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Theophylline formulation by supercritical antisolvents

Christelle Roy *, Arlette Vega-González, Pascale Subra-Paternault

Laboratoire d'Ingénierie des Matériaux et des Hautes Pressions (LIMHP), C.N.R.S., Institut Galilée, Université Paris 13, 99 Avenue Jean-Baptiste Clément, 93430 Villetaneuse, France

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Abstract

Supercritical antisolvent precipitation has been used to produce theophylline particles as pure crystals or as a solid formulation with poly lactic acid (PLA). Experiments were carried out as batch or semi-continuous process and with carbon dioxide (CO_2) or trifluoromethane (CHF_3) as antisolvent. Both modes led to micronized theophylline, but the semi-continuous produced smaller particles than the batch. The particle morphology was sensitive to the antisolvent, since CO_2- and CHF_3-samples exhibited hexagonal and triangular shape, respectively. The most distinguish feature of CO_2-powders was that theophylline exhibited a different crystal lattice than the crude- or the CHF_3-materials, accredited by new diffraction peaks in the XRD patterns not attributable to hydration of samples. However, neither the shape nor the new crystal lattice influenced the dissolution kinetics of Theophylline. For co-precipitation with PLA, the successful recovery of a powder occurred only in conditions of CHF_3-batch and CO_2-semi continuous. The powder retained the shape and the crystallinity of the corresponding pure processed theophylline, indicating that PLA did not interfere with the precipitation behavior of the drug. Moreover, only the CO_2-semi continuous products exhibited a decreased release rate and a prolongation of the total release time of the drug.

Keywords: Theophylline; Supercritical fluids; Fluoroform; Formulation; Controlled release; Crystals

1. Introduction

In pharmaceutical industry, the method for producing the drug is a critical step since crystal habit, crystal size distribution and polymorphic form play a large role in the therapeutic behavior. The development of effective particle technologies is thus keeping up with the need for increasingly smaller crystals of increased purity, that allow to reduce the drug dosage whilst keeping the therapeutic benefits, and to minimize the side effects. Crystallization using supercritical fluids (SCF) was mostly developed as an alternative approach to conventional liquid crystallization, challenging for a better control of the particle size and a lower concentration of residual solvent in drugs, besides the environmental advantage of reducing the liquid solvent wastes. Although many fluids could be chosen for crystallization purposes, carbon dioxide (SC-CO_2) is the most extensively used when processing pharmaceutical compounds, owing to its mild critical conditions of temperature and pressure (31.05 °C and 7.28 MPa, respectively) and its non-reactive characteristic that makes it attractive towards sensitive drugs.

Crystallization by addition of a liquid antisolvent is widely used in the chemical, food, and pharmaceutical applications, specially as a batch operation. In the corresponding SCF-crystallization technique, CO_2 acts as the antisolvent for the compound of interest. Its addition to a liquor solution that contains the compound, decreases the solvation ability of the original solvent and causes the solute precipitation. Although there are numerous articles dealing with supercritical fluid micronization of single component, examples of drug delivery formulations by SCF are scarce (Perrut et al., 2005), and only few go up to the comparison of the dissolution or release profiles between raw and processed materials. In 2003, Dehghani and Foster have demonstrated the feasibility of dense gas antisolvent (GAS) technologies to produce drug formulations with various systems. Lately, protein-loaded polymeric particles below 1 μm in size were obtained from insulin/poly(lactic acid)/poly(ethylene glycol)s solutions by GAS operation (Caliceti et al., 2004); the morphology and biopharmaceutical properties of the product were affected mostly

* Corresponding author. Tel.: +33 149403431; fax: +33 149403414.
E-mail addresses: roy@limhp.univ-paris13.fr, subra@limhp.univ-paris13.fr (C. Roy).

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by the poly(ethylene glycol)s concentration and its molecular weight. Besides poly(lactic acid) (Taki et al., 2001), various biodegradable polymers, such as ethyl cellulose, poly(methyl methacrylate), cellulose acetate, PLA as well, or waxes (Boutin et al., 2004) were coprecipitated with the diuron herbicide in order to prepare sustained release systems; PLA was found to be the best coating substance, since the formulation was recovered as non-aggregated particles and provided a better efficient release of the herbicide. Supercritical antisolvent technique is developped as a semi-continuous or a batch operation. Both modes were tested to prepare ophthalmic drug delivery systems of acetazolamide and Eudragits (Duarte et al., 2007); the produced formulations allowed to slow down the drug release, but the semi-continuous mode was found to produce smaller particles and less aggregated than the batch operation.

In addition, co-precipitation could be investigated to improve the dissolution rate of a poorly water-soluble drug. Contrary to the previous sustained release formulations, the excipient has to exhibit a hydrophilic character. Dispersions of indomethacin and poly(vinylpyrrolidone) were produced by supercritical batch process and the produced formulations were exhibiting a rapid release (Gong et al., 2005). However, in case of hydrocortisone and poly(vinylpyrrolidone), dissolution of products prepared by supercritical batch mode was not faster than dissolution of systems processed by conventional techniques (Corrigan and Crean, 2002).

In this work, Theophylline was selected as model drug to prepare a controlled release matrix by precipitation assisted by supercritical antisolvent. Theophylline is a methylxanthine derivative widely used for its bronchodilatory effects in asthma and pulmonary diseases therapy. Besides a low solubility in water (below 0.9% at 23 °C Zacchigna et al., 2003), the drug exhibits dose-dependent pharmacokinetics (Parvez et al., 2004) and shows a narrow therapeutic range (10–20 μg/mL), i.e. a small difference between therapeutic benefits and toxicity effects (Mengozzi et al., 1998). Over 80% of individuals with serum theophylline levels of 20–30 μg/mL show toxic symptoms (Gohel et al., 1997). It is thus difficult to maintain the theophylline plasmatic levels necessary for the induction of its bronchodilating effect. The drug action could be improved through formulations as controlled delivery systems. Theophylline is therefore often selected as model compound to develop new formulations or improved methods: direct compression of drug/polymer mixtures (Ceballos et al., 2005), immobilization of theophylline into polymer hydrogels (Antal et al., 1997; Liu et al., 2005), dip-coating of drug tablets by polymer solutions (Lin and Lee, 2003), incorporation of theophylline into the aqueous phase of stable multiple emulsions (Leadi Cole and Wateley, 1997), spray drying of drug/polymer aqueous solutions (Asada et al., 2004), ultrasonic spray-congealing method (Albertnini et al., 2004) or preparation of microcomposites with hydrogenated palm oil (Rodrigues et al., 2004). Recently, precipitation by supercritical antisolvent was investigated to prepare theophylline and hydroxypropylmethylcellulose matrices (Moneghini et al., 2006).

The first objective of this work was to ascertain the feasibility of the supercritical antisolvent technique as a route to produce controlled release mixtures, that, in the specific case of theophylline delivery design, would prevent the initial burst of the delivery and sustain the drug release to avoid the toxicity level. Due to the fast observed dissolution rate of theophylline, the biodegradable Poly(lactic acid) was selected as candidate to slower the release. It is also well known that dissolution properties could be manipulated through physico-chemical properties of the drug, as particle size, modification of crystal habit or polymorphism (Leuner and Dressman, 2000), thus, special attention was paid to the micronization of the pure drug. Finally, and due to the observed crystal singularity of the CO_2-processed samples, Trifluoromethane was selected as a new compressed antisolvent. Compared to CO_2, the fluid has lower critical conditions of temperature and pressure (25.6 °C and 4.84 MPa, respectively) and a higher polarity susceptible to influence the process performances, and consequently, the product characteristics. CHF_3, also known as fluoroform or R-23 as refrigerant, is one of the haloalkanes with zero ozone depletion as it does not contain chlorine atoms, and is thus acceptable towards environmental concerns.

