A THREE STEP SUPERCRITICAL PROCESS TO IMPROVE THE DISSOLUTION RATE OF EFLUCIMIBE

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INTRODUCTION

One of the most important challenges to be faced by the pharmaceutical industry is the lack of water solubility of new drugs since it is linked with their bio-availability. Ensuring an improved dissolution rate of these molecules is therefore a major goal for researchers. Various methods can be used to increase the dissolution rate like micronisation by comminution, crystallisation leading to finely divided powder or complexation with cyclodextrins, for example. Indeed, cyclodextrins are a family of well known cyclic oligosaccharides which can form inclusion complex with large organic molecules [1]. The resulting complex may improve the stability, solubility and bio-availability of the host molecule. Usually, inclusion occurs in an aqueous solution or in presence of large water content.

Recently, the use of supercritical carbon dioxide has appeared as a new complexation medium for the preparation of inclusion complexes using its properties of mass transfer. Depending on the solubility of the host molecule different techniques can be applied: Extraction and percolation through a cyclodextrin packed bed [2-3], co-crystallisation by SAS process [4] or complexation using a static method [5].

Eflucimibe is an active compound (M = 469,73 $g.mol^{-1}$) provided by "Laboratoires Pierre Fabre" [6] and poorly soluble in supercritical CO_2 ($5.71\ 10^{-7}$ mol/mol at 274 bars and 308 K) [7]. In a previous paper, we have shown that supercritical particle generation processes like SAS could positively improve the dissolution rate of eflucimibe by increasing tenfold the specific surface area of the powder [8]. This improvement has proved however to be insufficient in a scaling-up perspective, and association with γ-cyclodextrins has been developed and a new process is proposed in this paper. It is composed of three consecutive steps, a crystallisation by SAS, a batch step leaving the powder in a supercritical CO_2 medium and finally a solvent stripping step.

I – EXPERIMENTAL SET-UP, MATERIALS AND CHARACTERISATIONS

The experimental set up and the different experimental configurations.

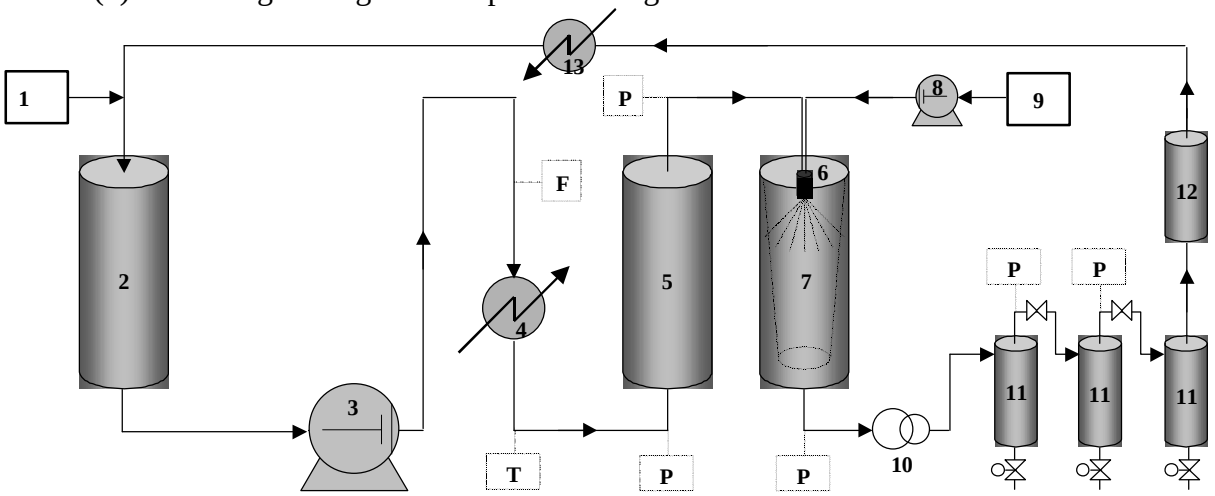
The experiments were carried out in a versatile pilot plant (Separex, France). A schematic diagram of the apparatus is shown in figure 1. Carbon dioxide is cooled and stored in a liquid CO_2 storage tank (2). It is circulated by a membrane pump (Lewa, Germany) (3). Compressed CO_2 passes then through a heat exchanger (4) and becomes supercritical.

