COCRYSTALLIZATION OF 5-AMINOSALICYLIC ACID AND NICOTINAMIDE BY CO_2 ANTISOLVENT

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ABSTRACT

Formation of cocrystals is a new strategy in drug development to alter the chemical and physical solid-state properties of a pharmaceutical molecule. In this work, coprecipitation from solution by using compressed CO_2 as antisolvent was investigated for mesalazine (5ASA) and nicotinamide (NCTA), varying NCTA concentration as operating parameter. Recrystallization by CO_2 was found to influence the morphology of each compound compared to raw materials and the increasing NCTA concentration led to increase the 5ASA precipitation yield. However, whatever the conditions, the produced powder was a mix of 5ASA and NCTA homocrystals, with no new crystal lattice evidenced. Cocrystals were hence never produced.

INTRODUCTION

Since they largely influence functionality (e.g. solubility, stability, bioavailability) and process handling (e.g. flowability, compressibility, tableting), the control of physicochemical properties of active pharmaceutical ingredients (APIs) is a major concern for the pharmaceutical industry [1]. These properties result from the molecular arrangement within the crystal. Thus, the formation of salts, polymorphs, hydrates, solvates and more recently cocrystals are various strategies to alter the chemical and physical solid-state properties of APIs [2].

Cocrystals are solid molecular complexes formed by the API and a cocrystal former, called coformer, through intermolecular interactions. Although interactions are necessary to envisage cocrystal formation, they are insufficient to predict if a cocrystallization will be successful. So far, cocrystals are mostly obtained by cogrinding or solution crystallization methods [3,4]. Recently, the use of supercritical CO_2 to produce cocrystals has been proposed. Although the supercritical techniques lead to solvent-free products, only few articles in literature report fabrication of cocrystals by this route [5,6].

Mesalazine, or 5-aminosalicylic acid, is one of the cornerstones in modern treatment regimens of ulcerative colitis. However, this API is hardly soluble in biological fluids [7] and although it is generally well tolerated, adverse reactions such as nephrotoxicity and mesalazine precipitation in the urinary tract have been reported [8].

In this work, the CO_2-antisolvent technique was investigated in attempts to produce cocrystals of 5-aminosalicylic acid (5ASA) and nicotinamide (NCTA). As model compounds for cocrystal formation, 5-ASA and NCTA were selected because of their complementary hydrogen bond donor and acceptor sites. To author's best knowledge, cocrystals of 5ASA and NCTA have never been reported, neither by the supercritical route nor by any of the conventional crystallization methods.

MATERIALS AND METHODS

Materials

5-amino salicylic acid, (5-amino-2-hydroxybenzoic acid, > 99 %, 5ASA) and nicotinamide (pyridine-3-carboxamide, 99.5 %, NCTA) were supplied by Sigma Aldrich (France). Carbon dioxide (CO_2, 99.5 %) was from Air Liquide (France). Acetone (99.5 %, Scharlau), DMSO (99.9 %, Scharlau), acetonitrile (HPLC grade), potassium dihydrogenophosphate and phosphoric acid were supplied by Atlantic Labo (France). Water was obtained from a Milli-Q water purification system.

Recrystallization by CO_2 antisolvent

The experimental set-up is the home-made GAS (Gaseous Anti Solvent) equipment described elsewhere [9]. During the GAS process, pressurized carbon dioxide is added to the solution of API and conformer. Since their solubilities decrease in the supercritical solvent/CO_2 phase as the carbon dioxide molar fraction increases, both compounds precipitate. Because GAS is a semi-batch process and two species are involved, the produced powder could contain homocrystals of NCTA and 5ASA besides cocrystals.