2. Materials and methods

2.1. Materials

Carbon dioxide (CO_2, purity 99.5%) and trifluoromethane (CHF_3, 100% CAS number 75-46-7)) were supplied by Air Liquide (France). Ethanol (EtOH, 99.5%) and methylene chloride (DCM, 99.5%) were from VWR International. Anhydrous theophylline (THEO, minimum 99%) was purchased from Sigma–Aldrich and poly(lactic acid) (L-PLA, $M_w > 100{,}000$) from Galactic Laboratories (Belgium). PLA was stored at −18 °C as received. Fresh solutions of theophylline, pure or

Table 1
Some physical properties of materials

Materials	M (g/mol)	T_m (°C)	T_b (°C)	T_c (°C)	P_c (MPa)	ρ (g/mL)
Theophylline	180.2	270–74	–	–	–	–
l-PLA	>100000	170–76	–	–	–	–
DCM	84.9	−95	40	237	6.3	1.336
EtOH	46.1	−117	78.5	243	6.1	0.789
CO_2	44.0	−57	−78	31.05	7.28	0.713
CHF_3	70.0	−155	−82	25.60	4.84	0.670

M: molecular weight, T_m: melting temperature, T_b: normal boiling point, T_c: critical temperature, P_c: critical pressure, ρ: liquid density at 25 °C. Solubility of theophylline in (EtOH:DCM): 32.7 mg/mL at 36 °C and atmospheric pressure (Subra et al., 2005).

mixed with the polymer, were prepared for immediate use without further purification. Some properties of materials are listed in Table 1.

2.2. Equipment and procedure

According to previous studies (Subra et al., 2005), recrystallization of theophylline was carried out from an ethanol:methylene chloride solution (EtOH:DCM, 1:1 in volume), since both the drug solubility and the CO_2 antisolvent effect were higher in this mixture than in pure solvents individually. Moreover, the medium was also ensuring the dissolution of the polymer.

2.2.1. Batch process

The set-up is presented schematically in Fig. 1. The basic equipment and procedure were previously described (De Gioannis et al., 2004). Briefly, the crystalliser was a high pressure vessel of 490 mL, with sapphire windows to allow visual observations of the solids formation, and equipped with baffles to preclude the generation of a central vortex upon stirring. The temperature was measured by a type K thermocouple and controlled by electric resistances at ±1 °C. The pressure was measured by a pressure sensor (Asco) with an accuracy of 0.1 MPa. The stirring was provided by an electric motorized magnetic stirrer with a eight-bladed Rushton-type turbine of 25 mm in diameter. A stainless steel filter of 2 μm in porosity and a membrane of 0.22 μm were located at the vessel bottom to collect the precipitated crystals. The CO_2, firstly cooled at 0 °C by circulation through a chiller, was delivered by a high pressure pump and gradually introduced into the stirred solution through the turbine; the CO_2 flow at the pump inlet was monitored by a Flow-meter (Bronkhorst, France) and integrated over time through Labview software. The reactor was not purged from the air initially present, since its configuration did not allow for purging without sweeping some of the organic liquor.

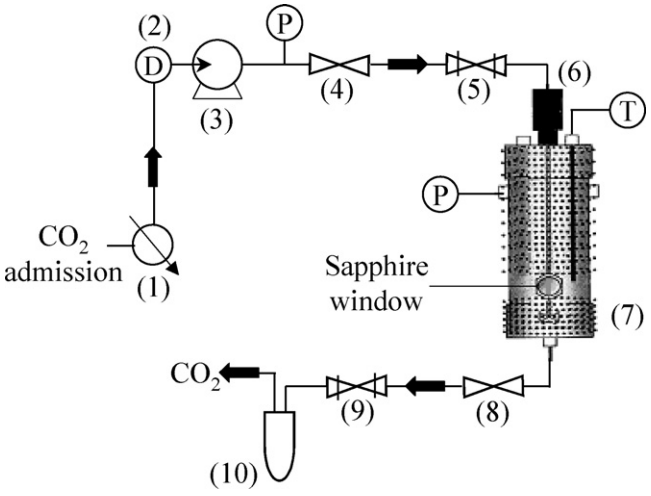

Fig. 1. Batch set-up. (1) Cooling exchanger, (2) flow meter, (3) CO_2 pump, (4 and 8) valve, (5 and 9) micrometering valve, (6) stirrer, (7) precipitator and (10) separator.

Theophylline was recrystallized from 85 mL of EtOH:DCM mixture at 36 °C, adding CO_2 up to a final pressure of 10 MPa under stirring (varied between 100 and 500 rpm). For co-precipitation experiments, the polymer was dissolved in the solution before loading the vessel. After stabilization of the vessel temperature, CO_2 was introduced into the solution up to the desired pressure; once the pressure was attained, the discharge of the unprecipitated species by the exit line and the drying of crystals were carried out immediately, by flowing fresh CO_2 to compensate for the exit flow, during 120 min. After depressurization, crystals were collected on filter, walls and stirrer for subsequent analysis; particles were collected without discriminating their location in the vessel, so, characteristics reported below are representative of the whole produced powder. The amount of collected powder ratioed to the initial amount of component(s) defined the process yield.

The experiments carried out with CHF_3 instead of CO_2 were performed by the same procedure.

2.2.2. Semi-continuous process

The experimental set-up and procedure were described in previous works (Subra and Jestin, 1999; Vega-González et al., 2004) and schematically presented in Fig. 2. The precipitator consisted of a stainless steel vessel of 490 mL equipped with three sets of sapphire windows, in order to get a visual observation of both the spray and the precipitation. The liquor solution was co-currently introduced into the continuous flow of CO_2 and constantly discharged from the vessel bottom. The solution was sprayed at the top of the chamber through a swirl nozzle (Lechler) that had an exit diameter of 100 μm. The pressure inside the vessel was regulated downstream by a micro-metering valve and the temperature was controlled by heating jackets at ±1 °C. The separation of the solvents from the fluid occurred across the metering valve in a homemade cyclonic separator. A stainless steel frit of 2 μm pore size and a membrane of 0.22 μm were placed at the vessel bottom to retain the precipitate.

A typical experiment started by delivering CO_2 to the vessel until the desired pressure is attained. Once the operating pressure, temperature and flow rate (about 67 mL/min at 0 °C) were stabilized, ~40 mL of the Theophylline (+PLA)–EtOH:DCM solution was sprayed at ~3 mL/min into the precipitator through

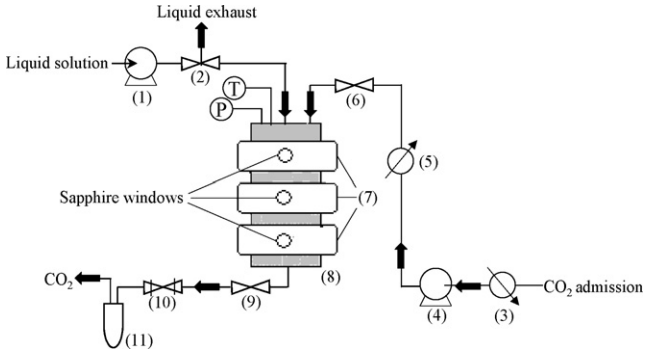

Fig. 2. Semi-continuous set-up. (1) Liquid solution pump, (2, 6 and 9) valve, (3) CO_2 cooling bath, (4) CO_2 pump, (5) CO_2 heating bath, (7) heating jackets, (8) high-pressure vessel, (10) micrometering valve and (11) separator.

