric core. Microspheres are polymer microparticles in which the active agent is uniformly dispersed at molecular level or in aggregate form (matrix system). The rate of drug release is determined either by the degradation of the polymer (erosion), the diffusion of the drug through the matrix, or the swelling of the polymer and the consequent diffusion of the agent solubilizing the drug [272-275]. In some cases, other compounds different from biopolymers are used. These include mainly lipids or phospholipids [276].

Cyclodextrins are bucket-shaped oligosaccharides produced from starch. As a result of their molecular structure and shape, they possess a unique ability to act as molecular containers by entrapping guest molecules in their internal cavity and altering the physical, chemical, and biological properties of guest molecules. Cyclodextrin are hydrophilic on the outside surface, leading to a very fast dissolution in aqueous media, but hydrophobic on the inside, permitting a stable inclusion of poorly soluble molecules of a suitable size [241,277]. Formulation of therapeutic proteins in dry form is often required to improve their stability and excipients, such as sugars, are used to improve the stability and to drive the particle formation and morphology [189,245,278-283].

3 AIMS OF THE STUDY

The general aim of this research was to attest the feasibility of micronization of some pharmaceuticals and food ingredients using SCF-based techniques. At the same time a valid guidelines for performing SCF micronization of compounds with different natures was found. In particular, several objectives were intended.

- To obtain controlled particle size and morphology of the micronized compounds using the SAS technique, and investigate the reproducibility of the process at different physical plants, different micronization modes (batch and semi-continuous) and different scale plants (laboratory and semi-industrial). The possible modifications induced to the compounds by the process, in terms of polymorphism, pseudo-polymorphism and crystal habit have been monitored.
- To investigate the process in relation to the precipitation mechanism, and formulate and confirm some hypothesis of particle formation with the support of visual observation of the precipitation evolution. The study could help in tracing a direction for the micronization of other compounds.
- To explore the use of another SCF-based micronization technique, namely SAA, in case of compounds that cannot be processed using SAS, and compare the two techniques.

As a result of the whole study, the knowledge and the background acquired were intended to be used for transferring the SCF micronization technology to the food industry that still lacks in the application of this technique.

4 MATERIALS AND METHODS

4.1 Materials

4.1.1 Compounds

4.1.1.1 Pharmaceutical compounds (I, III-V)

Cromolyn sodium purity 98% (mw=512.34) was kindly given by Italchimici (Pomezia, Italy). The solubility of cromolyn sodium in water is of about 120 mg/mL at 24°C. The chemical structure is shown in Figure 1. It is an antiflammatory drug frequently used in the therapy of chronic asthma. As for many other drugs used for aerosol delivery, the problem of the control of particle size and distribution is one of the major concerns for an efficient delivery to the lung. Details can be found in (III).

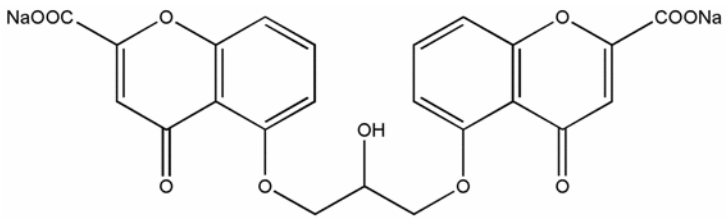

Figure 1. Chemical structure of cromolyn sodium

Nalmefene HCl purity of 99.9% (mw=375.9) was given by Biotie Therapies Corp. (Turku, Finland). The solubility profile of nalmefene HCl in liquid solvents was also obtained from Biotie Therapies Corp. The approximate solubility in EtOH is 10.9 wt% at room temperature. Untreated material had been crystallized from water and the crystals had a squared shape with the two main dimensions ranging between 70-90 µm and 90-120 µm respectively. The chemical structure is shown in Figure 2. Nalmefene is an opioid antagonist [284-286]. Details can be found in (I, IV).

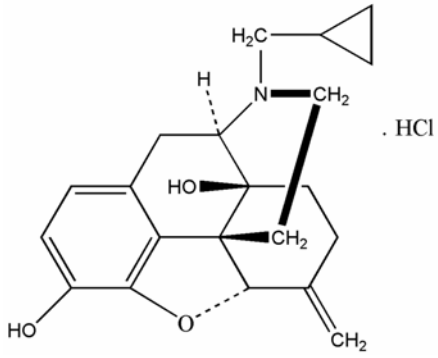

Figure 2. Chemical structure of nalmefene HCl

Rifampicin with a purity of 99.9% (mw=822.94) was supplied by Sigma–Aldrich (Italy). The approximate solubilities in DMSO, NMP, MeOH, EtAc and EtOH were measured at room temperature and were 120, 80, 60, 40 and 7 mg/mL, respectively. It is practically insoluble in water. The chemical structure is shown in Figure 3. Rifampicin is an antibiotic mainly used for the treatment of tuberculosis, but it is also used in the therapy of meningitis and infections of the biliary routes.

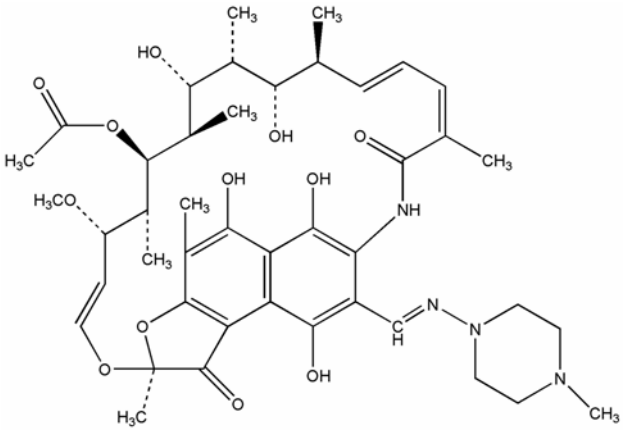

Figure 3. Chemical structure of rifampicin

Salbutamol sulphate (mw=576.7) was given by Orion Pharma (Espoo, Finland). The approximate solubilities of salbutamol sulphate were measured at room temperature in different solutions and they were 0.5 mg/mL in EtOH, 2.3 mg/mL in EtOH/water 95:5 v/v, and 5.8 mg/ml in EtOH/water 90:10 v/v, respectively. The chemical structure is shown in Figure 4. Salbutamol sulphate is a drug used in the treatment of asthma.

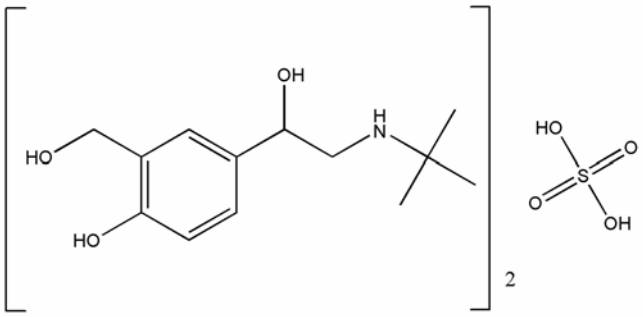

Figure 4. Chemical structure of salbutamol sulphate

4.1.1.2 Biopolymer (II)

Polyvinyl alcohol (PVA) with an hydrolization grade of 99.8% (mw=30'000-70'000) was supplied by Sigma–Aldrich (Milan, Italy). It is a water-soluble polymer made by hydrolysis of a polyvinyl ester. The approximate solubility of PVA in DMSO at room temperature is

about 22 wt%. Untreated PVA is a fine powder formed of irregular particles with size ranging from about 50 to 250 μm. The chemical structure of the monomer is shown in Figure 5. Among biopolymers, PVA is widely recommended for pharmaceutical applications as a carrier of active compounds, as in the case of production of capsules or spheres for medical purposes. Details can be found in (II).

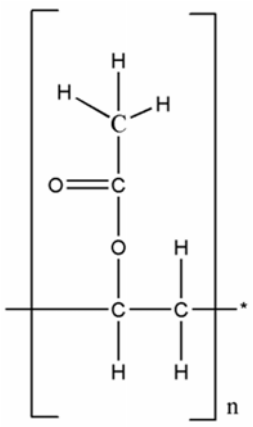

Figure 5. Chemical structure of PVA

4.1.1.3 Food ingredient (V)

Adipic acid purity 99.5% (mw=146.14) was supplied by Sigma-Aldrich (Italy). It is also called hexanedioic acid and is a white, crystalline compound of C6 straight-chain dicarboxylic acid, which is slightly soluble in water and soluble in EtOH and acetone. The chemical structure of adipic acid is shown in Figure 6. Adipic acid is used in plastic processing (production of nylon 6.6), as excipient in drugs (tablet lubrificant, constituent of tablet coatings) and in food production (as flavouring, leavening, and pH control agents with E-number E355). Details can be found in (V).

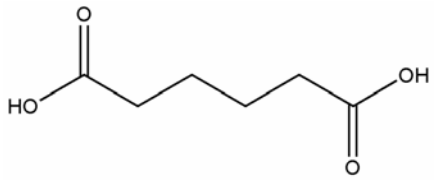

Figure 6. Chemical structure of adipic acid

4.1.2 Liquid solvents (I-V, unpublished results)

The liquid solvents were supplied by Sigma-Aldrich (Milan, Italy). EtOH, DMSO, MeOH with purity of 99.9% acetone with purity of 99.8% and EtAc with purity of 99.5% were used in the SAS process. Distilled water was used in the SAA process (III). Isopropyl alcohol

anhydrous with purity of 99.8% and MeOH with purity of 99.9% were used for the analyses (III, IV). The solvents used in the micronization processes are classified by the International Conference on Harmonization (ICH) as follows: EtOH, DMSO, EtAc, acetone Class 3 solvents with residual solvent limit in drug substances fixed at 5000 ppm; and MeOH Class 2 solvent with residual solvent limit fixed at 10 ppm [111].

4.1.3 Other materials (I-V)

Carbon dioxide (CO_2) purchased from SON (Napoli, Italy) and by AGA (Helsinki, Finland), and nitrogen (N_2) purchased from SON (Napoli, Italy), were used in the micronization processes (I-V). Ammonium thiocyanate (supplied by Sigma-Aldrich, Milan, Italy), NaCl purity 99% (supplied by Fluka, Sigma-Aldrich Milan, Italy), and triethylamine p.a. grade (supplied by Fluka, Sigma-Aldrich Finland Oy, Helsinki, Finland) were used in the analyses (III, IV). Urea purity 99.5%, purchased from Sigma-Aldrich (Italy), was used as habit modifier in SAS precipitation of adipic acid (V).

4.2 Apparatuses

4.2.1 Micronization by supercritical antisolvent (SAS) technique

4.2.1.1 SAS laboratory plant (I, II, IV, V)

The laboratory apparatus used for semicontinuous antisolvent precipitation is located at the University of Salerno (Italy) and is represented schematically in Figure 7. In the SAS experiments, the liquid solution containing the solute is delivered by an HPLC pump to the heated high pressure precipitation vessel (P) through a stainless steel nozzle. SC-CO_2 is pumped by a diaphragm high-pressure pump, pre-heated to the process temperature and delivered through another inlet port located on the top of the precipitation vessel. The pressure in the precipitation vessel is regulated by a micrometering valve. The produced powder is collected on a stainless steel frit located at the bottom of the vessel. The liquid solvent is recovered in a second collection vessel (S) located downstream, at lower pressure, regulated by a backpressure valve. Manometers, flow-meters, temperature controllers and thermocouples complete the apparatus. A more detailed description of the plant can be found in (I, II, IV, V) and some information about the plant are also available elsewhere [287,288].

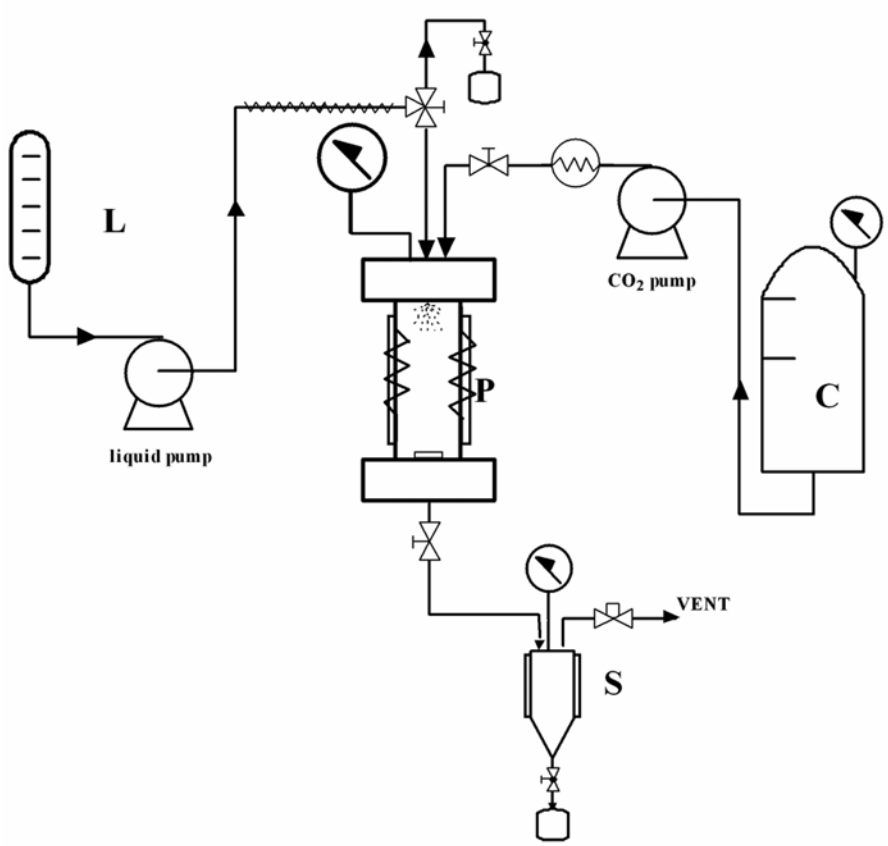

Figure 7. Schematic representation of SAS laboratory scale apparatus: C) CO_2 storage vessel; L) liquid solution; P) precipitator; S) liquid separator.

The windowed SAS apparatus (II) differs from the one described above only as regards the precipitator, which consists of a stainless steel cylindrical vessel with two quartz windows put along two longitudinal sections (Figure 8). The windows allow visual observation of the evolution of the precipitation process [22,103].

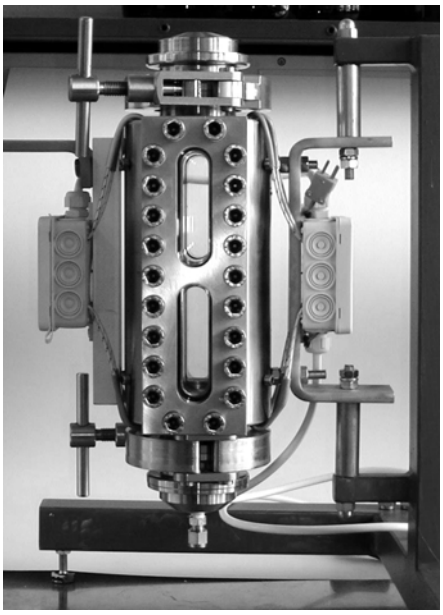

Figure 8. SAS quartz windowed precipitator

In GAS experiments (II), a vessel with the same characteristics as the one used for SAS experiments was used. The inlet port of CO_2 is located at the bottom of the precipitation vessel and the outlet is on the top. The GAS windowed plant has the same characteristics as the SAS windowed plant.

4.2.1.2 SAS pilot plant (I)

The pilot plant is located at MTT Agrifood Research Finland, Jokioinen (Finland) and is represented schematically in Figure 9. It is a closed loop plant consisting of a CO_2 storage vessel (C), an extraction unit (E), a precipitation unit (P), two liquid separators (S_1, S_2), two pumps and a heat exchanger. A stainless steel basket is located inside the precipitator and at its bottom a filter allows the collection of the product. Gaseous CO_2 is cooled in a shell and tube heat exchanger, condensed and recirculated to the storage vessel. The liquid solution is delivered to the precipitator by a piston pump. Liquid CO_2 is delivered using a diaphragm pump. The liquid solution and SC-CO_2 are fed to the precipitator through a tube-in-tube injection system. Similar injection devices have been used for general purpose atomization and by several authors for SAS processing [21,177,289]. The liquid solvent is recovered in the separators. During a precipitation test the pressure in the precipitator is regulated by a micrometering valve located upstream the separator. A more detailed description of the plant can be found in (I).

