SUPERCRITICAL FLUIDS AS NEW MEDIA FOR THE GENERATION OF PHARMACEUTICAL COCRYSTALS – PHARMACEUTICAL CHARACTERIZATION OF THEOPHYLLINE-SACCHARIN COCRYSTALS

L. Padrela, M. A. Rodrigues, V. André, M. Teresa Duarte, S. Velaga[1], A. C. Fernandes, H. A. Matos, E. G. Azevedo*
Department of Chemical and Biological Engineering
Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal
[1] Department of Health Sciences, Luleå University of Technology,
S-971 87 Luleå, Sweden.

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Topic: Material processing in SCFs

ABSTRACT

The crystal form adopted by the respiratory drug theophylline was modified using supercritical fluids together with crystal engineering approaches in order to search for a solid material with improved physical stability. Cocrystals of theophylline and saccharin were generated from ethanol solutions by a supercritical fluid enhanced atomization (SEA) process. In this process, high pressure N_2 and CO_2 have been used as supercritical media. The cocrystals morphology was analysed by SEM (Scanning Electron Microscopy) and the potential cocrystalline phase was characterized by DSC (Differential Scanning Calorimetry) and PXRD (Powder X-Ray Difraction). The theophylline-saccharin (TPL-SAC) cocrystals were subjected to relative humidity challenges in order to assess their stability relatively to the pure theophylline anhydrate. This cocrystal demonstrated superior humidity stability to theophylline anhydrate under the conditions examined: after 1 day at 90% of relative humidity , the pure theophylline anhydrate converted into theophylline monohydrate while the theophylline-saccharin cocrystals remained stable still after one month.

INTRODUCTION

One challenging task in the pharmaceutical industry is the improvement of the physicochemical properties of active pharmaceutical ingredients (APIs). Nearly 40% of new chemical entities launched in the pharmaceutical market during the last decade failed to achieve their full potential activity (and most didn't even get into to the marketplace) because of solubility or stability issues. Issues ranging from poor solubility and inadequate dissolution properties to lack of crystallinity and instability affect the industry and do have significant impact on the productivity of drug research [1].
The selection of the pharmaceutical crystalline form can be used to optimize drug properties because solid form influences relevant physicochemical parameters such as solubility, dissolution rate, chemical stability, melting point and hygroscopicity, which can result in

* Corresponding author. Tel + 351-21 841 9394. E-mail: egazevedo@ist.utl.pt

solids with far superior properties. Some fundamental strategies to improve the aqueous solubility, or manipulation of technical properties of drugs, include the manipulation of solid state structure (polymorph changes or amorphous form) and the formation of a salt. Crystalline forms of APIs have traditionally been limited to salts, polymorphs, and solvates.
Over the last years, a new class of crystalline solids, named as pharmaceutical cocrystals, has been driving much interest from both the academic and industrial environments because their physical and pharmacokinetic properties (e.g. solubility, stability) can be different and improved as compared to the pure APIs [2, 3]. Pharmaceutical cocrystals, also referred as crystalline molecular complexes, may be defined as materials which are formed between a molecular or ionic API and a pharmaceutically acceptable co-crystal former, that is a solid at room temperature. They contain therefore two or more discrete molecular entities in the crystal lattice. APIs constitute complex chemical structures with functional groups that could involve in the molecular recognition events. It is the presence of these functional groups, such as amides and carboxylic acids, that provides an ability to engage in supramolecular event with co-crystal formers having complementary hydrogen bond donor and acceptor sites, thereby forming pharmaceutical cocrystals.
The use of Supercritical Fluids (SCF) in the generation of pharmaceutical cocrystals has been addressed for the first time in the literature over the last year [4, 5]. Using Supercritical Anti-Solvent (SAS) or Supercritical Enhanced Atomization (SEA) processes brings a new platform for the single-step generation of microparticles of these novel crystalline forms.
The API under consideration in the current report is theophylline, a drug commonly used in the treatment of asthma and other respiratory diseases. From a physicochemical standpoint, theophylline represents a challenge to formulators in that it is known to interconvert between crystalline anhydrate and monohydrate forms as a function of relative humidity (RH). Cocrystallization of theophylline with dicarboxylic acids (e.g., oxalic acid, malonic acid, etc.) had been shown to improve the stability of the drug towards moisture [6].
We address in this work the stability under high humidity conditions of a recently reported theophylline-saccharin cocrystal [5, 7]. Theophylline-saccharin cocrystals were generated by using supercritical N_2 and CO_2 as a new media for the cocrystallization.