Table 2
Conditions and results of experiments performed with pure theophylline

Run#	Process	Antisolvent	T (°C)	P (MPa)	χCO 2 (%)	Yield (%)	Particles shape	Size (μm)	
								SEM	PSD
1	Batch	CO_2	36	10.0	84*	64	Hexagon	20–100 × 5–20	54
2		CO_2	36	10.0	87*	54	Hexagon	40–100 × 15–25	64
3		CHF_3	36	10.0	–	41	Triangle	60–100	81
4		CHF_3	30	6.5	–	53	Triangle	12–50	55
5	SAS	CO_2	36	10.0	96.4**	77	Rhomb + hexagon	40–95 × 10–40	58
6		CO_2	60	10.5	96.9**	85	Rhomb + hexagon	5–30 × 2–10	18
7		CO_2	60	16.0	96.8**	87	Rhomb + hexagon	20–50 × 10–40	49

$C \sim 24$ mg/mL in all cases. For the batch process, $R \sim 500$ rpm for the runs #1 and #4, 300 rpm for the run #2 and 100 rpm for the run #3. Yield is the ratio of collected amount to initial amount of THEO dissolved in the liquor. χCO_2 is the CO_2 content (mole fraction) in the CO_2 + solvent mixture at pressure P, calculated from *flow-meter data in batch or **solution and CO_2 flows in SAS. D_{50} is the mean value of the particle size distribution measured by laser diffraction set-up.

the nozzle. During the spray, the solution and CO_2 merged as a new mixture in which the solute was less soluble, and consequently, precipitation was induced. At the end of the solution spraying, the CO_2 flow was maintained through the vessel for about 30 min, in order to wash out the residual content of organic solvents. The crystalliser was then gently depressurized at the working temperature and the precipitates were collected on walls and membrane as for the batch process.

2.3. Characterization

2.3.1. Scanning electron microscopy

Scanning electron micrographs (SEM) were used to document the changes in morphology, size and aggregation of the produced powder. Micrographs were obtained with a Leica S440, after gold-palladium coating (∼4 min).

2.3.2. Particle size distribution

PSD were analyzed with a Malvern Instrument laser diffraction instrument (MS2000, accurate in the region of 0.02–2000 μm) after dispersion of powders in petroleum.

2.3.3. X-ray powder diffractometry

Powder X-ray diffraction analysis (XRD) was performed with a INEL XRG 3000 diffractometer, Cu $K\alpha_1$ radiation, a voltage of 40 kV and a current of 20 mA. The grazing angle was of 3°; intensities were measured with a curved multichannels detector over a 2θ range of 5–90°.

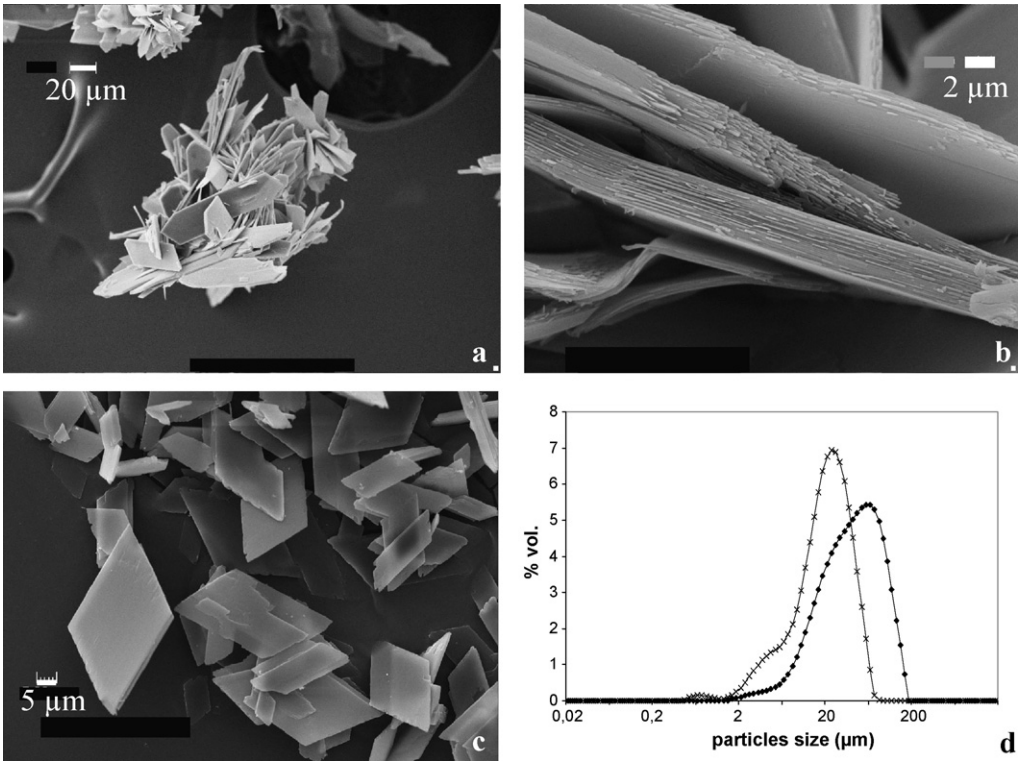

Fig. 3. SEM images of powders obtained with CO_2 by batch operation (a and b: run #2 at different scales) or by SAS precipitation (c: run #7), and particles size distribution (vol.%) of CO_2-processed theophylline (d: run # (♦) and (×)).

2.3.4. Dissolution and release profiles

Dissolution and release profiles were established from powders, by a basket method and in a home-made device settled according to USP 28. Thirty milligrams of powder put in a filter was placed into a basket and immersed in 900 mL of distilled water at 37 °C upon a stirring of 100 rpm. Theophylline content was determined by regular samplings of the aqueous solution and subsequent analysis by reversed phase chromatography. HPLC conditions were: methanol: acetic acid: water (30:2:68, v:v:v) as mobile phase, a column of 150 mm × 4.6 mm, Nucleosil 100-5 C18 Macherey-Nagel, $T = 35$ °C, $F = 1.0$ mL/min, UV detection at 275 nm, $V_{inj} = 10$ μL. Theophylline eluted at 4.5 min as a single symmetric peak.

3. Results and discussion

3.1. Pure theophylline

Pure theophylline was recrystallized using CO_2 as antisolvent by the two modes, i.e. batch and semi-continuous, the range of operations being selected owing our previous experience on the two processes (De Gioannis et al., 2004 for the batch; Subra et al., 2005; Vega-González et al., 2004 for the SAS). We reported here only the experiments performed as a pre-step of the co-precipitation with polymer; the detailed influence of several parameters upon the process performances is out of scope and could be find elsewhere (Roy et al., 2005; Subra et al., 2005). When CHF_3 was used, experiments were carried out only in the batch mode, since the higher fluid consumption of the semi-continuous mode would have exceeded the CHF_3 reservoir capacity. Conditions of experiments and size results are summarized in Table 2. Experiments were all carried out with an initial theophylline concentration of 24 mg/mL.

