STUDY OF THE MATURATION OF A KETOPROFEN/β-CYCLODEXTRIN MIXTURE, ASSISTED BY SUPERCRITICAL FLUID

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ABSTRACT

Supercritical fluids have found many applications due to their versatile physicochemical properties. They can be used as media for the formation of inclusion complexes, for helping encapsulation of an active substance into cyclodextrin (CDs) cavities. In addition, several active pharmaceutical substances such as ketoprofen, are not very polar, thus not very water-soluble, and in order to increase their rate of dissolution, such drugs can be included in a molecular cage, such as CD with the aid of supercriticalfluid processing.

Several inclusion processes in supercritical media have been developed and the maturation process, in which supercritical carbon dioxide (SC-CO_2) is generally used, is among the most recent. It consists of using supercritical CO_2 to bring the active substance, the cyclodextrin and a quantity of water in contact one with another over certain duration, in order to obtain an inclusion complex. We have also studied the influence of several operating parameters on the inclusion rate e.g pressure, temperature, duration of maturation, density of the SC-CO_2, agitation and factors related to the physical mixture, the amount of water, the molar ratio β-cyclodextrin/ketoprofen and the mass ratio SC-CO_2/mixture, as well as the effect of the method of preparation of the physical mixture on inclusion.

We found that pressure, temperature, duration of maturation, agitation and density of SC-CO_2 all had a positive effect on inclusion of ketoprofen (KP) in β-cyclodextrin (β-CD). The stoichiometry of inclusion of KP in β-CD is 1:2. The method of preparation of the powder mixture and mass ratio SC-CO_2/mixture has an effect on inclusion.

INTRODUCTION

Several processes using supercritical fluids for the formation of the inclusion complexes have been already developed. They are based on the solvent and transfer properties of supercritical fluids. The principal processes used in supercritical medium are: the RESS Process (Rapid Expansion of Supercritical Solution), the SAS Process (Supercritical Antisolvent) and the PGSS® Process (Particle from Gas-Saturated Suspension/Solution) [1]. The supercritical maturation process, in which supercritical carbon dioxide (SC-CO_2) is generally used, is among the most recent. It consists of holding in contact the active substance, the CD and some water, in supercritical medium over certain duration, in order to obtain an inclusion complex. This process was first developed in 1999, by the team of Pr. Delattre from Liège University (Belgium). Previous work using this process involves the inclusion of a nonsteroidal anti-inflammatory drug (NSAID), Piroxicam [2].

Our work tries to clarify the phenomena involved in the inclusion process using the supercritical maturation process. We also studied the influence of the process parameters and those related to the physical mixture composition and method of preparation.

I – MATERIALS AND METHODS

1-1-The experimental set-up

The tests of maturation are carried out in a multifunction pilot plant (Separex, France). Figure 1 presents the configuration of the plant for maturation. CO_2 is initially stored in liquid form at 50 bars and 0°C in reservoir (2). Liquid CO_2 is then pumped by the membrane pump (3), to be pressurized up to 350 bars. The cooling of the pump head makes it possible to maintain solvent in the liquid state and avoid cavitation. Compressed CO_2 then passes through a heat exchanger (4) to become supercritical. The mass flow of CO_2 is fixed at 20 kg/h. The autoclave (5) with a capacity of 2 litres is electrically heated and contains the mixture to be maturated. Digital and analog indicators of CO_2 flow, pressure and temperature, on the control panel give direct reading of these process parameters.

We carried out some maturation tests using a Labsize pilot, also built by Separex, to determine the effects of agitation and of the mass ratio SC-CO_2/mixture on the inclusion process. The Labsize pilot is similar to the main pilot but the maturation autoclave has a capacity of only 0.5 litre and is fitted with a mechanical stirrer.

1-2-Operating mode

To perform complexation in the supercritical medium, we first prepared a physical mixture of the components. This involves manually mixing with a spatula, a certain quantity of KP, β-CD and water. The physical mixture is then placed in a basket closed by two sintered steel porous plates, which is then placed in the maturation autoclave (5). CO_2 flows in, until the desired pressure and the temperature is reached. The autoclave is then isolated for the duration of maturation. At the end of the experiment, the apparatus is depressurized by sending SC-CO_2 to the vent.

