CRYSTAL PHASE/HABIT AND CO_2 ANTISOLVENT PROCESS

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The paper deals with recrystallization of two compounds using CO_2 as anti-solvent. For tolbutamide, comparison between CO_2- processed and evaporation precipitates clearly indicated the major role of the liquor solvent on the drug form. In acetone, batch and continuous versions produced mostly Form III, with a narrow window for the SAS to add traces of Form I; when co-processed, it was shown that the carrier can induce a modification of the crystal form. Theophylline displayed a different behaviour, with a modification of the crystalline structure upon processing by CO_2 compared to evaporation. The occurrence of this modified structure was dependent of the processing conditions, as pressure for instance, and was not affected by the co-processing with PLA..

INTRODUCTION

Crystallization is a key operation in powder technology since characteristics of produced particles will influence the material final properties, and more specifically bioavailability or stability when active pharmaceutical ingredients (API) are considered.

Differences in the visual or morphologial appearance of a substance do not necessary reflect polymorphism [1]. A compound can produce crystals with different morphologies without changing the crystalline structure, by preferentially growing in different directions. Different solid phases are polymorphs, amorphous, or solvates/hydrates (known as pseudo-polymorph). Polymorphs have the same chemical composition but different crystal structure due to different crystal packing arrangements or conformations. As a result, they exhibit different physical properties, including intermolecular interaction, particle density, change in free energy, thermodynamic activity, solubilility, dissolution rate, stability and bioavailability.

Precise knowledge of thermodynamic stability and relationship between different solid phases is a prerequisite for understanding the crystallization process. Since polymorphs have different lattice energies, the more energetic ones seek to revert to the most stable form. On a general rule, the form which is thermodynamically stable at a given pressure and temperature is that which has the lowest free energy and the poorest solubility. In crystallization process, it is quite common for a metastable form to appear first and then transform into the stable polymorph (Ostwald rule). Hence, although thermodynamics point the direction of the solid-solid transition, the kinetic aspects impact the time scale of the transition. The control of a crystallization process of a polymorph is thus challenging, since it involves many mechanisms of nucleation and growth of each form but also the transition of metastable to the stable form.
For a pharmaceutical compound, crystallinity or polymorphism is susceptible to influence bioavailability because of the differences in free energy of the various forms. However, the difference (in the range of few kJ/mol) might be too small to induce a breakthrough in

dissolution rates, considering also that the effect could be obscured by particle aggregation and crystal size distribution[2]. A higher impact on bioavailability can be expected by the amorphous form of a molecule, since the amorphous state, characterized by a non-ordered organization, shows a higher free energy than the crystalline phase, and thus shows a potentially enhanced dissolution rate. However, because of their high reactivity, amorphous pahses develop a propensity to crystallize over time, specially in presence of humidity.

Few studies have been dedicated to polymorphic behaviour in CO_2-process (see [3,4] as reviews) and many of them focused on Carbamazepine. Although pure α, β, γ forms were obtained by manipulating process variables as solvent in combination with pressure and temperature[5], another study [6] concluded that the transformation of Form III into Form I was due to solvents rather to the CO_2 treatment. In attemps to produce drug delivery systems, CBZ was co-processed with polymers. Co-precipitates were mostly produced in case of CBZ-PEG 8000[7], whereas with PVP 30[8], CBZ was present in amorphous form due probably to the interaction of the drug with PVP. Plasticization of PVP and increased diffusion of the drug into the polymer matrix could also account for the reduction of crystallinity of CBZ when processed by liquid CO_2[9].

Manipulating the form by changing pressure, temperature, solvent or antisolvent variants was evidenced for other drugs. Fluconazole [10] recrystallized by continuous SAS was identified as Form II when prepared at 80°C (8 and 12 MPa), and as Form I when prepared at 40°C and 8 MPa ; pressure was found to give a preferred orientation to crystal arrangement but did not realize the polymorphic conversion, whilst the use of ethanol instead of DCM or acetone indeed led to Form II, even at 40°C and 8 MPa. The batch and the continuous versions were developped for processing Sulfamethoxazole [11] (acetone, 35°C, 12 or 10 MPa); the batch mode led to crystals of form II between 20 and 60µm in size, whereas the continuous mode produced particles of Form I below 10µm. The influence of solvent and fluid composition was investigated in case of sulfathiazole by continuous SAS [12]. From methanol, three pure forms were obtained (I, III, IV) by manipulating temperature and in a lesser extend, the fluid composition; in acetone, only amorphous, I and IV forms were produced, with a temperature effect minimal against the composition effect.