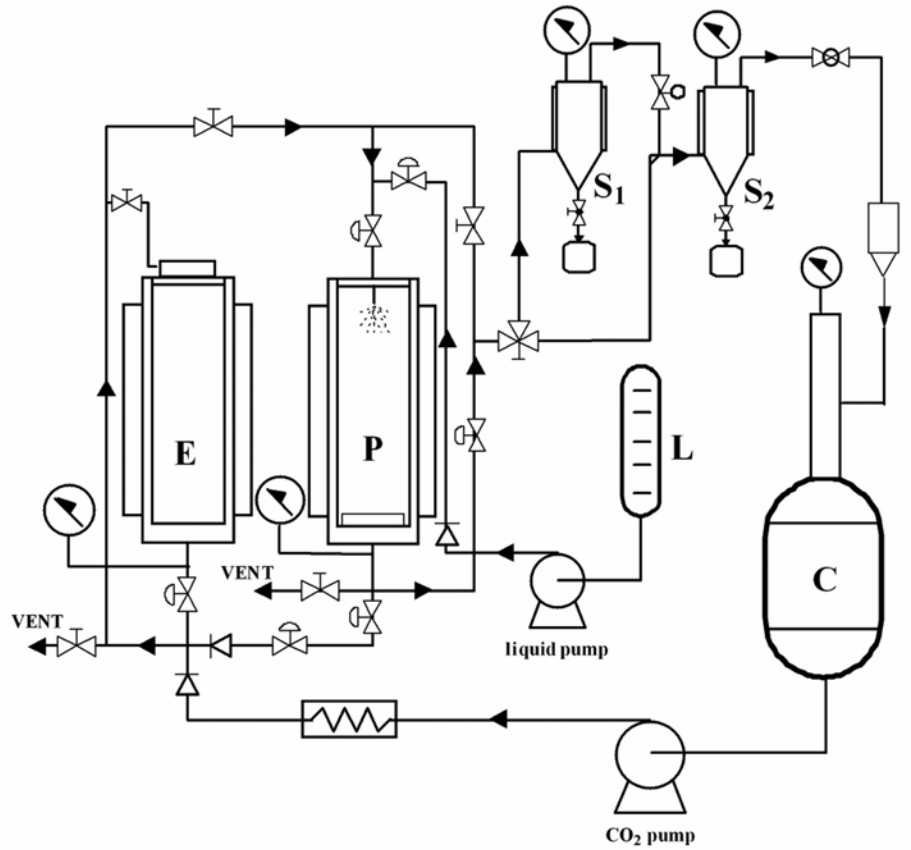

Figure 9. Schematic representation of SAS semi-industrial scale apparatus: C) CO_2 storage vessel; L) liquid solution; E) extraction vessel; P) precipitation vessel; S_1, S_2) liquid separators

4.2.1.3 SAS process description

An SAS experiment begins by delivering CO_2 to the precipitation vessel until the desired pressure is reached and a steady antisolvent flow is established. Pure liquid solvent is then sent through the nozzle to the vessel at the operating flow rate. This procedure is aimed at obtaining steady-state composition conditions during the solute precipitation. Then the liquid solution is delivered through the nozzle at the same flow rate. The experiment ends when the delivery of the liquid solution to the vessel is interrupted. However, supercritical CO_2 continues to flow for a certain time to wash out from the vessel the residual content of solvent solubilized in the supercritical antisolvent. When the washing process is completed, the CO_2 flow is stopped, and the vessel is depressurized down to atmospheric pressure.

The GAS procedure is different from the SAS experiments: the liquid solution is placed inside the vessel that is then filled with supercritical CO_2 up to the desired pressure. The first part of the process is operated in batch mode allowing CO_2 to pressurize and diffuse inside the sample; then, the micrometering valve is opened and the operation is performed

with a constant CO_2 flow rate. A more detailed description of the procedure of SAS experiments can be found in (I, II, IV, V).

4.2.2 Micronization by supercritical assisted atomization (SAA) technique (III)

4.2.2.1 SAA laboratory plant

The SAA apparatus is represented schematically in Figure 10 and consists of two high-pressure pumps that deliver the liquid solution and CO_2 to a heated bath and then to the saturator. The saturator, or mixer, (M) is a high-pressure vessel loaded with stainless steel perforated saddles. The solution produced in the saturator is sprayed through a thin-wall 80 μm diameter injection nozzle into the precipitator (P). N_2 is taken from a cylinder (N), heated in an electric heat exchanger (H) and sent to the precipitator to assist evaporation of the liquid droplets. The precipitator is an electrically heated stainless steel vessel operating at atmospheric pressure. A stainless steel frit located at the bottom of the precipitator allows the powder collection. A condenser (S) located after the precipitator is used to recover the liquid solvent. Manometers, temperature controllers and thermocouples complete the apparatus. Further details are described in (III) and some information about the plant can also found elsewhere [230,247,252,290].

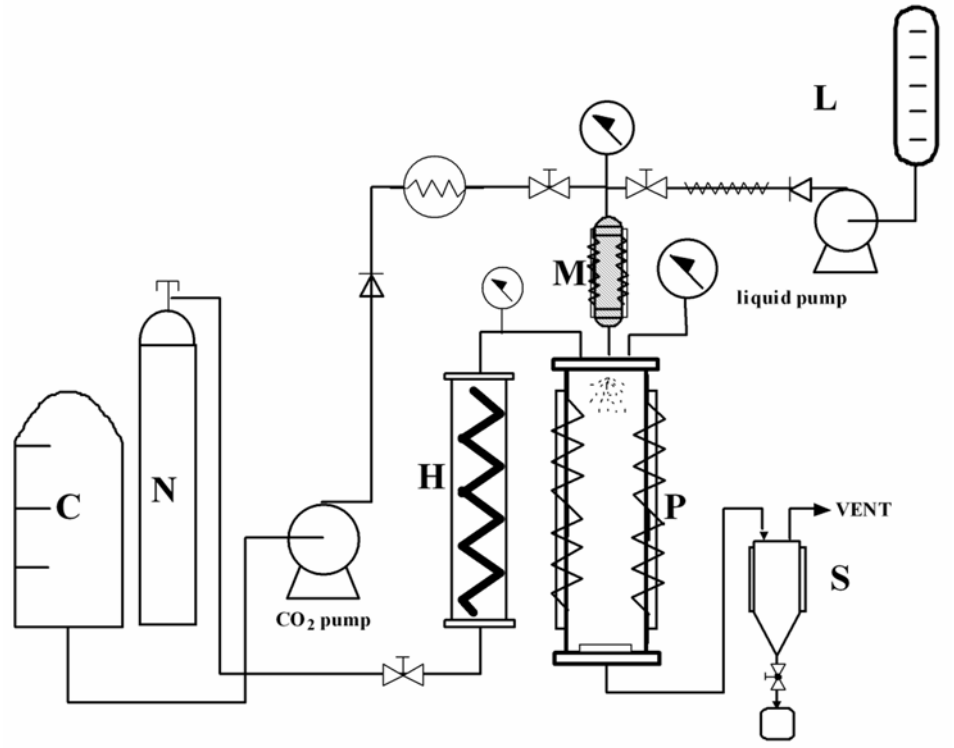

Figure 10. Schematic representation of the SAA apparatus: C) CO_2 cylinder; L) liquid solution; N) N_2 cylinder; H) heat exchanger; M) saturator; P) precipitator; S) condenser.

4.2.2.2 SAA process description

The SAA process is based on the use of a packed saturator characterized by a large contact surface between liquid solution and CO_2 and a long residence time. Thus, the dissolution of the gaseous stream in the liquid solution is promoted up to near-saturation conditions. In the saturator, SC-CO_2 dissolves in the liquid solution (formed by an organic solvent or water and a solid solute) before the atomization in a thin wall injector, and forms the ternary solution.

SAA takes advantage of two mechanisms of atomization, namely pneumatic atomization and decompressive atomization illustrated in Figure 11. *Pneumatic atomization* takes place when a high-speed fluid flow pushes the solution into the injector. Droplet dimensions depend on the saturator pressure, injector diameter and geometry as well as some parameters, such as flow rates, viscosities and surface tensions of the two fluids. This is also the principle of the generic gas atomization process [289]. *Decompressive atomization* is caused by the mixing between CO_2 and the solvent, and the efficiency of the atomization is due to the fast release of CO_2. The rapid depressurization, occurring while passing from the high-pressure saturator into the low-pressure precipitator, leads the CO_2 moving from the liquid solution in which was dissolved into the gaseous state. The fast expansion from inside to outside of droplets enhances their fragmentation. The secondary droplets are then rapidly dried by warm N_2 causing the micrometric and submicrometric particle precipitation.

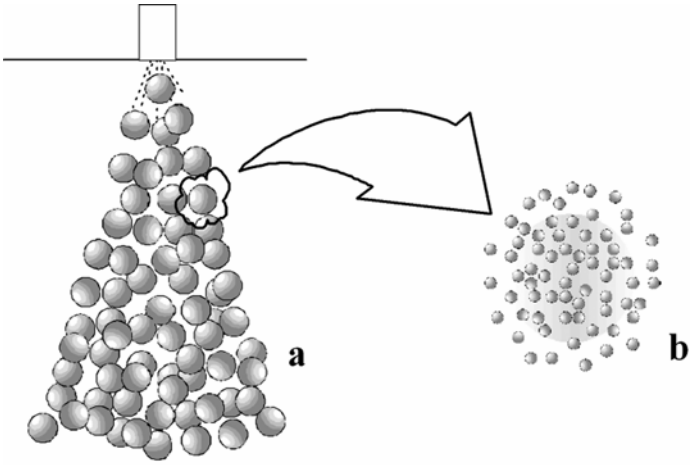

Figure 11. (a) pneumatic atomization; (b) decompressive atomization

A more detailed description of the procedure of SAA experiments can be found in (III).

4.3 Analytical methods

4.3.1 Powder morphology

4.3.1.1 Scanning Electron Microscopy (I-V)

Samples of the powder precipitated in all the experiments were observed by a Scanning Electron Microscope (SEM). Several SEM images from different parts of the precipitation vessel were taken for each run to verify the powder uniformity. Details can be found in (I-V).

4.3.2 Particle size distribution

4.3.2.1 Image analysing (I-V)

Particle size and distribution of all the compounds were evaluated from SEM images using the Sigma Scan Pro Software. Histograms representing the particle size distributions were best fitted using Microcal Origin Software. Details can be found in (I-V).

4.3.2.2 Laser scattering diffraction (III)

Particle size analysis of cromolyn sodium was also performed by a laser diffractometer using the laser scattering (LS) technique. The smallest particle diameter detectable with the instrument is 0.05 μm. Measurements on each sample were repeated at 2 min intervals for 20 min. Details and the procedure can be found in (III).

4.3.3 Drug purity and degradation

4.3.3.1 Liquid chromatography (III, IV)

Purity and degradation of nalmefene HCl was evaluated by HPLC (high performance liquid chromatography) analysis (IV). Duplicate analyses were performed on each batch of SAS processed nalmefene HCl. Degradation of cromolyn sodium was evaluated by HPLC-UV/vis analysis of the untreated material and SAA processed powder (III), using a method according to Radulovic et al. [291,292]. Details and methods can be found in (III, IV).

4.3.3.2 Headspace gas chromatography (IV)

EtOH residue in untreated and micronized nalmefene HCl was measured by a headspace (HS) sampler coupled to a gas chromatograph (GC) equipped with a flame ionization detector. GC conditions were as described in the USP [293] with some minor modifications (IV). Analyses were performed on each batch of processed drug in triplicate. Details and methods can be found in (IV).

4.3.4 Drug crystallinity and structure

4.3.4.1 X-ray analysis (I, III-V)

The X-ray diffractograms of the powder samples were recorded using a diffractometer operating at 40 kV and 20 mA with Ni-filtered CuKα radiation operating at room temperature. Samples were placed in the holder and flattened with a glass slide to ensure a good surface texture. The measuring conditions varied depending on the compound analysed. Details and methods can be found in (I, III-V).

4.3.4.2 Differential scanning calorimetry (I-V)

Thermograms of processed compounds were recorded on a differential scanning calorimeter (DSC) using Mettler STAR^e system. The temperature axis and cell constant of DSC were previously calibrated with indium standard materials. Powder sample was accurately weighed into an aluminum pan and an empty aluminum pan was used as a reference. Different analytical conditions were used depending on the compound. Details and methods can be found in (I-V).

4.3.4.3 Thermogravimetric analysis (IV, V)

The presence of solvates or hydrates in the powders was determined by thermogravimetric analysis (TGA). The solvent/water loss was determined by placing the sample in alumina crucibles and heating it with a temperature program different for each compound. The thermobalance was coupled to a mass spectrometer (V). Details and methods can be found in (IV, V).

4.3.4.4 Fourier transform infrared spectroscopy (V)

The spectra of adipic acid were obtained by using a FT-IR (Fourier transform infrared) spectrometer at a resolution of 0.5 cm^{-1}. Scan signals were averaged to reduce the noise. The samples were ground and mixed thoroughly with potassium bromide (KBr) as an IR transparent matrix, and prepared in form of compressed discs. Details and method can be found in (V).

5 RESULTS AND DISCUSSION

The research work presented here has been carried out using SCF-based micronization techniques, where mainly SC-CO_2 has been used as antisolvent or alternatively as co-solute. The SAS process has been reproduced with different plants and at different scales, and the process has been scaled-up from the laboratory scale to the semi-industrial scale. The application to pharmaceutical or related compounds and a food ingredient as well as the use of different solvents are presented. In addition, the possible modifications of the micronized compounds have been studied in terms of particle size and distribution, polymorphism, crystallinity and amorphousness.

5.1 Control of particle size and spherical morphology in SAS process (I, II)

A successful micronization by SAS requires that the compound to be micronized is not soluble in SC-CO_2 and that the solvent used to solubilize the solid compound has a good affinity with SC-CO_2. For nalmefene HCl it was possible to verify in a previous study that it is not soluble in SC-CO_2 using a variable-volume view-cell [294]. Moreover, the precipitation of crystals from the system already at 45°C and 100 bar was observed, which provided also the information that for the given ternary system, antisolvent precipitation takes place in the liquid phase. When this kind of study cannot be performed, it is necessary to perform some micronization experiments in order to scan the different operating conditions corresponding to different phases in the thermodynamic equilibrium of the system. The VLEs of the binary system solvent-antisolvent are a good starting point for identifying the phase equilibrium at which the precipitation takes place and the corresponding operating conditions [295,296]. The more common VLE behaviour of two components in a mixture is represented by a type I system, according to the classification of Van Konynenburg and Scott. It is usually reported on isothermal pressure-molar fraction diagrams (P-x, as in Figure 12 and Figure 14) in which a closed loop envelops the two-phase region. The maximum of the two-phase equilibrium curve is the mixture critical point (MCP).

In the micronization of nalmefene HCl (I) it was supposed that nano-particles were obtained in the supercritical region well above the MCP of the mixture solvent-antisolvent. Indeed, the irregular spherical shape of the particles suggests a generation by a gas-to-particles mechanism in absence of liquid surface tension. The coalescence of the particles supported this hypothesis, and it may be related to the particle-to-particle interactions developed in the colloidal suspension during the precipitation (I, II). The behaviour of nalmefene HCl presented in this work, has been also discussed with similar behaviour of

other compounds by Reverchon et al. [101]. In the case of PVA (II) the windowed precipitator allowed to observe the precipitation of this kind of particles from a "fully developed" supercritical phase. The colour of the fluid phase was transparent when only solvent and antisolvent were present and changed to orange when precipitation started. The colour transition is related to the sudden change of the refractive index that is characteristic of the passage through a critical point. Moreover, the intensity of the colour depends on how close the conditions are to the MCP. The same phenomenon can be observed also for pure CO_2 [297]. The same behaviour was observed for the binary system DMSO-CO_2 when some experiments with a variable volume cell were performed [298]. The jet at the exit of the injector was very short (1-2 mm) and sometimes not visible at all.

Submicro- and microparticles of nalmefene HCl and PVA were produced when the SAS operating point was near the corresponding MCP at given temperature, and their spherical regular shape suggests that they were formed from droplets produced by the atomization of the liquid jet and characterized by their surface tension. The particles were dried during the precipitation by the supercritical medium (I, II). Using the windowed vessel, the hypothesis of precipitation from near-critical or sub-critical conditions developed for nalmefene HCl (I) was confirmed for PVA (II). The powder precipitated from a homogeneous transparent gas phase, and the fluid became dark during the precipitation. A liquid jet was visible near the injector and the large quantity of particles drifting in the system was responsible of the obscuration of the fluid phase.

A different kind of precipitation takes place when the operating point is positioned in the two-phase region of the VLE diagram. At these conditions, there are two phases in equilibrium: a liquid phase and a gas phase. The solute is partitioned between the two phases in relation to its affinity with them. From the gas phase, spherical submicro- and microparticles with the same characteristics as those produced near the MCP are formed. From the liquid phase either crystals can be formed, like for nalmefene HCl (I) or a cake of material, like for PVA (II). The windowed vessel allowed to see the distinct meniscus between the liquid phase on the bottom and the gas phase above it. Moreover, by varying SC-CO_2 flow rate it was also possible to see how the volumetric ratio between the two phases changed. The precipitation of the solute was observed from both phases.

For the formation of a "droplet confined" liquid expanded solution [102], balloons of nalmefene HCl and PVA of several tenths of microns in size were generated at sub-critical conditions far from the MCP. The liquid surface tension keeps the droplet shape that expands until super-saturation is reached on the surface, where the precipitation starts (I, II). The mass transfer in and out the droplet is slower than at supercritical conditions.

Droplets are therefore first expanded and then dried [16]. Using the windowed vessel it was noted that during the precipitation the fluid is grey also in this case, as was observed in the case of formation of microparticles, but the colour is darker. The obscuration of the fluid may be due to the formation of larger particles.