MATERIALS AND METHODS

Materials
Theophylline (minimum 99% chemical purity) was purchased from BioChemika (USA) and saccharin from Sigma Aldrich (purity >99.9%). Absolute ethanol (99.5%) was supplied by Panreac, and carbon dioxide and nitrogen (99.998%) by Ar Líquido (Portugal).

Feed solution preparation for SEA processing
For TPL-SAC cocrystal production, a 1:1 mixture of theophylline (22.5 mg or 0.12 mmol) and saccharin (24.3 mg or 0.12 mmol) was dissolved in 10 g of ethanol.

Particle production by SEA
The SEA apparatus was configured for the co-crystallization as described schematically in Figure 1.

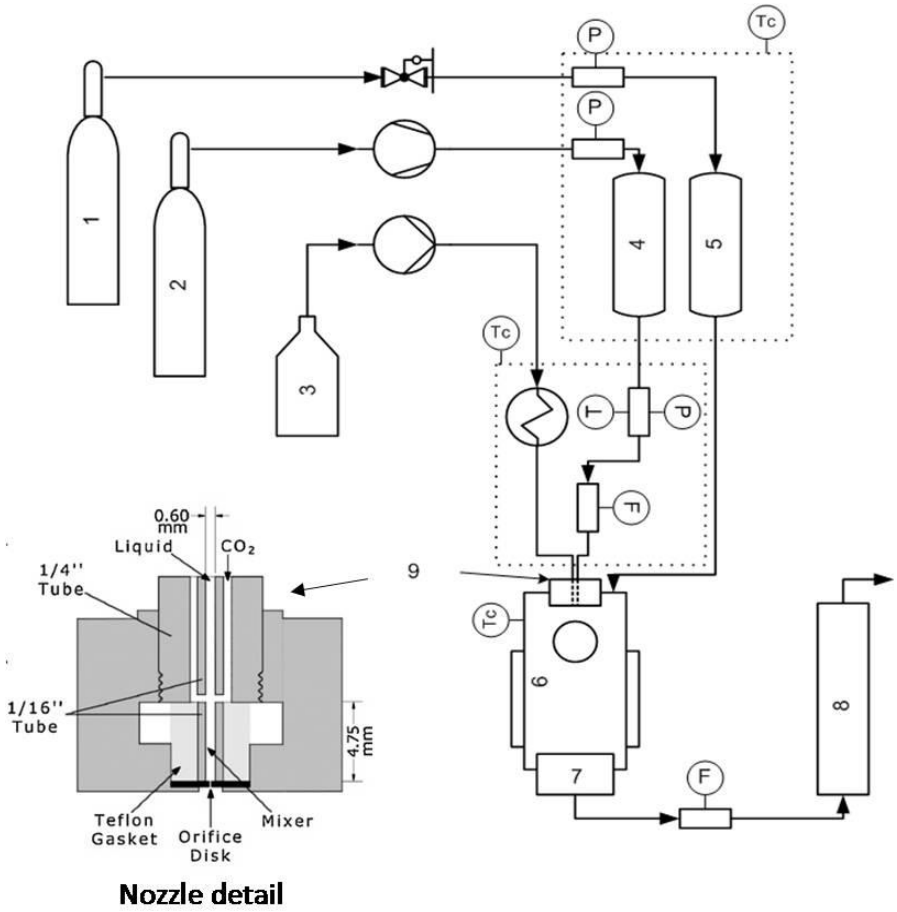


Figure 1 - Schematic diagram of the SEA apparatus. 1: N_2 cylinder; 2: CO_2 cylinder; 3: liquid solution flask; 4: temperature controlled CO_2 storage cylinder; 5: temperature controlled N_2 storage cylinder; 6: precipitator; 7: filter; 8: solvent trap; 9: nozzle cap.