To summarize, literature points out several operating parameters susceptible to orientate the crystal form, as temperature, fluid composition, mono- or bi-phasic conditions, sursaturation, mixing time (batch/continuous), contact time, carriers (for the crystalline to amorphous transition).

MATERIALS AND METHODS

Tolbutamide is an oral hypoglycemic agent which exhibits four polymorphs (I to IV); theophylline is a bronchodilator, found as forms I and II. Re-crystallization experiments were carried out using CO_2 as anti-solvent, in the two modes of the techniques (batch and continuous); main characteristics of the two equipments can be found elsewhere [13,14].

TOLBUTAMIDE CRYSTALLIZATION

In batch mode and from acetone solutions, Tolbutamide was recovered as agglomerates built up with particles of polyedric shape; the yield of precipitation, between 45 and 58%, indicated a partial extraction via solubilization of TBM in CO_2-acetone mixtures. The mean size and PSD of TBM was influenced by both the stirring and the introduction rates, as illustrated in Table 1; smallest sizes and narrowest distribution were consequently obtained at higher rates

of 900 rpm and 1.2MPa/min, with values of 63, 119 and 210µm as d_{10}, d_{50} and d_{90} respectively. Due to the high solubility in solvents, batch experiments were performed at ambient temperature. An increase of temperature to 39°C led to a decrease of the precipitation yield (in coherence with an higher solubilization of TBM) but to a particle size distribution in the same range. Although particles size were sensitive to parameters, the X-Rays diffraction patterns of all samples produced from acetone overlaid and matched the referenced Form III pattern, regardless the stirring/introduction rates and temperature.

Table I. Influence of operating parameter on crystal mean size (d_{50}), size distribution (d_{10}-d_{90}) production yield [R] and Form, of Tolbutamide processed by batch mode from acetone.

Operating parameter	d_{50} µm	[d_{10}-d_{90}] µm	Yield (%)	Form
Stirring rate (I = 0.4 MPa/min)				
R: 35 → 900 rpm	302 → 167	[141-583] → [85-285]	45 → 51	III
Introduction rate (R = 510 rpm)				
I : 0.4 and 1.4 MPa/miin	254 → 153	[170-360] → [94-234]	51 → 60	III
Temperature (R=900rpm;I= 0.4 MPa/min)				
T : 28°C → 39°C	167 → 148	[85-285] → [73-274]	50 → 43	III

In attempts of polymorphic change and/or effect on size, the initial solvent was changed [13]; since the few crystals produced from ethanol were of Form I, mixtures of acetone and ethanol were further used as liquors for TBM recrystallization. Similarly, mixtures of acetone and ether were investigated. Results are overviewed in **Table II**.

Table II. Influence of solvent on crystal mean size (d_{50}), size distribution (d_{10}-d_{90}) production yield and Form, of Tolbutamide processed by batch mode at 28°C. C_{TBM} ~ 58 mg/ml, excepted in run[a], where C ~11 mg/ml.

solvent	d_{50} µm	[d_{10}-d_{90}] µm	Yield (%)	Form
acetone	250	[138-420]	58	III
ethanol	87	[25-325]	10	I
Acetone-ethanol X_{etOH} 0.15→0.52	122 → 59	[36 -291] → [16 - 198]	43 → 21	III+εI → I + ε III
Acetone-ether X_{ether} 0.45→0.83[a]	199 → 67	[108-338] → [21- 193]	54 → 23	III→ I + III

As the content of ethanol increased in the liquor, needle-like particles appeared in the product besides the polyedre population. X-Rays patterns and thermal events from DSC enlighted the occurrence of Form I besides the Form III charateristic of the recrystallization from acetone. When ethanol content is increased above 29% molar, Form I is predominant. With ether instead of ethanol, even a 70% of ether in acetone still led to predominance of Form III, with form I being at traces level. A comparison with conventional crystallizations by evaporation showed that, excepted traces of Form IV in EVA samples, the forms found in CO_2-samples and EVA-samples were alike, indicating that the solvent is predominant to the orientation of the polymorph.