5.2 Understanding of the modification of the VLE by the solute in SAS process (I, II, IV, V)

Predicting particle formation on the basis of binary VLE diagrams is not conclusive, because when the third component is added to the system, it contributes to the VLE with different influences depending on the structure of the solute itself and the kind of solvent used. The choice of solvent is also related to this observation, and the use of DMSO in many works (see literature review chapter) is not causal; indeed, it has a good affinity with SC-CO_2 and does not have a strong interaction with a relevant number of solid compounds. However, also in this case the solute may modify the VLE diagram [103].

The influence of PVA on the system DMSO-CO_2 was investigated by visual observation through the windowed precipitation vessel (II). In particular, in the experiment performed at 120 bar 60°C, X_{CO_2}=0.98 precipitation from one homogeneous subcritical phase was observed, although the operating point falls in the two-phase region of the VLE diagram of the binary system (Figure 12). The adding of the solid compound enlarges the two-phase region, because the affinity solvent-antisolvent is decreased. Increasing the percentage of PVA in the system, this behaviour is intensified and it is evident that the MCP of the system moves to higher pressures (II). In Figure 12, which reports data previously calculated [298], the continuous line represents the interpolation of experimental data for DMSO-CO_2 VLEs at 60°C. The discontinuous lines represent the hypothesized equilibrium lines formed when the third, solid component, PVA is added to the system. The symbols are the operating points at different process conditions and, depending on the concentration, the operating points have to be considered with respect to the correspondent curve. In particular it is interesting to note that at 140 bar, 60°C, X_{CO_2}=0.99 (▲) the point changes its position compared to the MCP and moves away from it at the increase of concentration. In the same way at 120 bar, 60°C, X_{CO_2}=0.99 (x) decreasing the concentration, the point is nearer to the two-phase region. At very low concentration, the operating point could be positioned inside the envelope.

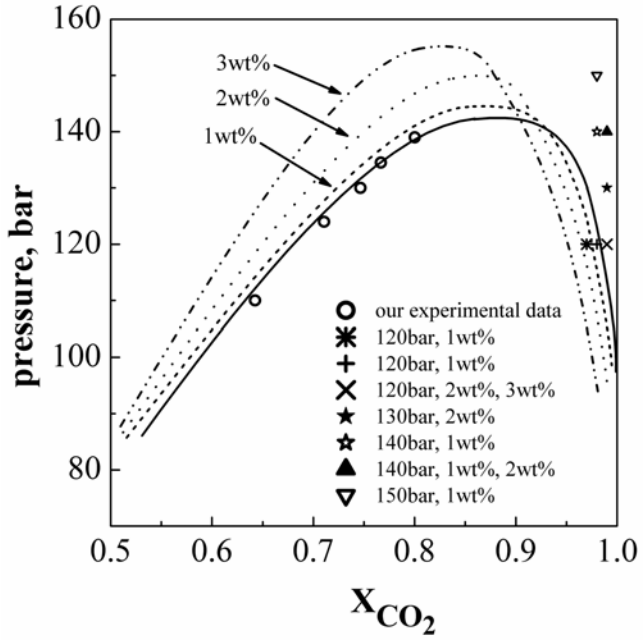

Figure 12. Interpolation of experimental data for DMSO-CO_2 VLEs at 60°C (continuous line) [298] and curves at different concentrations of PVA in DMSO (discontinuous lines) at the same temperature. The symbols indicate operating points of some experiments performed in (II) and the molar fraction can be read on the abscissa.

EtOH is not often used in the SAS technique because of its strong interaction with the solute; indeed, during the micronization of nalmefene HCl (I, IV) two main observations are evinced. First, the VLE of the system EtOH-CO_2 is modified by the solute and the equilibrium curve corresponding to 60°C moves towards the curve corresponding to higher temperatures (I). Second, the solvent modifies nalmefene HCl form from hydrate to anhydrous (IV).

The influence of adipic acid on the system acetone-CO_2 is different and cannot be explained with the simplified VLEs used in the other cases (V). Large crystals are obtained at 40°C and different pressures, corresponding also to operating points far from the two-phase region where supercritical or subcritical conditions were expected (Figure 14). A possible explanation of this fact is that due to the partial solubility of adipic acid in the mixture SC-CO_2 – acetone, adipic acid is partly solubilized in the fluid phase until supersaturation occurs. This kind of high pressure ternary system can be defined as an expanded liquid [109,299,300]. In this hypothesis, adipic acid precipitates from a ternary solution solvent-antisolvent-solute, and a liquid-like nucleation and growth process is obtained. The process is mainly controlled by the residence time of the solute in the precipitator and is different from the one that produces nanoparticles from supercritical solutions (I, II) [101].

The same behaviour is noted using EtOH as solvent (unpublished data). At the same operating conditions at which nalmefene HCl precipitated as spherical microparticles, that is 150 bar and 40°C (I), adipic acid precipitated in the form of needle-like crystals several millimetres long (Figure 13). The same results were obtained at different pressures, as in the SAS precipitation form acetone.

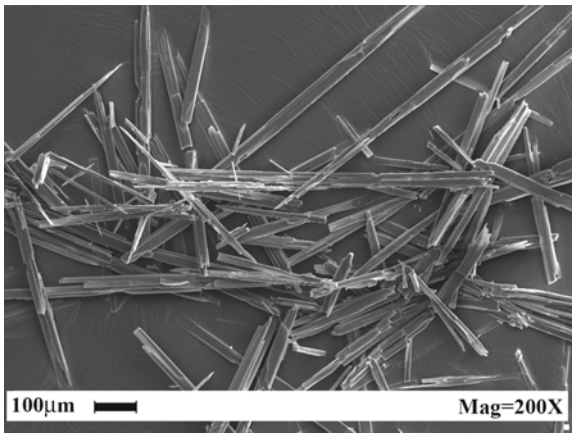

Figure 13. SEM image of adipic acid precipitated by SAS from EtOH at 40°C, 150 bar.

The binary systems EtOH-CO_2 and acetone-CO_2 are different, but in both cases, at the operative conditions used in the experiments, the system should be in supercritical conditions well above the MCP (Figure 14). The adipic acid influences the systems in the same way, and a high pressure expanded liquid region is formed in both cases (V).

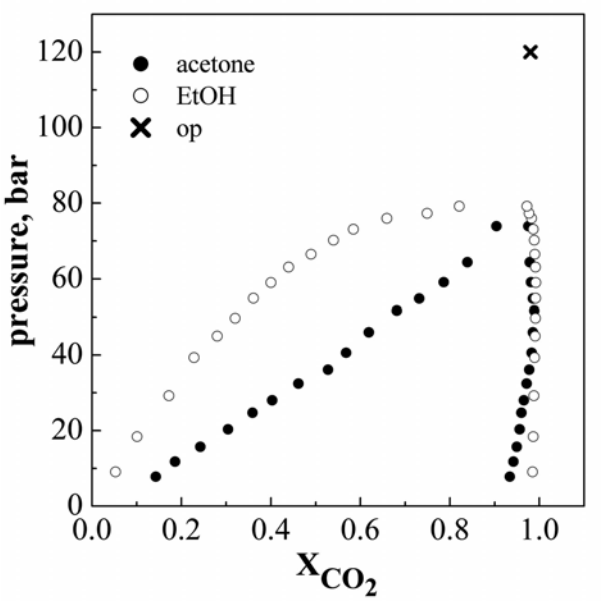

Figure 14. Original representation of experimental data from Day et al. [301] for EtOH-CO_2 and acetone-CO_2 VLEs at 40°C; op = operating point.

5.3 Role of the injector in the succesful particle production by SAS process (I, II)

In the SAS process, the injector has a key in successful micronization because it increases the contact surface between the liquid solution and the antisolvent, thus improving the mass transfer.

In the case of PVA, if the micronization was performed at supercritical conditions, the shape and the size of the injector had no influence on the final size of the particles because no droplets were formed (II). If the micronization was performed in the region near the MCP, the size of the injector influenced the particle size and distribution as a consequence of the droplets formed and dried. When the injector had not the proper diameter size, there was no jet break-up and filaments were formed (II). In Figure 15 the different kinds of observed jets are reported. To obtain jet break-up using a larger injector, CO_2 flow rate has to be increased. This is sometimes difficult with a laboratory scale plant, but can be easily performed when operating on a larger scale. In the pilot plant used for nalmefene HCl micronization, a tube-in-tube injector is used which is 10 times larger than the one on the laboratory plant, and a CO_2 flow rate of more than one order of magnitude higher than in the laboratory scale plant is used. Nalmefene HCl micronized particles have been successfully obtained, which have a larger particle size and distribution when compared to the one obtained using the laboratory plant, mainly because of the different efficiency of the tube-in-tube device (I).

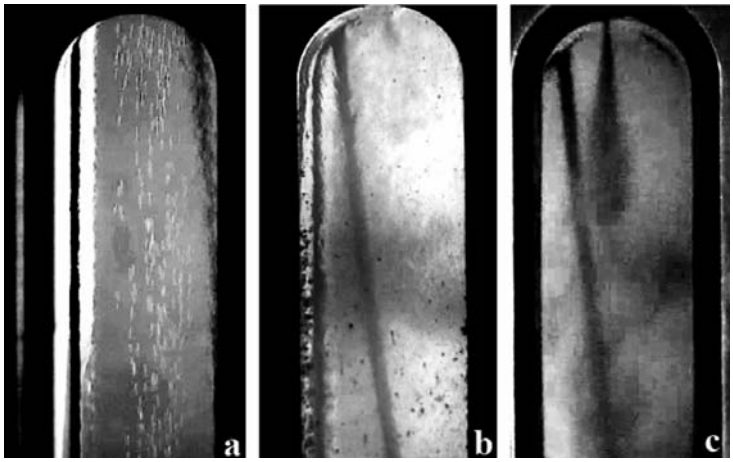

Figure 15. Jet-break up at different conditions: (a) droplets of liquid solution in absence of atomization; (b) short spray only just visible at supercritical conditions; (c) long spray visible at subcritical conditions.

If no injector is used in the antisolvent process, the atomization step is not performed, as in the case of the batch process (GAS) used for the PVA micronization. No droplets are

generated, because the particles are obtained by supersaturation of the expanded liquid (II). It can be described as the formation of a disperse phase rich in polymer in an expanded liquid continuous phase. In this case the particles are spherical like in the SAS semi-continuous precipitation, since the disperse phase is organized in droplets. The formation of particles with a narrow particle size distribution is related to the absence of the typical droplet size distribution obtained in a spray and to the stability of the growth environment during the expansion.

5.4 Control of degree of crystallinity in SAS process (I, II, IV, V)

The particles produced by the SAS micronization technique presented different degrees of crystallinity depending on the operating conditions, the micronized compound and the solvent used. PVA, which is a polymer, did not modify its structure and maintained the semicrystalline form in all the obtained particles: nanoparticles, submicro- and microparticles and balloons (II).

Nalmefene HCl, on the other hand, showed a high crystallization rate in EtOH. At subcritical conditions and conditions near the MCP, particles with different degrees of crystallinity were formed (from amorphous to 70% crystallinity), depending on the process temperature (I) or the CO_2 molar fraction (IV). These two factors influence the crystallization rate of nalmefene HCl in EtOH. High temperatures and high CO_2 molar fractions seem to favour the kinetics. In supercritical conditions, amorphous particles of nalmefene HCl were formed, because the precipitation was so fast that the particles could not organize in regular crystalline structure. The formation of amorphous particles is typical for SAS micronization [9].

Adipic acid processed using acetone (V) and EtOH as liquid solvents was always obtained as crystalline needle-like particles, independently of the solvent or the operating conditions. This fact might be related to the precipitation performed in the same phase conditions, because of the influence on the system already discussed in the previous paragraph.

5.5 Polymorphic, pseudo-polymorphic and crystal habit modifications in SAS process (IV, V)

SAS micronization also affected the crystalline structure of nalmefene HCl particles and pseudo-polymorphic modifications have been observed. The system CO_2-EtOH showed to have the property to modify the monohydrate form of nalmefene HCl to anhydrous form during the SAS process (IV). A possible explanation is that the water molecules linked to the monohydrate nalmefene HCl were involved in the system solvent-antisolvent during

the antisolvent process, and were removed perhaps because EtOH can solubilize a small amount of water. The solvent-antisolvent ratio, that is the relative amount of CO_2 and EtOH in the system, affects the role played by the solvent during the micronization. By decreasing the quantity of CO_2, the influence of the solvent on the final compound form increases. At low X_{CO_2}, a solvate form can be obtained, which has a different pseudo-polymorphic form (IV).

Similar results were obtained in the case of rifampicin, micronized using several liquid solvents and varying the operating conditions (Table 6). At different operating conditions, and using EtOH as liquid solvent, rifampicin with different crystal habits was obtained [302].

Table 6. Operating conditions used for micronization of rifampicin by SAS [302]

solvent	**Conc** ***(wt%)***	**T** ***(°C)***	**P** ***(bar)***	$\mathbf{X_{CO_2}}$	$\boldsymbol{\rho_{CO_2}}$ ***(kg/m³)***	**Flow rate sol** ***(mL/min)***	**Flow rate** $\mathbf{CO_2}$ ***(kg/h)***
EtOH	0.63	60	130	0.98	505.62	1.5	1.78
	0.63	60	125	0.98	473.13	1.5	1.78
	0.63	60	150	0.98	602.51	1.5	1.78
	0.63	40	150	0.98	780.06	1.5	1.78
EtAc	2.22	40	150	0.98	780.06	1.5	1.78
MeOH	2.53	60	150	0.98	602.51	1.5	1.78

The effect of the operating pressure on rifampicin crystalline structure has been studied at 60°C and the experiments have been performed at 125, 130, 150 bar. Pressure affected the crystal habit of the product; in particular spherical particles have been obtained at 130 bar (Figure 16b), and at 150 bar, two different crystal habits coexisting in the same precipitate powder were produced (Figure 16c). The DSC and the XRPD analyses showed no differences in the traces of the micronized powders obtained at the conditions reported. Thus, the pressure did no affect the polymorphism of the micronized rifampicin.

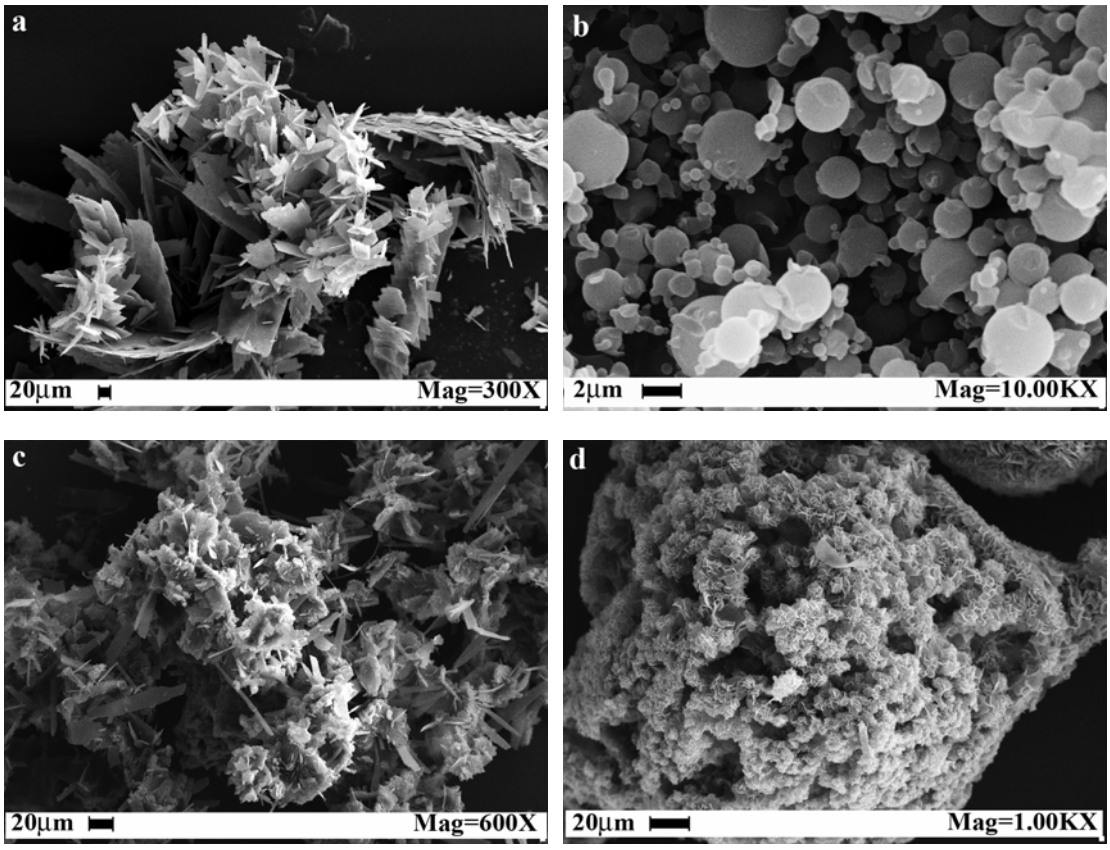

Figure 16. SEM images of rifampicin precipitated from EtOH at 60°C, 20 mg/mL and (a) 125 bar, (b) 130 bar, (c) 150 bar; (d) 40°C, 20 mg/mL and 150 bar.