The solution containing both substances (theophylline and saccharin) is pumped (by a TSP metering pump, model 2396-74) through a coaxial nozzle (where it mixes with the supercritical fluid in a small mixing chamber prior to its depressurization into a precipitator vessel. The gas (N_2 or CO_2) is compressed by a Newport Compressor (model 46-13421-2) to an atomization pressure of 8 MPa and the supercritical mass flow through the nozzle was measured by a Rheonik flow meter (model RHM007). The droplets formed during the spray atomization were dried inside a temperature -controlled precipitator at 323 K and near atmospheric pressure (0.1 MPa to 0.4 MPa). The solution flow rate was 1 g/min while the SC-CO_2 flow rate was 15 g/min and the N_2 flow rate was 25 g/min. The CO_2 was compressed by a Newport Compressor (model 46-13421-2) to an atomization pressure of 8 MPa. The particles are collected in the precipitator walls and from a filter at the precipitator exit. The samples were stored in a desiccator before their analysis.
A smaller nozzle orifice was used for CO_2 processing to limit the CO_2 flow rate and nozzle freezing due to strong depressurizations. A nozzle with a 200 μm orifice was used in the experiment with N_2, whereas a nozzle with a 100 μm orifice was used in the experiments with CO_2.

Particle and solid-state characterization
The particle size and morphology were analyzed by a Scanning Electron Microscope (SEM)

Hitachi S2400. Particle samples were coated prior to measurement with a gold film by electrodeposition in vacuum.
Thermal analyses (DSC) of the samples were performed using a differential scanning calorimeter DSC 121 (Setaram) which was calibrated for temperature and cell constants using indium and sapphire. Samples (6-7 mg) were crimped in non-hermetic aluminium pans and scanned at 5°C/min under an argon purge.
Powder X-ray diffraction patterns for different samples were collected on a Bruker D8 Advanced powder diffractometer using Cu Kα radiation (1.54056 Å) in a Bragg Brentano geometry. The tube voltage and amperage was 40 kV and 40mA, respectively. The divergence slit and antiscattering slit settings were variable for illumination of the 20 mm sample. Each sample was scanned with 2θ between 5º and 35º with a step size of 0.02º and 0.5 s at each step.

Relative humidity (RH) stability experiments
Relative humidity conditions were achieved at ambient temperature (20ºC) within sealed glass desiccator jars containing P_2O_5 for the 0% RH condition and a saturated solution of $CuSO_4.5H_2O$ for 90%. Relative humidity conditions were monitored with humidity-indicator cards (Sigma–Aldrich Co. Ltd.). In order to compare the stability of theophylline anhydrate to that of TPL-SAC cocrystals, samples of each were evaluated for physical stability at the conditions of 0% and 90% RH for time periods of 1 day, 3 days, 1 week and 1 month. Open paper dishes containing 120 mg of powder were stored in the RH chambers at ambient temperature. A dish was removed for each cocrystal material at each time point. Upon removal from the chamber, the samples were promptly evaluated for any form change by PXRD.

RESULTS

Solid state characterization of cocrystalline materials
Cocrystalline phase characterization and identification was carried out using DSC and PXRD. As Figure 2 shows, TPL-SAC cocrystals produced by the SEA technique have a single melting transition at ~198.1ºC that is different from those of the corresponding pure components (theophylline and saccharin) suggesting a successful cocrystalline phase formation.

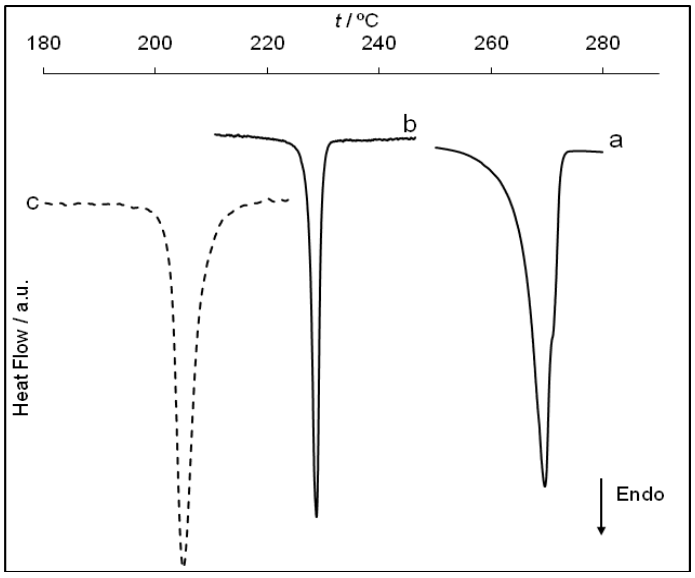
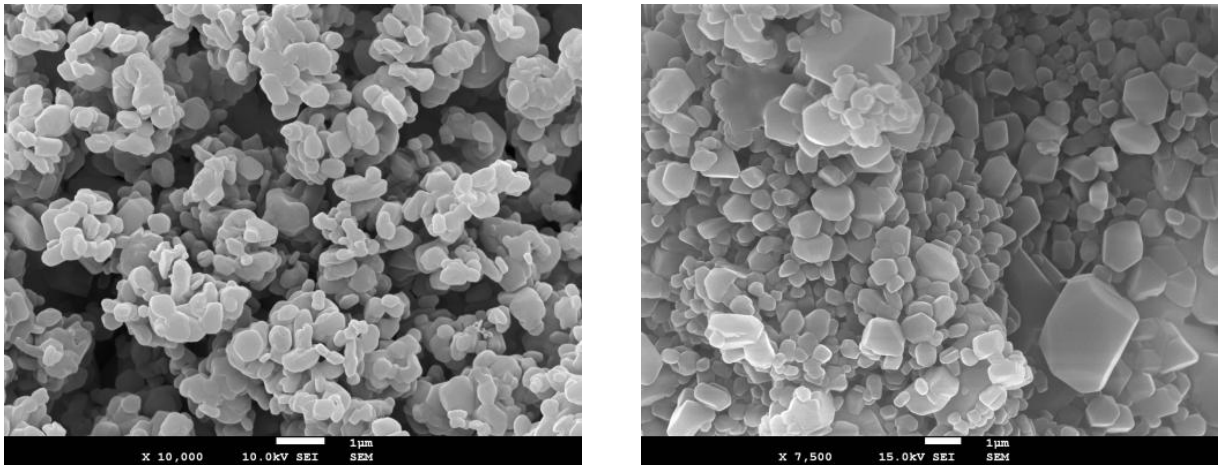