API (5ASA) and coformer (NCTA) were dissolved in 40 mL acetone : DMSO solution (70:30 vol %) at various concentrations and molar ratios. The vessel is equipped with a magnetically-driven impeller fit with a Rushton turbine that disperses the pressurized CO_2 in the solution. At the bottom, a stainless steel filter overtopped by two 0.2 µm pore size membranes prevents the particles to exit from the vessel when the solution is flush out. The vessel temperature was kept constant to 36°C. Stirring rate was set to 500 rpm. CO_2 was introduced by a LEWA (EM1, Lewa, Germany) or an ISCO (Model 260D, Teledyne Isco, USA) pump at a constant pressurization rate of 20 g/min. Once the desired pressure (11 MPa) was reached, the CO_2-solvent solution was withdrawn from the vessel by compensating with fresh CO_2 to maintain the pressure at 11 MPa. CO_2 was continuously flown through the vessel at 25 g/min for 90

min to remove solvent traces from crystals. Thereafter, the vessel was depressurized through the exit line and particles were collected, weighed and characterized.

Particle characterization

The product morphology was characterized by optical microscopy (Olympus BX51TF and camera ColorView U-CMAD3). The powder composition was determined by high performance liquid chromatography (HPLC) after dissolution in acetone : DMSO (70:30 vol %). An Agilent 1200 system (Agilent Technologies) equipped with a diode array detector (DAD, G1315A), an autosampler (G1329A) and a Chemstation software was used with a ZORBAX Eclipse Plus C18 column (4.6 x 100 mm, 3.5 µm, Agilent Technologies) at 1.2 mL/min flow rate, 25 °C. Elution was performed under isocratic conditions with phosphate buffer (20 mM, pH 3.3) and acetonitrile (35:65 vol %). 5ASA and NCTA were assayed at 300 nm and 263 nm respectively and calibration curves were obtained in the range 0.4 – 4 mg/mL for each compound. From the product composition, recrystallization yield of 5ASA was calculated.

The presence of interactions between 5ASA and NCTA, that would evidence cocrystal formation, was assessed by infrared spectroscopy. ATR-FTIR spectra on diamond crystal (GoldenGate) were recorded with NEXUS 870 FTIR ESP spectrometer from Nicolet (Madison, USA) equipped with a liquid nitrogen cooled mercury-cadmium-telluride detector. Analyses were performed at room temperature between 800 and 4000 cm^{-1} with a resolution of 2 cm^{-1} and 100 scans.

RESULTS

Product morphologies

For the pure compounds processed alone, the GAS recrystallization yielded products of completely different morphologies. Whereas raw 5ASA consisted in off-white to grey expanded cottony powder, the CO_2 recrystallized 5ASA was a fine beige to tan powder. At microscopic scale, the 5ASA crystal shape varied from long needles (length up to 69µm) to few micron spheres before and after processing. The contrary was observed for NCTA: initial spherical powder was transformed into large needles. Thus, recrystallization by CO_2 as antisolvent led to products of completely different shape, which means probable different physical properties as well.

When 5ASA and NCTA were coprocessed with increasing amounts of NCTA in the initial solution, the macroscopic appearance of produced powders evolved as well to more needles and brightened.

Recrystallization yields and product composition

The influence of NCTA concentration in initial solution upon both 5ASA crystallization yield and NCTA content (w %) in final product was addressed. Concentration of 5ASA was set to 17 mg/mL, i.e. close to its solubility in the acetone:DMSO mixture, whereas NCTA concentration was varied from 0 to 57 mg/mL. The recrystallization yield of 5ASA was found to increase linearly with NCTA concentration (Fig.1a), indicating that the addition of an extra specie in the solution promoted the precipitation of the drug. Regarding the produced powder composition (Fig.1b), more NCTA in the initial solution yielded more NCTA in the final product, which could explain the macroscopic change of the powder towards more needles.