The effect of the operating temperature on rifampicin crystalline structure has been studied at 150 bar by performing the experiments at 40°C and 60°C. By decreasing the temperature, different crystals than those previously discussed have been obtained (Figure 16d). They are very thin lamellar crystals joined together in a rose-like shape. DSC thermograms showed one additional peak at 140°C for the micronized powder compared to the untreated, and it can be hypothesized that at 40°C hydrated rifampicin has been produced. XRPD analyses, reported in Figure 17, showed that the particles precipitated by EtOH as solvent had a lower degree of crystallinity compared to the untreated drug.

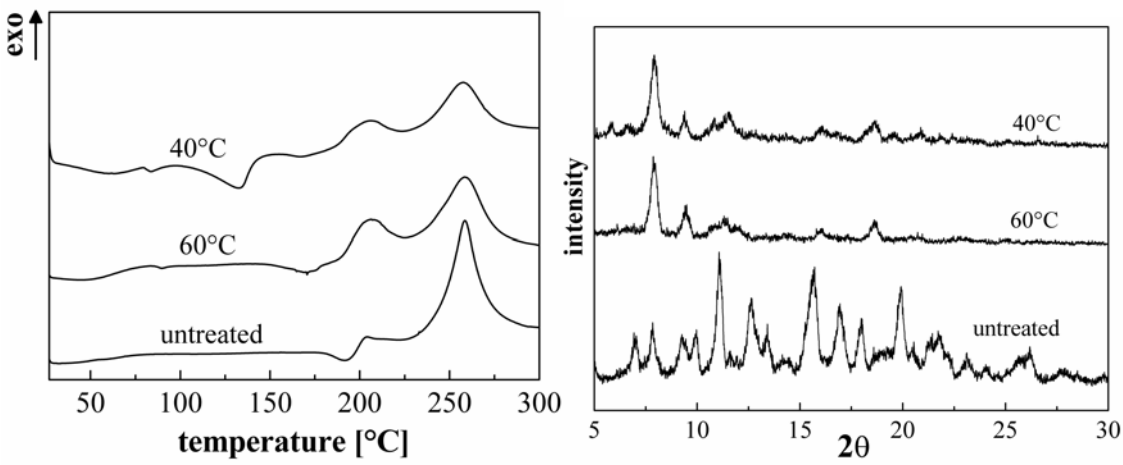

Figure 17. DSC thermograms (left) and XRPD diffractograms (right) of rifampicin untreated and powders precipitated from EtOH at 150 bar and different operative temperatures.

Different solvents proved to play different roles in the micronization of rifampicin by SAS. Indeed, a monohydrate form was obtained by using EtOH, a dihydrate form was obtained by using MeOH and an anhydrous form was obtained by using EtAc. The differences can be noted in the DSC and XRPD results reported in Figure 18. Different operating conditions have been chosen to work in the same region of the VLE diagram, which is different for different solvent-antisolvent systems.

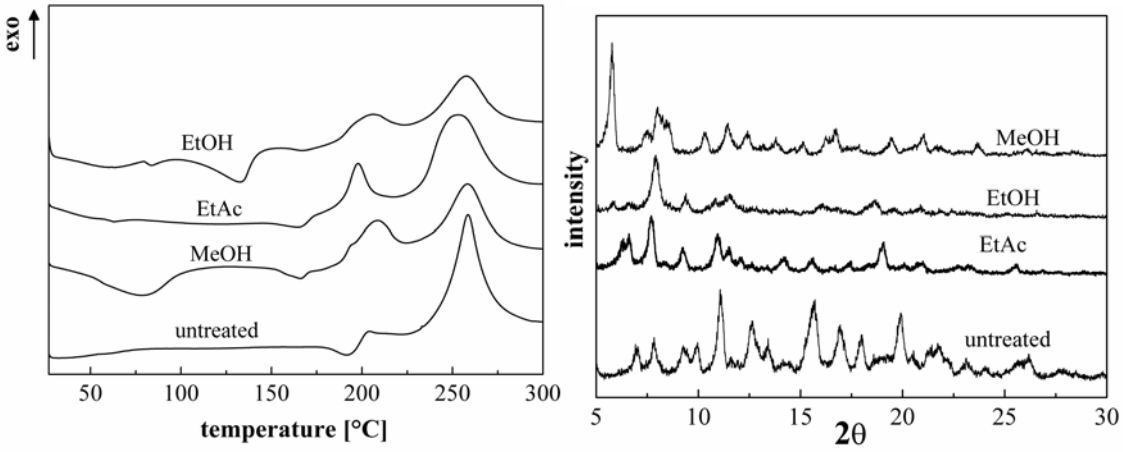

Figure 18. DSC thermograms (left) and XRPD diffractograms of rifampicin precipitated by SAS from different solvents.

The effect of using EtOH as solvent is opposite to the one observed for nalmefene HCl (IV). In the SAS micronization of rifampicin, it favoured the formation of a monohydrate, while in the case of nalmefene HCl, the formation of the anhydrous was obtained. This clearly suggests that the pseudo-polymorphic modifications that might take place during SAS

micronization are related to the specific solvent-antisolvent-solute system and may vary with the different compounds to micronize.

The effect of the SAS process on adipic acid crystals was to modify their shape and to produce more regular structures (V). Untreated adipic acid was composed of boulder-like crystals with irregular shapes and a large size distribution. With SAS micronization, using acetone (V) and EtOH (unpublished data, Figure 13) as solvents, regular needle-like shaped crystals were obtained. The addition of urea as crystallization modifier to the system modified the crystal habit and the polymorphism of the precipitated adipic acid. Urea inhibited the growth of the needles. Prism-like crystals, with the smallest size obtained at an optimal urea concentration of 12.5 wt%, or spherical particles, with the surface composed of very small crystals, were formed. The choice of urea as crystal modifier was due to its capability to form hydrogen bonding with carboxylic groups of adipic acid, thus inhibiting the growth of the crystals along the direction of such groups. In Figure 19 the morphology, faces and orientation of adipic acid crystals are schematized following the indications in literature [303]. The C face is made of carboxylic groups and is the one that grows in the needles formation. Urea, attaching to these groups, facilitates the growth of the faces A and B, usually slower compared to face C, with the formation of prismatic crystals. The crystal size is reduced from a length ranging between 100 and 750 μm to 2.5-30 μm with a mode of 7.5 μm. The width is approximately one order of magnitude smaller than the length (V).

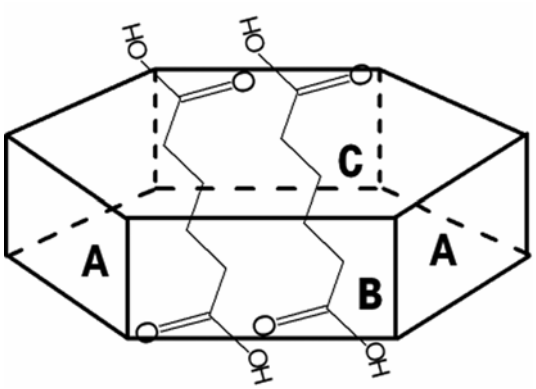

Figure 19. The morphology, faces and orientation of the molecules with respect to the faces of adipic acid crystals, original interpretation from Michaelis [303]. The linear six-carbon chains are perpendicular to the C face so that the face is made up entirely of carboxylic groups.

In the case of spherical particle formation, urea acts as growth inhibitor of adipic acid during the "droplet confined" crystallization, producing particles with the surface formed by small prismatic crystals of less than 1 μm in length. Upon crystallization, urea is partially extracted by CO_2 in the final washing step of the process, and the remaining part forms a

solid mixture with adipic acid. The urea is not incorporated into the crystal structure, but adipic acid is probably transformed to a new crystal polymorph (V).

5.6 Scale-up of the SAS process from the laboratory to the semi-industrial scale (I, unpublished results)

Scale-up can be considered successful if a compound micronized with the laboratory scale plant can be micronized with the pilot scale plant with complete reproducibility of the results. Moreover, the productivity has to be high and increased when compared to the smaller plant.

The scale-up of the SAS process has been performed in a semi-industrial plant mainly used for SCF extraction processes. Hence, it has been necessary to modify it for the SCF micronization process. The plant was already constructed with a view to this possibility. Consequently, few changes have been done. There is also the added advantage that the versatility of the plant was increased. The two vessels used for the extraction were modified by isolating one of them from the rest of the plant and adding the tube-in-tube injection device to the other one. A pump for liquid delivery was added to deliver the liquid solution in the line terminating with the inlet of the tube-in-tube injector. CO_2 was delivered to the outer part of the tube-in-tube injector using the existing line. The outlet of CO_2 was also changed: it was previously on the top of the vessel (Figure 20a), but this configuration generated hard to control fluidodynamics, with the formation of vortexes and stagnation points inside the vessel. Therefore, the outlet was placed on the bottom of the vessel, below the filter for the deposition of the powder (Figure 20b).

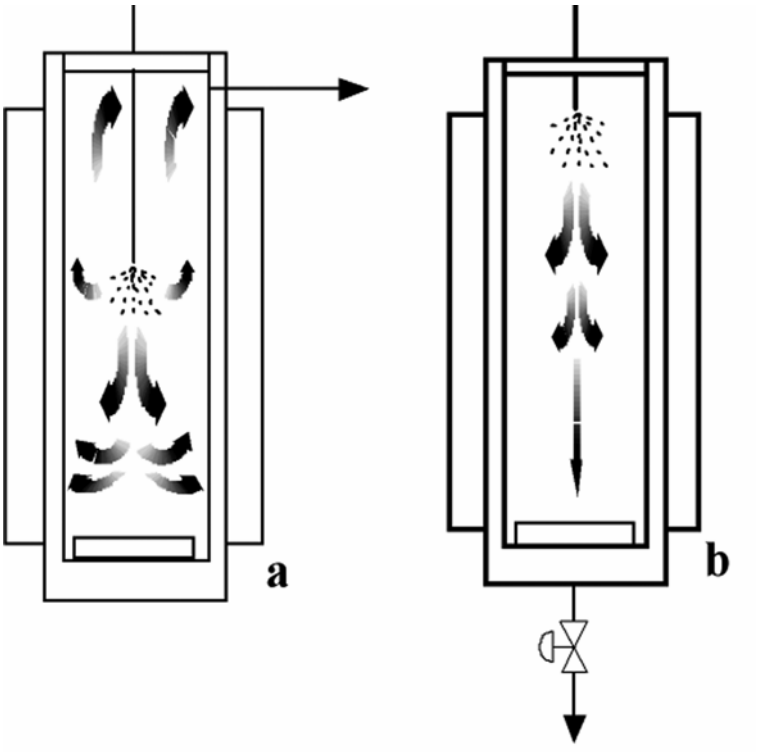

Figure 20. Precipitation vessel of the semi-industrial SAS apparatus before (a) and after (b) the modifications. Representation of the flow.

From a design point of view, the most relevant differences between the laboratory and the semi-industrial plant are the precipitation vessel volume, which is about 20 times bigger, the L/D ratio, which is about 1.3 times larger, and the injector device, which has a final injection section that is 3.75 times larger in the semi-industrial plant. A related difficulty was the big difference between laboratory and semi-industrial apparatuses in terms of size; indeed, the process had to be scaled-up 10 times. This might lead to problems in maintaining uniform temperature in the precipitation vessel and constant pressure during the process. An electronic valve was used to control the pressure in the vessel and CO_2 flow rate in relation to the delivery pump, assuring a precise and accurate control. The precipitation vessel was heated by a mineral oil jacket, which guarantees uniform temperature during the precipitation.

The scale-up of the SAS micronization process was performed by choosing the operating conditions that have given the best results on the laboratory scale plant. For the scale-up, the feed ratio solution/antisolvent (R) was the constant parameter, and the CO_2 flow rate was increased by about 12 times according to the capacity of the semi-industrial plant compared to the laboratory plant and the increased section of the injector (I). As a consequence, the solution flow rate was increased according to R.

All the above-mentioned differences between the laboratory scale and the semi-industrial scale had an influence on one of the important parts of the SAS process: the washing step, that is, the flowing of CO_2 in the vessel after the micronization to remove the residual solvent from the vessel. The time required for this step is related to the volume of the precipitation vessel and to the CO_2 flow rate and on the large scale has to be recalculated. The precipitation vessel was considered as a continuous stirred tank reactor (CSTR) [288] and the washing time (1) was calculated as 98% of the total time to completely eliminate the solvent from the vessel.

$$t_r = -\tau \cdot \ln(0.02) \qquad (1)$$

$$\tau = \frac{V}{Q}$$

$\tau = stabilization\ time$

$V = vessel\ volume$

$Q = flow\ CO_2\ from\ vessel$

The washing time was calculated per each experiment, because it depends on the CO_2 flow rate. The scale-up was successful (I) and at the end of the experiments the powder was distributed in the precipitation section:

- on the walls of the inner vessel
- on the bottom
- blocked in filter bottom meshes and slits.

When the quantity of nalmefene HCl processed was increased, it was noted that the same amount of material was always lost. Increasing the production could then make the percentage of loss negligible.

After the successful reproduction of nalmefene HCl powder at semi-industrial scale, it was interesting to reproduce also data found in literature. Salbutamol sulphate was micronized by Reverchon et al. [192] by SAS on laboratory scale using several solvents. The test performed at 145 bar, 50°C and 1 mg/mL (salbutamol sulphate in solution of EtOH/water 90:10 v/v) has been reproduced with the semi-industrial plant and the same shape of crystals has been obtained (Figure 21).

It was proved that the parameters used to scale-up the process in the case of nalmefene HCl could be applied also to other compounds.

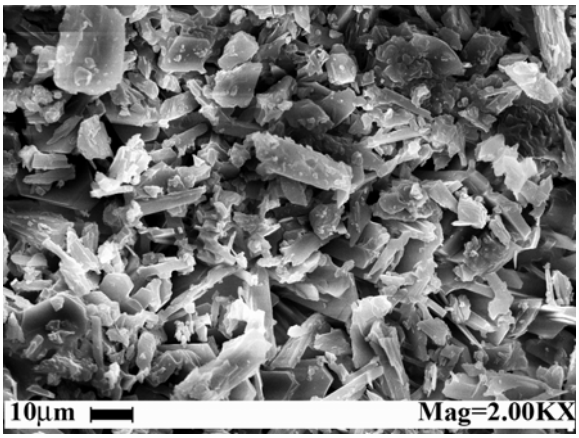

Figure 21. SEM image of salbutamol sulphate precipitated from EtOH by SAS at 145 bar, 50°C, 1 mg/mL using the semi-industrial plant.

5.7 Micronization by SAA as complement to SAS (III)

The choice in this thesis to work also with the SAA technique, which may be considered as complementary to SAS, was linked to the need to have a more complete understanding of SCF-based micronization techniques. These two techniques have been already used to micronize the same compound, the pigment disperse red 60 [304]. It has been shown that with SAS nanometric particles were produced with a low yield, while near-micrometric crystalline particles were produced with SAA with higher yield. From a general point of view, the results were considered complementary.

Cromolyn sodium belongs to the category of compounds that cannot be successfully micronized using SCF antisolvent techniques, because it is slightly soluble in organic solvents, but freely soluble in water (see chapter on materials and methods). Cromolyn sodium was micronized with the SAS technique in other works, using a mixture water/MeOH (6%wt) and the obtained particles, with a size of 0.1-20 µm, required post processing in the oven for 20 hr to eliminate the residual solvent [156,305]. In the present work, using the SAA technique (IV), amorphous spherical particles were obtained, and it was possible to control the particle size and distribution in the range used for aerosol delivery systems without any post processing of the final product. The use of water guarantees a real solvent-free process, which can be applied to pharmaceutical compounds but also to food ingredients that also have strict regulations about the use of solvents in their processing [7,112-114].

The precipitation temperature is a parameter to be carefully controlled during the process. Indeed, too low temperatures do not guarantee the drying of the particles and too high temperatures can degrade the compound, as in the case of cromolyn sodium processed at

140°C (III). The temperature chosen depends on the solvent used and on the compound to be micronized. Varying the concentration of the compound in water is possible to control particle size and distribution: with an increase in concentration, particle size increases and particle size distribution enlarges. Cromolyn sodium particles obtained by SAA were amorphous at all the operative conditions tested, differently from the SAS technique, in which it was possible to control the degree of crystallinity by varying the operating conditions (see paragraph on crystallinity). The reason for this is that the precipitation by SAA is so fast that the material has no time enough to rearrange on both the nanometric and the micrometric scale. This fact tends to stabilize the amorphous character of the powders. The amorphous structure of the powders was stable in closed vials at room temperature for at least 12 months, which is the time period that has been monitored until now.

The SAA process, similarly to SAS, had an effect on the solid state of cromolyn sodium, in particular on the amount of water molecules entrapped in the structure. Cromolyn sodium has the peculiar attitude to form non-stochiometric hydrates and to absorb and liberate water in a continuous manner. A variable number of molecules of water (as many as nine) can be accommodated in the crystalline structure, depending on the relative humidity [306,307]. Water molecules can accommodate in different ways: part in the interstitial space that can be readily lost, and part combined with sodium ions that are more difficult to be removed [308]. While untreated cromolyn sodium contains water in both positions in the structure, the micronized powders contains water only in the interstitial positions.