Figure 2 - DSC heating curves of (a) pure theophylline, (b) pure saccharin and (c) TPL-SAC cocrystals produced by the SEA technique.

Particle characterization of cocrystals

Theophylline-saccharin cocrystal particles produced by SEA and SAS techniques are represented by Figure 3.



(a) (b)

Figure 3 - SEM images of theophylline-saccharin cocrystals produced by supercritical (a) N_2 and (b) CO_2.

As Figure 3 illustrates, TPL-SAC cocrystals produced by N_2 and CO_2 have similar size and morphologies. TPL-SAC cocrystals were produced as microparticles having a block-shaped particle morphology.

Relative humidity (RH) stability experiments

The RH challenges comprised the storage of the samples (pure theophylline anhydrate and TPL-SAC cocrystals) and subsequent PXRD analysis at two specific RH levels (0% and 90% RH) across four different time points (1 day, 3 days, 1 week and 1 month). A comparison of the RH stability of theophylline-saccharin cocrystals to that of theophylline anhydrate was performed in order to assess whether these cocrystals offer enhanced physical stability profiles.

Figure 4 shows the corresponding powder X-ray difractograms of the samples that were subjected to 90% RH after 1 day.

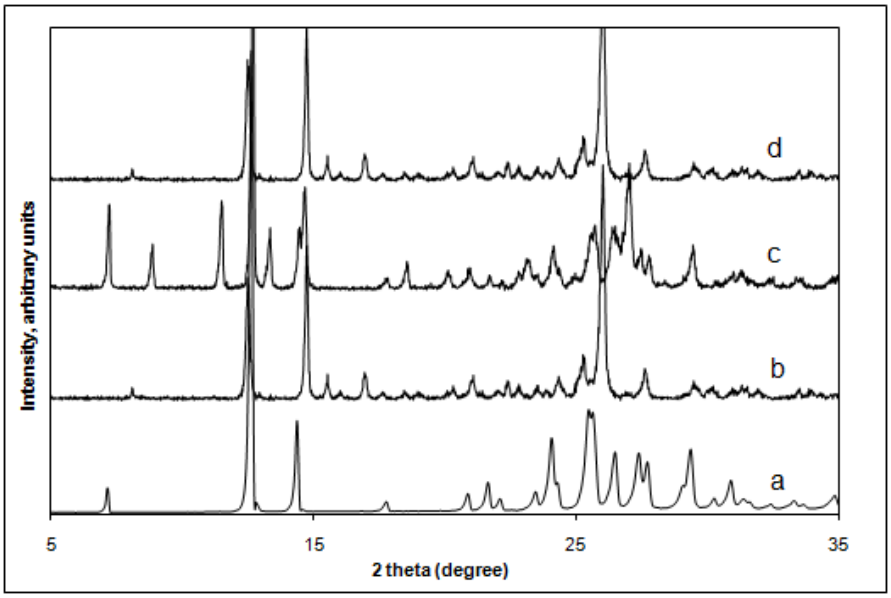


Figure 4 – PXRD patterns of (a) pure theophylline, (b) TPL-SAC cocrystals processed by SC-CO_2, (c) theophylline after 1 day at 90% RH and (d) TPL-SAC cocrystals processed by SC-CO_2 after 1 day at 90% RH.

