USE OF SUPERCRITICAL FLUIDS FOR PHARMACEUTICALS APPLICATIONS

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Today 30% of discovered drugs are water insoluble and only 1% of water insoluble drug become marketed products. Indeed, insolubility of a drug can induce low bioavailability, lack of dose proportionality, sub-optimal efficacy, food effect, etc…

Enhancing the bioavailability or the solubility of an Active Pharmaceutical Ingredient (API) gives rise to many advantages. The dramatic reduction of the quantity of API in the final drug leads, beyond the important cost reduction, to a decrease in the potential side-effects. For the patient comfort, minimising the amount of API can mean new formulations. For example, we could envisage the development of oral forms with flash effect. The inherent patentability of supercritical carbon dioxide processes leads to a mechanism for enhanced product life cycle extension.

COMPLEXATION WITH FORMULPLEX®

Most of new molecules in the pharmaceutical industry is almost water-insoluble. As water is the basis of any biological fluid, one challenge is to produce formulation in such a way that the dissolution rate of API is increased, thus improving their bioavailability for living organisms.

FORMULPLEX® is a supercritical carbon dioxide batch process that permits to obtain inclusion complex of insoluble drug, increasing its oral bioavailability. Other applications are under developpement: stabilisation of API in a formulation, taste masking, etc…

Cyclodextrins, a family of oligosaccharides, have the ability to form inclusion complexes with organic molecules. The most commonly used are alpha-, beta- and gamma-cyclodextrins. Some other cyclodextrins have been developed, which differ from the standard cyclodextrins in their higher solubility, due to the chemical modification of some hydroxyl groups, and in their price.

Inner part of the cyclodextrin macromolecule is hydrophobic, whereas outer part is hydrophilic. This configuration enables the complexation of a water-insoluble API to take part in the cavity. Once the inclusion complex has been completed, we observe an enhancement of the API's solubility. It has been proved that this has a significant effect on the permeation of some APIs through biological membranes.

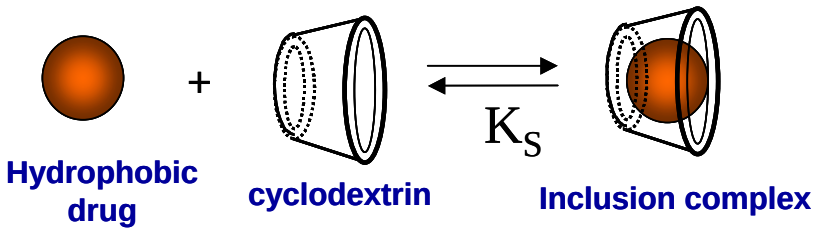

Figure 1. Complexation Equilibrium

The use of supercritical fluids has many advantages compared to traditional methods, such as kneading, freeze drying or grinding. First of all, it appears in some cases, that complexation phenomenon is observed only in supercritical medium, and not by conventional methods. The process leading to inclusion complex is totally “green” using only carbon dioxide and a few amount of water. The latter is neither time- nor energy- consuming.

MATERIAL

This paper presents a preclinical study on rats focused on a Non Steroidal Anti Inflammatory Drug (NSAID). Beta cyclodextrin (CAVAMAX W7 Pharma, WACKER) and a amino acid ternary agent : L-Lysin (KYOWA HAKKO) were used. The molar ratio of each component is 1:1:1.

FORMULPLEX® [1] is a batch process consisting in placing in supercritical carbon dioxide (150 bar, 60°C) a mixture of NSAID with cyclodextrin and ternary agent wetted with a few amount of water.

During this 2 hours maturing step, complexation occurs. Then powder is dried in a convective dryer. The inclusion complex of the API in cyclodextrin is called FORMULPLEX®.

METHODS AND RESULTS

Thermals analysis: For the characterisation of the solid complexes, powder is analysed by differential scanning calorimetry (DSC). The DSC patterns of sample (5 mg) were obtained between 0 to 100 °C at a heating rate of 5 °C/min under a gas stream. By integrating the

melting peak of drug containing in the powder, knowing the real drug content, we can deduced the content of non crystalline drug (amorphous or included in cyclodextrins).

In Figure 2, After supercritical treatment , an amorphous and dry powder is obtained.The inclusion ratio is complete.

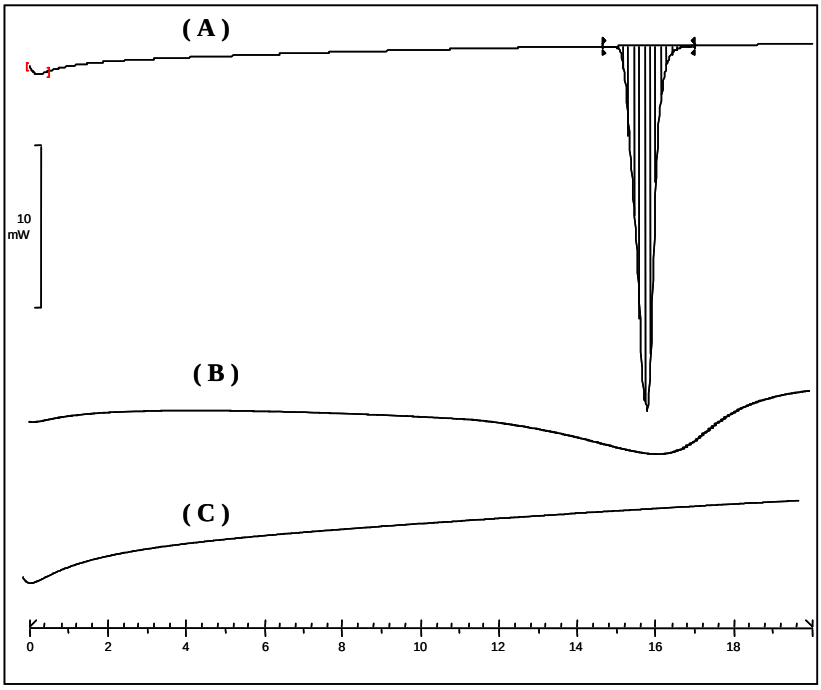

Figure 2. Thermal profile of API before treatment (A) and beta- cyclodextrin (B) and FORMULPLEX® (inclusion complex API / cyclodextrin/ Lysin) (C).

