Production of Naproxen-Nicotinamide cocrystals by CO_2 antisolvent: comparison of SAS and GAS

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ABSTRACT

A recent strategy to alter properties of a pharmaceutical compound is to form cocrystals, i.e. molecular complexes of two entities in a solid state. Cocrystallization assisted by supercritical fluids is in its infancy with only few systems investigated so far. In this work, we used CO_2 as antisolvent to prepare cocrystals of Naproxene and Nicotinamide from acetone solutions. The two process variants, GAS (Gaseous Anti-Solvent) and SAS (Semi-continuous Supercritical Anti-Solvent) were investigated. Since homocrystals can be produced besides cocrystals, it is of high importance to deeply characterize the produced powders. X-Rays diffraction, Infra-Red spectroscopy and liquid chromatography were used to assess the presence of cocrystals and its stoichiometry and to quantify the cocrystals content. Impact of process variables such as pressure or solution concentrations were studied for SAS. Comparison of SAS and GAS results shows that GAS is more amenable to produce, in a robust way, powders of 100% cocrystals that are moreover of smaller sizes thanks to the solution stirring.

INTRODUCTION

Altering the placement and/or interactions between the molecules of a crystalline material can, and usually does, have a direct impact on the properties of the particular solid. Forming a cocrystal of an active pharmaceutical ingredient (API) and an excipient is an emerging strategy to modify API properties such as solubility and dissolution, stability over time, hygroscopicity.

Cocrystals are molecular complexes in a solid state that are mostly prepared by solid-state grinding or evaporative and cooling crystallization. Cocrystal studies pertain mostly to functional group compatibility, so that a tremendous literature can be found on molecular aspects. Although molecular interactions are necessary to envisage cocrystal formation, they are insufficient to predict if a cocrystallization will be successful. Thus, cocrystallization must be carried out under various conditions with different techniques to produce the molecular complex [1-2].

Supercritical technologies have beneficed from the growing consciousness for better, safer products, especially in food and pharmaceutical sectors. For particles generation, supercritical CO_2 has for long demonstrated its ability at tuning particles size thanks to the various precipitation processes (RESS, SAS, GAS, SEDS…). However, its capability at modifying the crystal lattice is investigated more marginally. So far, only few publications report cocrystals fabrication by supercritical routes [3]. To authors's knowledge, none of them compares the performances of the two antisolvent variants, nor prepares cocrystals of naproxen (NPX) and nicotinamide (NCTA) that were the pharmaceutical ingredient and the coformer selected in this work. Naproxen is a non-steroidal anti-inflammatory compound that belongs to BCS Class II, i.e. of high permeability and low solubility. For such compound, a cocrystal with a water-soluble coformer is a potential way for improving its solubility. Literature background has demonstrated that NPX and NCTA form cocrystal in a 2NPX:1NCTA stoichiometry [4-5]. We also have already produced NPX and NCTA cocrystal by the GAS supercritical technique [3], but not by the SAS process. The objective of this work is to compare the quality of crystals produced by the two process variants when starting from acetone solutions containing both components.

MATERIALS AND METHODS

Materials

Naproxen ((+)-(S)-2-(6-methoxynaphtalen-2-yl)propanoic acid, 98%, NPX) and nicotinamide (pyridine-3-carboxamide, 99.5%, NCTA) were supplied by Sigma–Aldrich (France). Carbon dioxide (CO_2, 99.5%) and acetone (99.5%) were supplied by Air Liquide and Scharlau, respectively.