Because the process is based on the formation of a spray of the solution and a subsequent explosion of the droplets for the decompressive action of CO_2, the droplet size distribution is responsible of the final particle size. Consequently, it is easier to obtain submicrometric and micrometric particles than nanoparticles. The production of nanoparticles would be possible if some aspects of the process are further improved. For example, it could be possible to vary the operating pressure in the mixer to improve the secondary atomization and to study the high pressure VLEs that are formed for the system solvent-cosolute-solute.

The knowledge of both techniques is important, because the borderline between the antisolvent, co-solvent and co-solute role of SC-CO_2 in the micronization process is sometimes very smooth and completely dependent on the system considered. The antisolvent effect has to be avoided in SAA, otherwise the compound could precipitate in the mixer, and the co-solvent effect has to be avoided in SAS, as otherwise the compound would more likely be extracted than precipitated.

6 CONCLUSIONS

The supercritical antisolvent technique is proved to be an effective micronization technique for pharmaceuticals, food ingredients and related compounds. The rich literature collected shows that the interest in this field is in continuous development, and there is a tendency to apply the technique to more specific fields, for example encapsulation of pharmaceuticals and nutraceuticals or "ready to use" formulations. However, supercritical fluid processing is at present mainly an experimental field, and the real-life process applications are most often only variations from those studied in detail in previous research projects.

In this study, the compounds processed with SAS have been also used as model compounds for the determination of some general considerations regarding the micronization process. The visual observation clearly showed that the morphology and the size of the particles are directly dependent on the high pressure VLEs generated in the system. This finding can be considered generally valid for several systems and can be used to predict the results and to plan preliminary micronization tests. The prediction cannot be complete and definitive, mainly because the interaction between solvent-antisolvent, which can be thermodynamically measured by variable-volume view-cell, is affected by the compound to be micronized. However, with some preliminary specific experiments and some considerations on these, it is possible to guide the process towards the desired target. The possibility to obtain the desired results by adding an additive, for example to control the growth during particle formation, improves the versatility and enlarges the application field of the process.

The SAS process showed a tendency to influence not only the particle size, particle size distribution and morphology of the product, but also the crystallinity, the crystal habit and the pseudo-polymorphism. In particular, solvents such as EtOH, MeOH and EtAc may have a role in controlling the solvation or desolvation processes that may take place during the micronization. One of the most important results of this study is that the SAS process can be performed using physically different plants and that this technology can be transferred from the bench scale to the industrial scale, obtaining the reproducibility of the results, with a large production and an high process yield.

The co-solute SCF-based micronization techniques, in particular SAA, seem to be a very good complement to the antisolvent techniques. Water-soluble compounds and compounds that cannot be processed by SAS, can be successfully micronized by SAA. In this study, amorphous particles of a compound that was difficult to micronize by SAS technique (cromolyn sodium), with a size in the range for inhalation delivery, have been obtained. No

relevant change was observed in the polymorphism. The technique is very promising and it is worth it to explore other solvents than water, also as co-solvent combinations, as well as different operating conditions, to investigate the potentiality of this technique and develop a model.

With this research work some concepts about SCF micronization have been settled and some fundamental knowledge, together with general interpretations, have been presented. Further investigations are needed in the future to find the still missing complete model and the process equations that may convert these experimental techniques into unit operations used in the industrial field. The general rules found for the antisolvent micronization techniques can be transferred to other SCF-based techniques that use the same principle. In particular, in the food field the antisolvent technique may be used to fractionate solid compounds by performing a selective precipitation, combining also that process with the already industrially used extraction processes.

The knowledge of both antisolvent and co-solvent techniques allows to process a more wide variety of compounds, pharmaceuticals, food ingredients, bio-polymers and others, both hydrophilic and hydrophobic. Moreover, the principles on which these complementary techniques are based can be compared and developed to find more practical applications in the industrial field in the future. The food industry can take advantage of the SCF antisolvent and cosolvent micronization mechanisms for the development of products with special functionality. Starting from the first step of the production (which might be the extraction of the material of interest), through the manufacturing steps (which may include purification or fractionation), until the final product (which might be encapsulated or suspended in a drink or in some specific formulation), the principles widely discussed in this thesis can be used. The added advantage is the use of clean, cheap and versatile techniques.

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REFERENCES

1. Gonda, I. (1992). Targeting by deposition. In: Hickey, A.J. (Eds.), Pharmaceutical Inhalation Aerosol Technology. Marcel Dekker, New York, 365 pp.
2. York P. (1983). Solid-state properties of powders in the formulation and processing of solid dosage forms. *Int. J. Pharm.* 14, 1-28.
3. Marshall P.V. and York P. (1989). Crystallization solvent induced solid-state and particulate modifications of nitrofurantoin. *Int. J. Pharm.* 55, 257-63.
4. Sanguansri P. and Augustin M.A. (2006). Nanoscale materials development - a food industry perspective. *Trends Food. Sci. Tech.* 17, 547-556.
5. Weiss J., Takhistov P., McClements D.J. (2006). Functional materials in food nanotechnology. *J. Food Sci.* 71, R107-R116.
6. Council Directive 1999/13/EC (1999). On the limitation of emissions of volatile organic compounds due to the use of organic solvents in certain activities and installations.
7. Council Directive 88/344/EEC (1988). On the approximation of the laws of the Member States on extraction solvents used in the production of foodstuffs and food ingredients.
8. Chang C.J., Randolph A.D., Craft N.E. (1991). Separation of beta-carotene mixtures precipitated from liquid solvents with high-pressure carbon dioxide. *Biotechnol. Prog.* 7, 275-278.
9. Reverchon E. (1999). Supercritical antisolvent precipitation of micro- and nano-particles. *J. Supercrit. Fluids* 15, 1-21.
10. Jung J. and Perrut M. (2001). Particle design using supercritical fluids: Literature and patent survey. *J. Supercrit. Fluids* 20, 179-219.
11. Thiering R., Dehghani F., Foster N.R. (2001). Current issues relating to anti-solvent micronisation techniques and their extension to industrial scales. *J. Supercrit. Fluids* 21, 159-177.
12. Shariati A. and Peters C.J. (2003). Recent developments in particle design using supercritical fluids. *Curr. Opin. Solid St. M.* 7, 371-383.
13. Yeo S.-D. and Kiran E. (2005). Formation of polymer particles with supercritical fluids: A review. *J. Supercrit. Fluids* 34, 287-308.
14. Reverchon E. and Adami R. (2006). Nanomaterials and supercritical fluids. *J. Supercrit. Fluids* 37, 1-22.
15. Lengsfeld C.S., Delplanque J.P., Barocas V.H., Randolph T.W. (2000). Mechanism Governing Microparticle Morphology during Precipitation by a Compressed Antisolvent: Atomization vs. Nucleation and Growth. *J. Phys. Chem. B* 104, 2725-2735.
16. Werling J.O. and Debenedetti P.G. (1999). Numerical modeling of mass transfer in the supercritical antisolvent process. *J. Supercrit. Fluids* 16, 167-181.
17. Werling J.O. and Debenedetti P.G. (2000). Numerical modeling of mass transfer in the

supercritical antisolvent process: miscible conditions. *J. Supercrit. Fluids* 18, 11-24.

18. Elvassore N., Cozzi F., Bertucco A. (2004). Mass Transport Modeling in a Gas Antisolvent Process. *Ind. Eng. Chem. Res.* 43, 4935-4943.
19. Rantakylä M., Jantti M., Aaltonen O., Hurme M. (2002). The effect of initial drop size on particle size in the supercritical antisolvent precipitation (SAS) technique. *J. Supercrit. Fluids* 24, 251-263.
20. Jarmer D.J., Lengsfeld C.S., Randolph T.W. (2004). Nucleation and growth rates of poly(L-lactic acid) microparticles during precipitation with a compressed-fluid antisolvent. *Langmuir* 20, 7254-7264.
21. Reverchon E., De Marco I., Caputo G., Della Porta G. (2003). Pilot scale micronization of amoxicillin by supercritical antisolvent precipitation. *J. Supercrit. Fluids* 26, 1-7.
22. Reverchon E., Caputo G., De Marco I. (2003). Role of Phase Behavior and Atomization in the Supercritical Antisolvent Precipitation. *Ind. Eng. Chem. Res.* 42, 6406-6414.
23. Reverchon E. (20030116). Process for the production of micro and/or nano particles, WO A1 2003004142.
24. Hanny J.B. and Hogarth J. (1879). *Proc. R. Soc. London* 324-326.
25. York P. (1999). Strategies for particle design using supercritical fluid technologies. *Pharm. Sci. Technol. To.* 2, 430-440.
26. Lee M.L. and Markides K.E. (1987). Chromatography with supercritical fluids. *Science (Washington, DC, United States)* 235, 1342-7.
27. McHugh M.A. and Krukonis V.J. (1986) Supercritical Fluid Extraction: Principles and Practice, 512 pp.
28. Brunner G. (1994) Gas Extraction: An Introduction to Fundamentals of Supercritical Fluids and the Application to Separation Processes, 387 pp.
29. Kerrola K. (1995). Literature review: Isolation of essential oils and flavor compounds by dense carbon dioxide. *Food Rev. Int.* 11, 547-73.
30. Reverchon E. (1997). Supercritical fluid extraction and fractionation of essential oils and related products. *J. Supercrit. Fluids* 10, 1-37.
31. Reverchon E. and De Marco I. (2006). Supercritical fluid extraction and fractionation of natural matter. *J. Supercrit. Fluids* 38, 146-166.
32. Perrut M., Jung J., Leboeuf F. (2005). Enhancement of dissolution rate of poorly-soluble active ingredients by supercritical fluid processes. *Int. J. Pharm.* 288, 3-10.
33. Pasquali I., Bettini R., Giordano F. (2006). Solid-state chemistry and particle engineering with supercritical fluids in pharmaceutics. *Eur. J. Pharm. Sci.* 27, 299-310.
34. Chow A.H.L., Tong H.H.Y., Chattopadhyay P., Shekunov B.Y. (2007). Particle Engineering for Pulmonary Drug Delivery. *Pharm. Res.* 24, 411-437.
35. Tomasko D.L., Li H., Liu D., Han X., Wingert M.J., Lee L.J., Koelling K.W. (2003). A review of CO_2 applications in the processing of polymers. *Ind. Eng. Chem. Res.* 42, 6431-6456.
36. Jovanovic N., Bouchard A., Hofland G.W., Witkamp G.-J., Crommelin D.J.A., Jiskoot W. (2004). Stabilization of Proteins in Dry Powder Formulations Using Supercritical Fluid Technology. *Pharm. Res.* 21, 1955-1969.
37. Tomasko D.L., Han X., Liu D., Gao W. (2003). Supercritical fluid applications in

polymer nanocomposites. *Curr. Opin. Solid St. M.* 7, 407-412.

38. Tom J.W. and Debenedetti P.G. (1991). Particle formation with supercritical fluids - a review. *J. Aerosol Sci.* 22, 555-84.
39. Petersen R.C., Matson D.W., Smith R.D. (1986). Rapid precipitation of low vapor pressure solids from supercritical fluid solutions: the formation of thin films and powders. *J. Am. Chem. Soc.* 108, 2100-2.
40. Matson D.W., Fulton J.L., Petersen R.C., Smith R.D. (1987). Rapid expansion of supercritical fluid solutions: solute formation of powders, thin films, and fibers. *Ind. Eng. Chem. Res.* 26, 2298-306.
41. Smith R.D. (19860415). Supercritical fluid molecular spray film deposition and powder formation, US A 4582731.
42. Smith R.D. (19880329). Method of making supercritical fluid molecular spray films, powder and fibers, US 4734227.
43. Smith R.D. (19880329). Supercritical fluid molecular spray thin films and fine powders, US A 4734451.
44. Knez Z. and Weidner E. (2003). Particles formation and particle design using supercritical fluids. *Curr. Opin. Solid St. M.* 7, 353-361.
45. Sun Y.-P. and Rollins H.W. (1998). Preparation of polymer-protected semiconductor nanoparticles through the rapid expansion of supercritical fluid solution. *Chem. Phys. Lett.* 288, 585-588.
46. Kropf C., Dolhaine H., Forster T., Schaber K., Türk M., Cihlar S., Christophliemk P. (20000323). Method for producing nanoparticles by expansion of supercritical solutions, WO A1 2000015329.
47. Foerster T., Fabry B., Hollenbrock M., Kropf C. (20000420). Use of nanoscale sterols and sterol esters for producing cosmetic and/or pharmaceutical preparations, WO A1 2000021490.
48. Kropf C., Fabry B., Foerster T., Wachter R., Reil S., Panzer C. (20000817). Use of nanoscale chitosans and/or chitosan derivatives, WO A1 2000047177.
49. Reverchon E., Della Porta G., Taddeo R., Pallado P., Stassi A. (1995). Solubility and micronization of griseofulvin in supercritical CHF_3. *Ind. Eng. Chem. Res.* 34, 4087-91.
50. Pathak P., Meziani M.J., Desai T., Sun Y.-P. (2004). Nanosizing Drug Particles in Supercritical Fluid Processing. *J. Am. Chem. Soc.* 126, 10842-10843.
51. Thakur R. and Gupta R.B. (2005). Rapid Expansion of Supercritical Solution with Solid Cosolvent (RESS-SC) Process: Formation of Griseofulvin Nanoparticles. *Ind. Eng. Chem. Res.* 44, 7380-7387.
52. Thakur R. and Gupta R.B. (2006). Rapid expansion of supercritical solution with solid cosolvent (RESS-SC) process: Formation of 2-aminobenzoic acid nanoparticle. *J. Supercrit. Fluids* 37, 307-315.
53. Thakur R. and Gupta R.B. (2006). Formation of phenytoin nanoparticles using rapid expansion of supercritical solution with solid cosolvent (RESS-SC) process. *Int. J. Pharm.* 308, 190-199.
54. Türk M., Hils P., Helfgen B., Schaber K., Martin H.-J., Wahl M.A. (2002). Micronization of pharmaceutical substances by the Rapid Expansion of Supercritical Solutions (RESS): a promising method to improve bioavailability of poorly soluble

pharmaceutical agents. *J. Supercrit. Fluids* 22, 75-84.

55. Chiou A.H.-J., Yeh M.-K., Chen C.-Y., Wang D.-P. (2007). Micronization of meloxicam using a supercritical fluids process. *J. Supercrit. Fluids* 42, 120-128.
56. Letourneau J.-J., Vigneau S., Gonus P., Fages J. (2004). Micronized cocoa butter particles produced by a supercritical process. *Chem. Eng. Process.* 44, 201-207.
57. Ksibi H., Ben Moussa A., Baccar M. (2006). Powder structure transition under the recrystallization conditions in the RESS process. *Chem. Eng.Technol.* 29, 868-874.
58. Debenedetti P.G., Tom J.W., Kwauk X., Yeo S.D. (1993). Rapid expansion of supercritical solutions (RESS): fundamentals and applications. *Fluid Phase Equilib.* 82, 311-21.
59. Türk M. (1999). Formation of small organic particles by RESS: experimental and theoretical investigations. *J. Supercrit. Fluids* 15, 79-89.
60. Helfgen B., Türk M., Schaber K. (2000). Theoretical and experimental investigations of the micronization of organic solids by rapid expansion of supercritical solutions. *Powder Technol.* 110, 22-28.
61. Shaub G.R., Brennecke J.F., McCready M.J. (1995). Radial model for particle formation from the rapid expansion of supercritical solutions. *J. Supercrit. Fluids* 8, 318-28.
62. Weber M., Russell L.M., Debenedetti P.G. (2002). Mathematical modeling of nucleation and growth of particles formed by the rapid expansion of a supercritical solution under subsonic conditions. *J. Supercrit. Fluids* 23, 65-80.
63. Weber M. and Thies M.C. (2007). A simplified and generalized model for the rapid expansion of supercritical solutions. *J. Supercrit. Fluids* 40, 402-419.
64. Bettini R., Rossi A., Lavezzini E., Frigo E., Pasquali I., Giordano F. (2003). Thermal and morphological characterization of micronized acetylsalicylic acid powders prepared by rapid expansion of a supercritical solution. *J. Therm. Anal. Calorim.* 73, 487-497.
65. Van Nijlen T., Brennan K., Van Den Mooter G., Blaton N., Kinget R., Augustijns P. (2003). Improvement of the dissolution rate of artemisinin by means of supercritical fluid technology and solid dispersions. *Int. J. Pharm.* 254, 173-181.
66. Domingo C., Berends E., Van Rosmalen G.M. (1997). Precipitation of ultrafine organic crystals from the rapid expansion of supercritical solutions over a capillary and a frit nozzle. *J. Supercrit. Fluids* 10, 39-55.
67. Shinozaki H., Oguchi T., Suzuki S., Aoki K., Sako T., Morishita S., Tozuka Y., Moribe K., Yamamoto K. (2006). Micronization and polymorphic conversion of tolbutamide and barbital by rapid expansion of supercritical solutions. *Drug Dev. Ind. Pharm.* 32, 877-891.
68. Gosselin P.M., Thibert R., Preda M., McMullen J.N. (2003). Polymorphic properties of micronized carbamazepine produced by RESS. *Int. J. Pharm.* 252, 225-233.
69. Subra P., Berroy P., Vega A., Domingo C. (2004). Process performances and characteristics of powders produced using supercritical CO_2 as solvent and antisolvent. *Powder Technol.* 142, 13-22.
70. Kröber H., Teipel U., Krause H. (2000). Manufacture of submicron particles via expansion of supercritical fluids. *Chem. Eng.Technol.* 23, 763-765.
71. Kröber H. and Teipel U. (2004). Micronization of organic substances by supercritical fluid processes. *Chem. Eng.Technol.* 27, 510-514.