In vitro test : In order to estimate the solubility improvement, dissolution studies were performed. The equivalent of 100 mg of Active Pharmaceutical Ingredient was placed in 100 ml aqueous solution (Phosphate buffer pH= 3; European Pharmacopea. 4000500) at 37°C under stirring. At different time, 2 ml of solution was taken and the concentration of drug was evaluated by HPLC.

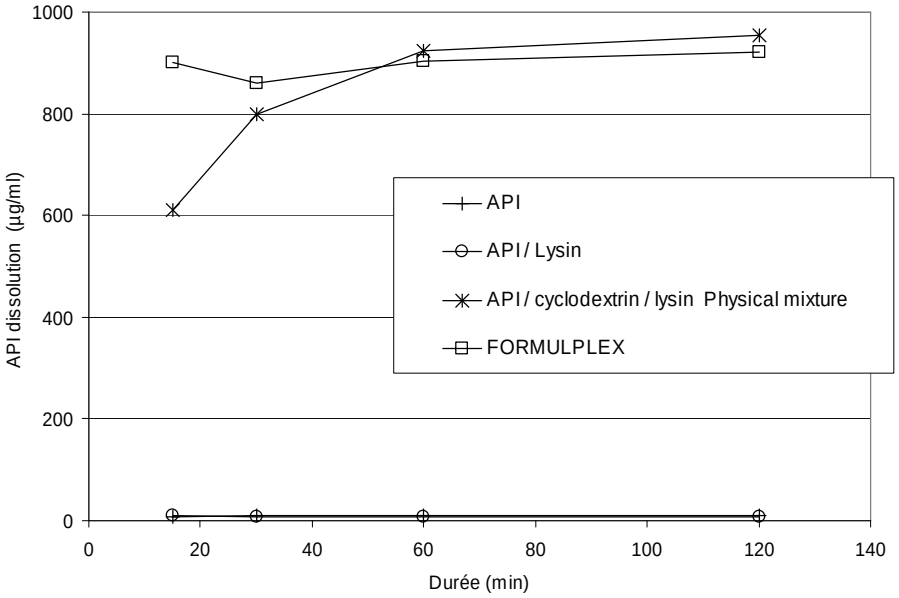

Figure 3. Dissolution profile of API, Physical mixture and inclusion complex obtained by supercritical carbon dioxide

Dissolution profile obtained (cf. Figure 3) shows improvement of apparent solubility of API in presence of cyclodextrin in the aqueous medium This is linked to the existence of an equilibrium of complexation. In case of physical mixture, one hour is necessary to obtain this equilibrium value. In case of FORMULPLEX®, the inclusion complex is already made, so the dissolution is instantaneous.

In vivo test : In order to estimate the improvement of relative bio-availability, a comparative pharmacokinetic study was realised with animals (Rats, 6 groups, n = 3, single dose).Oral administration of 20 mg/kg, blood samples are taken at 0.25, 0.5, 1, 2, 4, 6 et 24 hours post-administration.

Plasmatic concentrations are analysed by HLPC-MS. Pharmaco-kinetic parameters were deduced by software KINETICA®. Results are presented in the table bellow:

- Cmax (ng/ml) corresponds to maximal plasmatic concentration of NSAID

- Tmax (hours) is the post administration time for reach the Cmax.

- AUC (ng.h/ml) corresponds to the Area Under Curve of C_{API} = f (time) until 8 hours post-administration. Concentration of sample effected after 24 h is bellows quantitative limits.

- Half-life (hours) corresponds to the time necessary for organism to eliminate half of the drug in plasma.

	API	API + Lysin	API + β-cyclodextrin	FORMULPLEX®
Cmax (ng/ml)	2276	12089	11744	41813
Tmax (h)	0.5	0.5	1	0.25
AUC (ng.h/ml)	7188	53251	29064	63314
Half life $t_{1/2}$	1.76	3.04	3.23	1.62
Cmax / $Cmax_{INITIAL}$	1	5.3	5.2	18.4
AUC / $AUC_{INITIAL}$	1	7.4	4.0	8.8

Table 1.

On this table, we can say that FORMULPLEX®improves in a considerable way the intestinal absorption to the rat by oral route: improvement of Cmax, shorter Tmax. Consequently, the half-life of drug is diminishing, because the clearance of drug is deeper.

Plasmatic exposition of drug is also improved with FORMULPLEX®: The AUC is increased by a factor: 8.8.

DISCUSSION AND FOLLOW UP

In-vivo studies were executed in order to demonstrate the improvement of drug bioavailability. On a first approach, mainly for regulatory and economic issue, tests were realised on animal models: rats. This model was chosen regarding the pK data already published in the literature. These results are positive but it's clearly insufficient for predict final result on human race. So, clinical trials are now realized with FORMULPLEX® versus different commercial formulations actually available: stability studies and clinical study on 36 healthy volunteers are now under progress.

Several batches of 20 kg have been already produced on our pilot scale production area . It has been designed in order to manufacture clinical and commercial batches in compliance with BPF and cGMP Reproducibility and batch homogeneity (composition, physico-chemical properties) was also verified.

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METHOD FOR THE PREPARATION OF MOLECULAR COMPLEXES
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