For the SAS experiments, the solvent used was DMSO and the antisolvent was CO_2. γ-cyclodextrin, and eflucimibe were both dissolved in DMSO. The solution was injected by an HPLC pump (8) (GILSON, 307 piston pump) into the CO_2 stream (9).

Both flows were mixed in the mixing chamber of the nozzle (*Spraying system, France*) and the resulting mixture was sprayed into the expansion vessel. The powder formed was collected in a porous bag (7). Then, CO_2-solvent mixture was depressurised (10) and separated in cyclonic separators (11). After purification through an active carbon bed (12) and cooling (14), the condensed CO_2 ran back to the liquid CO_2 storage tank (2).

In the batch step configuration, 7g of eflucimibe/cyclodextrin powder has been wet by 2,33 g of ultrapure water and placed in the two litres extraction vessel (5). Vessel is filled with supercritical CO_2 at the desired pressure and temperature and left during several hours. Then vessel is slowly depressurised and the powder taken away.

During the solvent stripping step supercritical CO_2 flows through a stainless steel basket (5) containing the organic compound during two hours.



1 : CO_2 feed
2 : Liquid CO_2 storage tank
3 : Membrane pump
4 : Heater
5 : Extractor
6 : Nozzle
7 : Particule formation vessel
8 : Liquid pump (SAS)
9 : Solution (SAS)
10 : Back pressure regulator
11 : Cyclonic separators
12 : Adsorption bed
13 : Condenser
F : Flowmeter
P : Manometer
T : Thermometer

Figure 1 : Experimental set-up

Materials.

CO_2 (purity 99,995%) was supplied by Air liquide S.A., dimethylsulfoxide (DMSO) (purity > 99%) was obtained from Aldrich. γ-cyclodextrin with an initial mass water content of 8.7% was supplied by WACKER (Cavamax W8 pharma).

Characterisations

Powders obtained were characterised by a Scanning Electron Microscope (XL30 ESEM FEG, Philips, Netherlands) and the specific surface area was measured using the BET method (ASAP, 2010 Micromeritics).

After each step, composition of the powder obtained was determined. Eflucimibe content was measured by HPLC, residual DMSO content by GPC, water content with a Karl Fisher titrator. γ-cyclodextrin content was then deduced from all these results.

To estimate the improvement of the dissolution rate, *in vitro* dissolution studies were performed at 37°C. The equivalent of 100 mg of eflucimibe was put in 100 ml of aqueous solution containing sodium-dodecyl-sulfate, SDS (5% m/v). Periodically 2 ml of solution were sampled and the concentration of dissolved drug was measured by HPLC.

The DSC patterns of the samples (2-3 mg) were obtained between 40 °C to 140°C at a heating rate of 5 °C/min under a N_2 gas stream. The melting point of the eflucimibe occurs at T = 129.6°C and $\Delta H_{melting}$= 76±1 J/g. By integrating the melting peak of drug in DSC thermograms which is generated by crystalline form of the powder and knowing independently the total drug content, we can deduce the content of non-crystalline eflucimibe (amorphous or included in cyclodextrins).

II – RESULTS AND DISCUSSIONS

Main results and suggested interpretations

As for the initial physical mixture, its solubility at 20 hours (Figure 4,a) in the dissolution test is near 100 µg/ml, with the slowest kinetics of dissolution. The DSC curve (Figure 3, a) of the physical mixture shows a broad endotherm between 50°C and 100°C corresponding to the water loss of the γ-cyclodextrin followed by endothermal peaks corresponding to solid / solid transition of the eflucimibe at 111 °C and to the melting peak at 130°C. Besides, the initial drug powder is a rather pure polymorphic form.

The resulting powder of co-crystallisation of the drug and γ-cyclodextrin by SAS is illustrated on figure 2,I where we can clearly identify cyclodextrin particles and drug fibres deposited on them. Most of the drug remains in its initial crystalline form (Figure 3, b) but a small part crystallises in another polymorphic form, as evidenced by the DSC peak at 119°C. The dissolution rate is higher (Figure 4, b) in the first hours. The co-crystallisation process may have two effects on the drug. First, it ensures a good and fine dispersion of the hydrophobic drug in a hydrophilic matrix of cyclodextrin. Second, a part of eflucimibe contained in the mixture after co-crystallisation is not visible by DSC suggesting the formation of an amorphous or microcrystalline form. The study of the influence of mass ratio of CO_2 to DMSO, varying between 50 and 300, on the composition and dissolution rate of the resulting powder reveals that this parameter has no effect. On the contrary, decreasing the molar ratio of eflucimibe to cyclodextrin in the initial mixture from 1/1 to 1/3 increases the drug yield in the resulting powder from 40% (w/w) to 70 % (w/w). The cyclodextrin may have a stabilising effect on eflucimibe.