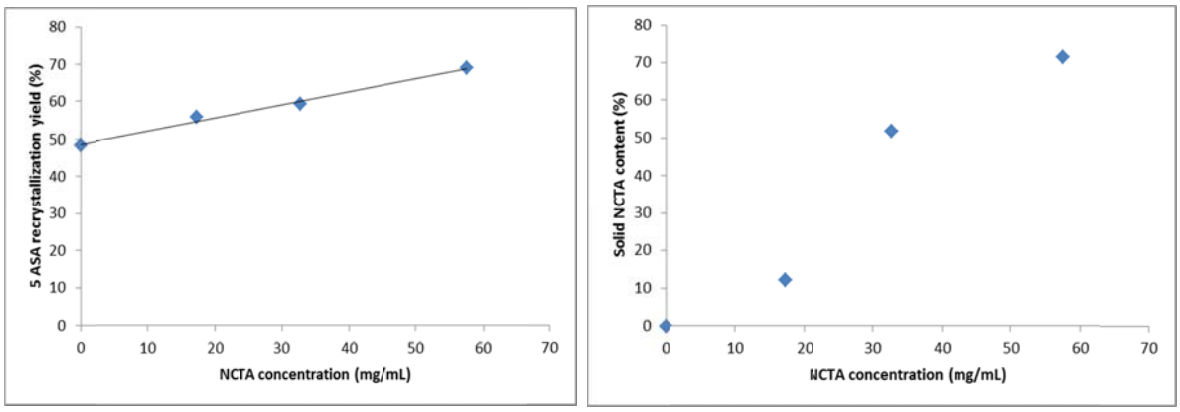

Figure 1: influence of NCTA concentration on the recrystallization of 5ASA. (a) 5ASA precipitation yield (b) NCTA content of produced powders. GAS recrystallization from acetone:DMSO at 36°C and Pfinal of 11 MPa. Solution of 5ASA (C=17 mg/mL) and NCTA (0 to 57mg/mL).

Cocrystallization assessment

Powders produced by CO_2 recrystallization of 5ASA+NCTA were analyzed by ATR-FTIR in order to detect interactions between components at solid state that would sign cocrystal formation. Indeed, in a cocrystal, interactions would have modified the spectrum either as shifts for some characteristic bands or/and apparition of new bands [6]. Spectrum of powder produced from processing 5ASA+NCTA mixture is given Figure 2, to be compared with spectra of raw species. The 5ASA+NCTA spectrum exhibited the same bands than individual compounds indicating that no new interaction in the solids has occurred. Therefore, the powder was made of a physical mixture of 5ASA and NCTA homocrystals rather than of a new crystalline solid entity, i.e. a cocrystal.