3.2. Morphology

The CO_2-processed theophylline was usually recovered as a free-flowing powder (the particulate powder was easily flowing in the storage flask, by opposition of cotton-like or microfibers systems) and was made of agglomerated particles with a morphology of hexagonal flake (Fig. 3a); higher magnitude allowed to detail that the flake morphology resulted from a piling of smaller units (Fig. 3b). For conditions reported here, theophylline particles had a longer axis between 5 and 100 μm, a width between 5 and 40 μm and a thickness from 1 to 2 μm. The semi-continuous mode seems to produce particles with smaller size than the batch process (morphology given in Fig. 3c), as illustrated by the particles size distribution (PSD) reported in Fig. 3d for run #6 (semi continuous) and run #1 (batch). Generally, the PSD profiles were asymmetric with a large scattering of the small sizes, so produced powders were quite dispersed in sizes. Previous investigations had been carried out in attempts to reduce particle size and/or particle size distribution. The semi-continuous mode was found to be more suitable to modify the size characteristics of the powder, specially by exploring both single-phase or two-phases conditions of CO_2 + solvent equilibria (Subra et al., 2005). In the batch mode, the stirring speed is known to influence the particle size (De Gioannis et al., 2004); however, in case of theophylline, the stirring did not influence notably nor the morphology nor the size, i.e. no clear deviation from the reproducibility range was obtained. Thus, among the CO_2-essays reported hereby, the smallest particles were produced by a semi-continuous operation carried out at 60 °C and 10.5 MPa, with an observed range of particle sizes of 5–30 μm × 2–10 μm, in agreement with the mean diameter D_{50} of 18 μm provided by the size analyser.

When CHF_3 was used as antisolvent, theophylline was also recovered as a free-flowing powder, with particles highly agglomerated. The morphology was however different, as illustrated by the SEM images (Fig. 4a); the plate-like particles exhibited a triangular shape, whose side length was between 60 and 100 μm when particles were obtained at 36 °C and 10 MPa. Contrary to the CO_2-processed samples, the flakes were sensitive to the operating conditions of temperature and pressure, with a side that decreased to 12–50 μm at 30 °C and 6.5 MPa; however, particles seemed more welded together. The particles size distribution of run #3, reported in Fig. 4b, showed a more symmetric profile although some spreading of smaller sizes was still noticeable. Compared to run performed with CO_2, the use of CHF3 did not improve significantly nor the particle sizes (sizes provided by SEM or reported D_{50} values were in the same range whatever the fluid) nor the yield. CHF3 is by far more expansive than CO_2 (€100/kg versus €4.5/kg for CO_2 and the lack of

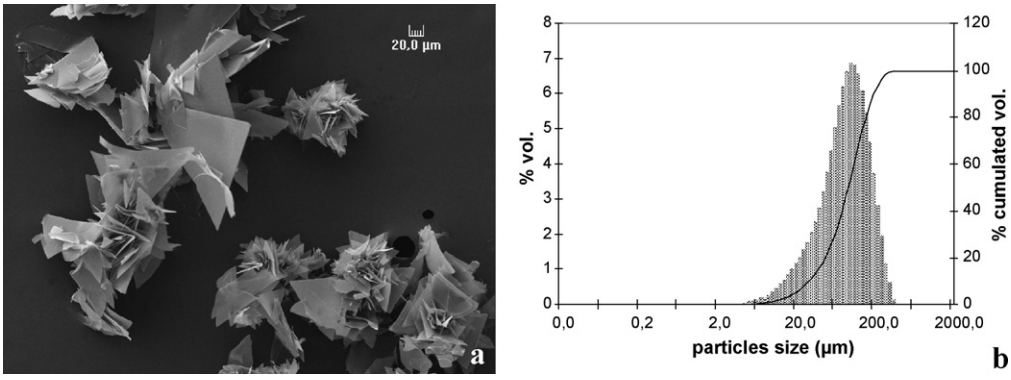

Fig. 4. SEM images and particles size distribution (vol.%) of a powder obtained with CHF_3 by batch operation (run #3).

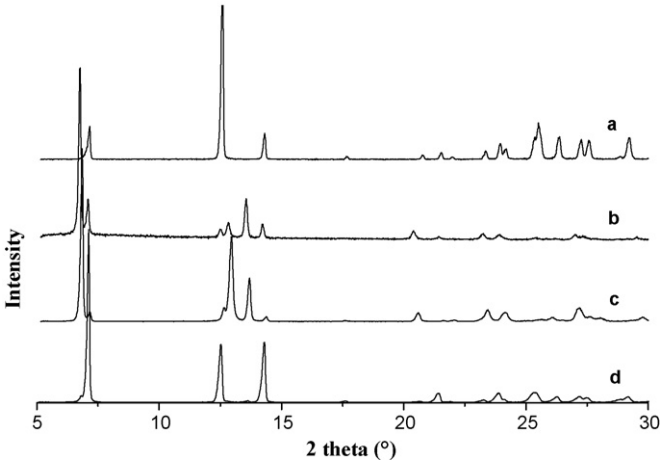

Fig. 5. X-ray diffraction patterns of crude theophylline (a) and of some powders obtained by semi-continuous operation (b: run #5), by batch precipitation with CO_2 (c: run #2) or with CHF_3 (d: run #3).

significant improvement of process performances explained the limited number of experiments carried out with this fluid.

3.2.1. Crystallinity

Powder X-ray diffraction patterns (XRD) of theophylline, raw or SCF-processed, are given in Fig. 5. With typical peaks at 7.1° 12.6° and 14.3° 2θ, the XRD pattern of unprocessed theophylline (Fig. 5a) closely matched the pattern reported by Airaksinen et al. (2003) for anhydrous theophylline, or the pattern of orthorombic theophylline anhydrate provided by the diffraction library (powder diffraction file 27-1977, ICDD, USA). Compared to unprocessed theophylline, the pattern of the CO_2-processed samples (Fig. 5b for the semi-continuous, c for the batch) showed different characteristic peaks at 6.8°, 12.9° and 13.6° 2θ, besides significant differences in the 20°–30° 2θ range. All CO_2-processed samples (including those obtained in conditions not reported in this study) had similar patterns, with only little variations in the peaks relative intensity but not in the peak position. Most samples exhibited, in addition to these new peaks, those of unprocessed theophylline but with a lower intensity; this could indicated that although the new pattern is privileged, part of theophylline recrystallized in the usual crystal lattice. Literature has already pointed theophylline polymorphism (Airaksinen et al., 2004; Phadnis and Suryanarayanan, 1997), but X-rays peaks did not match our new peaks. Complementary analysis are in progress to determine if the occurrence of the usual crystal form in the produced powders depends on the experimental conditions, and more specially, on the phase regime where the precipitation occurred (mono- or biphasic domain).