TBM was recrystallized by the continuous mode, with the expectation that more intensive mixing and final composition richer in CO_2 might promote the orientation of a polymorph, as observed for Sulfamethoxazole. With a pre-mixer + spray nozzle, neither the pressure, the flow rates combination, nor the concentration induced significantly the occurrence of Form I.

The largest modification came from the use of a capillary co-axial nozzle instead of a pre-mixing chamber + spray nozzle (Table III). TBM was produced as a mixture of needles and powder, made of particles of irregular shape, less faceted than those obtained through the pre-mixed design (Fig.1). Although XRD patterns showed peaks attribuable to Form I, the form III was always predominant in the produced samples. Thus, within the range of operating conditions investigated, Form III was produced, pure or with Form I as minor polymorph when a capillary coaxial nozzle was used.

Table III. Selected conditions and properties of TBM recrystallized by SAS from acetone.

Operating conditions	d_{50} µm	$[d_{10}$-$d_{90}]$ µm	Yield (%)	Form
Pre-mixer nozzle				
Pressure and flow rates (ml/min) 10 MPa 2/62 →1/15	55 → 55	[13 -250] → [17- 150]	34 → 64	III→III + εI
Capillary co-axial nozzleof 130µm				
Pressure 7.6 → 10 MPa, 1/12	116 → 122	[60-248] → [55 - 325]	77 → 54	III → III+I
Capillary diameter (10MPa, 1/12) 130 → 500µm	122 → 250	[55 - 325] →[90-600]	53→ 47	III+I

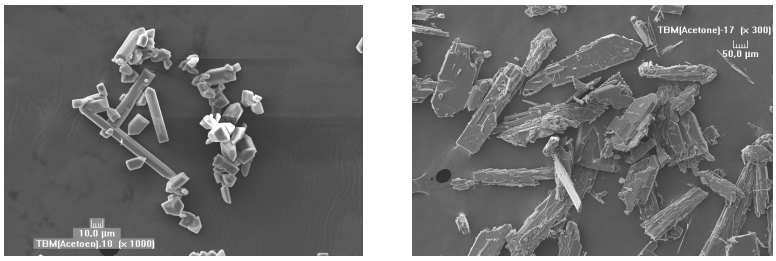


Fig. 1.TBM produced at 10 MPa, 36°C, 1/15 ml/min for F_{liq}/F_{CO2}, with a pre-mixed + spray nozzle of 200µm (left) with a co-axial capillary nozzle of 130µm (right).

