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SUPERCRITICAL FLUID IMPREGNATION OF PHARMACEUTICALS INTO STARCH: EXPERIMENTS AND PARAMETERS OPTIMIZATION

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alla mia famiglia e a Federico

Abstract

1. *Die Planeten bewegen sich auf Ellipsen, in deren einem Brennpunkt die Sonne steht.*
2. *Die Verbindungslinie Sonne-Planet überstreicht in gleichen Zeiten gleiche Flächen.*
3. *Die Quadrate der Umlaufszeiten zweier Planeten verhalten sich wie die Kuben ihrer großen Bahnhalbachsen.*

1. *The orbits of the planets are ellipses, with the Sun at one focus of the ellipse.*
2. *The line joining the planet to the Sun sweeps out equal areas in equal times.*
3. *The ratio of the squares of the revolutionary periods for two planets is equal to the ratio of the cubes of their semimajor axes.*

(Johannes Kepler. *Taught in Graz, 1594-1600*)

The aim of this thesis is to study the possibility of using starch for drug impregnation, using supercritical carbon dioxide.

Experiments have been performed on two starch types, tapioca and corn starch, and with two model drugs: ibuprofen sodium salt and caffeine. Ibuprofen sodium salt has low solubility in carbon dioxide, whereas caffeine is more soluble.

Impregnation has been studied by changing temperature, pressure and treatment time.

All the experiments performed showed that it is not possible to achieve high drug impregnation of the starch matrix. For this reason, starch does not seem to be a proper material for this technology.

However, based on observations done during this study, another interesting technique has been developed, which exploits a drug melting point depression, due to CO_2 pressure, to produce a solid dispersion, using starch as the substrate, without need of organic solvents.

Encouraging results have been found and other experiments and analysis are suggested to better develop this idea.

Riassunto

1. *Die Planeten bewegen sich auf Ellipsen, in deren einem Brennpunkt die Sonne steht.*
2. *Die Verbindungslinie Sonne-Planet überstreicht in gleichen Zeiten gleiche Flächen.*
3. *Die Quadrate der Umlaufszeiten zweier Planeten verhalten sich wie die Kuben ihrer großen Bahnhalbachsen.*

1. *I pianeti si muovono intorno al Sole lungo ellissi, di cui il Sole occupa uno dei fuochi.*
2. *La linea che va dal Sole al pianeta spazza aree uguali in tempi uguali.*
3. *Il quadrato del periodo orbitale di un pianeta è proporzionale al cubo del semiasse maggiore.*

(Giovanni Keplero. *Insegnò a Graz, 1594-1600*)

L'obiettivo di questa tesi di ricerca è di studiare la possibilità di usare amido per l'impregnazione di prodotti farmaceutici, sfruttando la tecnologia dell'anidride carbonica supercritica. La tesi, sviluppata presso la University of Technology di Graz, si inquadra all'interno del panorama della ricerca nel campo dei fluidi supercritici. Lo scopo dell'utilizzo dell'anidride carbonica supercritica è quello di sviluppare delle tecnologie più pulite, in sostituzione di quelle attualmente utilizzate.

La seguente trattazione analizzerà infatti l'impregnazione con un fluido supercritico, una tecnologia che consente di ottenere delle forme farmaceutiche senza l'uso di solventi organici. In letteratura esistono numerosi studi sull'impregnazione di polimeri con farmaci. Esempi tipici di polimeri utilizzati sono policaprolattone e polivinilpirolidone. Questa tecnica è particolarmente utile nel caso in cui si voglia sviluppare un sistema a rilascio controllato o aumentare la biodisponibilità di un farmaco, modificandone la velocità di solubilizzazione.

Tra i numerosi eccipienti utilizzati in campo farmaceutico, l'amido rappresenta un componente interessante, essendo atossico ed economico. Per queste sue caratteristiche è infatti gia utilizzato in altre tecnologie, come lo spray-dry, e nella

produzione di compresse. Il suo impiego assieme all'anidride carbonica supercritica, invece, non è ancora stato preso in considerazione, perciò sono disponibili poche informazioni in letteratura circa il comportamento dell'amido in CO_2 supercritica.

Per questo studio, si sono considerati due tipi diversi di amido:

- amido di tapioca, non poroso;
- amido di mais, poroso.

La scelta di usare due amidi con caratteristiche diverse si propone di valutare se la presenza di porosità può aumentare il grado di impregnazione, aiutando l'anidride carbonica satura di farmaco a penetrare all'interno della matrice dell'amido.

Per questi esperimenti si sono scelti due farmaci modello:

- sale sodico di ibuprofene,
- caffeina.

Altri farmaci più adatti allo scopo potrebbero essere utilizzati, in particolare farmaci già usati in altri studi di impregnazione, in modo da consentire un confronto. La scelta tuttavia è limitata dalle disponibilità del laboratorio presso cui si è svolto il lavoro.

La caffeina è scelta perchè particolarmente solubile in anidride carbonica e perchè sono disponibili dati di solubilità in CO_2 supercritica. Il sale sodico di ibuprofene, invece, è scelto in sostituzione dell'ibuprofene, che è un acido debole comunemente utilizzato nelle formulazioni. In letteratura si trovano una serie di studi di impregnazione dell'acido di ibuprofene, mentre sono scarsi i riferimenti sul sale sodico di ibuprofene. Di questo farmaco non sono disponibili dati di solubilità in anidride carbonica supercritica, ma si ipotizza che sia meno solubile rispetto all'acido.

Entrambi i farmaci, come anche l'amido, sono polveri bianche, composte da particelle di dimensioni variabili.

Per l'impregnazione, si considerano diverse variabili operative, con l'obiettivo di ottimizzare i risultati ottenuti: temperatura e pressione si sono rivelate le due più importanti. Altri aspetti considerati sono la configurazione dell'impianto e il tempo di impregnazione.

Gli studi sono stati effettuati in un impianto batch senza mescolamento, considerando due differenti configurazioni. La configurazione che mostra i risultati migliori prevede che, all'interno del reattore di 120 cm^3 di volume, sia posta una rete metallica, su cui poggia il farmaco. Al di sopra di questa, distanziata di 7 cm, è posta un'altra rete metallica, su cui poggia l'amido. Il farmaco e il substrato, quindi, non sono in contatto all'interno del reattore. Questa configurazione è tra quelle tipicamente utilizzate per impregnazione con CO_2 supercritica.

La concentrazione di farmaco ottenuta nell'amido è stata analizzata mediante spettroscopia UV. I campioni sono stati inoltre studiati al microscopio ottico.

Prima di procedere alle prove di impregnazione, si presentano alcune osservazioni preliminari. Queste sono state fatte in parte con l'impianto utilizzato per l'impregnazione, in parte con una cella visibile, in modo da poter vedere che fenomeni si verificano all'interno del reattore, in presenza di anidride carbonica supercritica.

Dalle osservazioni preliminari, si è notato che:

- l'amido non subisce evidenti variazioni a seguito del trattamento con CO_2 supercritica, tuttavia, una discreta percentuale di anidride carbonica potrebbe ugualmente solubilizzarsi al suo interno, ma senza dare effetti permanenti;
- la caffeina è visibilmente solubile in anidride carbonica, a conferma di quanto riportato in letteratura;
- quando posto a contatto con anidride carbonica supercritica, il sale sodico di ibuprofene presenta un abbassamento del punto di fusione a 40°C e 70 bar;
- la solubilità del sale di ibuprofene non è determinabile con gli strumenti a disposizione, ma dalla semplice osservazione sembra molto inferiore rispetto alla solubilità della caffeina.

La scoperta della presenza di un abbassamento del punto di fusione per il sale sodico di ibuprofene, ha suggerito l'idea di sfruttare questo fenomeno per la formazione di una dispersione solida. Si tratta di un effetto notevole, dato che a pressione atmosferica il farmaco fonde attorno ai 200°C. Questa tecnologia, benchè diversa dall'impregnazione, si pone come ulteriore possibilità offerta dall'anidride carbonica supercritica. Consente infatti il trattamento dei farmaci che fondono in presenza di CO_2 supercritica, evitando l'uso di alte temperature o di solventi organici. La presenza di tale fenomeno in altri farmaci è riportata in letteratura.

Anche per questa tecnica si è studiato l'effetto delle variabili operative, principalmente temperatura, pressione e rapporto tra farmaco e substrato. In questo caso, il mixing risulta fondamentale per ottenere un composto omogeneo.

I campioni ottenuti sono stati analizzati mediante spettroscopia UV e osservati al microscopio ottico ed elettronico.

Per quanto riguarda l'impregnazione dell'amido, si sono tratte le seguenti conclusioni:

- l'impregnazione dipende particolarmente dalla solubilità del farmaco in CO_2;
- per i due farmaci considerati, i migliori risultati si ottengono alle temperature e pressioni maggiori, nella fattispecie a 60°C e 300 bar;

- aumentando il tempo di impregnazione, la concentrazione di farmaco all'interno dell'amido aumenta inizialmente, per poi saturare ad un valore costante;
- i risultati ottenuti con amido di mais sono leggermente migliori rispetto a quelli ottenuti con amido di tapioca. Questo può essere giustificato dal fatto che l'amido di mais, a differenza della tapioca, è poroso e consente un maggior contatto con la CO_2;
- in tutte le prove effettuate, la concentrazione di farmaco ottenuta è bassa, in confronto ai dati normalmente riportati in letteratura per esperimenti di impregnazione;
- i valori risultano bassi per entrambi i farmaci ed entrambi i tipi di amido.
- la causa della scarsa impregnazione è attribuibile alla bassa solubilità dell'anidride carbonica all'interno della matrice dell'amido. Questo provocherebbe un rigonfiamento della matrice insufficiente a consentire una buona impregnazione.

Come risultato significativo, si riporta il miglior valore ottenuto, che è 0.55% in peso, per la caffeina a 60°C e 300 bar, dopo 14 ore di impregnazione.

Riguardo agli esperimenti condotti per l'ottenimento di una dispersione solida, si conclude che:

- le variabili operative sono state ottimizzate, individuando come ottimale la dispersione condotta a 200 bar e 40°C;
- la pressione di 200 bar è necessaria per abbassare la viscosità del composto e consentire un mescolamento adeguato;
- il rapporto tra farmaco e substrato massimo lavorabile è di 1:1; frazioni massive superiori di farmaco non consentono di ottenere un prodotto con caratteristiche uniformi;
- la dispersione solida ottenuta alle condizioni ottimali è una polvere scorrevole, composta da particelle di amido rivestite di farmaco.

In sintesi, si può affermare che l'amido non è un materiale adatto per l'impregnazione di farmaci solubilizzati in anidride carbonica supercritica.

Per quando riguarda la dispersione solida, invece, l'amido consente di ottenere una polvere uniforme e scorrevole. Si ritiene inoltre che questo composto consenta un miglior controllo della biodisponibilità del farmaco, aumentandone la velocità

di solubilizzazione. Non è stato possibile effettuare misure al riguardo, ma questo comportamento è stato ipotizzato, in relazione a quanto suggerito in letteratura.

Contents

Introduction

One of the challenges of modern chemical engineering is to deal with the problem of pollution and try to find green alternatives to already existing processes. In the last decades new practicable ideas and technologies have been developed.