1: Condenser
2: Liquid CO_2 tank
3: Membrane pump
4 : Heat exchanger
5: Autoclave of maturation
T : Thermometer
F: Flowmeter
P: Manometer

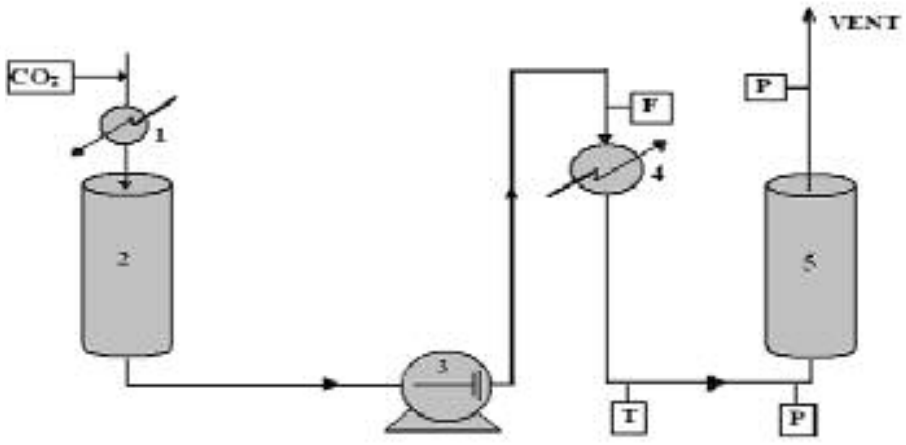

Figure 1 : Experimental set up

1-3-Materials

Four different substances, CO_2, KP, β-CD and water were used in the experiments.

• ***CO_2***

CO_2 (purity 99.995 %), is supplied by Air Liquids S.A.

• ***Ketoprofen***

The active compound (KP) was supplied by Boehringer-Ingelheim. KP belongs to the class of NSAID (non-steroidal anti-inflammatory drug), derived from aryl-carboxylic acid. It has an activity in the treatment of inflammatory rhumatoid diseases and for the relief of acute pain. It is effective against period pains, pain after surgery, and fever. Its chemical formula is $C_{16}H_{14}O_3$ (2-(m-Benzoylphenyl) propionic acid) having a molecular weight of 254.28, a melting point of 95 °C. The solubility of KP in SC-CO2 was determined. It was found that, at 200 bars and 65 °C corresponding to a SC-CO_2 density of 0.661 g/l, the solubility of KP is 0.15 g/ml ($y_{KP} = 4{,}1\ 10^{-5}$). According to the literature, at 220 bars and 58.35 °C, the molar fraction of KP in SC-CO_2 is $y_{KP} = 15\ 10^{-4}$ and at 100 bars and 39.3 °C $y_{KP} = 4\ 10^{-6}$ [3]. In addition, KP is not very water-soluble, its solubility at 25 °C is found to be 0.13 g/l [4]

• ***β-cyclodextrin***

β-CD Cavamax W7 Pharma was provided by Wacker. β-CD is insoluble in SC-CO_2. Comparative to other natural CDs, β-CD is the least water-soluble. At 20 °C and atmospheric pressure, the water solubility of β-CD is 16.4 g/l; it reaches 18.5 g/l at 25 °C and 196.6 g/l at 80 °C [5].

• ***Water***

Distilled water was used. The evolution of the solubility of water at 50 °C in CO_2 for various pressures can be found in [6]

1-4-Analytical methods

• ***Differential Scanning calorimetry (DSC)***

The DSC allows the determination of the percentage of included active compound. A Perkin-Elmer DSC-7, was used to measure the heat absorbed by the sample when non-included active compound melts by increasing the temperature from 20 to 120 °C at 2 °C/min, under nitrogen flow. The sample sizes were 4.5-5 mg. The comparison of the thermogram corresponding to the fusion of non-included or free KP with that of pure KP enables us to calculate the percentage of inclusion. To determine the percentage of inclusion of KP in CD, we have used the equation (1):

$$\%AC\ complexed = 1 - \frac{(\Delta H_{f1} / \Delta H_{f2})}{(M_1 / M_2)} \qquad (1)$$

% AC complexed: is the mass percentage of the complexed active compound (KP). ΔH_{f1} is the enthalpy of fusion of non-included KP after complexation (J/g). ΔH_{f2} is the enthalpy of fusion of pure KP (J/g). M_1 is initial mass of KP contained in the physical mixture (g) before maturation. M_2 is the mass of physical mixture (g) before maturation. This thermal characterization method for inclusion compounds is now a standard method as confirmed in the review by Giordano and al. [7].