(I) **(II)** **(III)**

Figure 2. SEM picture of g-cyclodextrin/eflucimibe after co-crystallisation (I); after co-crystallisation and static step (II); after final stripping step (III).

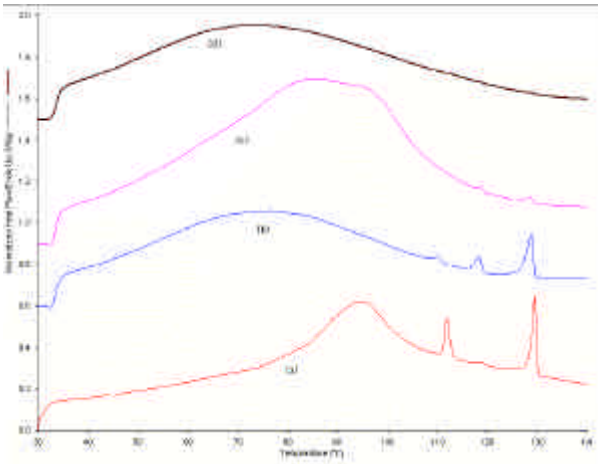


Figure 3. Typical DSC curve (a) physical mixture ; (b) co-crystallised powder ; (c) powder after co-crystallisation and static step ; (d) powder after co-crystallisation, static step and stripping

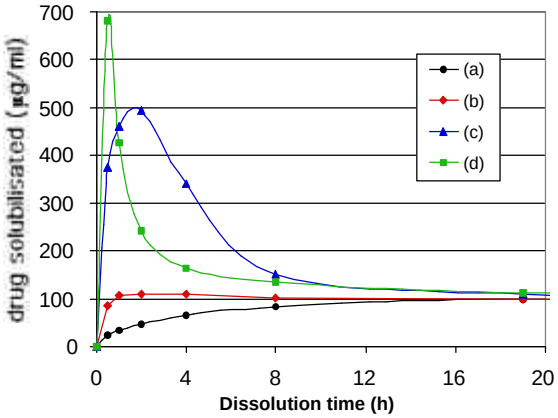


Figure 4. Dissolution curve of (a) physical mixture ; (b) co-crystallised powder ; (c) powder after co-crystallisation and static step ; (d) powder after co-crystallisation, static step and stripping

After the static step, drug fibres are not so clearly distinguishable from cyclodextrin particles, as seen on figure 2,II. Furthermore, we observe only a very small melting peak (Figure 3, c) and an increase up to 500µg/ml in the dissolution curve of the drug (Figure 4, c). We may have here formation of an inclusion complex of eflucimibe/γ-cyclodextrin. Its dissolution begins by its dissociation thus generating a drastic increase of the dissolution rate and ends with the recrystallisation of eflucimibe in its initial crystalline form. The study of the influence of CO_2 density and viscosity shows that non-crystalline drug content increases when CO_2 density and viscosity decrease. In other words the formation of complex is limited by mass transfer. In addition, varying the operating time of this static step shows that the composition and dissolution kinetics of the powder remains constant from 6 hours. Finally, we tried to performed the static step without adding any water and it failed, that is to say that we had no modification of the initial mixture.

After the stripping step, we observe an homogeneous powder on figure 2,III, a sharper and higher dissolution peak on figure 4,d and the complete disappearance of the melting peak on figure 3,d. The aim of the stripping step was to decrease the solvent content, but in fact we have also extracted some drug not involved in an inclusion complex. In addition, the stripping step dehydrates the cyclodextrin, which explains the fast initial dissolution rate due to its enhanced hygroscopicity.