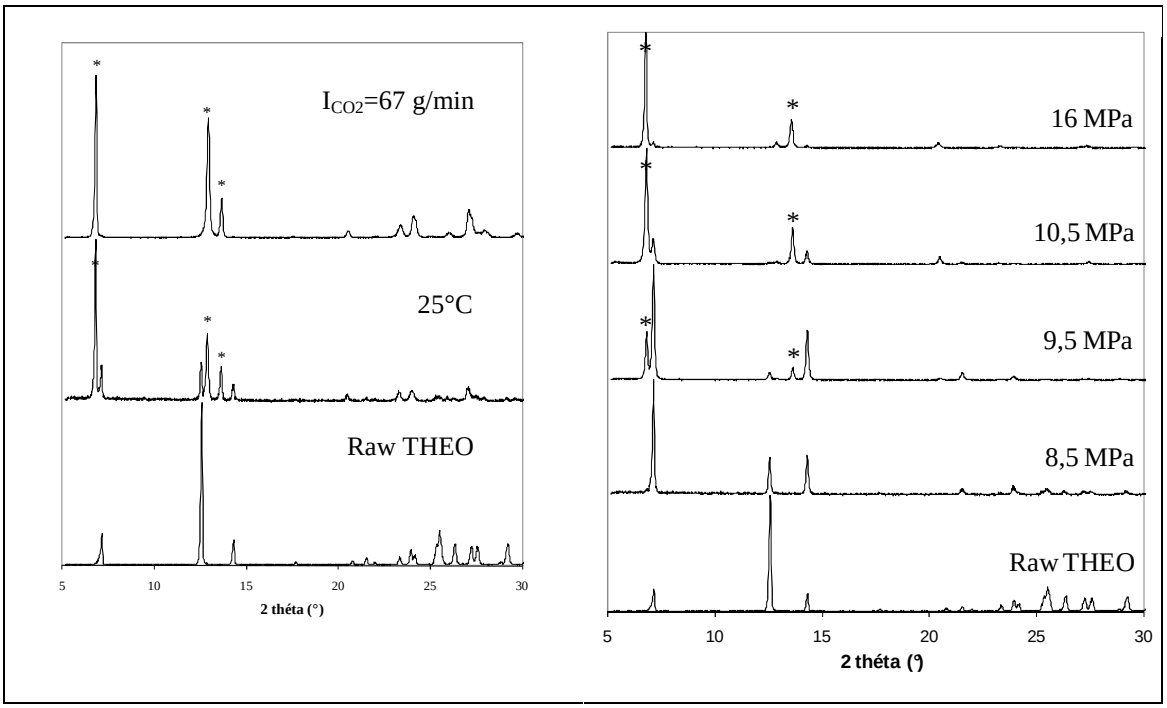
Finally, TBM was coprocessed with PEG 8000, in attempts to realize the crystalline to amorphous conversion. Operating conditions in SAS were a CO_2 flow of 15ml/min, 40°C and 7.6 – 8 MPa, and the co-axial capillary nozzle. The solution flow rate was varied, in order to vary the final composition of the fluid. For low content in acetone, produced samples were a mixture of Forms I and III, whilst similar conditions for pure TBM gave mostly Form III. At $X_{acetone}$ above 9%, the unusual Form IV was obtained, together with I or III; the additional I or III polymorph was obtained during the reproducibility experiments. Although it pointed out the difficulty of processing concentrated solutions, the appearance of Form IV was specific to the conjunction of polymer and 10% of acetone; indeed, processing pure TBM in same conditions led to pure Form III. Hence, coprocessing TBM and PEG did not produce a solid dispersion with amorphous drug, but induced the re-crystallization of TBM as a new Form IV, providing that a mole fraction in solvent was settled above 10%.

THEOPHYLLINE CRYSTALLIZATION

Theophylline was recrystallized from a mixture of ethanol and methylene chloride $(1:1)_{vol\%}$.
In batch mode, THEO particles were shaped as hexagonal flakes, whose sizes were quite insensitive to stirring and CO_2 introduction rates. Regarding cristallinity, XRD analysis revealed that THEO preferentially recrystallized in a different crystal lattice after CO_2 treatment (**Fig. 2**). Indeed, all batch samples exhibited new diffraction peaks, alone or together with those of raw THEO; the new peaks did not match the peaks of the two forms

already identified. To determine the cause of this new structure, crystallizations by evaporation were performed on CO_2 processed and raw THEO samples, after solubilization in the EtOH:DCM mixture; both XRD patterns exhibited peaks of raw THEO. Therefore, unlike Tolbutamide, the new structure was not induced by the solvent but came from the CO_2 treatment. Moreover, X-rays data were refined by the Rietveld method; although still under investigations, first results indicated a slight deformation of the crystal lattice, causing a modification of the diffraction peaks position.