II-EXPERIMENTS: PROCESS PARAMETERS AND RESULTS

2-1-Process parameters

The parameters varied are : Process parameters: pressure, temperature, duration of maturation, density of SC-CO_2 and agitation; Composition mixture parameters: amount of water, molar ratio KP/β-CD, mass ratio SC-CO_2 /mixture ; Method of preparation of the physical mixture: water added to the KP/β-CD mixture, water added to β-CD before the addition of KP.

2-2-Main results

For the same complex, obtained at 150 bars and 65 °C, we made three DSC of three different samples. The standard deviation (defined here as the ratio of the mean deviation from the mean value to the mean value) on the measured heat of fusion is 0.7%. The inclusion of KP into CDs can be considered to be homogeneous in the entire complexed mixture**.** Three experiments were also performed for the same mixture composition, at 150 bars, 65°C, 2 hours of maturation and a molar ratio KP/β-CD 1:2. The standard deviation on the measured heat of fusion of non included KP is 0.9%. The process therefore has a satisfactory reproducibility.

2-2-1-Effects of the operating variables

It is found that temperature has a positive effect on inclusion. This positive effect can be explained by the fact that an increase in the temperature accelerates the kinetics of formation of the complex. The temperature has also a negative effect on the solubility of the KP in the SC-CO_2 at a fixed pressure. Hence, if SC-CO_2 is a transfer medium for water and KP; it is less efficient when increasing the temperature. However, the positive effect of the temperature on inclusion kinetics may prevail. Increasing the contact time between SC-CO_2 and the physical mixture is also favorable. Indeed, for one and a half hours maturation, the inclusion is 47.62 %, reaches 65.19 % after 2 hours and 75.16 % after 5 hours of maturation. In other words, time of contact has a very important effect on inclusion up to 2 hours of maturation and becomes less significant for longer durations. The increase in the time of contact in SC-CO_2 allows increasing the transfer in SC-CO_2 of dissolved KP and water and the diffusion of KP and water through the mixture. This would suggest that the process is mass-transfer limited. A higher density of SC-CO_2 has a positive effect on inclusion (see Figure 2). By increasing the density of SC-CO_2 the quantities of water and solubilized KP in SC-CO_2 will be more important. Consequently, the transfer of KP and water towards the βCDs, which can be done via CO_2 medium, will be more important (there will be more opportunity for contact between β-CD, water and KP). Figure 2, given for a fixed temperature, indicates also a positive effect of pressure on inclusion. We carried out two tests on the Labsize pilot. The first made without agitation and the percentage of inclusion is 60.22 %, the second made using the mechanical agitator gives percentage of inclusion of 72.55 %. Thus there is an increase in inclusion of 12.33% when the mixture is stirred at an agitation speed of 350 rpm. Agitation makes it possible to increase the transfer of KP and water in CO_2, as well as the transfer of KP in the mixture, thus obtaining a more homogeneous mixture.