Anhydrous theophylline is known to transform into theophylline monohydrate on contact with water molecules, as example during the wet granulation process (Rasanen et al., 2001; Airaksinen et al., 2003), during the storage of tablets under elevated humidity conditions (Ando et al., 1992), or during crystallization from solvent + water mixtures (Zhu et al., 1996). Despite the fact that traces of water could have been introduced into the precipitator as air moisture or solvent impurities, patterns of CO_2-processed samples were clearly distinct from the monohydrate curve reported by Airaksinen et al. To assess whether the crystallization medium (EtOH:DCM) or CO_2 affected the crystal lattice, unprocessed theophylline and a CO_2-processed sample were dissolved in EtOH:DCM, and recrystallized by cooling. Both samples showed only the characteristic peaks of anhydrous theophylline. Consequently, the distinct patterns of the CO_2-processed samples were specific to the use of CO_2 as antisolvent and did not come from the solvents used as liquor. The specificity was also accredited by comparison with the XRD pattern of a CHF_3-processed samples, as given in Fig. 5d. Contrary to the CO_2-samples, the CHF_3-samples showed only the characteristic peaks of the unprocessed and anhydrous theophylline (7·1°, 12.6° and 14.3° 2θ). As function of the process operating conditions, only the relative intensity of peaks changed, their position remained the same and no new peaks appeared. The precipitation of theophylline by supercritical SAS process has been carried out by Moneghini et al. (2006). The CO_2-processed pattern clearly exhibited peaks of the anhydrous form, but few peaks not attributable to any polymorphic forms of the drug were detected in the same range that our new peaks; as those peaks occured when theophylline was co-processed with polymers, authors suggested that they could sign the existence of interactions between the drug and carriers. In our experiments, the new peaks were detected on pure processed theophylline. To elucidate the CO_2-samples singularity, XRD analysis such as Rietveld Refinement are under investigation, but they should be more extensively carried out to provide straightfull conclusions. Other techniques were used to go further in the product characterization. Indeed, pseudopolymorphic changes could be detected using infrared spectroscopy, thermal methods, optical and electron microscopy, nuclear magnetic resonance spectroscopy and X-ray diffraction. HPLC was first performed to confirm that theophylline was indeed produced; raw and CO_2-processed samples prepared in same concentrations gave same peak areas, i.e. areas exhibited a deviation in the range of reproducibility. The differential scanning calorimetry (DSC) analysis of raw theophylline and of a CO_2-processed sample closely resembled, revealing an endothermic peak at 266 and 265 °C, respectively. The technique suggested that the degree of crystallinity of both samples were high. Vibrational spectroscopy techniques usually provide an excellent method for probing solid-state bonding interactions between molecules including polymorphs and solvates. Raman and Infra Red spectra of raw and CO_2-processed theophylline were monitored; for the CO_2-processed sample, a shift from 2 to 8 cm^{-1} of several Raman bands and a shift of the IR peak from 1187 to 1182 cm^{-1} were noticed. These slight differences in IR and Raman spectra might suggest modifications in the bonding interactions between molecules but differences are too small to evidence a pseudopolymorphism by CO_2-insertion for example.

Based on the various characterization techniques (XRD, HPLC, IR, DSC, Raman), it can be concluded that crystallization assisted by CO_2 produced crystals which exhibit a high degree of internal regularity, but with a different crystal lattice than the starting material, and might sign a new crystalline form

Table 3
Conditions and results of co-precipitation of theophylline and polylactic acid

Run#	Process	Antisolvent	Concentrations (mg/mL)		Conditions		χCO_2 (%)	Product yield (%)	Macroscopic aspect	Size (μm)	
			THEO	PLA	T (°C)	P (MPa)				SEM	PSD
8	Batch	CO_2	12	12	36	10.0	88*	35	Coarse film	nd	nd
9		CHF_3	24	10	32	6.5	–	64	Powder	13–25	98
10		CHF_3	20	10	29	6.5	–	66	Powder	10–20	137
11	SAS	CO_2	12	12	36	10.0	96.8**	81 ± 5	Powder	12–54 × 15–100	75 ± 6
12		CO_2	12	24	36	10.0	96.5**	80	Powder	14–40 × 16–66	53
13		CO_2	24	12	36	10.0	96.5**	91 ± 9	Powder	7.5–45 × 12–55	25 ± 10

For batch conditions, $R \sim 500$ rpm. Yield is the ratio of collected amount to initial amount of THEO + PLA dissolved in the liquor. χCO_2 and D_{50} have same definition than in Table 2 (nd: not determinable).

of the theophylline, although extra-investigations are required to accredit this assumption. Like adsorbed water, one can imagine that CO_2 is able to penetrate into the structure whilst the crystal growths, letting the crystal with a modified lattice in the final arrangement.

3.2.2. Theophylline and polylactic acid formulations

Co-precipitation of theophylline and polymer was carried out with two objectives. The first one was to assess the ability of the techniques to produce formulations of theophylline with sustained release; the second objective was to use the polymer to prevent the growth of theophylline particles during the antisolvent process, in an attempt to decrease the product particle size. The polymer was expected to precipitate onto the drug nuclei, and as a barrier, to limit the diffusion of the incoming drug molecules that nourish the crystal growth. Conditions of processing are summarized in Table 3.

3.2.3. Batch precipitation

An experiment was performed by CO_2-batch operation at 36 °C and 10 MPa with a (THEO:PLA) ratio of 1:1, and with a concentration of 12 mg/mL in each component. The product was recovered as a film that coated and adhered firmly to the vessel walls and to the filter. Since the objective was to produce a powdered formulation, the run was discarded, and we further focused on CHF_3 as antisolvent.

Experiments were carried out in conditions of pressure and temperature that were the most favorable to pure theophylline processing, i.e. 6.5 MPa and ∼30 °C; free flowing powders were recovered with satisfying yields of about 65%. The morphology of the precipitates is viewed by the SEM pictures given in Fig. 6a and b. Aggregates were formed with plate-like particles of a triangular shape that closely resembled to particles obtained by processing pure theophylline. To the periphery of some triangular particles, small spheres can be seen, that could reasonably be assigned to the polymer. The presence of those two distinct populations, even in unequal proportions, attested that co-precipitation of theophylline and PLA had occurred in these conditions, as further confirmed by XRD analysis. Indeed, besides characteristic peaks of anhydrous theophylline, the patterns of the CHF_3-processed mixture (Fig. 9c) exhibited a large peak at ∼16.6° 2θ, corresponding to PLA (Fig. 9a).

3.2.4. Semi-continuous precipitation

Experiments were performed at 36 °C and 10 MPa with special emphasis to THEO:PLA ratio. Although the conditions were not the best for pure theophylline precipitation, the lower temperature of 36 °C was privileged in co-precipitation experiments, owing to the tendency of PLA to film onto the vessel at 60 °C. In those conditions, precipitates were collected as free flowing powders with yields in the range of 80–90%. The morphology was assessed through the SEM pictures, as reported in Fig. 7 for the three runs. The powders were made of two distinct populations: one of plate-like particles similar to those observed with pure theophylline that exhibited sizes in the range of 7.5 × 12–40 × 100 μm, and the second population made of small spheres of ∼0.5–4 μm in diameter, attribuable to PLA by analogy of the particle shape recovered when the polymer

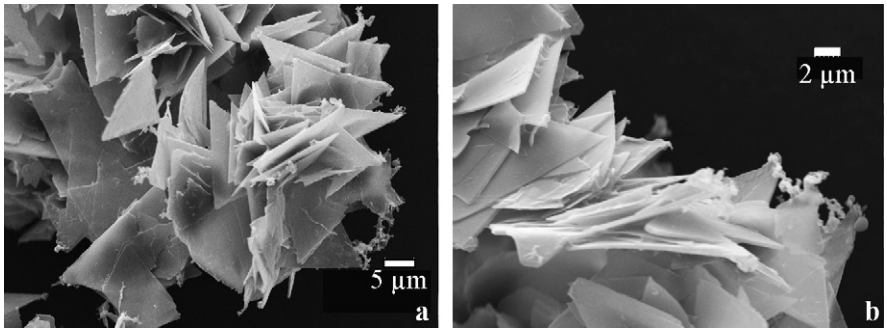

Fig. 6. SEM images of powders obtained by co-precipitation with theophylline and PLA in batch with CHF_3 (a and b: run #9 at different scales).

