COCRYSTAL SCREENING USING SUPERCRITICAL FLUID-ASSISTED PROCESSES

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ABSTRACT

Supercritical fluid technology has been used together with crystal engineering approaches to produce cocrystals of different APIs. Indomethacin, theophylline, caffeine and sulfamethazine cocrystals, using the FDA-approved sweetener saccharin (SAC) as a cocrystal former, were generated from ethanol solutions by the Atomization and Anti-solvent (AAS) technique. In this process, supercritical CO_2 was used as a screening media. The cocrystals morphologies were analysed by SEM (Scanning Electron Microscopy) and the potential cocrystalline phase was characterized by DSC (Differential Scanning Calorimetry) and PXRD (Powder X-Ray Diffraction). The results of this work show the feasibility of the AAS technique to generate pharmaceutical cocrystals in a micro- and nanosized range.

INTRODUCTION

The improvement of the physicochemical properties of active pharmaceutical ingredients (APIs) has become an important challenge in the pharmaceutical industry. Pharmaceutical cocrystallization is a relatively recent technology and offers a platform, together with crystal engineering approaches, for improving physicochemical and biopharmaceutical properties of APIs through the development of new class of crystalline solids, called pharmaceutical cocrystals [1]. The choice of pharmaceutical crystalline form of an API can be used to optimize drug properties, such as solubility and stability and therefore cocrystals are emerging as new and interesting alternatives [2]. Cocrystals are often prepared by grinding or using a traditional solution crystallization approach, such as solvent evaporation, cooling or anti-solvent addition. In addition to these traditional methods of cocrystal preparation, the use of SCFs (Supercritical Fluids) offers a new platform that may enhance the rate of cocrystal formation. This alternative process offers a solvent-free product, allowing a single-step generation of microparticles that are difficult or even impossible to obtain by traditional techniques, particularly for substances with thermal sensitivity or structure instability [3], and providing a media for new molecular recognition events [4]. The generation of pure and dried new cocrystals with unique stoichiometry or polymorphs of cocrystals can be anticipated due to unique properties of SCFs by using the supercritical fluid technique AAS [5] (Atomization and Anti-Solvent). In this work, several cocrystal systems were studied with different APIs (e.g. indomethacin, theophylline, caffeine and sulfamethazine) and saccharin as a cocrystal former by using supercritical CO_2 as a screening media.

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MATERIALS AND METHODS

Materials

Indomethacin (γ-form) and saccharin were purchased from Sigma Aldrich, Stockholm, Sweden (purity of these chemicals was >99.9%). Theophylline (minimum 99% chemical purity) was purchased from BioChemika (USA), caffeine from Fluka (purity >99.0%) and sulfamethazine from Acros Organics (purity >99.0%). Absolute ethanol (99.5%) was supplied by Panreac, and carbon dioxide and nitrogen (99.998%) by Ar Líquido (Portugal).

Feed solution preparation

In the AAS process, the cocrystal components were dissolved in 10 g of ethanol. In the IND-SAC cocrystal preparation, a 1:1 mixture of indomethacin (28.2 mg or 0.08 mmol) and saccharin (15.3 mg or 0.08 mmol) was used. For TPL-SAC cocrystal production, a 1:2 mixture of theophylline (22.5 mg or 0.12 mmol) and saccharin (48.6 mg or 0.27 mmol). For CAF-SAC cocrystal production, a 1:1 mixture of caffeine (27.2 mg or 0.14 mmol) and saccharin (24.3 mg or 0.14 mmol) was used. Finally, for SFZ-SAC cocrystal production, a 1:1 mixture of sulfamethazine (22.3 mg or 0.08 mmol) and saccharin (15.3 mg or 0.08 mmol) was used. In the grinding process, the cocrystal components were ground together during 15 minutes using a mortar and a pestle. For IND-SAC cocrystal production, a 1:1 mixture of indomethacin (282 mg or 0.8 mmol) and saccharin (153 mg or 0.8 mmol) was used and for TPL-SAC cocrystal production a 1:2 mixture of theophylline (225 mg or 1.2 mmol) and saccharin (486 mg or 2.7 mmol) was used.