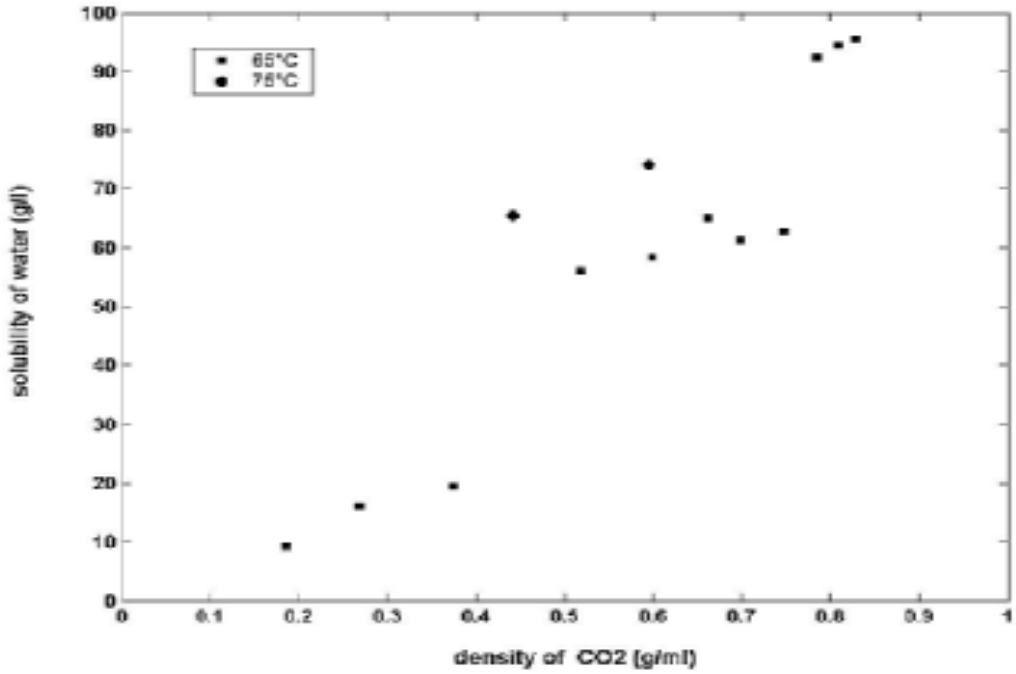

Figure 2 : The effect of the density of CO_2 on inclusion

2-2-2-Effects of the mixture composition

It should be noted that the addition of water is necessary for inclusion to occur (see table 2). With a physical mixture prepared without any additional water (thus just with water adsorbed on initial β-CD), we obtain 0 % of complexation and for 25 % of added water; we have more than 95 % of complexation. The water added to the physical mixture solubilizes the surface of β-CDs and cracks them, thus increasing their contact surface. It also destabilizes the water initially present in the cavities of CDs. This internal water becomes unstable with a higher energy state. The thermodynamic changes occuring during maturation will tend to restabilize the system. This could be achieved when the destabilized water leaves the CD cavities to allow access by the KP molecules. The included KP molecules in the cavities of CDs form hydrophobic links with the glucosidic groups of CDs. This exchange and hence this destabilization phenomenon is a key-contribution to the inclusion process. We also studied the stoichiometry of inclusion of KP in β-CD. For the molar ratio KP/CD 1:1, the percentage of inclusion is 30.81%, and goes to 73.96 % for a ratio 1:2 and then varies no more using a ratio 1:2.5. Thus the stoichiometry of inclusion of KP into β-CD is 1:2, i.e. a KP molecule is included in two molecules of CDs. To study the influence of the mass ratio CO_2/mixture, we did maturation tests with both pilots. The volume of the autoclave of the first pilot is 2 liters. In the second pilot the volume of the autoclave is 0.5 liter. The percentage of inclusion seems to increase when the mass ratio SC-CO_2 /mixture decreases. Hence, for the same density of SC-CO_2 and similar experimental conditions, at the end of the experiment, the total quantity of KP, which remains solubilized in SC-CO2, is more important in the larger autoclave than in the smaller one. Thus the quantity included in CDs is more important in the smaller autoclave than in the larger one. Therefore, for small quantities of the physical mixture, it is more interesting to use not too great a quantity of SC-CO_2, so as to minimize the diluting effect of SC-CO_2.

2-2-3- Method of preparation of the physical mixture

In order to find out if there is an incidence of the method of preparation of the mixture on inclusion, we prepared the mixture by two different methods. In the first case, we mixed CD with KP and then we added water and mixed all together. In the second case, we mixed CD with water and then we added KP and mixed a second time. Then we studied the effects of pressure, temperature and duration of maturation.
When we add water after KP, the pressure and the temperature have a positive effect on inclusion. By adding water before KP, the results of the complexation show that, at 65 °C, the pressure does not have any effect on inclusion. However, at 75 °C, the increase in the pressure generates an increase in inclusion, as for the first method. The addition of water before KP makes this water structured in a more stable way on the CDs surface and in the cavities of CDs. It is more difficult to destabilize to allow substitution by KP. Moreover KP was badly distributed onto CD surfaces. For a higher temperature, here 75 °C, water in the cavities becomes less stable, and it is thus more likely to leave the cavities and to be replaced by KP. The pressure has then a positive effect on the solubility of KP. This additional limitation for the inclusion process has to be compensated by increasing temperature. The higher temperature will then favour the destabilization of the internal water and then allow the pressure on the inclusion process to be more effective.

III – CONCLUSION

This work confirms that the batch maturing process using supercritical CO_2 is an effective and straightforward process of encapsulation by CDs. However, the phenomena at play are not so simple and need to be further investigated, for instance the role of the added water has not yet been totally elucidated. To implement the work done on this complexation process, we tried to put in light the effect of some parameters that have not yet been widely studied such as the mass ratio SC-CO_2/mixture and the method of preparation of the physical mixture. It may be concluded that the initial physical mixture must be carefully prepared by only introducing water at the end of the mixing, and that conditions of temperature, ambient humidity and stirring have to be controlled. The use of great volumes of CO_2 causes the dilution of KP in SC-CO_2, which decreases the quantity included in CDs. Therefore, a high powder density in the autoclave favours inclusion.

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