Supercritical carbon dioxide -scCO$_2$- has to be numbered among those, since it can be a valid substitute for organic volatile compounds. Several applications of supercritical fluids have been developed and some of them are already used in industrial plants.

This work deals with a pharmaceutical application, that is impregnation of suitable materials with a drug, achieved using scCO$_2$ as a solvent. In particular, impregnation of starch with two model drugs is considered. Drugs studied are ibuprofen sodium salt and caffeine. Operative conditions such as temperature, pressure and impregnation time are taken into account to optimize the batch process.

Possible development of other interesting techniques, involving supercritical carbon dioxide, but different from impregnation, is also taken into account.

The work has been performed during a sixth month stay at the Institute of Chemical Engineering and Environmental Technology, Graz University of Technology, under the supervision of Prof. Thomas Gamse, engaged for long time in research.

This study is in four chapters, as here specified:

- Chapter 1 explains general aspects concerning supercritical carbon dioxide and its use in the most important technologies developed so far. Supercritical impregnation is then discussed in detail, focusing on advantages and constraints. Some important literature dealing with this technology is also presented.

- Chapter 2 offers a view on materials used for the study, that is starch, ibuprofen sodium salt and caffeine. Then, plants used and measurement methods are described.

- Chapter 3 better explains the aim of these experiments, thoroughly describes the experimental procedures followed and finally discusses some preliminary

observations done on starch and drugs, when treated with supercritical carbon dioxide. The idea of a solid dispersion technique is also here developed.

- Chapter 4 shows results obtained, which are then commented.

I would like to deeply thank the Institute of Chemical Engineering and Environmental Technology, University of Graz, and in particular Prof. Thomas Gamse, Prof. Peter Letonja and PhD student Nicola de Zordi, for helping me with this work.

Chapter 1

Supercritical carbon dioxide: theory and applications

1.1 Important theory

1.1.1 What is supercritical carbon dioxide?

A pure component is considered to be in a supercritical state, if its temperature and its pressure are higher than the critical values, but not too much. (Figure 1.1).

Moving from undercritical to supercritical conditions the change of component properties is continuous, but the magnitude of these variations can be really high. As a consequence, the effects on solutes and reactants moving near the critical point may be totally different. Similar effects are sometimes achieved for $P > P_c$ and $T < T_c$, at near critical temperatures in the liquid state of a substance.

In case of binary mixture (Figure 1.2 a), the critical pressure is always the upper limit of the two-phase area at constant temperature and a mixture is supercritical for all pressures higher than the critical pressure. On the other hand (Figure 1.2 b), critical temperature does not limit the two-phase region at constant pressure. For these reasons it would be more useful to talk about 'one-phase region' and 'two-phase regions' instead of 'supercritical mixtures' [Brunner, 1994].

For pure carbon dioxide, the critical data are T_c = 304.15 K = 31 °C and P_c = 7.38 MPa = 73.8 bar . Carbon dioxide is therefore subcritical at normal ambient conditions, but it is easy to bring it up to its critical point. To compare, the critical point of water is at T_c = 647.3 K = 374.15 °C and P_c = 22.12 MPa = 221.2 bar [NIST, 2011].

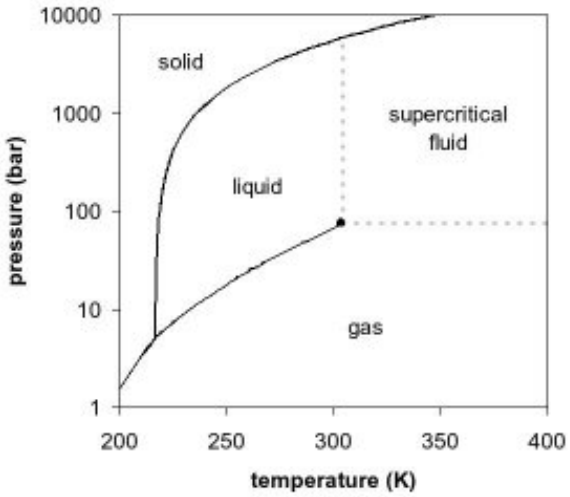

Figure 1.1: *Carbon dioxide pressure-temperature phase diagram.*

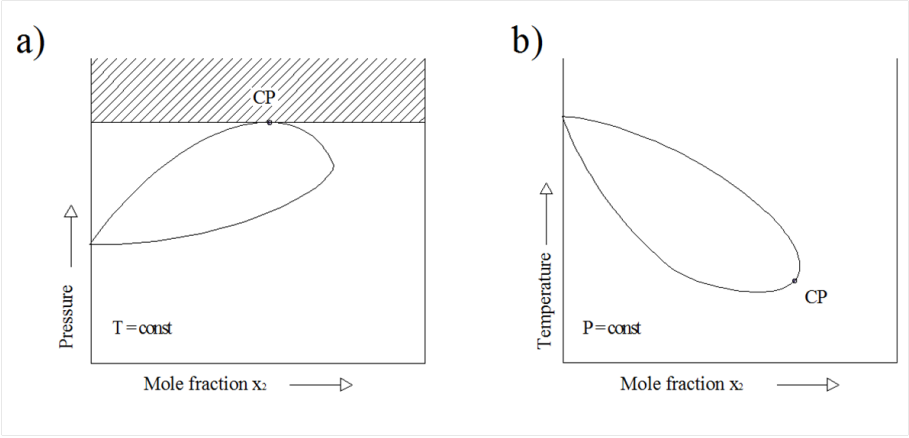

Figure 1.2: *Critical point in binary mixture:* ***(a)*** *diagram at constant temperature;* ***(b)*** *diagram at constant pressure.*

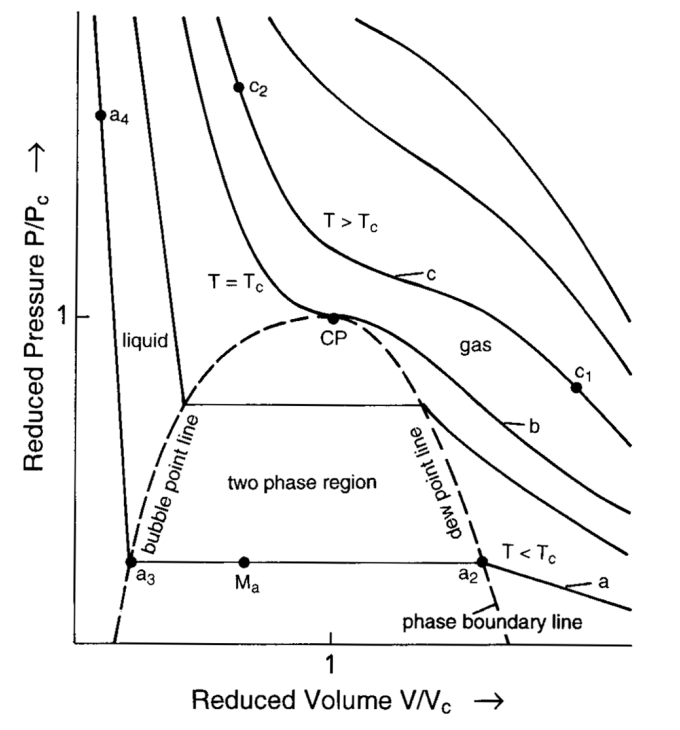

Figure 1.3: *Pressure volume diagram.*

1.1.2 Phase behavior: density

As it can be seen in Figure 1.3, in a gas phase the volume is strictly interdependent with temperature and pressure. Near the critical point this dependence varies strongly with conditions of state.

For temperatures lower than the critical temperature, the volume of a gas decreases rapidly increasing the pressure, until the two-phase-region is reached and liquid is formed. When the component is completely liquid, the volume change is negligible even if pressure is sharply increased.

For temperatures higher than the critical temperature, a gas can be compressed, but no phase change will occur.

At the critical temperature the two-phase-line is reduced to just a point and the isotherm has a horizontal inflection. Here the two branches of the phase envelope meet and the two phases (liquid and gas) become identical.

In this region the compressibility is high and small variations in pressure cause relevant variations in density and other related properties. These variations are exploited in supercritical fluids (SCFs) applications [Brunner, 1994].

Density can be either found in experimental data or calculated by means of an equation of state.

The behavior of a pure component can be summarized in a PVT (pressure-volume-temperature) diagram or can be usefully described by an equation of state (EOS).

The simplest relation is the *ideal gas EOS*, $PV = RT$ which is valid only at low pressures and can be used only to have a rough idea of the order of magnitude, when dealing with high pressure systems.

When accurate calculations are needed, it is necessary to use a more sophisticated equation of state. At the moment several EOS are available, but one of the most used is the Peng Robinson equation:

$$P = \frac{RT}{v-b} - \frac{a(T)}{v(v+b)+b(v-b)}. \tag{1.1}$$

For a pure fluid, constant b is given by:

$$b = 0.07780\frac{RT_c}{P_c} \tag{1.2}$$

while *a(T)* is function of temperature and is given by:

$$a(T) = a(T_c)\alpha(T) \tag{1.3}$$

$$a(T_c) = 0.45724\frac{(RT_c)^2}{P_c} \tag{1.4}$$

$$\alpha(T) = \left[1 + \beta\left(1 - \sqrt{\frac{T}{T_c}}\right)\right]^2 \tag{1.5}$$

$$\beta = 0.37464 + 1.54226\omega - 0.26992\omega^2, for: 0 \leq \omega \leq 0.5. \tag{1.6}$$

In the equations above:

P is the pressure;

T is the temperature;

v is the specific volume;

R is the universal gas constant;

P_c is the critical pressure of the component;

T_c is the critical temperature of the component;

ω is the acentric factor.

This is a generalized equation, with parameters linked to the critical point. For this reason it tends to be less accurate when approaching liquid state. When this equation has to be used to calculate a phase equilibrium, the values of a and b are referred to the mixture by means of mixing rules [Prausnitz et al., 1999].

1.1.3 Transport properties

Important features of supercritical CO_2 are the enhanced transport properties. Viscosity, thermal conductivity and diffusivity are strongly affected. Mass transfer is particularly important when considering applications such as impregnation or extraction. These properties are not calculated using thermodynamics, since they are not equilibrium properties.

Viscosity

Viscosity μ relates the shear stress τ applied to a fluid and the velocity gradient dv/dy obtained in the perpendicular direction:

$$\tau = \mu \frac{dv}{dy}. \tag{1.7}$$

Viscosity of a gas at $T >> T_c$ and $P << P_c$ is almost not affected by pressure. At constant pressure, viscosity will first decrease with temperature and then, after a minimum, it will increase. Increasing the pressure, the minimum will be at higher temperature.

In the generalized viscosity diagram, it can be seen that for $T_r \approx 1$ and $P_r \approx 1$ the curve has a minimum (Figure 1.4).

Viscosity for gases at high pressure is usually determined from viscosity at low pressure, at the same temperature: a correction is applied, in the same way as a departure function. For liquids, instead, it is necessary to rely on experimental data.

In a gas-liquid system, it is well known that the viscosity of the liquid will decrease with pressure, due to the higher amount of gas dissolved.

On the other hand, the viscosity of the gaseous phase will depend on the viscosity of the supercritical solvent and on the concentration of the low volatile compound. If the concentration is low, the viscosity can be approximated with that of the pure component. But if it is high, the deviation cannot be neglected.