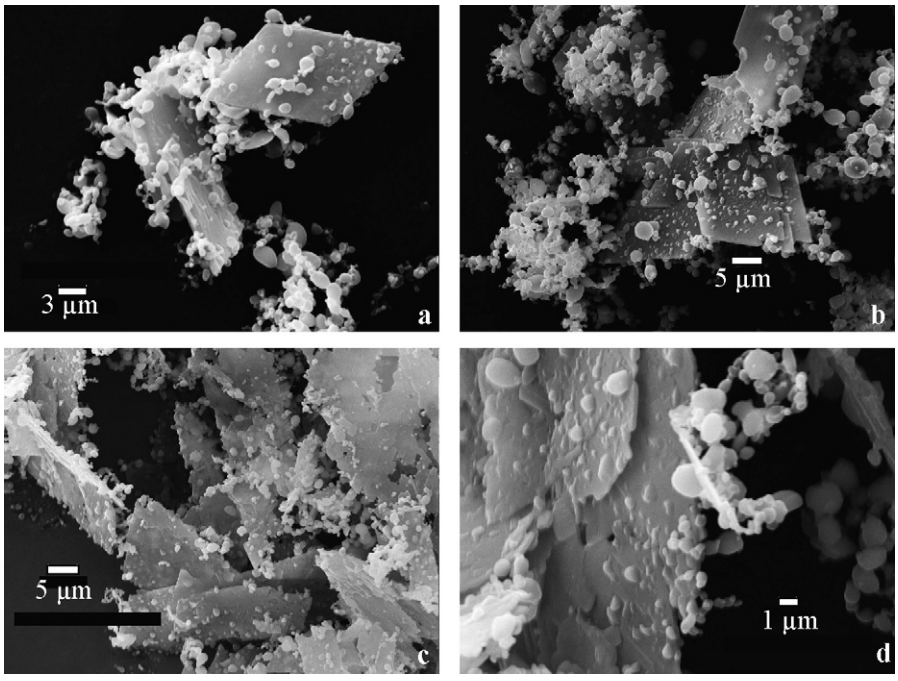

Fig. 7. SEM images of powders obtained by recrystallization of theophylline + PLA in semi-continuous with CO_2 (a: run #11, b: run #12, c and d: run #13 at different scales).

is processed alone. Pictures taken at higher magnitude (Fig. 7d) showed that semi-spheroid blisters existed at the surface of theophylline particles, surface which was not as smooth as when the pure drug was recrystallized. Thus, the co-processing of the drug and the polymer produced a powder made of coated theophylline particles together with spherical particles of PLA, these latest being susceptible to contain also theophylline.

The variation of THEO or PLA concentration in the initial solution has significant effect: an increase of the polymer concentration (#11 and 12) seemed to increase the population of individual spheres, whereas the increase of THEO concentration (#11 and 13) impacted the particle size and the size distibution as well (Fig. 8). It should be also noticed that, compared to the pure theophylline results (run #5 versus run #13), the addition of PLA favored a decrease of THEO particle size, as illustrated by SEM sizes reported in Tables 2 and 3, and D_{50} value as well.

Samples produced by semi-continuous operation were further characterized by XRD (Fig. 9); all powders showed peaks corresponding to the new crystal lattice of theophylline (new peaks at 6.7°, 12.8° and 13.5° 2θ), and the peak of PLA at 2θ of 16.6°. The coexistence of the new lattice with the usual one was however dependent on the processing conditions. Indeed, powders recovered from an initial drug concentration of 12 mg/mL (run #11 and #12) showed similar XRD-patterns without any

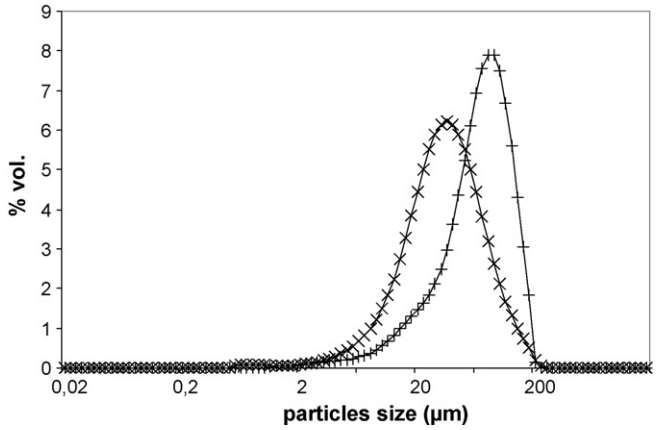

Fig. 8. Particles size distribution (vol.%) of powders obtained by co-precipitation with theophylline and PLA in semi-continuous with CO_2 (run #11 (+) and #13 (×)).

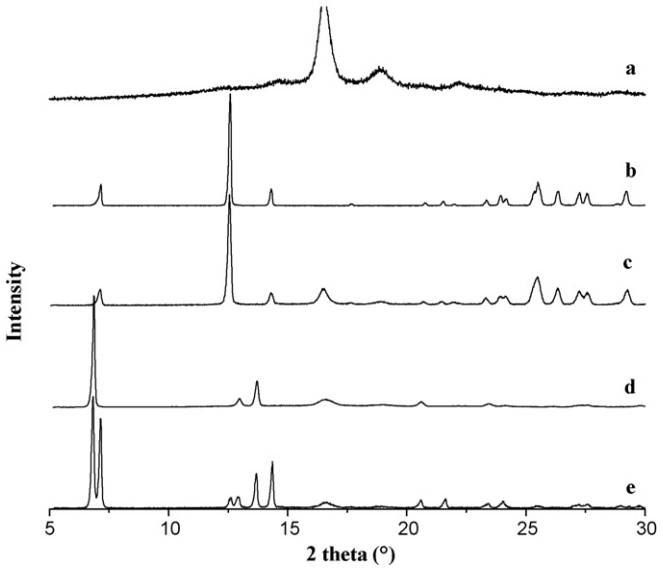

Fig. 9. X-ray diffraction patterns of unprocessed: (a) PLA and (b) theophylline, and of processed PLA + THEO mixtures: (c) CHF_3-batch run #9, (d) CO_2-semi continuous run # 12, (e) CO_2-semi continuous run #13.

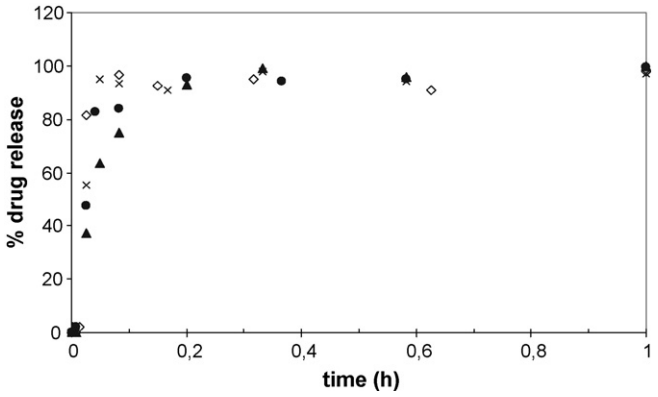

Fig. 10. Release profiles of pure theophylline prepared by batch operation with CO_2 (run #1: ●) or with CHF_3 (run #4: ◊) or obtained by semi-continuous operation with CO_2 (run #5: ▲); the dissolution profile of crude drug (×) is given for comparison.