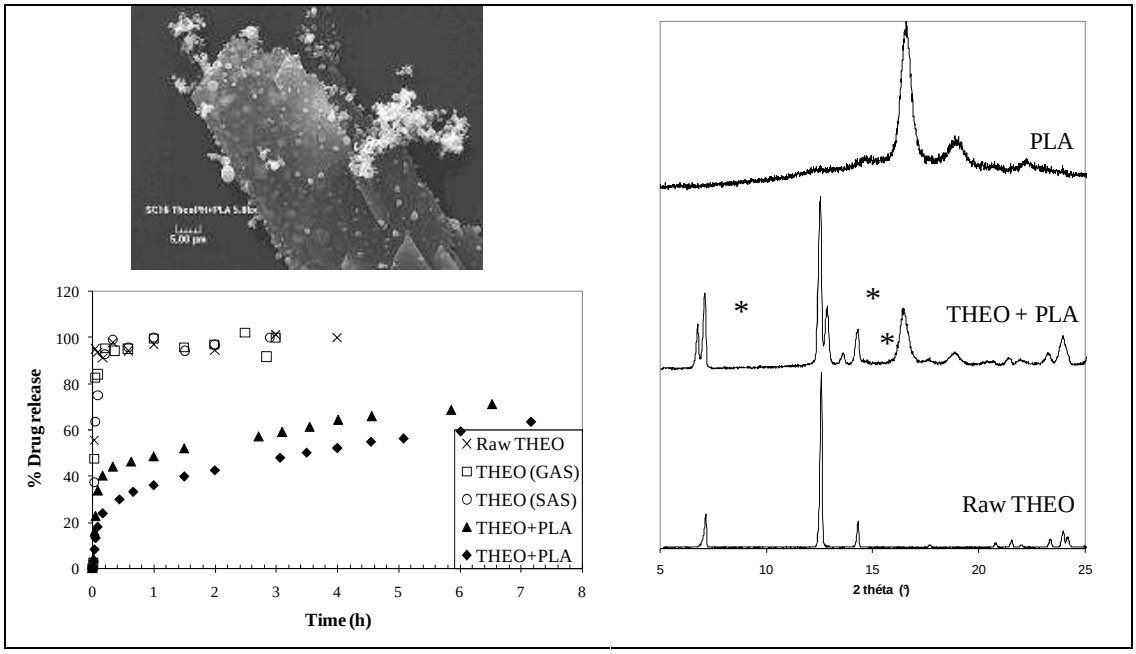
Fig 2: XRD patterns of THEO powders obtained by batch GAS process (left) or continuous SAS (right). (* : new diffraction peaks)



In continuous mode, particles sizes were dependent of pressure and temperature, according to phase behavior of the CO_2 + solvent equilibria [[14]. Both SEM and PSD analysis revealed a noticeable effect of pressure in the range of 8.5 to 16 MPa at 60°C or from 6.5 to 16 MPa at 36°C. As for GAS mode, the XRD patterns exhibited the new peaks charateristic of the CO_2-induced structure (Fig.2). Moreover, the relative intensity of these peaks increased with pressure, indicating that the new structure was preponderant as the pressure increased.

THEO was co-precipitated with l-PLA in attempts to produce drug delivery system. Due to the narrow therapeutic index of THEO, the aim is to deliver regularly the drug over time, thus PLA was selected as suitable carrier. The recovery of THEO- PLA as a powdered solid was only feasible by SAS. The produced powders were made of two distinct particles populations as shown in **Fig. 3**: the plate-like particles of THEO with embedded spheres, and spherical particles characteristic of PLA when CO2-processed. The XRD patterns exhibited the characteristic peaks of THEO (new crystalline form and/or initial structure, depending on experimental conditions) supplemented by peaks of the semi-crystalline PLA. The crystal lattice modification of THEO after CO_2 treatment occurred even in the presence of the carrier.

Fig 3: SEM picture and XRD patterns of a sample produced by co-precipitation in SAS mode (*: new diffraction peaks), and Dissolution profiles of THEO, pure (GAS- or SAS-processed) or co-precipitated with PLA (SAS-processed).



Dissolution properties of various samples were investigated. It can be seen in **Fig. 3**, that raw theophylline and samples produced by batch or continuous CO_2-process displayed a similar behavior. As mentioned in introduction, the crystal structure effect on dissolution rate might not be significant owing to the small difference of free energy between the polymorphs. Since the Rietveld refinement has indicated a small difference of packing arrangements between the CO_2-induced structure and the raw material, it is not surprising that samples behave similarly. With PLA, the co-processing indeed achieved a continuous release of the drug over time.

Acknowledgments: The financial support of the EU project NMP-CT-2005-013524 is greatly acknowledged.

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