In the case of impregnation of a solid, the solubility is always quite low, therefore the viscosity is approximated to that of the supercritical fluid [Brunner, 1994].

For example, viscosity of carbon dioxide is $7.68 \cdot 10^{-5}$ Pa· s at 333 K and 30 MPa and $1.66 \cdot 10^{-5}$ Pa· s at 333 K and 0.1 MPa [NIST, 2011].

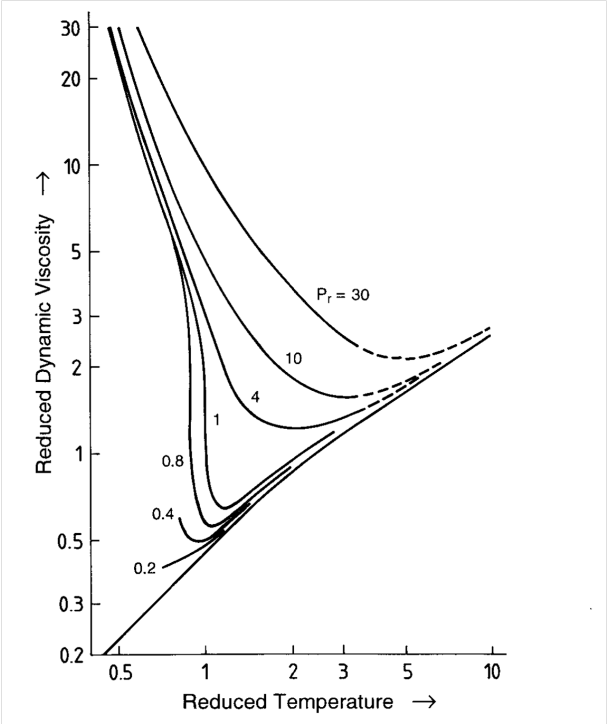

Figure 1.4: *Generalized viscosity behavior.*

Diffusion coefficient

Diffusion coefficient D is the proportionality constant between the molar flux J due to diffusion and the concentration gradient $\nabla\phi$, where ϕ is the molar concentration. Using Fick's first law it can be written as:

$$J = -D\nabla\phi. \tag{1.8}$$

Binary diffusion coefficients depend on the supercritical fluid and on the low volatile component. Increasing the pressure, the diffusivity decreases.

Diffusivity has been measured for several binary systems. Besides, some relations exist to estimate this value.

The system caffeine + CO_2 has already been studied and the binary diffusion coefficient can be found in literature. In the present work these data are not used, since no computer simulation is done. If the reader is interested, the work of Feist and Schneider is suggested [Feist and Schneider, 1982].

Surface tension

The surface tension γ is the ratio between the work to enlarge a surface A of an amount dA and the amount dA itself:

$$\gamma = \frac{dW}{dA}. \tag{1.9}$$

If the surface tension is high, two phases will tend to have the smallest possible surface between them. For example, a liquid will tend to form round droplets instead of a thin film when coming in contact with a solid or gas phase.

This aspect influences the wettability of a surface. In particular, this may be important when considering a process where a solid phase has to be wet by a fluid phase.

When a system with a single phase is reached, as in several $scCO_2$ applications, the problem of surface tension is avoided because no interface exists. In the case of impregnation, however, a surface still exists between the solid being impregnated and the supercritical solution.

Table 1.1 gives a general idea of the orders of magnitude of physical properties, when dealing with supercritical fluids.

Analyzing the properties of a supercritical fluid, it is clear that it behaves like something between a gas and a liquid. Its transport properties are similar to that of a gas, but its solvent power is much better. In fact, this characteristic is related to the density of a fluid. [McHardy and Sawan., 1998].

	Liquid	**Gas**	**Supercritical fluid**
Density $[kg/m^3]$	10^3	1	10^2
Viscosity $[mPa \cdot s]$	1	10^{-2}	10^{-2}
Diffusivity $[m^2/s]$	10^{-10}	10^{-5}	10^{-7}

Table 1.1: *Comparing physical properties: liquid, gas and supercritical fluid.*

1.1.4 Phase equilibrium

The impregnation process implies that a compound has a certain solubility in the supercritical fluid. This process is in fact limited by thermodynamic equilibrium between the different phases.

Equilibrium phase behavior in supercritical CO_2 has been studied for several binary mixtures and also for some ternary and quaternary mixtures. Depending on the compatibility between the compounds, miscibility gaps of different shape and extent are determined. Phase diagrams permit to fully understand the particular

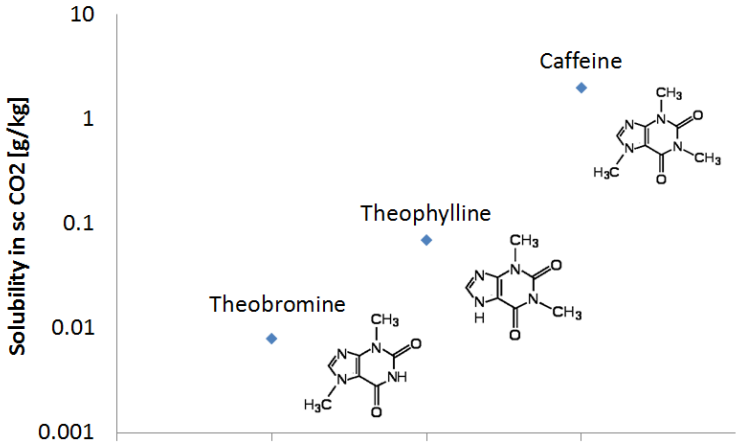

Figure 1.5: *Solubility of xanthines in supercritical CO_2 at 333 K and constant density ($800Kg/m^3$).*

behavior of a specific mixture (see [Prausnitz et al., 1999] and [Schneider et al., 1980]).

Unfortunately, such diagrams require too much time and work to be produced. For this reason they are not normally used, unless it is necessary. More economically, solubility data are usually measured and can be found in literature for several different molecules in $scCO_2$.

Solubility in supercritical carbon dioxide

Solubility of a low volatile solid compound in supercritical CO_2 depends on its molecular characteristics.

With higher molecular weight and higher polarity, the solubility is expected to be lower. If a polar group is present, its influence is important if the molecule is small, but it will be low if the molecule has a big apolar chain.

Solubility of different xanthines in $scCO_2$ is a meaningful example of how small differences in the chemical structure can change significantly the solubility (Figure 1.5).

For an impregnation process, solvent power is important. In particular, solubility of the component in the supercritical gas and solubility of the supercritical gas in the polymer are both to be considered [Prausnitz et al., 1999].

1.1.5 Mass transfer

The influence of pressure on mass transfer is important [Brunner, 1994]. The drug has to be dissolved in the gas and then absorbed in the polymer. In a batch op-

eration without stirring, there will be a forced convection while pressurizing and depressurizing. But during the static phase, only natural convection may occur. This is one of the drawbacks of working in static. Mass transfer is controlled by diffusion and not by convection. Therefore it is necessary to wait enough time, in order to reach equilibrium.

During static impregnation, natural convection occurs, since viscosity is low. However, this factor will be important only if there is a temperature gradient along the reactor. This is not the case of the experimental plant used, since the temperature is kept constant in the whole volume.

1.2 Applications

1.2.1 The choice of carbon dioxide

Several fluids are possible candidates for use as supercritical fluids. Hydrocarbons, hydro-fluorocarbons and common solvents as water and ammonia have been considered up to now.

The suitable solvent should be found in relation to the considered problem. For example, hydrocarbons C_3 and C_4 are frequently used in polymer treatment, thanks to their solvent power and critical temperatures around 100 - 150°C. Carbon dioxide and ethane are interesting for processing thermolabile chemicals, such as pharmaceuticals and flavors.

As a general rule, the critical temperature of a solvent increases with molecular weight, polarity and in the presence of intramolecular bondings (hydrogen bonding).

For this reason the critical temperature of methane is only 190.6 K, whereas that of n-heptane is 540.3 K and that of methanol is 513.1 K.

Another important example is water. Its critical temperature and pressure are extremely high, T_c = 647.4 K and P_c = 22.12 MPa, due to its strong hydrogen bonds (compare with ammonia: T_c = 405.4 K). These conditions make it technically difficult to use water as a supercritical fluid, because of engineering and safety problems [McHardy and Sawan., 1998].

Carbon dioxide is one of the most studied supercritical fluid and probably the most appealing. Some of the reasons are:

- the critical temperature is near ambient conditions and the critical pressure is acceptable. Consequently, the set up of a suitable equipment with suitable materials is not a problem.

- Supercritical carbon dioxide has a solvent power not much less than that of an hydrocarbon at ambient conditions. Hence it can be considered as a substitute.

- When dealing with solvents, the environmental point of view has to be considered. The problem of volatile organic compounds (VOC) is growing in importance. If possible, new technologies that do not imply the use of organic solvents should be considered. This is one of the strong point in favor of carbon dioxide: it is not a VOC.

- Unlike hydrocarbons, it is not flammable. This permits to simplify the processing procedures.

- Carbon dioxide is not toxic nor hazardous to health. Nevertheless, it is asphyxiating, since it does not contain oxygen.

- Carbon dioxide is naturally present in the atmosphere and is not to be considered a pollutant. Carbon dioxide produced by combustion can be recovered and used before venting it to atmosphere.

- Since it is not hazardous, no effort is needed to remove all traces of it from the final product. In any case, this task is easy to accomplish, due to the high volatility of carbon dioxide at room conditions.

- Comparing to other solvents, carbon dioxide is cheap and plentiful.

Being aware of these characteristics, it is clear why supercritical carbon dioxide is usually considered as a green alternative.

In spite of all these advantages, the basic limit of carbon dioxide is that it is completely apolar. Apolar substances are perfectly soluble in supercritical carbon dioxide, but, as the polarity increases, the solubility drops down.

To bypass this inconvenience, supercritical mixtures of carbon dioxide with other more powerful solvents have been used. However, in this case some advantages of using carbon dioxide are missing and the technology becomes less attractive.

1.2.2 Successful applications

For the past decades, commercial application of supercritical fluid technology remained restricted to few products due to high investment costs and because it was a new and unfamiliar operation.

Constant advances have been reached in process, equipment and product design and realization, for high added-value products. Therefore, industries are becoming more and more interested in supercritical fluid technology, for the potential profitability and the attractiveness of green processing.

Supercritical fluids gained public attention during the 1970s. They were a possible solution to address growing problems such as toxicity of solvents used in food processing, pollution control, increased purification demands for chemicals.

In some cases also a reduced energy consumption was achieved - for example if a difficult distillation was avoided.

The research around SCFs has covered many different fields and some outstanding applications were developed, that are now industrially used. Some of the most important applications of supercritical carbon dioxide will be now briefly presented.

It is easy to understand that $scCO_2$ is a key in extraction processes. Every time the separation of apolar or slightly polar compounds is an issue, carbon dioxide may be a green alternative. Supercritical carbon dioxide has been studied for an extremely high number of different applications.

Apart from the wide spread coffee decaffeination, one of the first industrial applications, described in 1978, is the regeneration of activated carbon filled with organic materials. Comparing to the thermal regeneration process, it requires less energy and the carbon loss is lower [McHung and Krukonis, 1986].