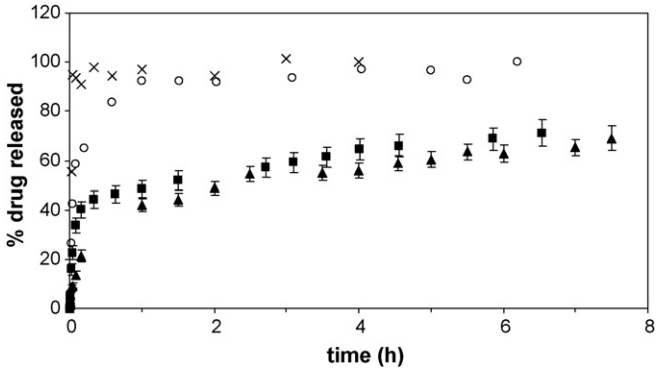

Fig. 11. Release profiles of the theophylline + polylactic acid prepared by batch operation with CHF_3 (run #10: ◯) or obtained by semi-continuous operation with CO_2 (run #11: ■, and #13: ▲); the dissolution profile of crude drug (×) is given for comparison.

characteristics peaks of the anhydrous theophylline. The pattern of powder produced from an initial concentration of 24 mg/mL (run #13) exhibited peaks of both anhydrous theophylline and of the new lattice (Fig. 9d and e); peaks of the anhydrous form were of higher relative intensities than those of the new form. An increase of the drug concentration promoted thus the recrystallization of theophylline in the usual anhydrous lattice. When theophylline was processed alone, (run #5, Fig. 5b), the peaks of the new form were of higher intensity than peaks of the anhydrous form. Since the reversed effect was observed in THEO + PLA powders, one can assume that PLA preclude the occurrence of the new crystal lattice. Further investigations are currently in progress, to accredit this hypothesis and to separate the two effects of theophylline concentration and PLA content, through a closer analysis of extra samples prepared by this method.

3.2.5. *Dissolution and release profiles of theophylline*

Since crystal lattice could influence dissolution properties, the dissolution profiles of single theopylline obtained with the two techniques and antisolvents were monitored (Fig. 10). The profiles indicated that the drug was completely dissolved in less than one hour whatever the process or the antisolvent used. Neither the particle shape (triangular for the CHF_3-material, or hexagonal for the CO_2-material), nor the singular crystal lattice of the CO_2-samples were found to originate significant changes in the dissolution rates. Thus, the produced crystals were not satisfying from a therapeutic point of view, since the theophylline toxicity threshold could be easily exceeded (when plasma concentration exceeds 20 mg/L, adverse effects can occur Nair and Watson, 2005).

Fig. 11 shows the release profiles of theophylline from PLA formulations. The sample obtained by CHF_3-batch processing showed a quasi-similar behavior than the pure drug: although the initial burst was slower, a drug release of 92% was obtained in 1 h. The coating of theophylline by the polymer, if it occurred, or their mixing was not efficient enough to sustain the release. For the CO_2-samples, it is obvious that the drug release was much more slower, since only ∼50% of drug was released after 2 h whereas the pure drug was totally dissolved in 1 h. The two formulations whose profiles are given in Fig. 11, were obtained from initial concentrations of 12:12 mg/mL and 24:12 mg/mL of THEO:PLA; bars showed the scattering of data for replicates. Both profiles exhibited similar behavior, i.e. an initial burst (attribuable to uncoated theophylline) and a quite monotonous subsequent release of the drug. However, the formulation issued from an initial THEO:PLA ratio of 2:1 (run #13) provided a slower release of the drug, with a 70% release in 8 h, compared to 6 h for the 1:1 THEO:PLA formulation (run #11).

As mentionned in the introduction section, a formulation of theophylline by the supercritical route has been already proposed in literature (Moneghini et al., 2006). The investigated polymer was hydroxypropylmethylcellulose (HPMC), and release profiles of obtained products were monitored. When profiles were obtained from powders, almost 90% of the drug was released in 3 min. When powders were compressed as tablets, the release was indeed delayed but was dependant of the polymer molecular weight. With HPMC-E5 tablets, 90% of theophylline was released in 60–85 min, and only the use of a higher molecular weight HPMC-K100 allowed to sustain significantly the theophylline release up to 8 h.

In order to enlighten the possible release mechanism, the kinetics below 60% of drug release was fit by the Power law (Siepmann and Peppas, 2001). The Power law consists in the representation of M_t/M_∞ versus t^n, where M_t is the amount of drug released at time t and M_∞ is the total amount of drug in the sample and n the release exponent. The comparison of the n exponent with established limits allows for proposing the release mechanism of the drug from the samples under consideration. The limits for n that allow to distinguish a transport by Fickian or anomalous diffusion, depend of the particle geometry. According to Sipmann and Peppas, values of 0.43, 0.45 and 0.5 are usually assessed for a release from a monodispersed population of spheres, cylinders and slabs, respectively, but they are susceptible to change if the population is polydispersed. As exemple, the n exponent was found to be of ∼0.30 when the release characterization was obtained from an hypothetical particle size distribution of 20% 20 μm, 60% 100 μm and 20% 500 μm (Ritger and Peppas (1987)), which

was significantly different from the $n = 0.43$ value obtained from a 100 μm monodispersed sample. Formulations #11 and #13 exhibited plate-like and spherical particles as well. However, the Power law was found to fit quite well the release profiles of all samples, with a release exponent n that was very close to the value of 0.45. The range of the experimental n value and its closeness to the limits that sign pure diffusion whatever the sample geometry, drove to the conclusion that the main release mechanism of theohylline from the formulations was Fickian diffusion.

4. Conclusions

Pure theophylline was successfully micronized as a free-flowing powder by supercritical process, and particles exhibited different shape according to the supercritical fluid used, i.e. triangular shape in case of CHF_3 or hexagonal/tablet-like morphology by CO_2. The distinguish feature of the CO_2 processing comes from the occurrence of a new crystal lattice accredited by new peaks in the X-rays diffraction patterns, whereas powders produced with CHF_3 were indisputably anhydrous theophylline.

Co-precipitation of theophylline and PLA was feasible only in the semi-continuous mode with CO_2, but also occurred in batch providing CHF_3 was used. Whatever the experimental conditions, powders were composed of two particle populations, with plate-like theophylline coated or not depending on the fluid, besides spherical particles of few microns in diameter. The drug was found to retain the specific crystallinity when processed by CO_2. The presence of polymer slightly prevented the growth of the theophylline particles, but was unable to reduce significantly their size down to limits acceptable for pulmonary delivery. Nevertheless, the rate of the drug release could be controlled to some extend by the preparation of these particles and furthermore, by varying the polymer content in the THEO:PLA ratio. The formulation of Theophylline with PLA allowed for significantly sustain the drug release over 14 h, providing that CO_2 was used as antisolvent. With CHF_3, the drug release was in the same range than pure theophylline, indicating that independent co-precipitation was likely to have occurred. When sustained, the release kinetics complied with the Power law, suggesting that the drug release was controlled by diffusion mechanism.

A controlled release system for the delivery of theophylline was therefore successfully prepared with satisfying yields using a CO_2 supercritical antisolvent process. However, the next step should be focused on the reduction of the particles size, and the use of coaxial nozzle to promote the solution atomization is forseen.