Particle production

The AAS apparatus was configured for the co-crystallization as described schematically in Figure 1.
The solution containing both substances (selected API 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 CO_2 is compressed by a Newport Compressor (model 46-13421-2).
Gas flows through the nozzle are measured by a Rheonik flowmeter (model RHM007) and pressures are measured by PX603 transducers from Omega. Temperatures are controlled in an air chamber or in a water bath by T-type thermocouples and Ero Electronic controllers (model LDS).
A secondary current of dry gas (N_2) is fed into the precipitator to enhance the solvent extraction from the particles. The particles are collected in a filter at the precipitator exit. A nozzle with a small orifice (100 μm) was used to limit the CO_2 flow rate and nozzle freezing due to strong depressurizations.

Particle 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.

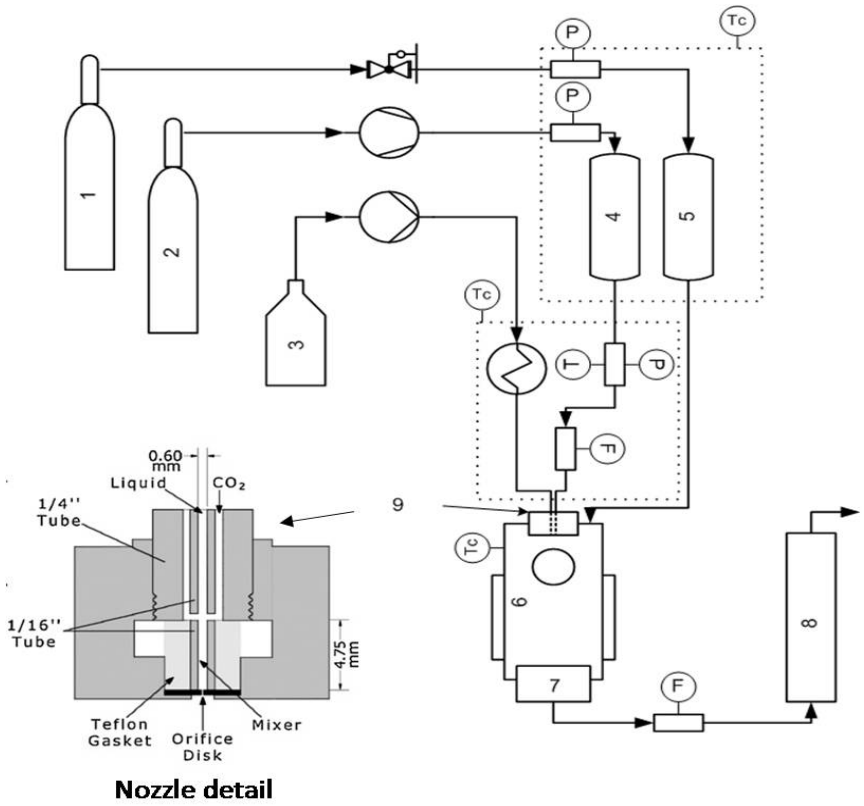

Figure 1: Schematic diagram of the AAS apparatus. 1: N_2 cylinder; 2: CO_2 cylinder; 3: liquid solution flask; 4: temperature controlled CO_2 storage cylinder; 5: temperature controlled N_2 storage cylinder; 6: precipitator; 7: filter; 8: solvent trap; 9: detail of the nozzle cap.

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.

RESULTS AND DISCUSSION

Particle morphology

The size and morphology of the cocrystal particles showed some differences between the ones produced by the AAS technique (IND-SAC 1 and TPL-SAC 1) and those produced by the grinding technique (IND-SAC 2 and TPL-SAC 2): the former having generally smaller sizes while the latter showing larger and aggregated particles (Figure 2).

Cocrystalline solid state characterization

Cocrystalline phase characterization and identification was carried out using DSC and PXRD (not presented). As Fig. 3 shows, TPL-SAC cocrystals produced by AAS have a single melting

Table 1. Experimental conditions for particle production. *P* is the pressure before the nozzle; *t* is the temperature in the mixing chamber; *R* is the mass flow-rate ratio of the aqueous feed to the supercritical fluid; t_{max} is the sample melting point; $\Delta_{fus}h$ is the enthalpy of fusion. IND - indomethacin; TPL - theophylline; CAF - caffeine; SFZ - sulfamethazine; SAC - Saccharin; AAS - atomization and anti-solvent technique; GRIND - grinding technique. Enthalpy of fusion data for pure caffeine it is not shown due to the degradation of caffeine during melting.