Supercritical fluids have been successfully applied in polymer processing. Carbon dioxide at supercritical conditions dissolves in the polymer. The dissolution is almost always less than 30% by weight and usually less than 10%. Carbon dioxide acts as a plasticizer, thus reducing the glass transition temperature T_g. It reduces the viscosity for a melted polymer and it is also a foaming agent. Well known technologies are ([Tomasko et al., 2003]):

- fractionation of polymers or synthetic oils to control the mass polydispersity, basically exploiting the adaptable dissolving and extracting power of $scCO_2$;
- extraction of unreacted oligomers to purify the polymer, thanks to the low viscosity and the plasticizing effect of $scCO_2$;
- extrusion assisted by supercritical CO_2 [Sauceaua et al., 2011];
- supercritical dyeing of polymers, where the advantage of using carbon dioxide is that there is no solvent residue at the end of the process;
- polymeric foams production, where CO_2 is a substitute of halogen - hydrocarbons or light hydrocarbons.

Supercritical chemical reaction is another interesting area: enhancement of selectivity and reaction rates has been proved. The key, in this case, is that a reaction can be carried out in a homogeneous phase. This is extremely favorable in case of a catalytic reaction, where the mass transfer between different phases is often the limiting rate step.

The separation step is no more a trouble, because traditional solvents are avoided in this way. The presence of $scCO_2$, reducing viscosity, makes mixing easier, thus helping temperature and product distribution control.

Oxidation and hydrogenation reactions have been mostly investigated [Hyde et al., 2001].

Particle technologies and crystal technologies have gained new important achievements thanks to supercritical fluids. The possibility of tuning solubility is exploited, thus permitting the production of fine crystals and micro-particles. This technology has been studied for various active pharmaceutical ingredients (API).

One of the main field for $scCO_2$ use is that of extraction of valuable soluble compounds, from coffee decaffeination, to essential oil extraction. This topic will be discussed in the next section.

1.2.3 Extraction: a well known application

Extraction has been particularly applied to food processing [Mohamed and Mansoori, 2002]. Supercritical extraction may be used as unit operation when a almost pure extract is required with good yield.

The advantage of using supercritical technology is that delicate flavor compounds are better preserved, comparing to other conventional techniques, such as steam distillation. Comparing to solvent extraction, no undesirable or toxic residue is left. If the extraction is diffusion limited, the extraction may be also more rapid than with usual systems [Henry and Yonker, 2006].

Extraction may have two different focuses [Gamse, 2010]:

1. extracting an undesired component from a raw material, maintaining its other characteristics;

2. extracting the main product from a material that may be pretreated.

In the first case, high selectivity is required, in order to extract only the chosen component, without changing flavor, appearance, smell and shape of the raw material.

Supercritical carbon dioxide permits to control solubility, in order to achieve this result. To improve the economic viability, the extracted compound may also be sold. This kind of process is usually referred to large-scale production.

The main application is the extraction of caffeine from green coffee beans, without loosing flavors. This system permits the production of decaffeinated coffee, using CO_2 instead of chlorinated organic solvents. Caffeine can be recovered at high purity and used in pharmaceutical and food industries.

In a similar way, green and black tea are decaffeinated. Other similar processes are defatting of cocoa press cake and removal of pesticides and toxins from cereals [Harvey et al., 2003].

In the second case, the extracted compound should be highly valuable, in order to cover the cost of raw material pretreating. The material has to be ground, in order to increase the extraction rate.

This technology is mainly used to produce hops extracts, that is the resin portions and essential oils used for brewing purposes, instead of natural hops [Vitzthum et al., 1978]. Other important applications are extraction of nicotine from tobacco, extraction of spice oleoresins, valuable flavors and oils for food industry and medical purposes.

Supercritical fluid extraction has proved effective in the separation of essential oils and its derivatives for use in the food, cosmetics, pharmaceutical and other related industries, producing high-quality essential oils with commercially more satisfactory compositions. However, commercial diffusion of supercritical extraction of pharmaceutical and food compounds is difficult, since the different method of extraction may change color, composition and taste.

To be extracted, the compound must be soluble in $scCO_2$ at moderate temperature and pressure. Extraction efficiency is limited by the maximum equilibrium solubility and by the mass transfer. To improve solubility, pressure needs to be increased. Sometimes, also increasing temperature, solubility will increase. This is not always true, because it depends on relative importance between two different contributions:

- increasing of vapor pressure,
- decreasing of supercritical fluid density.

To improve mass transfer, particle size of treated material may be reduced and higher flow rates may be used.

The material to be treated is loaded into a vessel, with an optimized amount of moisture and at a set temperature. Carbon dioxide is then let into the vessel in order to reach the required pressure. After extraction has reached a sufficient level, the compound is separated from the fluid. This can be achieved in different ways [Gamse, 2010]:

- decreasing pressure at constant temperature, thus reducing density and solvent power;
- increasing temperature, only if the solubility is actually reduced;
- with a combination of pressure decrease and temperature increase, which is the most used method and generally the most energy efficient;
- absorption on a suitable liquid or solid material.

1.2.4 Impregnation: a process opposite to extraction

Impregnation is a way to a add a compound to a matrix, by diffusion of the guest molecule from a gas or liquid solution. The molecule may deposit or dissolve in the matrix, depending on the specific interactions formed.

Supercritical carbon dioxide may replace organic solvents used in traditional methods. The strong point is that no following purification is needed. This simple consideration explains why this technique is desirable, when dealing with pharmaceuticals and food additives.

Furthermore, supercritical impregnation is an alternative for polymer treatment, when a component has to be added to the viscous matrix of a polymer.

The most important example is the dyeing of polymer fibers. Carbon dioxide, soluble in the matrix, can swell it and plasticize it, improving and accelerating the diffusion of the added molecules. The less crystalline is the polymer, the better results are achieved with $scCO_2$: it is generally accepted that sorption of gas occurs mainly in the amorphous regions.

Intermolecular interactions between segments and supercritical fluid are much more responsible for miscibility, than hydrostatic pressure. Chain flexibility aids dissolution. Carbonyl or ether groups in the backbone can specifically interact with CO_2, if they are accessible. Specific interactions in polymers containing carbonyl groups have been reported: they seem to act as electron donors towards carbon dioxide. These aspects explain why carbon dioxide is particularly soluble in polymers like polyethylene glycole and polymethyl methacrylate.

An extensive collection of data about solubility of carbon dioxide in polymers can be found in [Tomasko et al., 2003].

The impregnation process includes the following important steps:

1. dissolution of the solute in $scCO_2$;
2. exposition of the polymer to supercritical carbon dioxide for a period of time;
3. contact between polymer and solute-laden CO_2;
4. release of CO_2 in a controlled way, to trap the solute in the matrix.

These steps may be done together or separately, depending on the process technology chosen.

To fully understand this phenomenon, it is useful to be able to describe the ternary system thermodynamics (supercritical fluid, guest molecule, polymer) and the diffusion kinetics in the polymer. If data related to the specific considered system are not available, some general considerations are always possible. For example, swelling and chain mobility of the polymer will aid and accelerate impregnation.

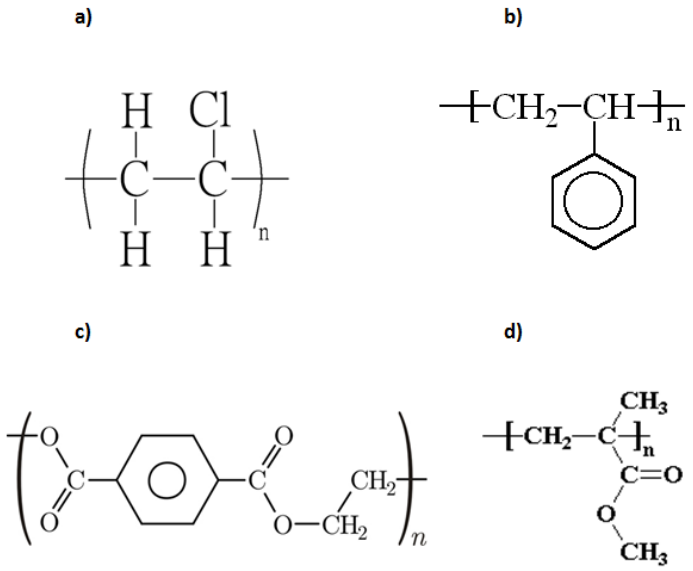

Figure 1.6: *Polymers used in impregnation processes:* ***(a)*** *PVC (atactic);* ***(b)*** *PS (atactic);* ***(c)*** *PET;* ***d()****: PMMA.*

In theory, every kind of molecule could be impregnated in a matrix. Yet, the applicability of this technology has two main issues [Tomasko et al., 2003]:

1. the compatibility and stability between solutes and polymer, which decides if the process is worth developing;

2. the transport rate of solutes, which narrows the effective possibilities.

Considering the first point, the possibility of following changes in the impregnated product, such as separation between solute and matrix, has to be taken into account.

Linked to the second issue, swelling extent has to be considered. Lesser swelling means relatively difficult impregnation. For example, PTFE is more difficult to impregnate, comparing to PVC or PC, because of its limited swelling.

A a general rule, highly crystalline polymers are not suitable for impregnation. The regular matrix has a smaller free volume, chain movements are hindered and therefore transport processes are definitely slower.

For these reasons, studies have been centered on highly amorphous polymers, or polymers with a specific interaction with CO_2. Polyethylene terephthalate (PET), polymethyl methacrylate (PMMA), polycarbonate (PC), polystyrene (PS) and poly vinyl chloride (PVC) have been successfully used (Figure 1.6).

Nevertheless, the idea of using such a technique for the treatment of other cheap and widely used polymers is interesting. This is the case, for example, of polyolefins.

In this frame, the possibility of applying this process to starch is noteworthy. The features of starch will be fully discussed in the second chapter. In any case, the knowledge collected up to now elucidates the difficulties for this kind of approach.

Another possible problem is the final appearance of the polymer after impregnation. Depending on the rate of decompression, residual CO_2 may be trapped inside the polymer, resulting in foaming. Depressurization has to be controlled, in order to achieve the desirable physical characteristic for the polymer [Tomasko et al., 2003].

So far, solutes that have been impregnated are of many different categories: from dyes, to metal complexes, to biological molecules, to pharmaceuticals.

A basic aspect to be considered is the partition of the molecule between the supercritical fluid and the polymer. The molecule will transfer from the fluid to the matrix, only if it is more soluble or compatible with the latter. This aspect makes high impregnation levels possible to reach, even if the molecule is not very soluble in carbon dioxide [Bertucco and Vetter, 2001].

Evidences indicate that:

- when the solubility in the supercritical medium is high, the molecule will be easily delivered inside a suitable matrix;
- when the solubility is low, the affinity of the molecule for the polymer is important.

1.2.5 Solid dispersion

Solid dispersion production is a technique used for pharmaceuticals, in order to enhance drug dissolution and controlled release. It is commonly used for poorly water soluble drugs.

Preparation techniques for a solid dispersion can be either the solvent method or the melting method, depending on the physicochemical properties of drug and carrier.

In the solvent method, solid dispersions are prepared by dissolving accurately weighted amounts of carrier and drug in an organic solvent, which will then be evaporated after a complete dissolution of drug and carrier. Subsequently, the solid mass is ground and passed through a sieve with a suitable mesh size.