72. Tandya A., Dehghani F., Foster N.R. (2006). Micronization of cyclosporine using dense gas techniques. *J. Supercrit. Fluids* 37, 272-278.

73. Sane A., Taylor S., Sun Y.P., Thies M.C. (2003). RESS for the preparation of fluorinated porphyrin nanoparticles. *Chem. Commun. (Camb)* 2720-1.

74. Sane A. and Thies M.C. (2005). The Formation of Fluorinated Tetraphenylporphyrin Nanoparticles via Rapid Expansion Processes: RESS vs RESOLV. *J. Phys. Chem. B* 109, 19688-19695.

75. Charoenchaitrakool M., Dehghani F., Foster N.R., Chan H.K. (2000). Micronization by Rapid Expansion of Supercritical Solutions to Enhance the Dissolution Rates of Poorly Water-Soluble Pharmaceuticals. *Ind. Eng. Chem. Res.* 39, 4794-4802.

76. Kayrak D., Akman U., Hortacsu O. (2003). Micronization of Ibuprofen by RESS. *J. Supercrit. Fluids* 26, 17-31.

77. Hermsdorf D., Jauer S., Signorell R. (2007). Formation and stabilization of ibuprofen nanoparticles by pulsed rapid expansion of supercritical solutions. *Mol. Phys.* 105, 951-959.

78. Huang J. and Moriyoshi T. (2006). Fabrication of fine powders by RESS with a clearance nozzle. *J. Supercrit. Fluids* 37, 292-297.

79. Alessi P., Cortesi A., Kikic I., Foster N.R., Macnaughton S.J., Colombo I. (1996). Particle production of steroid drugs using supercritical fluid processing. *Ind. Eng. Chem. Res.* 35, 4718-4726.

80. Moribe K., Tsutsumi S., Morishita S., Shinozaki H., Tozuka Y., Oguchi T., Yamamoto K. (2005). Micronization of phenylbutazone by rapid expansion of supercritical CO_2 solution. *Chem. Pharm. Bull.* 53, 1025-1028.

81. Reverchon E., Donsi G., Gorgoglione D. (1993). Salicylic acid solubilization in supercritical CO_2 and its micronization by RESS. *J. Supercrit. Fluids* 6, 241-8.

82. Yildiz N., Tuna S., Doeker O., Calimli A. (2007). Micronization of salicylic acid and taxol (paclitaxel) by rapid expansion of supercritical fluids (RESS). *J. Supercrit. Fluids* 41, 440-451.

83. Benedetti L., Bertucco A., Pallado P. (1997). Production of microparticles of a biocompatible polymer using supercritical carbon dioxide. *Biotechnol. Bioeng.* 53, 232-237.

84. Tom J.W. and Debenedetti P.G. (1991). Formation of bioerodible polymeric microspheres and microparticles by rapid expansion of supercritical solutions. *Biotechnol. Prog.* 7, 403-11.

85. Tom J.W., Debenedetti P.G., Jerome R. (1994). Precipitation of poly(L-lactic acid) and composite poly(L-lactic acid)-pyrene particles by rapid expansion of supercritical solutions. *J. Supercrit. Fluids* 7, 9-29.

86. Sane A. and Thies M.C. (2007). Effect of material properties and processing conditions on RESS of poly(L-lactide). *J. Supercrit. Fluids* 40, 134-143.

87. Matsuyama K., Mishima K., Umemoto H., Yamaguchi S. (2001). Environmentally Benign Formation of Polymeric Microspheres by Rapid Expansion of Supercritical Carbon Dioxide Solution with a Nonsolvent. *Environ. Sci. Technol.* 35, 4149-4155.

88. Ohgaki K., Kobayashi H., Katayama T., Hirokawa N. (1990). Whisker formation from jet of supercritical fluid solution. *J. Supercrit. Fluids* 3, 103-7.

89. Dehghani F. and Foster N.R. (2003). Dense gas anti-solvent processes for pharmaceutical formulation. *Curr. Opin. Solid St. M.* 7, 363-369.
90. Vemavarapu C., Mollan M.J., Lodaya M., Needham T.E. (2005). Design and process aspects of laboratory scale SCF particle formation systems. *Int. J. Pharm.* 292, 1-16.
91. Badens E., Boutin O., Charbit G. (2005). Laminar jet dispersion and jet atomization in pressurized carbon dioxide. *J. Supercrit. Fluids* 36, 81-90.
92. Hanna M. and York P. (19950112). Method and apparatus for the formation of particles, WO A1 9501221.
93. Subramaniam B., Said S., Rajewski R.A., Stella V. (19970904). Methods and apparatus for particle precipitation and coating using near-critical and supercritical antisolvents, WO A1 9731691.
94. Mawson S., Kanakia S., Johnston K.P. (1997). Coaxial nozzle for control of particle morphology in precipitation with a compressed fluid antisolvent. *J. Appl. Polym. Sci.* 64, 2105-2118.
95. Gupta R.B. and Chattopadhyay P. (20020103). Method of forming nanoparticles and microparticles of controllable size using supercritical fluids and ultrasound, US A1 2002000681.
96. Jarmer D.J., Lengsfeld C.S., Randolph T.W. (2003). Manipulation of particle size distribution of poly(L-lactic acid) nanoparticles with a jet-swirl nozzle during precipitation with a compressed antisolvent. *J. Supercrit. Fluids* 27, 317-336.
97. Boutin O., Maruejouls C., Charbit G. (2007). A new system for particle formation using the principle of the SAS process: The Concentric Tube Antisolvent Reactor (CTAR). *J. Supercrit. Fluids* 40, 443-451.
98. Suo Q.L., He W.Z., Huang Y.C., Li C.P., Hong H.L., Li Y.X., Zhu M.D. (2005). Micronization of the natural pigment-bixin by the SEDS process through prefilming atomization. *Powder Technol.* 154, 110-115.
99. He W.Z., Suo Q.L., Jiang Z.H., A S., Hong H.L. (2004). Precipitation of ephedrine by SEDS process using a specially designed prefilming atomizer. *J. Supercrit. Fluids* 31, 101-110.
100. He W.Z., Suo Q.L., Hong H.L., Li G.M., Zhao X.H., Li C.P., Shan A. (2006). Supercritical Antisolvent Micronization of Natural Carotene by the SEDS Process through Prefilming Atomization. *Ind. Eng. Chem. Res.* 45, 2108-2115.
101. Reverchon E., De Marco I., Torino E. (2007). Nanoparticles production by supercritical antisolvent precipitation: A general interpretation. *J. Supercrit. Fluids* 43, 126-138.
102. Reverchon E., De Marco I., Adami R., Caputo G. (2007). Expanded micro-particles by supercritical antisolvent precipitation: Interpretation of results. *J. Supercrit. Fluids*. In Press, doi:10.1016/j.supflu.2007.08.008.
103. Reverchon E. and De Marco I. (2004). Supercritical antisolvent micronization of Cefonicid: thermodynamic interpretation of results. *J. Supercrit. Fluids* 31, 207-215.
104. Perez de Diego Y., Pellikaan H.C., Wubbolts F.E., Borchard G., Witkamp G.J., Jansens P.J. (2006). Opening new operating windows for polymer and protein micronisation using the PCA process. *J. Supercrit. Fluids* 36, 216-224.
105. Sarup L., Servistas M.T., Sloan R., Hoare M., Humphreys G.O. (2000). Investigation of supercritical fluid technology to produce dry particulate formulations of antibody

fragments. *Food Bioprod. Process.* 78, 101-104.

106. Bustami R.T., Chan H.-K., Dehghani F., Foster N.R. (2000). Generation of microparticles of proteins for aerosol delivery using high pressure modified carbon dioxide. *Pharm. Res.* 17, 1360-1366.
107. Sarkari M., Darrat I., Knutson B.L. (2003). CO_2 and fluorinated solvent-based technologies for protein microparticle precipitation from aqueous solutions. *Biotechnol. Prog.* 19, 448-454.
108. Bouchard A., Jovanovic N., Jiskoot W., Mendes E., Witkamp G.-J., Crommelin D.J.A., Hofland G.W. (2007). Lysozyme particle formation during supercritical fluid drying: Particle morphology and molecular integrity. *J. Supercrit. Fluids* 40, 293-307.
109. Jessop P.G. and Subramaniam B. (2007). Gas-Expanded Liquids. *Chemical Reviews (Washington, DC, United States)* 107, 2666-2694.
110. Perrut M., Jung J., Leboeuf F. (2005). Enhancement of dissolution rate of poorly soluble active ingredients by supercritical fluid processes. *Int. J. Pharm.* 288, 11-16.
111. Federal Register (1997). International Conference on Harmonisation. Guidance on Impurities. Residual Solvents. *Fed. Regist.* 62, 67377-67388.
112. Council Directive 89/107/EEC (1988). On the approximation of the laws of the Member States concerning food additives authorized for use in foodstuffs intended for human consumption.
113. European Parliament and Council Directive 94/34/EC (1994). Amending Directive 89/107/EEC on the approximation of the laws of Member States concerning food additives authorized for use in foodstuffs intended for human consumption.
114. European Parliament and Council Directive 95/2/EC (1995). On food additives other than colours and sweeteners.
115. FIRST COMMISSION DIRECTIVE 81/712/EEC (1981). Laying down Community methods of analysis for verifying that certain additives used in foodstuffs satisfy criteria of purity.
116. Magnan C., Badens E., Commenges N., Charbit G. (2000). Soy lecithin micronization by precipitation with a compressed fluid antisolvent - influence of process parameters. *J. Supercrit. Fluids* 19, 69-77.
117. Badens E., Magnan C., Charbit G. (2001). Microparticles of soy lecithin formed by supercritical processes. *Biotechnol. Bioeng.* 72, 194-204.
118. Chen K., Zhang X., Pan J., Zhang W., Yin W. (2005). Gas antisolvent precipitation of Ginkgo ginkgolides with supercritical CO_2. *Powder Technol.* 152, 127-132.
119. Chattopadhyay P. and Gupta R.B. (2001). Production of griseofulvin nanoparticles using supercritical CO_2 antisolvent with enhanced mass transfer. *Int. J. Pharm.* 228, 19-31.
120. Chattopadhyay P. and Gupta R.B. (2002). Protein nanoparticles formation by supercritical antisolvent with enhanced mass transfer. *AIChE J.* 48, 235-244.
121. Perez Y., Wubbolts F.E., Witkamp G.J., Jansens P.J., de Loos Th.W. (2004). Improved PCA process for the production of nano- and microparticles of polymers. *AIChE J.* 50, 2408-2417.
122. Perez de Diego Y., Pellikaan H.C., Wubbolts F.E., Witkamp G.J., Jansens P.J. (2005). Operating regimes and mechanism of particle formation during the precipitation of

polymers using the PCA process. *J. Supercrit. Fluids* 35, 147-156.

123. Perez de Diego Y., Wubbolts F.E., Jansens P.J. (2006). Modelling mass transfer in the PCA process using the Maxwell-Stefan approach. *J. Supercrit. Fluids* 37, 53-62.
124. Weber A., Yelash L.V., Kraska T. (2005). Effect of the phase behavior of the solvent-antisolvent systems on the gas-antisolvent-crystallization of paracetamol. *J. Supercrit. Fluids* 33, 107-113.
125. Reverchon E. and De Marco I. (2006). Supercritical antisolvent precipitation of Cephalosporins. *Powder Technol.* 164, 139-146.
126. Palakodaty S. and York P. (1999). Phase behavioral effects on particle formation processes using supercritical fluids. *Pharm. Res.* 16, 976-985.
127. Sarkari M., Darrat I., Knutson B.L. (2000). Generation of microparticles using CO_2 and CO_2-philic antisolvents. *AIChE J.* 46, 1850-1859.
128. Dukhin S.S., Shen Y., Dave R., Pfeffer R. (2005). Droplet mass transfer, intradroplet nucleation and submicron particle production in two-phase flow of solvent-supercritical antisolvent emulsion. *Colloid Surf. A: Physicochem. Eng. Asp.* 261, 163-176.
129. Randolph T.W., Randolph A.D., Mebes M., Yeung S. (1993). Sub-micrometer-sized biodegradable particles of poly(L-lactic acid) via the gas antisolvent spray precipitation process. *Biotechnol. Prog.* 9, 429-35.
130. Carretier E., Badens E., Guichardon P., Boutin O., Charbit G. (2003). Hydrodynamics of Supercritical Antisolvent Precipitation: Characterization and Influence on Particle Morphology. *Ind. Eng. Chem. Res.* 42, 331-338.
131. Elvassore N., Bertucco A., Fermeglia M. (2002). Phase-equilibria calculation by group-contribution perturbed-hard-sphere-chain equation of state. *AIChE J.* 48, 359-368.
132. Martín A. and Cocero M.J. (2004). Numerical modeling of jet hydrodynamics, mass transfer, and crystallization kinetics in the supercritical antisolvent (SAS) process. *J. Supercrit. Fluids* 32, 203-219.
133. Chen A.-Z., Pu X.-M., Kang Y.-Q., Liao L., Yao Y.-D., Yin G.-F. (2006). Preparation of 5-fluorouracil-poly(L-lactide) microparticles using solution-enhanced dispersion by supercritical CO_2. *Macromol. Rapid Commun.* 27, 1254-1259.
134. Reverchon E. and Della Porta G. (1999). Production of antibiotic micro- and nano-particles by supercritical antisolvent precipitation. *Powder Technol.* 106, 23-29.
135. Reverchon E., Della Porta G., Falivene M.G. (2000). Process parameters and morphology in amoxicillin micro and submicro particles generation by supercritical antisolvent precipitation. *J. Supercrit. Fluids* 17, 239-248.
136. Kalogiannis C.G., Pavlidou E., Panayiotou C.G. (2005). Production of Amoxicillin Microparticles by Supercritical Antisolvent Precipitation. *Ind. Eng. Chem. Res.* 44, 9339-9346.
137. Tenorio A., Gordillo M.D., Pereyra C., Martinez de la Ossa E.J. (2007). Controlled submicro particle formation of ampicillin by supercritical antisolvent precipitation. *J. Supercrit. Fluids* 40, 308-316.
138. Wubbolts F.E., Bruinsma O.S.L., van Rosmalen G.M. (1999). Dry-spraying of ascorbic acid or acetaminophen solutions with supercritical carbon dioxide. *J. Cryst. Growth* 198/199, 767-772.
139. Kikic I., Alessi P., Eva F., Moneghini M., Perissutti B. (2006). Supercritical antisolvent

precipitation of atenolol: The influence of the organic solvent and of the processing approach. *J. Supercrit. Fluids* 38, 434-441.

140. He W.Z., Suo Q.L., Li Y.X., Hong H.L., Li G.M., Zhao X.H., Huang Y.C. (2007). Micronization of the official component baicalin by SEDS-PA process. *Cryst. Res. Technol.* 42, 631-638.
141. Bakhbakhi Y., Charpentier P.A., Rohani S. (2006). Experimental study of the GAS process for producing microparticles of beclomethasone-17,21-dipropionate suitable for pulmonary delivery. *Int. J. Pharm.* 309, 71-80.
142. Steckel H., Thies J., Muller B.W. (1997). Micronizing of steroids for pulmonary delivery by supercritical carbon dioxide. *Int. J. Pharm.* 152, 99-110.
143. Martin T.M., Bandi N., Shulz R., Roberts C.B., Kompella U.B. Preparation of budesonide and budesonide-PLA microparticles using supercritical fluid precipitation technology. *AAPS PharmSciTech* 3, E18.
144. Velaga S.P., Berger R., Carlfors J. (2002). Supercritical Fluids Crystallization of Budesonide and Flunisolide. *Pharm. Res.* 19, 1564-1571.
145. Steckel H., Pichert L., Mueller B.W. (2004). Influence of process parameters in the ASES process on particle properties of budesonide for pulmonary delivery. *Eur. J. Pharm. Biopharm.* 57, 507-512.
146. Lobo J.M., Schiavone H., Palakodaty S., York P., Clark A., Tzannis S.T. (2005). SCF-engineered powders for delivery of budesonide from passive DPI devices. *J. Pharm. Sci.* 94, 2276-2288.
147. York P., Wilkins S.A., Storey R.A., Walker S.E., Harland R.S. (20010308). Coformulation of drugs and oligomeric or polymeric excipients, WO A2 2001015664.
148. Edwards A.D., Shekunov B.Y., Kordikowski A., Forbes R.T., York P. (2001). Crystallization of pure anhydrous polymorphs of carbamazepine by solution enhanced dispersion with supercritical fluids (SEDS). *J. Pharm. Sci.* 90, 1115-1124.
149. Moneghini M., Kikic I., Voinovich D., Perissutti B., Filipovic-Grcic J. (2001). Processing of carbamazepine-PEG 4000 solid dispersions with supercritical carbon dioxide: preparation, characterization, and in vitro dissolution. *Int. J. Pharm.* 222, 129-138.
150. Moneghini M., Kikic I., Voinovich D., Perissutti B., Alessi P., Cortesi A., Princivalle F., Solinas D. (2003). Study of the solid state of carbamazepine after processing with gas anti-solvent technique. *Eur. J. Pharm. Biopharm.* 56, 281-289.
151. Debenedetti P.G., Lim G.B., Prud'homme R.K. (19930519). Formation of protein microparticles by antisolvent precipitation, EP A1 542314.
152. Yeo S.-D., Kim M.-S., Lee J.-C. (2003). Recrystallization of sulfathiazole and chlorpropamide using the supercritical fluid antisolvent process. *J. Supercrit. Fluids* 25, 143-154.
153. Liu Z., Wang J., Song L., Yang G., Han B. (2002). Study on the phase behavior of cholesterol-acetone-CO_2 system and recrystallization of cholesterol by antisolvent CO_2. *J. Supercrit. Fluids* 24, 1-6.
154. Kim M.-S., Lee S., Park J.-S., Woo J.-S., Hwang S.-J. (2007). Micronization of cilostazol using supercritical antisolvent (SAS) process: Effect of process parameters. *Powder Technol.* 177, 64-70.
155. Warwick B., Dehghani F., Foster N.R., Biffin J.R., Regtop H.L. (2002). Micronization of

Copper Indomethacin Using Gas Antisolvent Processes. *Ind. Eng. Chem. Res.* 41, 1993-2004.