Interest in coupling co-crystallisation and static step

Figure 5 shows the dissolved drug concentration after 2 hours in function of the type of powder mixture, before and after the static step. To perform this static step, three mixtures having the same mass composition are wetted and placed in the autoclave at 300 bars, 373K during 16 hours. The first mixture is composed of the initial drug and cyclodextrin, the second is composed of drug and cyclodextrin crystallised separately by SAS process and the third is composed of drug and cyclodextrin co-crystallised by SAS. Before the static step and comparing the first and the second mixtures, we confirm that the dissolved drug increases when specific surface increases [5]. If we compare now the second and the third mixtures, we see that the amount of dissolved drug increases when dispersion of drug in cyclodextrin matrix is enhanced. Then the static step increases the dissolved drug concentration in all cases. But the improvement of the compound dissolution is significantly higher for the co-crystallised powder. We can conclude from these results that coupling the co-crystallisation and static steps is very effective. A single mixing effect and a specific surface area increase can't alone explain this effect. To go a little further in our investigations, we have to enlighten what are the main mechanisms involved in each step.

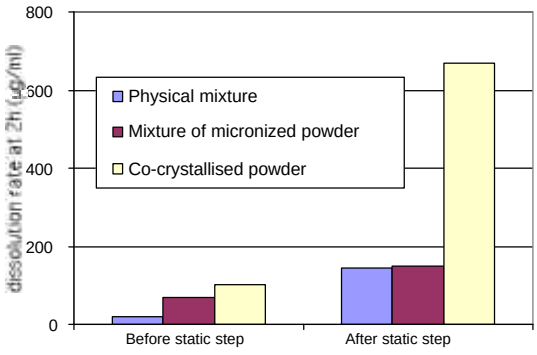


Figure 5. Dissolved drug concentration at 2 hours for different powder mixtures before and after the static step

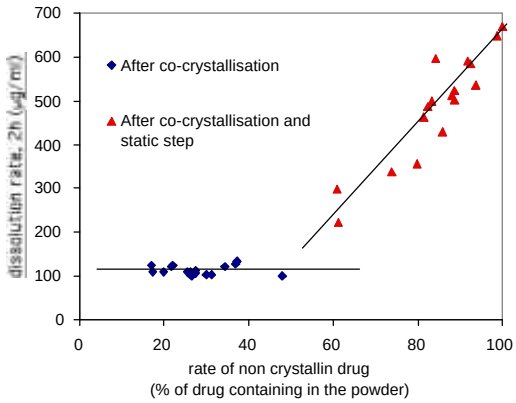


Figure 6. Dissolved drug concentration at 2 hours in function of the ratio of no-crystalline drug

Figure 6 shows the dissolved drug content after 2 hours (Y axis) in function of non-crystalline drug ratio (X axis) for all co-crystallisation and co-crystallisation/static step experiments performed. This ratio corresponds to the percentage of drug contained in the powder (measured by HPLC) that doesn't appear in DSC curves (melting peaks). For co-crystallised drug, its dissolution seems independent of the non-crystalline drug ratio. Hence we can hypothesise that the enhancement of initial dissolution rate is mainly due to the high degree of dispersion of drug into the cyclodextrin matrix. And the non-visible part of the drug in DSC

curve may be due to a molecular level dispersion. After the static step, this dissolution is correlated to the non-crystalline drug ratio which may be due here to the formation of an inclusion complex.
Hence we can suggest that a high degree of dispersion of the drug into the cyclodextrin is necessary to the formation of an inclusion but not sufficient. The addition of water to the powder performing the static step is another essential point. Further analyses have to enlighten the role played by this water.

CONCLUSION

In order to enhance dissolution rate of a drug: eflucimibe, a process was settled implying the formation of an inclusion complex of eflucimibe/γ-cyclodextrin. As we have no disputable evidence of a real complex, this expression is used to mean an entity, which develops an intimate contact between two compounds.
The process is composed of three steps. The first one is a co-crystallisation by SAS ensuring a very fine dispersion of the hydrophobic drug into the hydrophilic cyclodextrin powder. Reducing the molar ratio of eflucimibe to cyclodextrin favours the formation of drug powder. The second is a static step where the co-crystallised powder is wetted and remains under supercritical conditions a few hours leading to the formation of a complex. This formation is favoured at low CO_2 density and viscosity in which there are good mass transfer properties. Finally, a stripping step allows to extract residual solvent. The main novelty of this process lies in the coupling of these three steps, which is very effective in the improvement of the dissolution rate of the drug. Current investigations for a better understanding of the mechanisms involved in each step are under way.

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