For the melting method, the mixture of drug and carrier is completely melted at a certain temperature to obtain the final uniform melt, which is then cooled at room temperature or, more frequently, at a freezing temperature, followed by pulverizing and sieving.

Ethanol is commonly used and highly recommended because of the low risk of harm to the environment and personnel. Minimizing temperature and volume of

organic solvent is necessary to obtain a productive solid dispersion. A combination of the two methods may also be considered.

With the right drug-substrate combination, drug release rate can be controlled, accelerated or even slowed down [Tran et al., 2011].

Precisely, the term *solid dispersion* involves the formation of eutectic mixtures of drugs with water soluble carriers, by the melting of their physical mixtures. Once the solid dispersion is exposed to aqueous media and the carrier is dissolved, drug is released as very fine, colloidal particles. Because of greatly enhanced surface area obtained in this way, dissolution rate and bioavailability of poorly water-soluble drugs is expected to be high.

These technologies, studied since 1961, are now gaining particular attention, because of the availability of new types of vehicles and the development of new manufacturing technologies.

As long as the preparation method is concerned, limitations for commercial application of this systems are [Serajuddin, 1999]:

- high temperatures are needed, in order to melt drugs, which causes chemical stability problems;
- the melt has then to be hardened, in order to grind it;
- in case solvent is used, large volumes of solvents as well as heating may be necessary to complete dissolution of both components;
- complete solvent removal is an issue,

Other problems concerns reproducibility of phsycochemical properties, that can vary at a high degree, when manufacturing conditions are changed, such as cooling rate or drug:carrier ratio. For this reason, preparation method has to be standardized.

In this thesis, a technique similar to a solid dispersion is proposed. The main difference from a classical solid dispersion is that the carrier, that is starch, is not dissolved during preparation. In fact, starch is soluble only in hot water, but water will be avoided as all other possible solvents, except for carbon dioxide.

Since starch cannot be melted, it will not be possible to achieve an intimately dispersed compound.

On the contrary, as it will be explained in Chapter 3, ibuprofen sodium salt melts when exposed to carbon dioxide, at a certain temperature and pressure. This allows to disperse it on the starch surface. This technique will be discussed in Chapters 3 and 4.

Mechanisms that actually enhance dissolution are not yet fully understood. The currently accepted possible mechanisms are [Craig, 2002]:

- increase in the exposed surface area of the drug, due to size reduction;
- reduced aggregation;
- changes in physical properties such as degree of crystallinity and polymorphic form;
- carrier itself, that enhance solubility;

Since carbon dioxide is able to dissolve into polymer and also drugs, it can be considered as a possible solvent to be used for solid dispersion, alone or together with other solvents.

Exploitation of pharmaceutical melting under carbon dioxide pressure is not new. For example, PGSS (Particles from Gas Saturated Solutions) is a method where carbon dioxide is dissolved in the molten compound and the gas saturated liquid phase is further processed, by fast depressurization, in order to produce fine particles [Kerc et al., 1999]. In this thesis we have tried to apply a PGSS - related technique to produce the dispersion of starch loaded with a drug.

Chapter 2

Materials and methods

2.1 Introduction

2.2 Materials

2.2.1 Matrix

The matrix for the impregnation experiments is starch.

Characteristics

Starch is an edible food substance, produced by plants, and considered as a food ingredient and not as a food additive. It is essentially non toxic and non irritant. For these reasons it is widely used as an excipient in pharmaceutical formulations. Allergic reactions are extremely rare and usually limited to a specific variety of starch.

Starch is an odorless and tasteless, fine, white powder. It is formed by small, almost spherical, particles. Size and shape depend on botanical species.

Inside their crystalline structure, starch contains small quantities of lipids (0-0.8%) and proteins (0-0.5%). Contents are relatively stable, but differ dependently on the type of starch. Because of the differences between different starches, they are not automatically interchangeable in a given pharmaceutical application.

Starch is semicrystalline, as detected in wide-angle x-ray diffractometry, although the degree of cristallinity is low, estimated to be in the range of 15% -45%. The degree depends on the specific type. A decrease in cristallinity with increasing amylose content was also observed [Dendy and Dobraszczyk, 2001].

Starch is composed by two polysaccharides based on α (D) glucose: amylose (Figure 2.1), which is linear, and amylopectin (Figure 2.2), which is branched. Both polymers are organized in a semicrystalline structure. In particular, a granule is

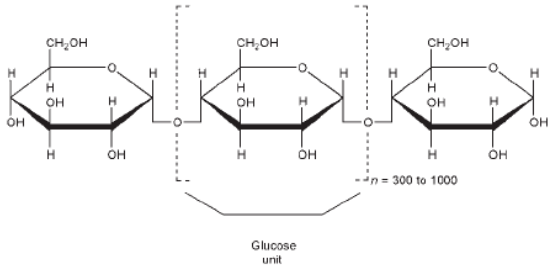

Figure 2.1: *Chemical structure of amylose.*

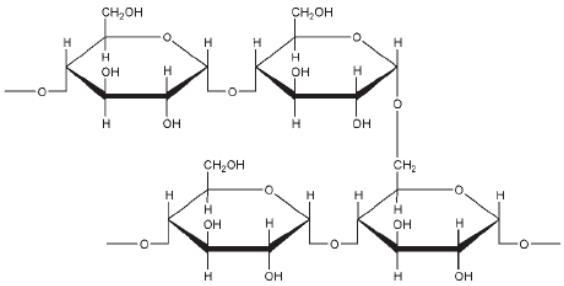

Figure 2.2: *Chemical structure of amylopectin.*

composed by three different regions: amorphous lamellae, crystalline lamellae and amorphous growth ring. In the starch grain, crystal lamellae are composed by amylopectine double helix formation, whereas amylopectin branch points form the amorphous region.

The empirical formula is $(C_6\ H_{10}\ O_5)_n$, where n can vary from 300 to 1000. Molecular weight depends on the origin and the nature of the starch. It can range between 50 to 500 million Da. Usually amylopectin has a higher molecular weight than amylose.

Amylose and amylopectin molecules do not seem to follow a specific pattern. At the lowest level of structure, the granule appears to be made up of alternating semi-crystalline and amorphous growth rings which are between 120 and 400 nm thick [Juszczak et al., 2002].

Due to their different cristallinity, these two molecules react in a different way with water. Amylose, being highly crystalline, tends to form insoluble adducts, whereas amylopectin shows slow jellification, forming highly viscous preparations.

Depending on the amylose content, starch has a different gelatinization temperature and may be used for specific purposes. Gelatinization causes the rupture of starch grains and the irreversible loss of structure of the starch particle.

The term gelatinization does not have a precise meaning. It includes collapse of molecular order, native crystallite melting, irreversible changes in granular swelling, loss of birifrengence and starch solubilization.

Gelatinization occurs over a temperature range that is typical of the starch, but that is also governed by water content and by the presence of other components, like salt and sugar. As a consequence of the loss of crystallinity and thus change in structure, starch granules pick up water and swell to several times their original size. Swelling is limited by the amylose content [Dendy and Dobraszczyk, 2001].

The existence of a glass transition in starch systems, perhaps at a temperature corresponding to the onset of gelatinization, has received increasing attention. The glass transition temperature T_g of starch is very sensitive to the water content and it decreases when the water content increases. I. e. water acts as a plasticizer.

Starch is currently used as a tablet and capsule diluent or disintegrant, a tablet binder or a thickening agent. It is primarily applied in oral solid dosage formulations. As a diluent, it allows to prepare mixtures with colorants or potent drugs. As a binder, a freshly prepared starch paste is used in wet granulation.

It is an important carrier for amorphous drug preparation, such as pellets with immediate or delayed drug release. Pellets may be obtained by melt extrusion and they can improve the bioavailability of poorly soluble drugs.

Pregelatinized starch is used as disgregant in tablets.

Starch is usually cohesive and has poor flow characteristics. Since flow properties depend on moisture content, drying can give a free flowing material. However, all starches are hygroscopic and absorb atmospheric moisture to reach equilibrium, thus the moisture content is typical for each type.

This material is practically insoluble in cold ethanol and in cold water. In water at 37°C it swells instantaneously about 5-10%. Above the gelatinization temperature, it becomes soluble in water. It is also partially soluble in dimethylsulfoxide and dimethylformammide.

Dry starch is practically inert toward chemical reactions and microbes, unless it is pretreated to activate it. Therefore, it is stable under normal storage conditions, that is in a dry and cool place. Starch solutions and pastes are physically and microbiologically unstable [Rowe et al., 2009].

Starch and carbon dioxide

Supercritical carbon dioxide has been recently considered for chemical and physical modification of polysaccharides like cellulose and starch. Using it for starch modification, it is believed that the extent of physical and chemical reactions are affected by the amount of dissolved CO_2 in the starch matrix.

Knowledge about the solubility of CO_2 in native starch is fundamental to explain the observed trends, to develop models and to aid new developments. Despite the importance that these data are gaining, at the moment only few studies are available and the range of conditions tested is limited.

It was found [Muljana et al., 2011]:

- a decrease of solubility with temperature;
- a maximum solubility near the critical region of carbon dioxide followed by a sharp decrease at higher pressures.

This behavior was found in both corn and potato starch. For potato starch, the CO_2 sorption reached a maximum of 29 mg_{CO_2}/g_{sample} at 8.2 MPa and 50°C [Muljana et al., 2011].

The occurrence of a maximum in the CO_2 solubility as a function of pressure has been reported also for other types of polymers. It may be explained by considering two opposing effects [Gupta and Schim, 2007]:

1. At low pressure (20 to 80 bar) carbon dioxide acts as a plasticizer for amorphous growth rings of the starch. This induces a longer average distance between chains and a higher mobility of the segments. As a result, the free volume increases, starch can swell and CO_2 can dissolve more easily.
2. When higher pressure is reached, however, the compressive effect dominates, the free volume in the matrix is reduced and the dissolution of carbon dioxide is hindered.

On the other hand, if the temperature is above the gelatinization temperature, the solubility has a monotonous trend and no maximum is observed. For potato starch a maximum sorption of 31 mg_{CO_2}/g_{sample} was found at 14.2 MPa and 120°C. Moreover, an increase of solubility was found in gelatinized corn starch, comparing to normal starch, irrespective of moisture content.

As comparison, in Table 2.1 some values of solubility of carbon dioxide in polymers are listed [Muljana et al., 2011]

Porosity

Other researches have focused on starch as an adsorbent for volatile compounds and porosity of different types of starch has been studied. For these applications, it has been noticed that microporous starches could be used with better results. Microporous starches are obtained from the native ones, hydrolyzed by α-amylases at temperatures lower than gelatinization temperatures [Sujka and Jamroz, 2007].