Preparation of cocrystal by GAS

The set-up is the home-made GAS (Gaseous Anti Solvent) equipment that comprises a crystallization vessel of 490 cm3 equipped with a stirrer [6]. GAS proceeds by loading the vessel with a volume of solution and adding CO_2. Solutions of various concentrations were prepared with a constant NPX:NCTA molar ratio of 2:1. When the operating temperature of approximately 38°C inside the vessel was stabilized, CO_2 was introduced through a Rushton turbine that plunges directly into the solution, by a LEWA (EM1, Lewa, Germany) or an ISCO (Model 260D, Teledyne Isco, USA) pump. Once the desired pressure of 10.0 MPa was attained, the formed CO_2-solvent solution was drawn down at the vessel bottom whilst fresh CO_2 flew through the vessel at 25 g/min for 90 min to maintain the pressure. The pressure in the vessel was controlled at 10.0 ± 0.5 MPa by an exit metering valve. A stainless steel filter overtopped by a 0.2 µm pore size membrane held back the produced particles whilst the mixture was evacuated. At the end of the period, the vessel was depressurized through the exit line and particles were collected, weighed and characterized.

Preparation of cocrystal by SAS

Details of the equipment can be found elsewhere [7]. The vessel of 0.49 L is equipped with three sets of sapphire windows over its 25 cm of length that allow for visualizing the liquid injection and to detect the presence of particles. Once the vessel was permanently set with CO_2 at controlled pressure, temperature and flow rate, the solution was injected via a 180 µm i.d. capillary. Solutions of NPX and NCTA dissolved in acetone at 2:1 molar ratio were sprayed. Injection flow rate was set at 7 ± 1mL/min and the CO_2 flow rate was kept at 54 ± 4 g/min, which gave an overall composition of the CO_2-solvent mixtures of 94mol%CO_2. The produced particles were held back at the vessel bottom by the same filter and membrane system as in GAS. At the end of the spraying, the CO_2 flow was maintained during 30 min to flush out the residual organic solvent. The vessel was then depressurized through the exit line and particles were collected, weighed and characterized.

Product characterization

The product morphology was documented by optical microscopy (Olympus BX51TF and camera ColorView U-CMAD3). The size distribution was measured by laser diffraction using a Mastersizer 2000 (Malvern) equipped with a low volume circulation unit and silicon oil as dispersion medium.

Crystallinity and phase identification were obtained by powder X-ray diffraction analysis (PXRD) performed on a D5005 Bruker/Siemens diffractometer using the theta/theta geometry with copper radiation. Data were collected for 2θ angles of 3-40°with a step size of 0.02°at a scanning speed of 0.015–0.06 °/min depending of the samples.

The product composition was determined by high performance liquid chromatography (HPLC, Agilent 1200 system (Agilent Technologies)), using a ZORBAX Eclipse Plus C18 column (10x0.46cm, d_p: 3.5 µm, Agilent) and a mobile phase of phosphate buffer (20 mM, pH 3.3) and acetonitrile (35:65 vol%) flowing at 1.2 mL/min, injection volume of 1 µL. Naproxen and nicotinamide content were assayed at 263 nm after calibration performed in the range of 3.0-30.0 mg/mL with a molar ratio of 2NPX:1NCTA. HPLC results were used to estimate the amount of cocrystals in the produced powder thanks to a set of mass balance equations described elsewhere [3].

RESULTS

Influence of pressure on cocrystal formation (SAS process)

The effect of pressure was first investigated. Injected solutions were prepared with a naproxen concentration of 31mg/mL and a nicotinamide concentration of 8mg/mL. Injections were conducted with a solution flow rate of 7 ± 1mL/min and CO_2 flow rate of 58 ± 5g/min, giving a global composition of 94%molCO_2. Different operating pressures were tested: above the critical pressure of CO_2-acetone mixture (SAS 4 and SAS 2) where precipitation of NPX and

NCTA occurred in a gaseous plume, and at subcritical or near-critical pressures (SAS 5 and SAS 4) where crystals formed into solution droplets dried by CO_2. Characteristics of powders obtained are summarized in Table 1.

Table 1 : Operating conditions and product characteristics of powders recrystallized by SAS from acetone solution. C_{NPX}=32mg/mL, C_{NCTA}= 8mg/mL, F_{sol} = 7±1mL/min, F_{CO2} = CO_2 flow rate = 58±5g/min. Yield = collected amount/injected amount, Cocrystal content = cocrystal amount/collected amount.