Figure 4 shows that when theophylline is exposed to high humidity conditions (90% RH), it converts to theophylline monohydrate. However, TPL-SAC cocrystals didn't convert into a hydrated cocrystal form nor to a theophylline hydrate.

TPL-SAC cocrystals were exposed to 90% RH during more time points. Figure 5 shows the corresponding analysis by PXRD.

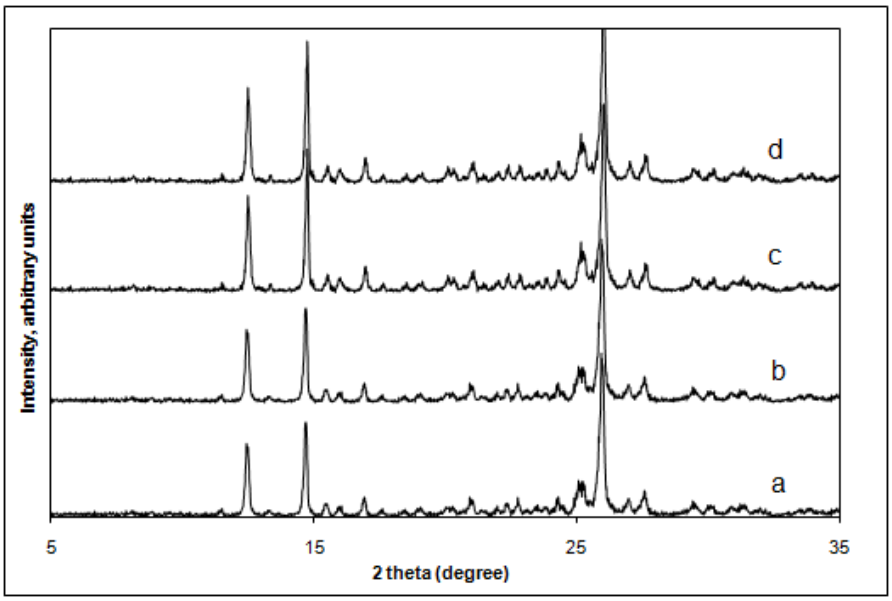


Figure 5 – PXRD patterns of TPL-SAC cocrystals (a) processed by SC-CO_2 after 3 days at 90% RH, (b) processed by SC-CO_2 after 1 week at 90% RH, (c) processed by SC-CO_2 after 1 month at 90% RH and (d) processed by N_2 after 1 day at 90% RH.

Figure 5 shows clearly that TPL-SAC cocrystals are stable at high humidity conditions still after one month. In considering these results, one observation of significant interest was that in no case was observed a hydrate of a given theophylline cocrystal to form. This may provide evidence that hydrate formation in cocrystals is less likely than in single-component systems. This enhanced physical stability of TPL-SAC cocrystals appears to suggest that saccharin successfully competes with water as a hydrogen bond donor with theophylline, thereby avoiding the formation of a hydrate.
Table 1 resumes the RH stability of the samples studied in this work.

Table 1 – Observed RH stability of TPL and TPL-SAC cocrystals (produced by CO_2 and N_2)

		Observed relative humidity stability*			
Samples	Condition (% RH)	1 day	3 days	1 week	1 month
TPL	0	+	+	+	+
	90	-	-	-	-
TPL-SAC (CO2)	0	+	+	+	+
	90	+	+	+	+
TPL-SAC 3 (N2)	0	+	+	+	+
	90	+	+	+	+

*The symbol (+) indicates that the crystalline material is stable at that condition and time point.
The symbol (-) indicates that the crystalline material exhibited physical instability.

CONCLUSION

In this study, neither of the TPL-SAC cocrystals generated by supercritical N_2 or CO_2 have convert into a hydrated cocrystal or theophylline monohydrate upon storage at high relative humidity. Furthermore, TPL-SAC cocrystals demonstrated superior humidity stability to theophylline anhydrate under the conditions examined. The results demonstrate the feasibility of pharmaceutical cocrystal design and furthermore show that avoidance of hydrate formation and improvement in physical stability is possible via cocrystallization of APIs using SCFs.

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