Polymer	**Pressure**	**Temperature**	**Solubility**
Name	MPa	°C	mg_{CO_2}/g_{sample}
Polystyrene	0 - 9.5	40	30.9 - 134
(PS)	2 - 19.8	100	12.7 - 121.8
Polyethylene terephthalate	0 - 39.5	80	0 - 38
(PET)	0 - 39.5	120	0 - 32.5
	5 - 30	120	9.1 - 37.2
Polypropylene	5 - 10	40	29.9 - 54.2
(PP)	5 - 10	120	22.6 - 43.8
Polybutylene succinate	1 - 9.9	50	9.8 - 73
(PBS)	2.3 - 19.9	120	21.7 - 176.1
Polycaprolactone (PCL)	8 - 20	50	29.3 - 1637.9
Poly L-lactide (PLLA)	10.73 - 29.9	50	190 - 699.5

Table 2.1: *Solubility of carbon dioxide in some polymers.*

Porosity is one of the characteristic features of starch. Porosity of solid materials is defined as the ratio between pore volume and solid volume. It is a network of one side and two sides opened pores and closed pores, combined with each others or not.

According to IUPAC (International Union of Pure and Applied Chemistry), pores are classified in three categories:

1. macropores, when the diameter is larger than 50 nm,
2. mesopores, when the diameter is between 2 and 50 nm,
3. micropores, when the diameter is smaller than 2 nm.

Starch pores have been studied in different researches and pores of different type and size have been reported. The presence of pores depends on the kind of starch [Fannon et al., 1992]:

- they have been found over the entire surface in corn, sorghum and millet;
- they have been found along the equatorial groove in wheat, rye and barley;
- no pores have been found in tapioca, rice, oat, canna and arrowroot;
- there is disagreement about potato starch granules.

Apart from surface pores, specific channels and cavities were observed in common corn starch, that seem to penetrate into the granule. Central cavities were found in corn, waxy corn and sorghum.

Pores are a natural feature of starch granules, but they may also be enlarged or increased in number during mechanical treatment for starch extraction from plants.

Macropores in corn starch seem to have diameters around 100 nm [Fannon et al., 1993].

The interaction between $scCO_2$ and a starch system has already been studied for gelatinized starch, with water content of 35-39% in weight. In fact, CO_2 injection or mixing in supercritical extrusion processes involving starch is a field of interest at the moment. As already said, water is able to plasticize starch, in order to get a molten matrix.

Even though the present study is not concerned in the use of gelatinized corn starch, it is interesting to analyze the results of the work by Singh et al. [Singh et al., 1996] and that by Chen and Rizvi [Chen and Rizvi, 2006].

Extruded gelatinized starch is a homogeneous mass of amylose and amylopectine molecules bound with water. It is a typical melt for cooking extrusion. It is known that it has a viscoelastic behavior and that the glass transition temperature is affected by the moisture content.

Singh et al. measured diffusivity of carbon dioxide in this molten matrix, in a range from 25 bar to 115 bar. They found that diffusivity decreased with the pressure, in a way typically not found in other polymers. In particular, diffusivity ranged from $7.5 \cdot 10^{-10} m^2/s$ at lower pressure and $0.9 \cdot 10^{-10} m^2/s$ at higher pressure. A conclusive explanation has not been found yet, also because too few data about starch are available to sustain a theory. No data relative to viscosity and melt density as a function of temperature and pressure exist.

What was measured, however, is that gelatinized starch at 343 K and 100 bar was 12-15% compressible. Such a high compressibility might decrease the hole free volume available for diffusion and decrease the observed diffusivity. It is possible, therefore, that also melt viscosity and density increase significantly with pressure.

Contrary to diffusivity, solubility of carbon dioxide do increase with pressure: it was found to be around 1.5% by weight at 115 bar. Considering that this value is referred to a molten system, it seems to be quite low. In [Chen and Rizvi, 2006], solubility was measured from 36 bar to 160 bar. Values up to 4% by weight were found. It was noted that solubility increased with pressure in a linear way up to 160 bar. Solubility of carbon dioxide in water alone, on the contrary, is not linear over this range of pressure, with a decreasing derivative.

This means that the solubility of carbon dioxide cannot be justified just by the presence of water: starch, if a sufficient amount of water is present, does contribute to the dissolution of carbon dioxide in the starch-water mixture.

However, it is pointed out that such solubility results are not to be expected from the sorption of carbon dioxide into anhydrous starch.

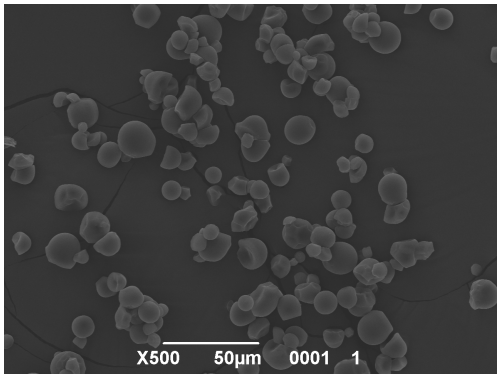

Figure 2.3: *Tapioca starch. SEM photo. Magnification: 500x.*

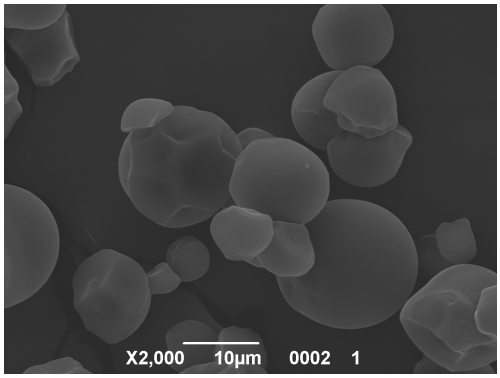

Figure 2.4: *Tapioca starch. SEM photo. Magnification: 2000x.*

In that work, diffusivity was found to range from approximately $2\cdot 10^{-8}$ m^2/s at 35 bar to $2\cdot 10^{-7}$ m^2/s at 150 bar. These results, completely different to those found by Singh [Singh et al., 1996], show that diffusivity increases with pressure. It is justified saying that an increase of the *'penetrant concentration'*, that is an increasing number of plasticizing molecules, seems to have a higher effect than the decrease of free volume due to the pressure.

The differences found in the two works considered may be justified by the use of different types of starch (even though in both cases corn starch has been investigated) or by the different methods used for measurements.

For sure, it can be concluded that the interactions between starch and carbon dioxide are not yet fully understood. Data relative to such systems should be used with caution.

Tapioca starch

Tapioca starch is derived from cassava. Its amylose content is around 17-20 %. Not many informations are specifically available for tapioca starch. Contrary to corn starch, no pores were found on the surface of tapioca starch [Fannon et al., 1992].

Precisely, starch used in this research is chemically modified food starch, derived from tapioca, *Capsul TA*, purchased from Food Innovation National Starch & Chemical GmbH.

Capsul TA is a starch specifically designed for the encapsulation of active ingredients, such as volatile flavor oils, natural oils, vitamins and herbal extracts. It is claimed to have excellent emulsification, film-forming properties and low viscosity, so that it can replace expensive gums and proteins, commonly used for encapsulation applications, such as spray-drying.

The following physical properties are reported:

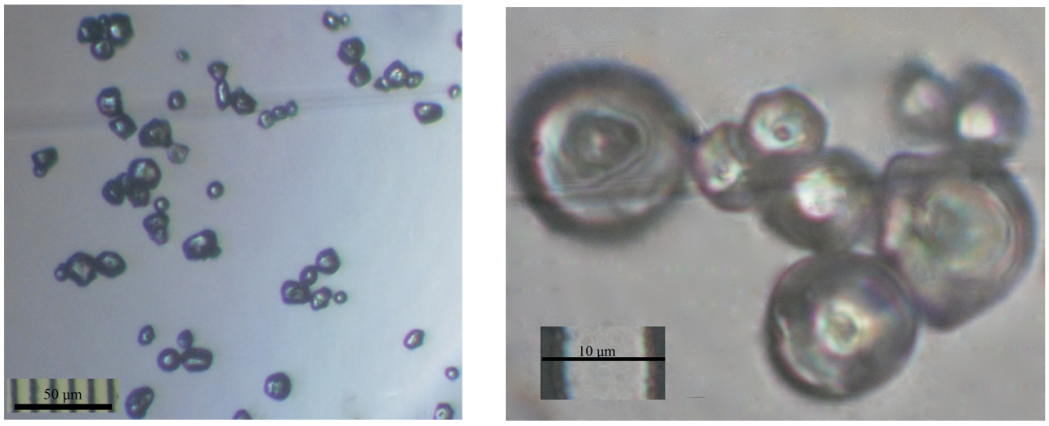

Figure 2.5: *Tapioca starch, seen with optic microscope. Photos taken with paraffine.*

- color: off-white;
- form: powder;
- moisture: approximately 5%;
- pH: approximately 3.

The measured moisture content is 5.85%.

A particular feature of this starch, when compared to other encapsulating agents, such as gum arabic, is its ability to form very stable oil-in-water emulsions. The fine particle size of the emulsion results in reduced losses during spray-drying.

The compatibility of *Capsul TA* with water insoluble substances permits the preparation of spray-dried powders with a higher level of active ingredients.

Spray-dried flavors prepared with tapioca starch are used in a variety of finished food products. These include dry beverage mixes, reconstituted by the addition of water and bakery dry mixes, such as cakes and cookies. It is also used to encapsulate other water insoluble liquid or solid substances such as vitamins and fatty esters. By using this starch, a free-flowing powder that readily disperses in water can be obtained. Typical formulations to use it in spray-dry technology are also given.

As it can be seen, it is not specifically designed for impregnation.

In spray dry technology, a micellar structure with the active compound inside is formed. In case of impregnation, the aim is to have something inside the starch matrix. Nevertheless, it could be interesting to have also a surface impregnation of the starch.

It is not possible to know in advance if this substrate will be effective for impregnation technology.

In Figure 2.3, Figure 2.4 and Figure 2.5 shapes of different granules are shown.