Reference samples	Molar proportion	Processing technique	*P* (MPa)	*t* (ºC)	*R* (g/g)	Melting Point t_{max} (ºC)	$\Delta_{fus}h$ (J/g)
IND	-	-	-	-	-	161.5	87.8
TPL	-	-	-	-	-	272.0	127.0
CAF	-	-	-	-	-	136.4	-
SFZ	-	-	-	-	-	198.1	102.1
SAC	-	-	-	-	-	227.8	144.5
IND-SAC	1:1	AAS	8.0	50	0.07	181.9	91.9
IND-SAC	1:1	GRIND	-	-	-	183.2	113.8
TPL-SAC	1:2	AAS	8.2	50	0.12	198.1	101.8
TPL-SAC	1:2	GRIND	-	-	-	199.8	112.1
CAF-SAC	1:1	AAS	8.0	50	0.06	152.5	63.1
SFZ-SAC	1:1	AAS	8.1	50	0.05	172.2	70.8

transition at ~198.1ºC that is different from those of the corresponding pure components suggesting a new cocrystalline phase formation. Similarly, IND-SAC cocrystals produced by the AAS technique show also a single melting transition (~183.5ºC) that is different from those of pure IND and pure SAC showing no residual peaks of their pure components.
Table 1 shows also the respective melting points and enthalpies of fusion for the two other cocrystal systems studied in this work, CAF-SAC and SFZ-SAC, which have been successfully cocrystallized, showing no residual peaks of their cocrystal components in the DSC thermograms.

CONCLUSIONS

This study addresses the ability of supercritical fluids to produce cocrystals of several APIs showing that supercritical media is an alternative process to produce pharmaceutical cocrystals. Specifically, IND-SAC and TPL-SAC cocrystals produced using the AAS technique evidenced similar thermal properties as those obtained by a classical pharmaceutical technique (e.g. grinding). Yet, the supercritical fluid based cocrystal technique