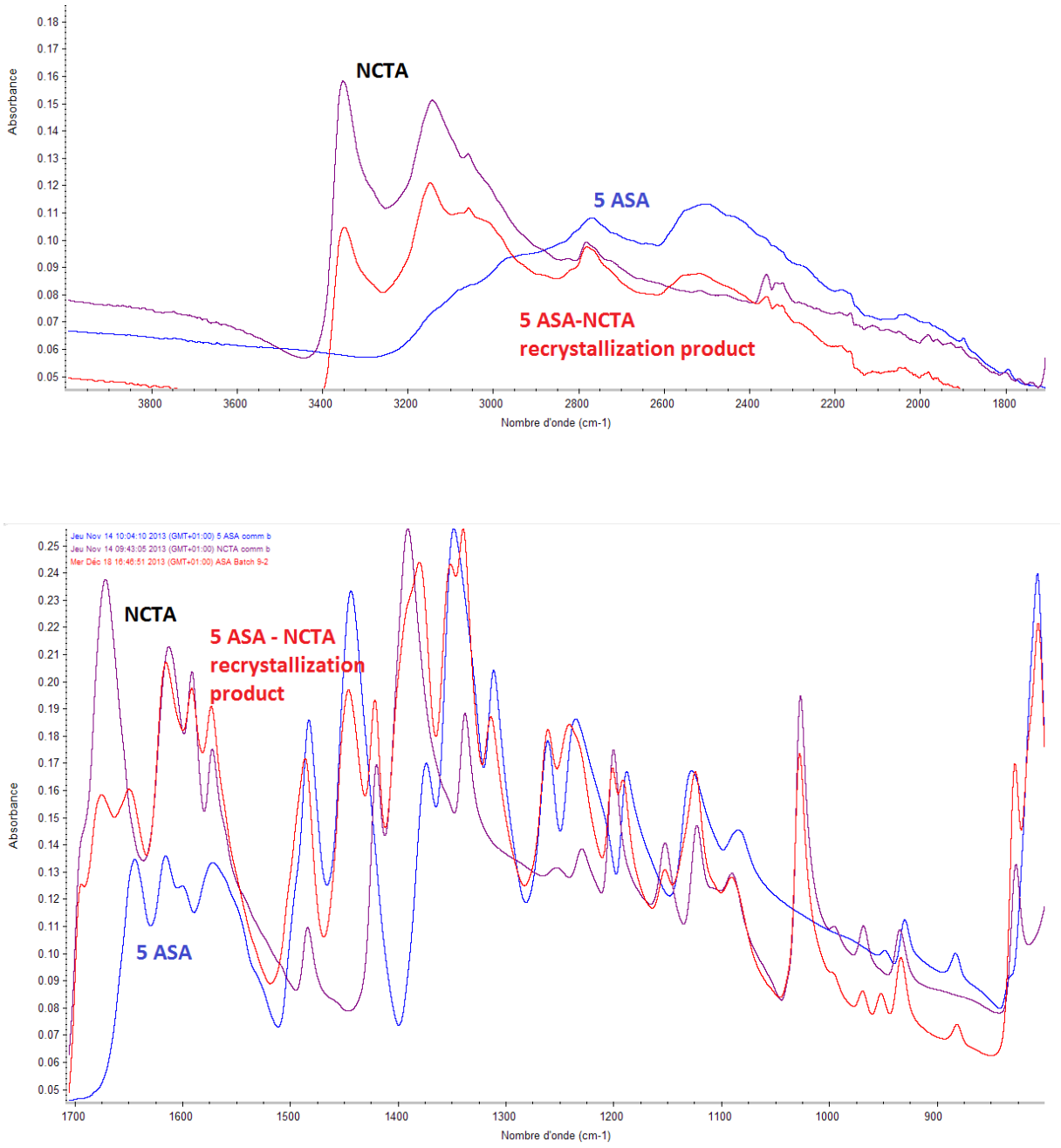

Figure 2: ATR-FTIR spectra of pure 5ASA, pure NCTA and 5ASA-NCTA powder obtained from a 5ASA +NCTA solution of 17 and 32 mg/mL, respectively. Top: 4000-1800 cm^{-1} region; bottom: 1700-800 cm^{-1} region.

Regarding the effect of NCTA concentration, all powders exhibited the same spectrum pattern with only variations in the band intensities according to the relative product composition (NCTA content in solid). Therefore, whatever the NCTA:5ASA ratio, cocrystals were never produced.

As mentioned in introduction, molecular interactions between API and coformer are necessary but not sufficient to envisage fabrication of cocrystal. In fact, contrary to many

APIs that give cocrystal, 5ASA is zwitterionic. The configuration of the molecule, with the hydroxyl and carboxyl groups in ortho position favours intramolecular hydrogen bonds, and the crystal packing is characterized by a complex two-dimensional network of hydrogen bonds [10]. The extra compound, NCTA, was obviously unable to compete with 5ASA for the molecular arrangement during crystallization.

CONCLUSION

Coprecipitation of 5ASA + NCTA mixture by CO_2 antisolvent technique was attempted to fabricate a 5ASA:NCTA cocrystal. When performed from solutions of pure compounds (either 5ASA or NCTA), this process led to a dramatic change in the product morphology. From 5ASA+NCTA solutions, it produced powders whose morphology varied according to the initial NCTA content, and lately, to the NCTA content in the final powder. Increasing NCTA initial concentration enhanced the 5ASA recrystallization yield and increased the product NCTA content. However, although 5ASA is a multiple hydrogen-bonding functionality molecule with carboxylic acid, amine, and phenol groups, potential interactions with NCTA were obviously insufficient to disrupt the strong interactions between the 5ASA molecules.

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