	P (Mpa)	T (°C)	Yield (wt%)	Cocrystal content (wt%)	Crystal morphology	d10 (µm)	d50 (µm)	d90 (µm)
SAS 5	7.6 ± 0,1	42 ± 3	61%	78%	Thin plates + needles	39	150	414
SAS 3	8.2 ± 0,1	40 ± 3	70%	78%	Thin plates + needles	66	241	525
SAS 4	10.1 ± 0,3	37 ± 1	63%	99%	Thin plates + needles	96	275	623
SAS 2	13.0 ± 1,6	40 ± 2	48%	81%	Thin plates + needles	82	286	584

The precipitation yield reached up to 70wt%, indicating that a fraction of the species was still soluble in the CO_2-acetone mixture, an observation in agreement with literature at least for NPX [8]. An average yield of 65 ± 5wt% was obtained for injection pressures below 10MPa, but was slightly lower between 10MPa and 13MPa. This tendency could be explained by a potential increase of solubility of the species at higher pressures.

For all tested pressures, SAS did produce the cocrystal, indicating that these process and conditions were suitable to fabricate the molecular arrangement and to recover it. The same cocrystal than that produced by more classical routes was recovered, i.e. with a molar stoichiometry of 2 NPX for 1 NCTA. However, cocrystals were not exclusively produced by the process, since NPX homocrystals (i.e. crystals of only NPX) were also identified in powders thanks to Infra-red spectroscopy and powder X-ray diffraction. The amount of NPX homocrystals varied from 1wt% to 22wt% depending on conditions. Therefore, pressure was found to affect in some extent the competition between cocrystals versus NPX homocrystals formation.

Regarding morphology and sizes, cocrystals have a typical plate-like shape and are very thin, whereas NPX homocrystals precipitated as needles (Figure 1).

(a) (b)

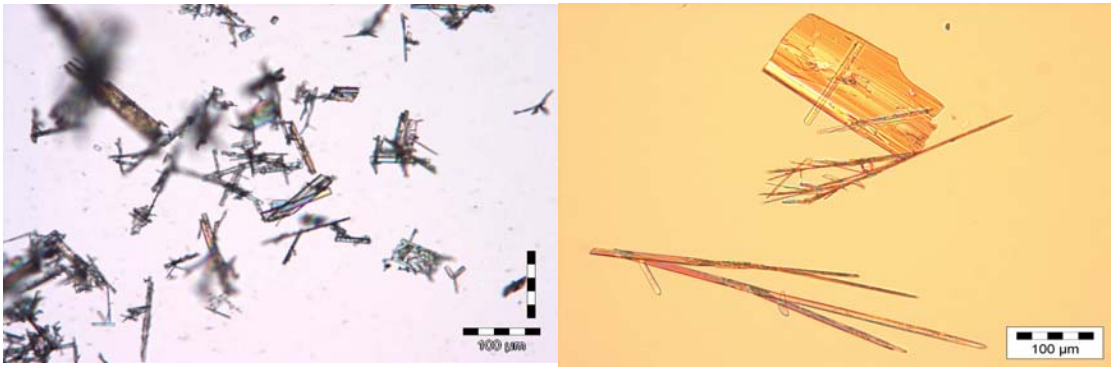

Figure 1 : Microscopy pictures of crystals precipitated from solutions with initial concentration C_{NPX}=20mg/mL and C_{NCTA}= 5mg/mL. (a) GAS-produced powder exhibiting cocrystals only. (b) SAS-produced crystals. The plate is constituted of cocrystal and needles are NPX homocrystals. Scale bar of 100 µm.

As for particle size distributions of powders (PSD), operating pressure was found to have only a minor impact unless a subcritical pressure is applied: the d_{90} of the distributions shifted from 603 ± 28µm when above critical pressure, to 414µm for the subcritical run. Precipitation at subcritical pressure has been already shown to yield smaller particles, probably because of the existence of solvent-rich droplets that confine the precipitation [9].