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References

Airaksinen, S., Karjalainen, M., Räsänen, E., Rantanen, J., Yliruusi, J., 2004. Comparison of the effects of two drying methods on polymorphism of theophylline. Int. J. Pharm. 276, 129–141.

Airaksinen, S., Luukkonen, P., Jorgensen, A., Karjalainen, M., Rantanen, J., Yliruusi, J., 2003. Effects of excipients on hydrate formation in wet masses containing theophylline. J. Pharm. Sci. 92, 516–528.

Albertnini, B., Passerini, N., Gonzalez-Rodriguez, M.L., Perissutti, B., Rodriguez, L., 2004. Effect on Aerosil® on the properties of lipid controlled release microparticles. J. Controlled Release 100, 233–246.

Ando, H., Ishii, M., Kayano, M., Ozawa, H., 1992. Effect of moisture on crystallization of theophylline in tablets. Drug Dev. Ind. Pharm. 18, 453–467.

Antal, I., Zelko, R., Röczey, N., Plachy, J., Racz, I., 1997. Dissolution and diffuse reflectance characteristics of coated theophylline particles. Int. J. Pharm. 155, 83–89.

Asada, M., Takahashi, H., Okamoto, H., Tanino, H., Danjo, K., 2004. Theophylline particles design using chitosan by the spray drying. Int. J. Pharm. 270, 167–174.

Boutin, O., Badens, E., Carretier, E., Charbit, G., 2004. Co-precipitation of a herbicide and biodegradable materials by the supercritical anti-solvent technique. J. Supercrit. Fluids 31, 89–99.

Caliceti, P., Salmaso, S., Elvassore, N., Bertucco, A., 2004. Effective protein release from PEG/PLA nano-particles produced by compressed gas anti-solvent precipitation techniques. J. Controlled Release 94, 195–205.

Ceballos, A., Cirri, M., Mastrelli, F., Certi, C., Mura, P., 2005. Influence of formulation and process variables on in vitro release of theophylline from directly compressed Eudragit matrix tablets. Il Farmaco 60, 913–918.

Corrigan, O.I., Crean, A.M., 2002. Comparative physicochemical properties of hydrocortisone-PVP composites prepared using supercritical carbon dioxide by the GAS anti-solvent recrystallization process, by coprecipitation and by spray drying. Int. J. Pharm. 245, 75–82.

De Gioannis, B., Jestin, P., Subra, P., 2004. Morphology and growth of griseofulvin recrystallized by compressed carbon dioxide as antisolvent. J. Crystal Growth 262, 519–526.

Dehghani, F., Foster, N.R., 2003. Dense gas anti-solvent processes for pharmaceutical formulation. Curr. Opin. Solid State Mater. Sci. 7, 363–369.

Duarte, A.R.C., Roy, C., Vega-González, A., Duarte, C.M.M., Subra-Paternault, P., 2007. Preparation of acetazolamide composite microparticles by supercritical antisolvent techniques. Int. J. Pharm. 332, 132–139.

Gohel, M.C., Jani, G.K., Amin, A.F., Patel, K.V., Gupta, S.V., 1997. Application of classical experimental design for the development of theophylline microspheres. J. Controlled Release 45, 265–271.

Gong, K., Viboonkiat, R., Rehman, I.U., Buckton, G., Darr, J.A., 2005. Formation and characterization of porous indomethacin-PVP coprecipitates prepared using solvent-free supercritical fluid processing. J. Pharm. Sci. 94, 2583–2590.

Leadi Cole, M., Wateley, T.L., 1997. Release rate profiles of theophylline and insulin from stable multiple w/o/w emulsions. J. Controlled Release 49, 51–58.

Leuner, C., Dressman, J., 2000. Improving drug solubility for oral delivery using solid dispersion. Eur. J. Pharm. Biopharm. 50, 47–60.

Lin, W.-J., Lee, H.-G., 2003. Design of a microporous controlled delivery system for theophylline tablets. J. Controlled Release 89, 179–187.

Liu, J., Lin, S., Liu, E., 2005. Release of theophylline from polymer blend hydrogels. Int. J. Pharm. 298, 117–125.

Mengozzi, G., Intorre, L., Bertini, S., Giorgi, M., Soldani, G., 1998. Comparative bioavailability of two sustained-release theophylline formulations in the dog. Pharm. Res. 38, 481–485.

Moneghini, M., Perissutti, B., Kikic, I., Grassi, M., Cortesi, A., Principalle, F., 2006. Preparation of theophylline-hydroxypropylmethylcellulose matrices using supercritical antisolvent precpitation: a preliminary study. Drug Dev. Ind. Pharm. 32, 39–52.

Nair, S.R., Watson, J.P., 2005. Suicidal ideation caused by theophylline toxicity—a case report. Respir. Med. Extra 1, 13–15.

Parvez, N., Ahmed, T., Monif, T., Saha, N., Sharma, P.L., 2004. Comparative bioavailability of three oral formulations of sustained release theophylline in healthy human subjects. Int. J. Pharm. 36, 29–33.

Perrut, M., Jung, J., Leboeuf, F., 2005. Enhancement of dissolution rate of poorly-soluble active ingredients by supercritical fluid processes. Part I: micronization of neat particles. Int. J. Pharm. 288, 3–10.

Phadnis, N.V., Suryanarayanan, R., 1997. Polymorphism in anhydrous theophylline—implications on the dissolution rate of theophylline tablets. J. Pharm. Sci. 86, 1256–1263.

Rasanen, E., Rantanen, J., Jorgensen, A., Karjalainen, M., Paakkari, T., Yliruusi, J., 2001. Novel identification of pseudopolymorphismic changes of theophylline during wet granulation using near infrared spectroscopy. J. Pharm. Sci. 90, 389–396.

Ritger, P., Peppas, N., 1987. A simple equation for description of solute release. I. Fickian and non-Fickian release from non-swellable devices in the form of slabs, spheres, cylinders or discs. J. Controlled Release 5, 23–36.

Rodrigues, M., Peiriço, 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.

Roy, C., Vega-González, A., Subra-Paternault, P., 2005. Batch and semi-continuous techniques for the precipitation of theophylline. In: ISASF Proceedings IBN 2-905267-47-X, 10th European Meeting on Supercritical Fluids, Colmar-France December.

Siepmann, J., Peppas, N.A., 2001. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv. Drug Delivery Rev. 48, 139–157.

Subra, P., Jestin, P., 1999. Powders elaboration in supercritical media: comparison with conventional routes. Powders Technol. 103, 2–9.

Subra, P., Laudani, C.-G., Vega-González, A., Reverchon, E., 2005. Precipitation and phase behavior of theophylline in solvent-supercritical CO_2 mixtures. J. Supercrit. Fluids 35, 95–105.

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.

Vega-González, A., Domingo, C., Elvira, C., Subra, P., 2004. Precipitation of PMMA/PCL blends using supercritical carbon dioxide. J. Appl. Polym. Sci. 91, 2422–2426.

Zacchigna, M., Di Luca, G., Cateni, F., Zorzet, S., Maurich, V., 2003. Improvement of physicochemical and biopharmaceutical properties of theophylline by poly(ethylene glycol) conjugates. Il Farmaco 58, 1307–1312.

Zhu, H., Yuen, C., Grant, D., 1996. Influence of water activity in organic solvent + water mixtures on the nature of the crystallizing drug phase 1. Theophylline. Int. J. Pharm. 135, 151–160.