156. Jaarmo S., Rantakylä M., Aaltonen O. (1997) Particle tailoring with supercritical fluids: production of amorphous pharmaceutical particles, Proceedings of the 4th International Symposium on Supercritical Fluids, Sendai, Japan, vol. A.
157. Thote A.J. and Gupta R.B. (2005). Formation of nanoparticles of a hydrophilic drug using supercritical carbon dioxide and microencapsulation for sustained release. *Nanomedicine* 1, 85-90.
158. Park H.J., Kim M.-S., Lee S., Kim J.-S., Woo J.-S., Park J.-S., Hwang S.-J. (2007). Recrystallization of fluconazole using the supercritical antisolvent (SAS) process. *Int. J. Pharm.* 328, 152-160.
159. Bouchard A., Jovanovic N., Hofland G.W., Mendes E., Crommelin D.J.A., Jiskoot W., Witkamp G.-J. (2007). Selective Production of Polymorphs and Pseudomorphs Using Supercritical Fluid Crystallization from Aqueous Solutions. *Cryst. Growth Des.* 7 (8), 1432 -1440.
160. De Gioannis B., Jestin P., Subra P. (2004). Morphology and growth control of griseofulvin recrystallized by compressed carbon dioxide as antisolvent. *J. Cryst. Growth* 262, 519-526.
161. Velaga S.P., Ghaderi R., Carlfors J. (2002). Preparation and characterization of hydrocortisone particles using a supercritical fluids extraction process. *Int. J. Pharm.* 231, 155-166.
162. Thakur R. and Gupta R. (2006). Production of hydrocortisone micro- and nano-particles using supercritical anti-solvent with enhanced mass transfer. *Chem. Eng. Commun.* 193, 293-305.
163. Schmitt W.J., Salada M.C., Shook G.G., Speaker S.M.I. (1995). Finely-divided powders by carrier solution injection into a near or supercritical fluid. *AIChE J.* 41, 2476-86.
164. Yeo S.D., Lim G.B., Debenedetti P.G., Bernstein H. (1993). Formation of microparticulate protein powders using a supercritical fluid antisolvent. *Biotechnol. Bioeng.* 41, 341-6.
165. Winters M A, Knutson B L, Debenedetti P G, Sparks H G, Przybycien T M, Stevenson C L, Prestrelski S J (1996). Precipitation of proteins in supercritical carbon dioxide. *J. Pharm. Sci.* 85, 586-94.
166. Thiering R., Dehghani F., Dillow A., Foster N.R. (2000). Solvent effects on the controlled dense gas precipitation of model proteins. *J. Chem. Technol. Biotechnol.* 75, 42-53.
167. Thiering R., Dehghani F., Dillow A., Foster N.R. (2000). The influence of operating conditions on the dense gas precipitation of model proteins. *J. Chem. Technol. Biotechnol.* 75, 29-41.
168. Snavely W.K., Subramaniam B., Rajewski R.A., Defelippis M.R. (2002). Micronization of insulin from halogenated alcohol solution using supercritical carbon dioxide as an antisolvent. *J. Pharm. Sci.* 91, 2026-2039.
169. Kim Y.H. and Shing K.S. Supercritical fluid-micronized ipratropium bromide for pulmonary drug delivery. *Powder Technol.* In Press, doi:10.1016/j.powtec.2007.04.009.
170. Hanna M. and York P. (19960111). Method and apparatus for the formation of particles, WO A1 9600610.

171. Palakodaty S., York P., Pritchard J. (1998). Supercritical fluid processing of materials from aqueous solutions: the application of SEDS to lactose as a model substance. *Pharm. Res.* 15, 1835-1843.

172. Amaro-Gonzalez D., Mabe G., Zabaloy M., Brignole E.A. (2000). Gas antisolvent crystallization of organic salts from aqueous solutions. *J. Supercrit. Fluids* 17, 249-258.

173. Muhrer G. and Mazzotti M. (2003). Precipitation of Lysozyme Nanoparticles from Dimethyl Sulfoxide Using Carbon Dioxide as Antisolvent. *Biotechnol. Prog.* 19, 549-556.

174. Moshashaée S., Bisrat M., Forbes R.T., Nyqvist H., York P. (2000). Supercritical fluid processing of proteins. I: Lysozyme precipitation from organic solution. *Eur. J. Pharm. Sci.* 11, 239-245.

175. Moshashaée S., Bisrat M., Forbes R.T., Quinn E.A., Nyqvist H., York P. (2003). Supercritical fluid processing of proteins: Iysozyme precipitation from aqueous solution. *J. Pharm. Pharmacol.* 55, 185-192.

176. Tu L.S., Dehghani F., Foster N.R. (2002). Micronization and microencapsulation of pharmaceuticals using a carbon dioxide antisolvent. *Powder Technol.* 126, 134-149.

177. Hanna M. and York P. (19980827). Method + apparatus for the formation of particles, WO A1 9836825.

178. Rehman M., Shekunov B.Y., York P., Colthorpe P. (2001). Solubility and precipitation of nicotinic acid in supercritical carbon dioxide. *J. Pharm. Sci.* 90, 1570-1582.

179. Moneghini M., Perissutti B., Vecchione F., Kikic I., Alessi P., Cortesi A., Princivalle F. (2007). Supercritical antisolvent precipitation of nimesulide: preliminary experiments. *Current Drug Delivery* 4, 241-248.

180. Majerik V., Charbit G., Badens E., Horvath G., Szokonya L., Bosc N., Teillaud E. (2007). Bioavailability enhancement of an active substance by supercritical antisolvent precipitation. *J. Supercrit. Fluids* 40, 101-110.

181. Shekunov B.Yu., Hanna M., York P. (1999). Crystallization process in turbulent supercritical flows. *J. Cryst. Growth* 198/199, 1345-1351.

182. Bristow S., Shekunov T., Shekunov B.Yu., York P. (2001). Analysis of the supersaturation and precipitation process with supercritical CO_2. *J. Supercrit. Fluids* 21, 257-271.

183. Fusaro F., Haenchen M., Mazzotti M., Muhrer G., Subramaniam B. (2005). Dense Gas Antisolvent Precipitation: A Comparative Investigation of the GAS and PCA Techniques. *Ind. Eng. Chem. Res.* 44, 1502-1509.

184. Fusaro F., Mazzotti M., Muhrer G. (2004). Gas antisolvent recrystallization of paracetamol from acetone using compressed carbon dioxide as antisolvent. *Cryst. Growth Des.* 4, 881-889.

185. Berends E.M., Bruinsma O.S.L., de Graauw J., van Rosmalen G.M. (1996). Crystallization of phenanthrene from toluene with carbon dioxide by the GAS process. *AIChE J.* 42, 431-9.

186. Bakhbakhi Y., Rohani S., Charpentier P.A. (2005). Micronization of Phenanthrene Using the Gas Antisolvent Process. 1. Experimental Study and Use of FTIR. *Ind. Eng. Chem. Res.* 44, 7337-7344.

187. Bakhbakhi Y., Rohani S., Charpentier P.A. (2005). Micronization of Phenanthrene Using the Gas Antisolvent Process: Part 2. Theoretical Study. *Ind. Eng. Chem. Res.* 44,

7345-7351.

188. Kulshreshtha A.K., Smith G.G., Anderson S.D., Krukonis V.J. (19980908). Process for sizing prednisolone acetate using a supercritical fluid anti-solvent, US A 5803966.
189. Bustami R.T., Chan H.-K., Sweeney T., Dehghani F., Foster N.R. (2003). Generation of Fine Powders of Recombinant Human Deoxyribonuclease Using the Aerosol Solvent Extraction System. *Pharm. Res.* 20, 2028-2035.
190. Reverchon E., De Marco I., Della Porta G. (2002). Rifampicin microparticles production by supercritical antisolvent precipitation. *Int. J. Pharm.* 243, 83-91.
191. Nesta D.P., Elliott J.S., Warr J.P. (2000). Supercritical fluid precipitation of recombinant human immunoglobulin from aqueous solutions. *Biotechnol. Bioeng.* 67, 457-464.
192. Reverchon E., Della Porta G., Pallado P. (2001). Supercritical antisolvent precipitation of salbutamol microparticles. *Powder Technol.* 114, 17-22.
193. Vatanara A., Rouholamini Najafabadi A., Gilani K., Asgharian R., Darabi M., Rafiee-Tehrani M. (2007). A Plackett-Burman design for screening of the operation variables in the formation of salbutamol sulphate particles by supercritical antisolvent. *J. Supercrit. Fluids* 40, 111-116.
194. Beach S., Latham D., Sidgwick C., Hanna M., York P. (1999). Control of the Physical Form of Salmeterol Xinafoate. *Org. Process Res. Dev.* 3, 370-376.
195. Shekunov B.Y., Feeley J.C., Chow A.H.L., Tong H.H.Y., York P. (2003). Aerosolisation behaviour of micronised and supercritically-processed powders. *J. Aerosol Sci.* 34, 553-568.
196. Yeo S.-D. and Lee J.-C. (2004). Crystallization of sulfamethizole using the supercritical and liquid antisolvent processes. *J. Supercrit. Fluids* 30, 315-323.
197. Kordikowski A., Shekunov T., York P. (2001). Polymorph control of sulfathiazole in supercritical CO_2. *Pharm. Res.* 18, 682-688.
198. Rehman M., Shekunov B.Y., York P., Lechuga-Ballesteros D., Miller D.P., Tan T., Colthorpe P. (2004). Optimisation of powders for pulmonary delivery using supercritical fluid technology. *Eur. J. Pharm. Sci.* 22, 1-17.
199. Kim Y.H., Sioutas C., Fine P., Shing K.S. Effect of albumin on physical characteristics of drug particles produced by supercritical fluid technology. *Powder Technol.* In Press, doi:10.1016/j.powtec.2007.06.008.
200. Chattopadhyay P. and Gupta R.B. (2001). Production of Antibiotic Nanoparticles Using Supercritical CO_2 as Antisolvent with Enhanced Mass Transfer. *Ind. Eng. Chem. Res.* 40, 3530-3539.
201. Subra P., Laudani C.-G., Vega-Gonzalez A., Reverchon E. (2005). Precipitation and phase behavior of theophylline in solvent-supercritical CO_2 mixtures. *J. Supercrit. Fluids* 35, 95-105.
202. Pallado P., Benedetti L., Callegaro L. (19961003). Nanospheres comprising a biocompatible polysaccharide, WO A1 9629998.
203. Engwicht A., Girreser U., Muller B.W. (1999). Critical properties of lactide-co-glycolide polymers for the use in microparticle preparation by the Aerosol Solvent Extraction System. *Int. J. Pharm.* 185, 61-72.
204. Reverchon E., Della Porta G., De Rosa I., Subra P., Letourneur D. (2000). Supercritical antisolvent micronization of some biopolymers. *J. Supercrit. Fluids* 18, 239-245.

205. Duarte A.R.C., Costa M.S., Simplicio A.L., Cardoso M.M., Duarte C.M.M. (2006). Preparation of controlled release microspheres using supercritical fluid technology for delivery of anti-inflammatory drugs. *Int. J. Pharm.* 308, 168-174.

206. Elvassore N., Baggio M., Pallado P., Bertucco A. (2001). Production of different morphologies of biocompatible polymeric materials by supercritical CO_2 antisolvent techniques. *Biotechnol. Bioeng.* 73, 449-457.

207. Ghaderi R., Artursson P., Carlfors J. (1999). Preparation of biodegradable microparticles using solution-enhanced dispersion by supercritical fluids (SEDS). *Pharm. Res.* 16, 676-681.

208. Ghaderi R., Artursson P., Carlfors J. (2000). A new method for preparing biodegradable microparticles and entrapment of hydrocortisone in dl-PLG microparticles using supercritical fluids. *Eur. J. Pharm. Sci.* 10, 1-9.

209. Costa M.S., Duarte A.R.C., Cardoso M.M., Duarte C.M.M. (2007). Supercritical antisolvent precipitation of PHBV microparticles. *Int. J. Pharm.* 328, 72-77.

210. Bleich J., Mueller B.W., Wassmus W. (1993). Aerosol solvent extraction system. A new microparticle production technique. *Int. J. Pharm.* 97, 111-17.

211. Bodmeier R, Wang H, Dixon D J, Mawson S, Johnston K P (1995). Polymeric microspheres prepared by spraying into compressed carbon dioxide. *Pharm. Res.* 12, 1211-7.

212. Bitz C. and Doelker E. (1996). Influence of the preparation method on residual solvents in biodegradable microspheres. *Int. J. Pharm.* 131, 171-181.

213. Thies J. and Mueller B.W. (1998). Size controlled production of biodegradable microparticles with supercritical gases. *Eur. J. Pharm. Biopharm.* 45, 67-74.

214. Breitenbach A., Mohr D., Kissel T. (2000). Biodegradable semi-crystalline comb polyesters influence the microsphere production by means of a supercritical fluid extraction technique (ASES). *J. Controlled Release* 63, 53-68.

215. Taki S., Badens E., Charbit G. (2001). Controlled release system formed by supercritical anti-solvent coprecipitation of a herbicide and a biodegradable polymer. *J. Supercrit. Fluids* 21, 61-70.

216. Kim M.Y., Lee Y.W., Byun H.-S., Lim J.S. (2006). Recrystallization of Poly(L-lactic acid) into Submicrometer Particles in Supercritical Carbon Dioxide. *Ind. Eng. Chem. Res.* 45, 3388-3392.

217. Coen E.M., Quinn J.F., Dehghani F., Foster N.R., Davis T.P. (2003). Molecular weight fractionation of poly(methyl methacrylate) using Gas Anti-Solvent techniques. *Polymer* 44, 3477-3481.

218. Cocero M.J. and Ferrero S. (2002). Crystallization of b-carotene by a GAS process in batch. Effect of operating conditions. *J. Supercrit. Fluids* 22, 237-245.

219. Miguel F., Martín A., Gamse T., Cocero M.J. (2006). Supercritical anti solvent precipitation of lycopene. Effect of the operating parameters. *J. Supercrit. Fluids* 36, 225-235.

220. Kröber H. and Teipel U. (2002). Materials processing with supercritical antisolvent precipitation: process parameters and morphology of tartaric acid. *J. Supercrit. Fluids* 22, 229-235.

221. Cooper A.I. (2000). Polymer synthesis and processing using supercritical carbon

dioxide. *J. Mater. Chem.* 10, 207-234.