Influence of initial solution concentration

Supersaturation of NPX and NCTA being the driving force of precipitation, concentration of components was expected to have effects on cocrystal formation in both SAS and GAS processes. While a molar ratio of 2 NPX for 1 NCTA was always respected for the solutions preparation, various concentrations of NPX ranging from 20 to 94mg/mL were prepared for GAS experiments. In SAS, to prevent the obstruction of the injector and overloading of the vessel, lower concentrations were investigated, from 10 to 40mg/mL in NPX. Consequently, NCTA concentrations were between 5 and 25mg/mL for GAS and between 3 and 10mg/mL for SAS processing. In GAS, stirring rate and CO_2 introduction rate were chosen according to previous experiments [3].

As displayed in Figure 2, precipitation yield of GAS and SAS followed the same predictable trend, that is higher yields are obtained with higher concentrations. A threshold concentration was however observed, i.e. concentrations higher than 10mg/ml in NPX should be prepared to get an effective precipitation, and by consequence, a powder to collect. A very good reproducibility (0.1%) was obtained in GAS experiments, whereas SAS were more prone to slight operating discrepancies that contributed to the worse reproducibility in yield (9%). Moreover, the light-weight crystals produced in SAS were harder to collect than the good-flowable powder from GAS, so less discarded materials in GAS contributed to higher yield.

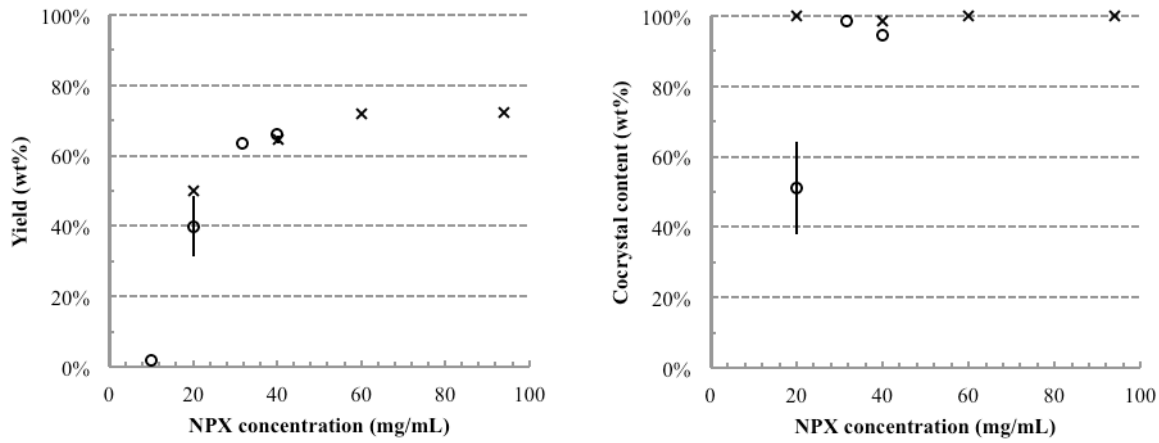

Figure 2 : Influence of initial concentration on the yield (collected amount/processed amount) and on cocrystal content of the collected powder. NPX concentration is taken as variable since solutions are prepared with a 2NPX:1NCTA molar ratio. Cross symbols (×) and circle symbols (o) represent GAS and SAS experiments, respectively. SAS-precipitated assay with C_{NPX}=10mg/mL did not yield enough powder to evaluate cocrystal content.

Regarding the type of produced crystals, Infra-red spectroscopy evidenced the same 2NPX:1NCTA cocrystals as before in every powder. HPLC and crystallographic data allowed for concluding that, whatever the initial concentrations tested, GAS trials gave only cocrystals without any homocrystal excess. Considering SAS results, almost pure cocrystals (content of 94%) were produced only if NPX concentration in the NPX:NCTA mixture equalled or exceeded 32mg/mL. Below that threshold and for instance at 20mg/ml, the cocrystal content was around 50%, indicating that half of NPX crystallized independently as homocrystals while the other half managed to cocrystallize with NCTA. Taking into account inherent operating parameters inaccuracies, crystals collect and quantification errors, this observation was repeated with a 13% margin error.