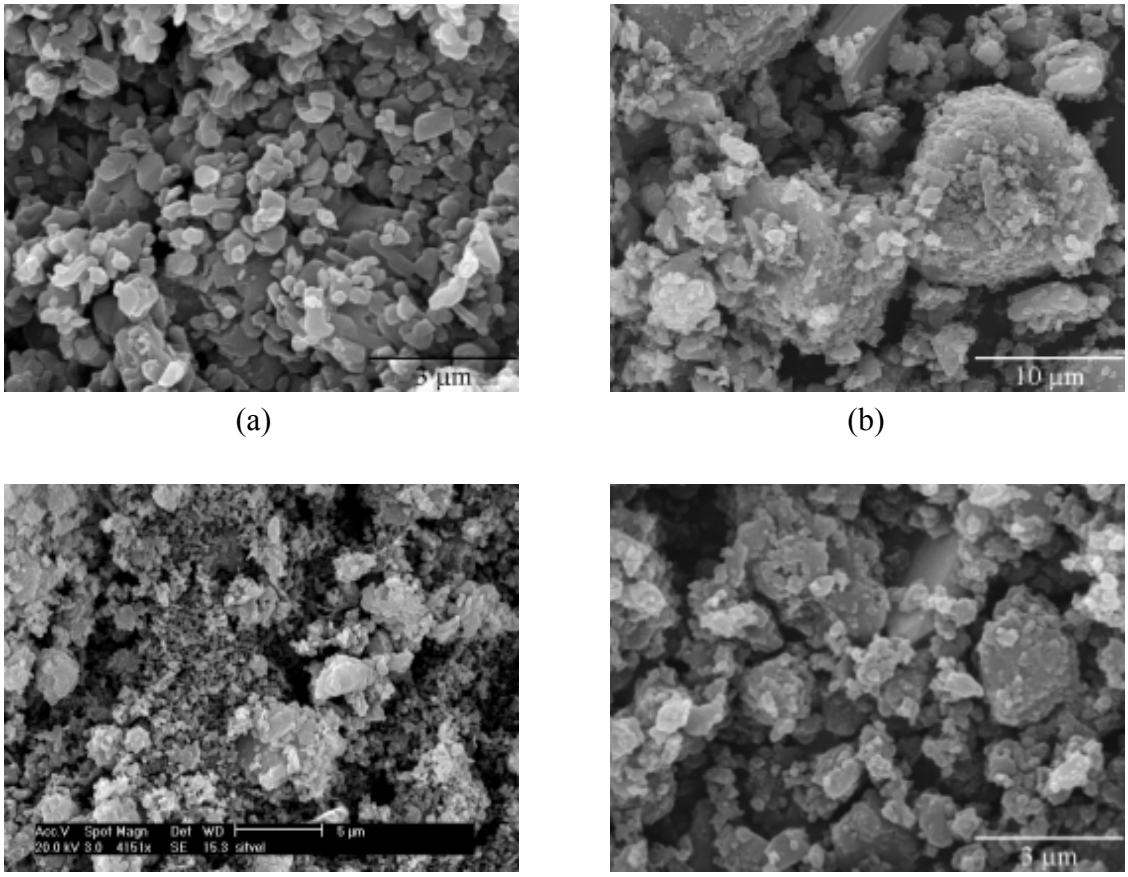

Figure 2: SEM images of processed cocrystal samples: (a) IND-SAC 1; (b) IND-SAC 2; (c) TPL-SAC 1; (d) TPL-SAC 2.

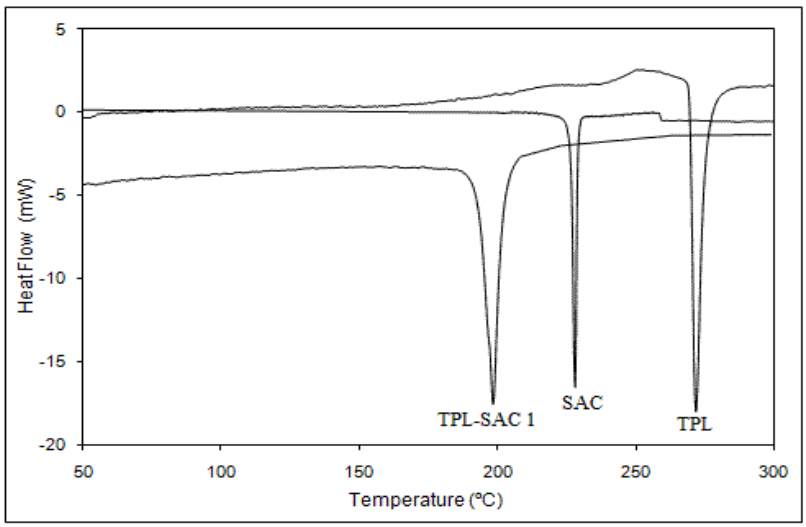

Figure 3: DSC heating curves of pure theophylline (TPL), pure saccharin (SAC), and TPL-SAC cocrystals produced by the AAS technique.

reported here allows the production of particles with a smaller size distribution than those produced using a classical technique.
Further work is currently in progress towards a thorough characterization of some of these cocrystals and analysis of their physicochemical properties, as well as the screening of other cocrystal systems.

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REFERENCES

[1] Almarsson, O., Zaworotko, M. J., *Crystal engineering of the composition of pharmaceutical phases. Do pharmaceutical co-crystals represent a new path to improved medicines?*, Chem. Comm., 17, **2004**, 1889-1896.

[2] Basavoju, S., Bostrom, D., Velaga, P. S., *Indomethacin–saccharin cocrystal: design, synthesis and preliminary pharmaceutical characterization*, Pharm. Res., 25, **2007,** 530-541.

[3] Rodrigues, M. A., Padrela, L., Li, J., Almeida, A., Matos, H. A., Azevedo, E. G., *Anti-solvent effect in the production of lysozyme nano- and microparticles by supercritical fluid-assisted atomization processes*, J. Supercrit. Fluids, 48, **2009**, 253-260.

[4] Padrela, L., Rodrigues, M., Velaga, S. P., Matos, H. A., Azevedo, E. G., *Generation of pharmaceutical cocrystals using supercritical fluid technology*, 10th Int. Chem. Biol. Engineering Conference, **2008**, Braga, Portugal.

[5] Padrela, L., Rodrigues, M., Velaga, S. P., Matos, H. A., Azevedo, E. G. *Formation of indomethacin-saccharin cocrystals using supercritical fluid technology*, Eur. J. Pharm. Sci., **2009** (under revision).