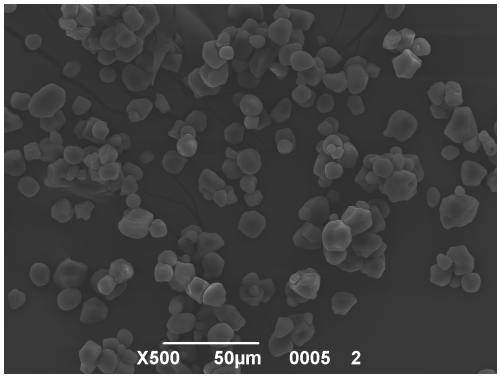

Figure 2.6: *Corn starch. SEM photo. Magnification: 500x.*

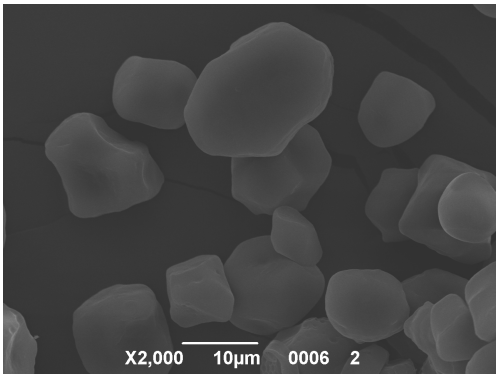

Figure 2.7: *Corn starch. SEM photo. Magnification: 2000x.*

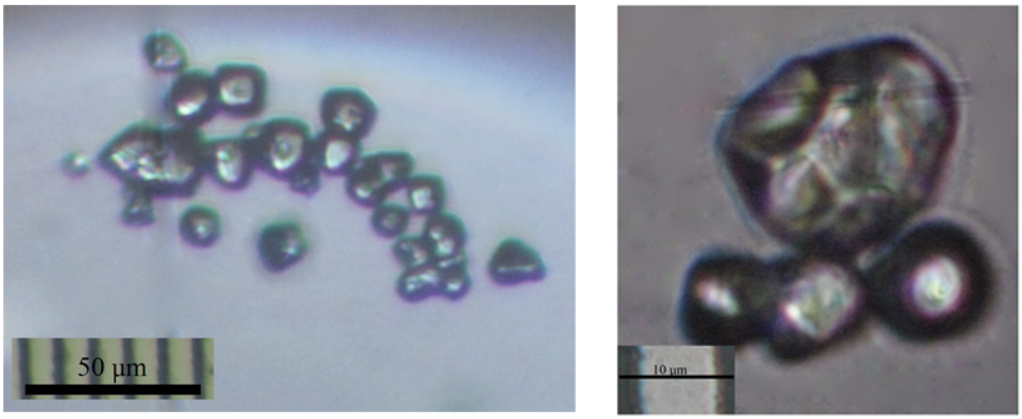

Figure 2.8: *Corn starch, seen with optic microscope. Photos taken with paraffine.*

Corn starch

Corn starch is derived from corn. The amylose content varies from 24% to 28%. Bulk density is around 0.45-0.48g/cm^3, tapped density is 0.69-0.77 g/cm^3 and true density is 1.478 g/cm^3. Gelatinization temperature is 71°C at 20% of weight in water. At 50% relative humidity, moisture content found in literature is 12%. Measured moisture content for the purchased starch is 12.44%. Particle diameter ranges from 2 to 32 μm, with an average value of 13 μm.

In Figure 2.6, Figure 2.7 and Figure 2.8 different shapes of granules are shown.

Corn starch used for this research is common commercial starch. This starch has been considered mainly to prove if the use of a surely porous starch could lead to better impregnation results.

Fannon [Fannon et al., 1993] offered evidence that the pores observed on the

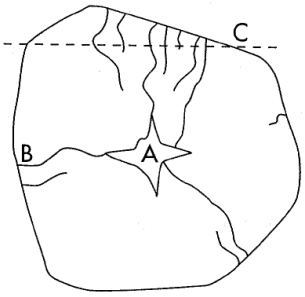

Figure 2.9: *Drawing of a possible architecture of a corn starch granule, as seen in cross-section.*

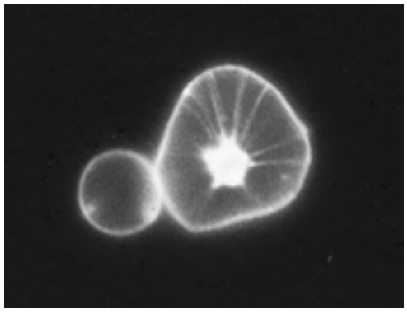

Figure 2.10: *Whole common corn starch granules treated with a methanolic solution of merbromin, mounted in immersion oil, and viewed by fluorescence microscopy.*

surface of corn starch granules are opening to serpentine channels that penetrate in a roughly radial direction. Moreover, starch granules are mostly conical in shape, with no bottle - shaped pores, or pores opened at both ends.

Figure 2.9 shows the drawing of a possible architecture of a corn starch granule, as seen in cross-section:

Central cavities (A) : they have a variety of shapes; star-shaped cavities are common.

Channels (B) : they are seen as lines being in the plane of the section, indicating that the cut was through the center of the granule.

Elsewhere cuts (C) : wen the cut is elsewhere, dots or short lines indicate cross-sections of channels or a channel slanting toward the center of the granule, respectively.

These pores have been detected in previous works [Huber and Bemiller, 1997] using fluorescence microscopy. An image is in Figure 2.10.

As long as pores dimension is considered, different results, due to different research methods, are found in literature. Table 2.2 reports results found for corn starch, using nitrogen adsorption. In corn starch a wide range of pore diameters was found. The prevalent group was around 10.0 - 14.4 nm, the second predominant group was between 10 and 30 nm [Juszczak et al., 2002].

2.2.2 Pharmaceuticals

Ibuprofen sodium salt

Ibuprofen sodium salt is the salt form of ibuprofen. Ibuprofen free acid is the commonly used form for this pharmaceutical. However, due to its low solubility in

BET method: mesopores		
Specific surface area	Volume	Average diameter
m^2/g	cm^3/g	nm
0.687 ± 0.015	1100	6.42

BJH method: pores from 1.7 to about 300nm		
Cumulative surface	Cumulative volume	Average diameter
m^2/g	cm^3/g	nm
0.440	1310	11.93

Table 2.2: *Pores diameter, surface and volume in corn starch. Data found using nitrogen adsorption, with BET and BJH methods [Juszczak et al., 2002]. BET method: Branauer, Emmett and Teller adsorption isotherm. BJH method: Barret, Jyner and Hallenda pore size distribution iterative method.*

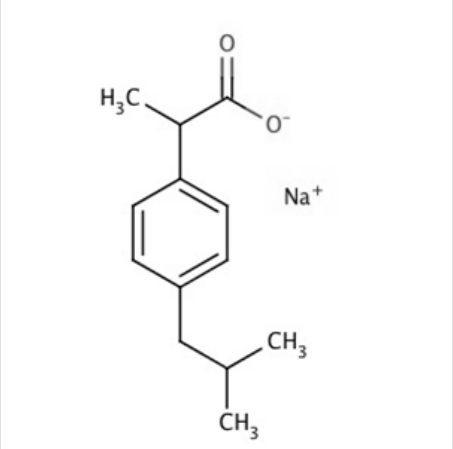

Figure 2.11: *Ibuprofen sodium salt: molecular structure.*

Name	Ibuprofen sodium salt
Formula	$C_{13}H_{17}NaO_2$
CAS Registry N	31121-93-4
Molecular weight	228.26 g/mol
Normal melting point	196-198 °C [Guay, 2010] or 220 °C [Martin et al., 2009]

Table 2.3: *Ibuprofen sodium salt: important information.*

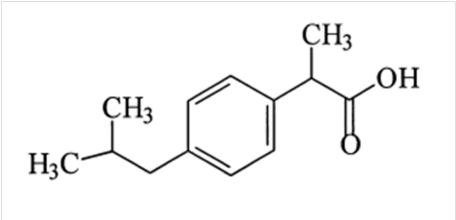

Figure 2.12: *Ibuprofen: molecular structure.*

aqueous media, dissolution and absorption rates are limited. The salt form overcomes this dissolution limit, because it is completely water soluble. This substance presents different polymorphic forms, as reported in [Martin et al., 2009]. The same article states that ibuprofen sodium salt is not enough soluble in carbon dioxide to perform a RESS (Rapid Expansion of a Supercritical Solution).

Figure 2.11 shows the molecular structure of ibuprofen sodium salt. Table 2.3 sums up some important information about the molecule.

Ibuprofen sodium salt has been purchased by Sigma Aldrich. The assay is $\geq$ 98% (GC). The declared solubility in water is 100 mg/mL.

Since really few literature is available referring to ibuprofen sodium salt, some data relative to ibuprofen are also reported, in order to add some useful information.

Ibuprofen

Even if ibuprofen itself is not used in this research, some important information are still reported, in order to understand some aspects of this research and to cover the lack of information about ibuprofen sodium salt. In fact, these two molecules are very similar for some important aspects, such as the pharmacological effect. Nevertheless, the presence of a sodium atom in the molecule changes characteristic such as melting point and solubility in carbon dioxide (Table 2.4).

Ibuprofen (Figure 2.12) is a non-steroidal anti-inflammatory drug. It is widely used as an analgesic, antipyretic, for relief of symptoms of rheumatoid arthritis and

Name	Ibuprofen
CAS Registry N	15687-27-1
Formula	$C_{13}H_{18}O_2$
Molecular weight	206.28 g/mol
Normal melting point	77-78 °C

Table 2.4: *Ibuprofen: important information.*

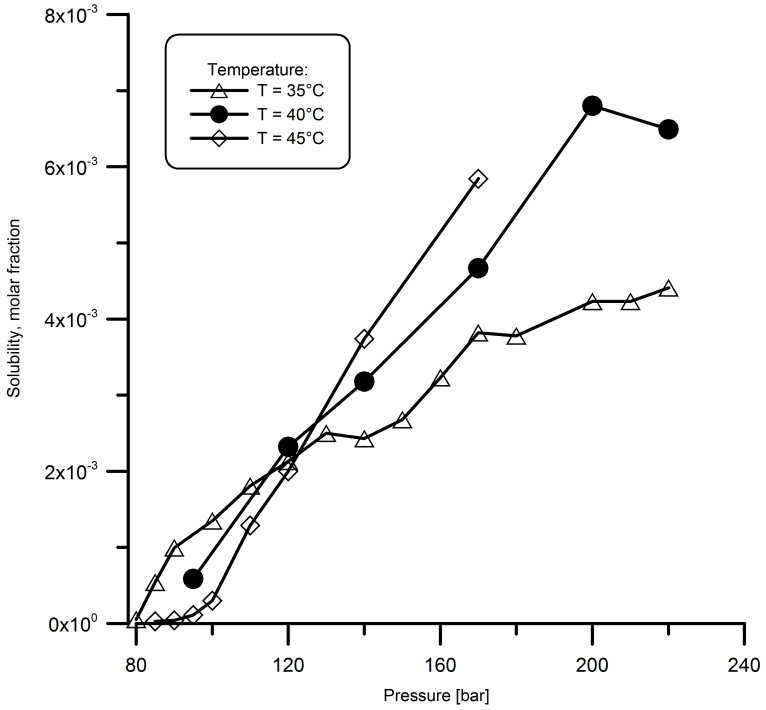

Figure 2.13: *Ibuprofen: solubility in carbon dioxide.*

for other indications.

Ibuprofen is a racemic mixture of (S)-(+) and (R)-(-) enantiomers. Only the (S)-(+) is responsible for the pharmacological effect. (S)-(+) enantiomer may be used alone in drugs (dexibuprofen). However, since the (R)-(-) enantiomer presents no toxicity problems, the racemic mixture is more commonly used.

As many other pharmaceuticals, ibuprofen exhibits poor solubility in water. This affects the dissolution rate in the gastrointestinal tract and the bioavailability of the drug.

Many studies have been done to manipulate solubility of drugs and various possibilities have been developed to enhance it. These include micronization, complexation with macromolecules and modification of physical or chemical properties of the drug, such as decreasing the cristallinity degree.

Ibuprofen is often taken into consideration as a model drug to study these different methods.

In particular, it has already been considered for supercritical impregnation of polymer matrices.

Polycaprolactone (PCL) has already been impregnated with ibuprofen, using an apparatus similar to the one of this work. PCL is a Food and Drug Administration (FDA) approved biodegradable polyester, used in tissue engineering applications. Temperatures and pressures considered are 35 and 40°C, 150 and 200 bar. PCL melts in these conditions and is swollen by carbon dioxide. In this way a considerable amount of ibuprofen can be absorbed in the polymer matrix. Drug loadings achieved vary from 1.83% to 27.16% by weigth. This system resulted effective for controlled drug delivery, with sustained drug release over several days [Yoganathan et al., 2010].