222. Weidner E., Knez Z., Novak Z. (19950817). Process for preparation of particles or powders, WO A1 9521688.
223. Sencar-Bozic P., Srcic S., Knez Z., Kerc J. (1997). Improvement of nifedipine dissolution characteristics using supercritical CO_2. *Int. J. Pharm.* 148, 123-130.
224. Kerc J., Srcic S., Knez Z., Sencar-Bozic P. (1999). Micronization of drugs using supercritical carbon dioxide. *Int. J. Pharm.* 182, 33-39.
225. Howdle S.M., Watson M.S., Whitaker M.J., Davies M.C., Shakesheff K.M., Popov V.K., Mandel F.S., Wang J.D. (2001). Supercritical fluid mixing: preparation of thermally sensitive polymer composites containing bioactive materials. *Chem. Commun.* 109-110.
226. Münüklü P. and Jansens P.J. (2007). Particle formation of edible fats using the supercritical melt micronization process (ScMM). *J. Supercrit. Fluids* 43, 181-190.
227. Elvassore N., Flaibani M., Bertucco A., Caliceti P. (2003). Thermodynamic analysis of micronization processes from gas-saturated solution. *Ind. Eng. Chem. Res.* 42, 5924-5930.
228. Li J., Matos H.A., Gomes de Azevedo E. (2004). Two-phase homogeneous model for particle formation from gas-saturated solution processes. *J. Supercrit. Fluids* 32, 275-286.
229. Strumendo M., Bertucco A., Elvassore N. (2007). Modeling of particle formation processes using gas saturated solution atomization. *J. Supercrit. Fluids* 41, 115-125.
230. Reverchon E. (2002). Supercritical-Assisted Atomization to produce micro- and/or nanoparticles of controlled size and distribution. *Ind. Eng. Chem. Res.* 41, 2405-2411.
231. Liu H. (2000) Science and Engineering of Droplets: Fundamentals and Applications, 225 pp.
232. Reverchon E. and Spada A. (2004). Crystalline Microparticles of Controlled Size Produced by Supercritical-Assisted Atomization. *Ind. Eng. Chem. Res.* 43, 1460-1465.
233. Reverchon E., Adami R., Caputo G. (2006). Supercritical assisted atomization: Performance comparison between laboratory and pilot scale. *J. Supercrit. Fluids* 37, 298-306.
234. Sievers R.E. and Karst U. (19970617). Methods for fine particle formation, US A 5639441.
235. Sievers R.E., Karst U., Milewski P.D., Sellers S.P., Miles B.A., Schaefer J.D., Stoldt C.R., Xu C.Y. (1999). Formation of aqueous small droplet aerosols assisted by supercritical carbon dioxide. *Aerosol Sci. Technol.* 30, 3-15.
236. Li Z., Jiang J., Liu X., Zhao S., Xia Y., Tang H. (2007). Preparation of erythromycin microparticles by supercritical fluid expansion depressurization. *J. Supercrit. Fluids* 41, 285-292.
237. Zhiyi L., Jingzhi J., Xuewu L., Yuanjing X., Shunxuan Z., Jian W. Preparation of tetracycline microparticles suitable for inhalation administration by supercritical fluid expansion depressurization. *Chem. Eng. Process.* In Press,doi:10.1016/j.cep.2007.04.011.
238. Ventosa N., Sala S., Veciana J., Torres J., Llibre J. (2001). Depressurization of an Expanded Liquid Organic Solution (DELOS): A New Procedure for Obtaining Submicron- or Micron-Sized Crystalline Particles. *Cryst. Growth Des.* 1, 299-303.
239. Ventosa N., Sala S., Veciana J. (2003). DELOS process: a crystallization technique using

compressed fluids. 1. Comparison to the GAS crystallization method. *J. Supercrit. Fluids* 26, 33-45.

240. Ventosa N., Sala S., Veciana J., Llibre J., Torras J. (2001) The DELOS process: a new eco-eficient tool for particle engineering, Proceedings of the 6th Conference on Supercritical Fluids and Their Applications, Maiori, Italy.
241. Reverchon E. and Antonacci A. (2006). Cyclodextrins micrometric powders obtained by supercritical fluid processing. *Biotechnol. Bioeng.* 94, 753-761.
242. Sievers R.E., Milewski P.D., Sellers S.P., Miles B.A., Korte B.J., Kusek K.D., Clark G.S., Mioskowski B., Villa J.A. (2000). Supercritical and Near-critical Carbon Dioxide Assisted Low-Temperature Bubble Drying. *Ind. Eng. Chem. Res.* 39, 4831-4836.
243. Reverchon E. and Antonacci A. (2006). Chitosan Microparticles Production by Supercritical Fluid Processing. *Ind. Eng. Chem. Res.* 45, 5722-5728.
244. Reverchon E. and Antonacci A. (2007). Polymer microparticles production by supercritical assisted atomization. *J. Supercrit. Fluids* 39, 444-452.
245. Sievers R.E., Huang E.T.S., Villa J.A., Engling G., Brauer P.R. (2003). Micronization of water-soluble or alcohol-soluble pharmaceuticals and model compounds with a low-temperature Bubble Dryer. *J. Supercrit. Fluids* 26, 9-16.
246. Reverchon E., Della Porta G., Spada A. (2003). Ampicillin micronization by supercritical assisted atomization. *J. Pharm. Pharmacol.* 55, 1465-1471.
247. Reverchon E. and Spada A. (2004). Erythromycin micro-particles produced by supercritical fluid atomization. *Powder Technol.* 141, 100-108.
248. Reverchon E., Della Porta G., Spada A., Antonacci A. (2004). Griseofulvin micronization and dissolution rate improvement by supercritical assisted atomization. *J. Pharm. Pharmacol.* 56, 1379-1387.
249. Della Porta G., De Vittori C., Reverchon E. (2005). Supercritical assisted atomization: a novel technology for microparticles preparation of an asthma-controlling drug. *AAPS PharmSciTech [Electronic Resource]* 6, E421-8.
250. Sellers S.P., Clark G.S., Sievers R.E., Carpenter J.F. (2001). Dry powders of stable protein formulations from aqueous solutions prepared using supercritical CO_2-assisted aerosolization. *J. Pharm. Sci.* 90, 785-797.
251. Reverchon E. and Della Porta G. (2003). Micronization of antibiotics by supercritical assisted atomization. *J. Supercrit. Fluids* 26, 243-252.
252. Reverchon E. and Della Porta G. (2003). Terbutaline microparticles suitable for aerosol delivery produced by supercritical assisted atomization. *Int. J. Pharm.* 258, 1-9.
253. Zhang G.G.Z., Law D., Schmitt E.A., Qiu Y. (2004). Phase transformation considerations during process development and manufacture of solid oral dosage forms. *Adv. Drug Deliv. Rev.* 56, 371-390.
254. Khoshkhoo S. and Anwar J. (1993). Crystallization of polymorphs: the effect of solvent. *J. Phys. D: Appl. Phys.* 26, B90-B93.
255. Shekunov B.Yu. and York P. (2000). Crystallization processes in pharmaceutical technology and drug delivery design. *J. Cryst. Growth* 211, 122-136.
256. Wille R.L. and Lutton E.S. (1966). Polymorphism of cocoa butter. *J. Am. Oil Chem. Soc.* 43, 491-6.
257. Tozuka Y., Kawada D., Oguchi T., Yamamoto K. (2003). Supercritical carbon dioxide

treatment as a method for polymorph preparation of deoxycholic acid. *Int. J. Pharm.* 263, 45-50.

258. Bettini R., Bonassi L., Castoro V., Rossi A., Zema L., Gazzaniga A., Giordano F. (2001). Solubility and conversion of carbamazepine polymorphs in supercritical carbon dioxide. *Eur. J. Pharm. Sci.* 13, 281-286.
259. Tong H.H.Y., Shekunov B.Yu., York P., Chow A.H.L. (2001). Characterization of two polymorphs of salmeterol xinafoate crystallized from supercritical fluids. *Pharm. Res.* 18, 852-858.
260. Vemavarapu C., Mollan M.J., Needham T.E. (2000). Crystal doping aided by rapid expansion of supercritical solutions. *AAPS PharmSciTech* 3, E29.
261. Jarmer D.J., Lengsfeld C.S., Anseth K.S., Randolph T.W. (2005). Supercritical fluid crystallization of griseofulvin: crystal habit modification with a selective growth inhibitor. *J. Pharm. Sci.* 94, 2688-2702.
262. Caputo G. and Reverchon E. (2007). Use of Urea as Habit Modifier in the Supercritical Antisolvent Micronization of Sulfathiazole. *Ind. Eng. Chem. Res.* 46, 4265-4272.
263. Montanari L., Fantozzi P., Snyder J.M., King J.W. (1999). Selective extraction of phospholipids from soybeans with supercritical carbon dioxide and ethanol. *J. Supercrit. Fluids* 14, 87-93.
264. Andersson M.B.O., Demirbueker M., Blomberg L.G. (1997). Semi-continuous extraction/purification of lipids by means of supercritical fluids. *J. Chromatogr. A* 785, 337-343.
265. Mukhopadhyay M. and Singh S. (2004). Refining of crude lecithin using dense carbon dioxide as anti-solvent. *J. Supercrit. Fluids* 30, 201-211.
266. Catchpole O.J., Grey J.B., Mitchell K.A., Lan J.S. (2004). Supercritical antisolvent fractionation of propolis tincture. *J. Supercrit. Fluids* 29, 97-106.
267. Aro H., Järvenpää E., Könkö K., Huopalahti R., Hietaniemi V. (2007). The characterization of oat lipids produced by supercritical fluid technologies. *J. Cereal Sci.* 45, 116-119.
268. Chattopadhyay P., Shekunov B.Y., Seitzinger J.S., Huff R.W. (20040115). Particles from supercritical fluid extraction of emulsion, WO A1 2004004862.
269. Shekunov B.Y., Chattopadhyay P., Seitzinger J., Huff R. (2006). Nanoparticles of Poorly Water-Soluble Drugs Prepared by Supercritical Fluid Extraction of Emulsions. *Pharm. Res.* 23, 196-204.
270. Chattopadhyay P., Huff R., Shekunov B.Y. (2006). Drug encapsulation using supercritical fluid extraction of emulsions. *J. Pharm. Sci.* 95, 667-679.
271. Tandya A., Mammucari R., Dehghani F., Foster N.R. (2007). Dense gas processing of polymeric controlled release formulations. *Int. J. Pharm.* 328, 1-11.
272. Park K. (1997). Controlled Drug Delivery: Challenges and Strategies, 629 pp.
273. Colombo, P., Santi, P., Bettini, R., Brazel C.S., Peppas, N.A. (2000). Drug release from swelling controlled systems. In: Wise, D.L. et al. (Eds.), Handbook of pharmaceuticals controlled release technology. Marcel Dekker, New York, 183-209.
274. Colombo, P., Santi, P., Bettini, R., Brazel C.S., Peppas, N.A. (2004). Drug release from swelling controlled systems. In: Handbook of controlled release. Wiley Science.
275. Antonacci A. (2007). Microparticle production for drugs controlled release by

supercritical assisted atomization, University of Salerno, Italy.

276. Rodrigues M., Peirico N., Matos H., Gomes de Azevedo E., Lobato M.R., Almeida A.J. (2004). Microcomposites theophylline/hydrogenated palm oil from a PGSS process for controlled drug delivery systems. *J. Supercrit. Fluids* 29, 175-184.
277. Toropainen T., Velaga S., Heikkila T., Matilainen L., Jarho P., Carlfors J., Lehto V.-P., Järvinen T., Järvinen K. (2006). Preparation of budesonide/γ-cyclodextrin complexes in supercritical fluids with a novel SEDS method. *J. Pharm. Sci.* 95, 2235-2245.
278. Velaga S.P. and Carlfors J. (2005). Supercritical fluids processing of recombinant human growth hormone. *Drug Dev. Ind. Pharm.* 31, 135-149.
279. Jovanovic N., Bouchard A., Hofland G.W., Witkamp G.-J., Crommelin D.J.A., Jiskoot W. (2006). Distinct effects of sucrose and trehalose on protein stability during supercritical fluid drying and freeze-drying. *Eur. J. Pharm. Sci.* 27, 336-345.
280. Jovanovic N., Bouchard A., Hofland G.W., Witkamp G.-J., Crommelin D.J.A., Jiskoot W. (2007). Stabilization of IgG by supercritical fluid drying: Optimization of formulation and process parameters. *Eur. J. Pharm. Biopharm.* In Press, doi:10.1016/j.ejpb.2007.05.001.
281. Jovanovic N., Bouchard A., Sutter M., Van Speybroeck M., Hofland G.W., Witkamp G.-J., Crommelin D.J.A., Jiskoot W. (2007). Stable sugar-based protein formulations by supercritical fluid drying. *Int. J. Pharm.* In Press, doi:10.1016/j.ijpharm.2007.06.013.
282. Bouchard A., Jovanovic N., Hofland G.W., Jiskoot W., Mendes E., Crommelin D.J.A., Witkamp G.-J. (2007). Supercritical fluid drying of carbohydrates: Selection of suitable excipients and process conditions. *Eur. J. Pharm. Biopharm.* In Press, doi:10.1016/j.ejpb.2007.06.019.
283. Sievers R.E., Quinn B.P., Cape S.P., Searles J.A., Braun C.S., Bhagwat P., Rebits L.G., McAdams D.H., Burger J.L., Best J.A., Lindsay L., Hernandez M.T., Kisich K.O., Iacovangelo T., Kristensen D., Chen D. (2007). Near-critical fluid micronization of stabilized vaccines, antibiotics and anti-virals. *J. Supercrit. Fluids* 42, 385-391.
284. Michel M.E., Bolger G., Weissman B.A. (1985). Binding of a new opiate antagonist, nalmefene, to rat brain membranes. *Methods Finds Exp. Clin. Pharmacol.* 7, 175-7.
285. Mason B.J., Ritvo E.C., Morgan R.O., Salvato F.R., Goldberg G., Welch B., Mantero-Atienza E. (1994). A double-blind, placebo-controlled pilot study to evaluate the efficacy and safety of oral nalmefene HCl for alcohol dependence. *Alcohol. Clin. Exp. Res.* 18, 1162-7.
286. Murthy S.S. and Brittain H.G. (1996). Stability of Revex, nalmefene hydrochloride injection, in injectable solutions. *J. Pharmaceut. Biomed.* 15, 221-226.
287. Reverchon E., Della Porta G., Di Trolio A., Pace S. (1998). Supercritical Antisolvent Precipitation of Nanoparticles of Superconductor Precursors. *Ind. Eng. Chem. Res.* 37, 952-958.
288. Reverchon E., Celano C., Della Porta G., Di Trolio A., Pace S. (1998). Supercritical antisolvent precipitation: A new technique for preparing submicronic yttrium powders to improve YBCO superconductors. *J. Mater. Res.* 13, 284-289.
289. Lavernia, E.J., Wu, Y. (1996). Spray atomization and deposition. In: John Wiley & Son, Chichester, England.
290. Reverchon E. and Della Porta G. (2003). Particle design using supercritical fluids. *Chem. Eng.Technol.* 26, 840-845.

291. Radulovic D., Kocic-Pesic V., Pecanac D., Zivanovic L. (1994). HPLC determination of sodium chromoglycate in pharmaceutical dosage forms. *Farmaco* 49, 375-6.

292. Lunn, G., Schmuff, N.R. (1997). HPLC Methods for Pharmaceutical Analysis.

293. USP 28 (2005). Organic Volatiles Impurities, 467. In: The United States Pharmcopeial Convention Inc. Rockville, MD, 2322-2332.

294. Adami R., Reverchon E., Järvenpää E., Huopalahti R. (2004) Criteria for drug processability by supercritical antisolvent technique, Proceedings of the 16th International Congress of Chemical and Process Engineering, Praha, Czech Republic.

295. Van Konynenburg P.H. and Scott R.L. (1980). Critical lines and phase equilibriums in binary Van der Waals mixtures. *Philosophical Transactions of the Royal Society of London, Series A: Mathematical, Physical and Engineering Sciences* 298, 495-540.

296. Sandler, S.I. (1993). Models for Thermodynamic and Phase Equilibria Calculations. Marcel Dekker Inc.

297. Laidler K.J. and Meiser J.H. (1982) Physical Chemistry, USA, p. 911.

298. Gentile S. (2003) Thermodynamic analysis of supercritical antisolvent micronization process using view cells, MSc Thesis, University of Salerno, Italy.

299. Lopez-Castillo Z.K., Aki S.N.V.K., Stadtherr M.A., Brennecke J.F. (2006). Enhanced Solubility of Oxygen and Carbon Monoxide in CO_2-Expanded Liquids. *Ind. Eng. Chem. Res.* 45, 5351-5360.

300. Anand M., Odom L.A., Roberts C.B. (2007). Finely Controlled Size-Selective Precipitation and Separation of CdSe/ZnS Semiconductor Nanocrystals Using CO_2-Gas-Expanded Liquids. *Langmuir* 23, 7338-7343.

301. Day C.-Y., Chang C.J., Chen C.-Y. (1996). Phase Equilibrium of Ethanol + CO_2 and Acetone + CO_2 at Elevated Pressures. *J. Chem. Eng. Data* 41, 839-843.

302. Adami R., Reverchon E., Järvenpää E., Huopalahti R. (2006) Drug polymorphs by Supercriticl Antisolvent micronization, Proceedings of the 8th International Symposium on Supercritical Fluids, Kyoto, Japan.

303. Michaels A.S. and Colville A.R.J. (1960). The effect of surface-active agents on crystal growth rate and crystal habit. *J. Phys. Chem.* 64, 13-19.

304. Reverchon E., Adami R., De marco I., Laudani C.G., Spada A. (2005). Disperse red 60 micronisation using supercritical fluid based techniques. *J. Supercrit. Fluids* 35, 76-82.

305. Rantakylä M.J. (2004) Particle production by supercritical antisolvent processing techniques, Helsinki University of Technology, Espoo, Finland.

306. Cox J.S.G., Woodard G.D., McCrone W.C. (1971). Solid-state chemistry of cromolyn sodium (disodium cromoglycate). *J. Pharm. Sci.* 60, 1458-65.

307. Chen L.R., Young V.G.Jr., Lechuga-Ballesteros D., Grant D.J.W. (1999). Solid-State Behavior of Cromolyn Sodium Hydrates. *J. Pharm. Sci.* 88, 1191-1200.

308. Stephenson G.A. and Diseroad B.A. (2000). Structural relationship and desolvation behavior of cromolyn, cefazolin and fenoprofen sodium hydrates. *Int. J. Pharm.* 198, 167-177.

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