Whatever the concentration, morphologies of produced powders were the same thin plates and long needles mixtures than seen previously at various pressures. But the powder size distribution was found to be sensitive to concentration. In SAS, when concentration increased from 20 to 30 and 40 mg/mL (the 10mg/mL NPX concentration did not yield enough powder for proper characterization), the d_{90} was found to decrease from 734µm to 623µm and to 534µm respectively. In GAS, the d_{90} decreased from 293µm to 146 ± 13 µm as concentration increased from 20 to 40mg/ml, but did not evolve notably when concentration raised more (to 60-94mg/mL). This evolution of particle size with solution concentration follows trends of classical crystallization, indicating higher nucleation rates over growth rates at higher concentrations.

The distribution of GAS and SAS powders produced from the same solution is compared in Figure 3. Looking as well to microscopic pictures in Fig.1, it is obvious that GAS process enables to obtain smaller particles than SAS, a trend that is rather unusual. Extra experiments in SAS are currently performed to comfort the particle size range at various solution and CO_2 flows.

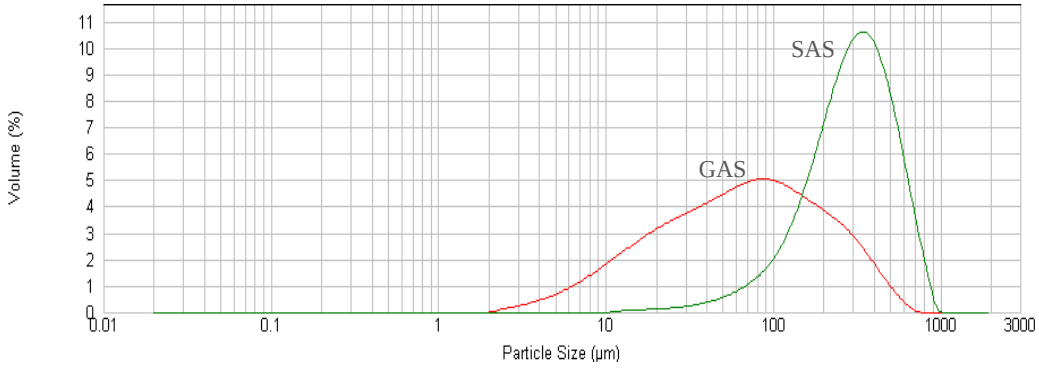

Figure 3 : Influence of precipitation process from solutions with initial concentrations C_{NPX}=40mg/mL and C_{NCTA}= 10mg/mL on particle size distribution. Red line and green line represent GAS and SAS experiments, respectively. GAS was conducted with a stirring rate of 500rpm and a CO_2 introduction rate of 20g/min. SAS conditions during injection: 37 ± 1°C and 10.3 ± 0.3 MPa, F_{sol} = 6 mL/min, F_{CO2} = 51 g/min.

CONCLUSIONS

The formation of naproxen-nicotinamide cocrystals was successfully achieved with the two CO_2-antisolvent processes GAS and SAS. Starting from solution prepared with a constant molar ratio of 2:1, the same crystalline structure and stoichiometry was obtained, i.e. 2NPX:1NCTA. Precipitation of excess naproxen homocrystals was only observed in SAS-produced powders, and for particular operating conditions. Operating pressure and precipitation regime during SAS solution injection was found to have little impact on cocrystal formation and size distributions. The influence of initial solution concentrations of species was found to be the same in both processes regarding precipitation yield and particle size, that is a larger amount of smaller particles is recovered for higher initial concentrations. At the lowest initial concentration tested and characterized, the SAS process was found to yield only half the mixture in cocrystals, the other half being naproxen homocrystals. Based on experiments detailed in this article, the best compromise between particle size and cocrystal powder purity was accomplished with the GAS process. It is likely that the presence of homocrystals is related to the solubility of the component in the CO_2-acetone mixture and to the hydrodynamics of the CO_2-solution mixture during the injection. Thermodynamic investigations are currently investigated [10-11].

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