In another work [Charoenchaitrakool et al., 2002], a complex was formed from ibuprofen and methyl-β-cyclodextrin, by using a different piece of equipment. The plant used, however, was different. An extraction cell, followed by a complex formation cell are adopted. Drug loaded CO_2 was flowed through the carrier for a set time and then left in static mode for some hours. Obtained drug loading varied from 8 to 10 % in weigth.

Ibuprofen has also been treated with RESS (Rapid Expansion of Supercritical Solution) technology, in order to control particle size and crystallinity degree [Charoenchaitrakool et al., 2000] [Cristini et al., 2003].

Ibuprofen is particularly soluble in supercritical carbon dioxide. Literature data are available, for both the racemic mixture and the enantiomers. A melting point depression has been reported close to 50°C. Solubility data cover temperatures from 35°C to 45°C, where ibuprofen should be always solid (Figure 2.13) [Charoenchaitrakool et al., 2000].

Caffeine

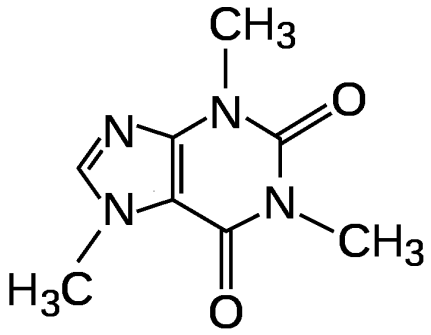

Figure 2.14: *Caffeine: molecular structure.*

Name	Caffeine
Formula	$C_6H_{10}N_4O_2$
CAS Registry N°	58-08-2
Molecular weight	194.19 g/mol
Normal melting point	238 °C

Table 2.5: *Caffeine: important information.*

Caffeine (Figure 2.14), also known as 1,3,7-trimethylxanthine, is a popular central nervous system stimulant. It is a drug commonly used as a mild stimulant, found in coffee, tea and cocoa. It seems that caffeine acts through adenosine receptors and monoamine neurotransmitter. It is used to reduce physical fatigue and restore mental alertness, when unusual weakness or drowsiness occurs. Pure caffeine is at the moment used in stimulant and diet pills. It also has a topical use for the treatment of cellulite. It appears as a white powder.

Caffeine has been purchased by Sigma Aldrich. Its minimum purity is 98%.

Some basic information are listed in Table 2.5.

Solubility in ethanol is approximately 15 mg/mL. Solubility in water at room temperature is around 16 mg/mL, but it notably increases with temperature.

As already discussed, caffeine is relatively soluble in supercritical carbon dioxide. In Table 2.6 data found in literature are reported [Johannsen and Brunner, 1994] [Gupta and Schim, 2007].

Caffeine molecular dimension is 145.9 $\dot{A}^3$. Approximating it to a sphere, the molecular diameter is 6.46 $\dot{A}$, that is 0.65 nm. To compare, corn starch pores have an average diameter of 12 nm and mesopores have an average diameter of 6.5 nm.

Temperature	**Pressure**	**Solubility**
[K]	[bar]	mole fraction $*10^6$
313	199	295
313	219	329
313	239	387
313	259	401
313	279	428
313	299	458
313	319	485
313	349	544
333	199	304
333	219	369
333	239	444
333	259	512
333	279	587
333	299	607
333	319	625
333	349	722
353	199	283
353	219	385
353	239	476
353	259	537
353	279	643
353	299	804
353	319	933
353	349	1130

Table 2.6: *Caffeine: solubility in carbon dioxide.*

2.2.3 Solvent

Carbon dioxide

Carbon dioxide for the experiments has been purchased by Linde AS GmbH. It is stored as a liquid in a vessel at a pressure of 60 bar. A cooling system is available in the case the ambient temperature becomes too high to store liquid carbon dioxide.The maximum capacity of the vessel is 2300 kg.

2.3 Equipment

2.3.1 Static plant

The static plant was used for the impregnation process. It is a *Spe-ed SFE system*, by *Applied Separations* (Figure 2.15), adapted to be used for supercritical impregnation.

The Spe-ed SFE has been specifically engineered for solvent-free extraction from natural products. The system can be utilized in subcritical or supercritical CO_2 applications. The Spe-ed SFE-NP has the largest extractor vessel size capacity of any bench scale instrument, has flexible collection for solids, liquids and volatiles, and has increased tubing size for higher throughput. The Spe-ed SFE-NP is ideal for extracting essential oils, pigments, flavors, fragrances, medicinal compounds and more from a variety of natural products.

The equipment is composed by:

- a vessel able to withstand high pressure;
- a oven where the vessel is located, by which temperature can be controlled;
- a reciprocating pump under the oven, to pump the CO_2 up to the desired pressure;
- a cooling water system before the pump, set at -20°C, in order to be able to pump CO_2 as a liquid;
- sensors for temperature and pressure measurement inside the vessel;
- manual valves at the entrance and at the exit of the vessel;
- an automatic releasing valve to control the pressure release at the end of the impregnation;
- a heater for the releasing valve;
- a computer connected to T and P sensors to record data and to control releasing valve;

Figure 2.15: *Spe-ed SFE system, as purchased from the company.*

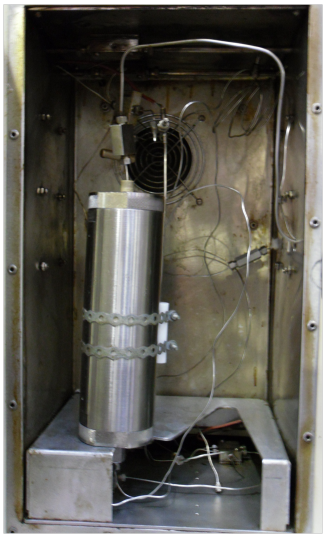

Figure 2.16: *Vessel assembled inside the oven of the Spe-ed SFE system .*

Model	(412)826-3939	**Manufacturer**	Thar design Inc.
Volume	300 mL	**Maximum pressure**	10000 psi (approx. 680 bar)
Internal diameter	2”	**External diameter**	3.5”
Internal length	5.87”	**External length**	11.42”

Table 2.7: *Detail data about the vessel used.*

- piping and valves necessary to connect the storage of CO_2 to the vessel;
- piping and valves necessary to connect pressurized air for pump and automatic valve.

Figure 2.16 is a photo of the vessel used. Some details about it are then listed in Table 2.7. For the rest of the equipment, details are to be found in Table 2.8.

Some other important data are:

- pressure of CO_2 from storage: 60 bar;
- pressure of compressed air for the automatic valve: 3 bar;
- range of pressure attainable in the vessel: 1 bar to 680 bar;
- range of temperature attainable in the vessel: ambient temperature to 240 °C;

Element	Model	Manufacturer	Other information
Automatic valve	11038-I/P	Kammer ventile	ND=0.25” K_{vs}=0.0004L
Electric heater	WRP203/20	Winkler	max 200°C
Valves	1.4401 NW1	Novaswiss	max 1000bar
Cooler	RTE-101	Neslab	range: [-30°C, 150°C]

Table 2.8: *Details about the equipment used.*

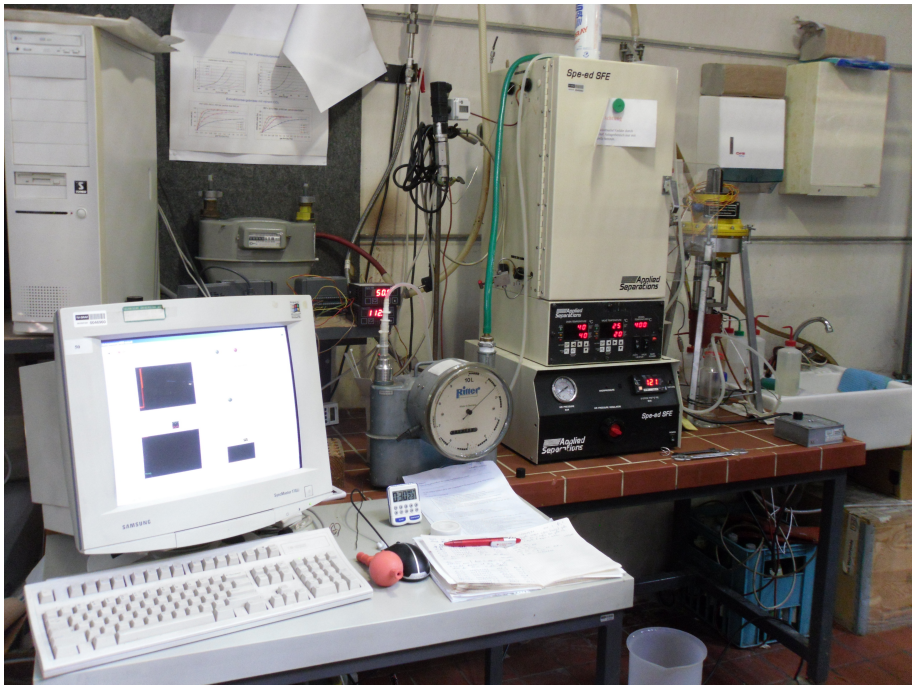

Figure 2.17: *Set up of the plant used for impregnation experiments.*

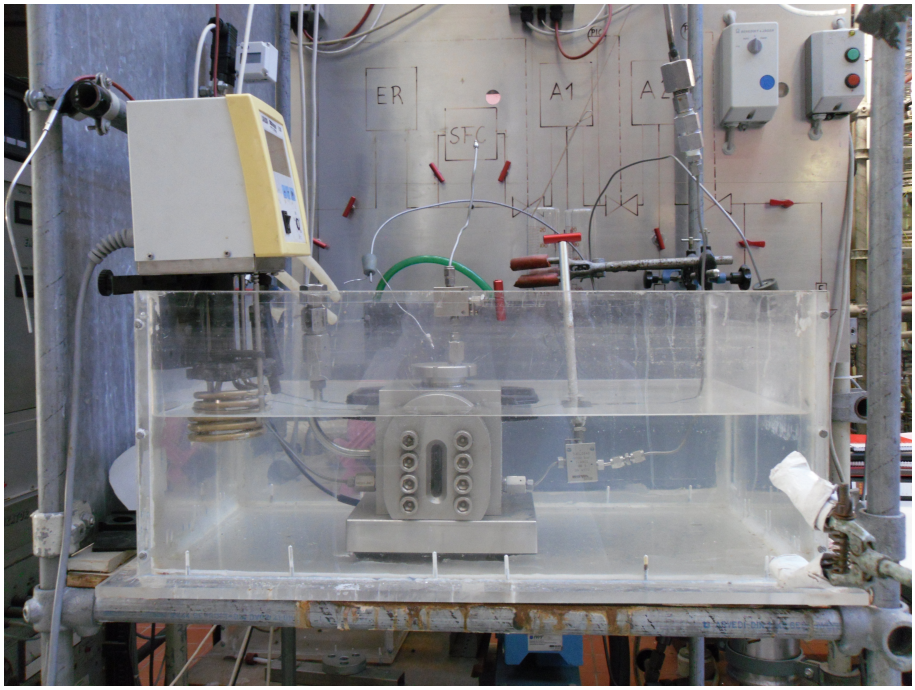

Figure 2.18: *Set up of the view cell. The pump and the T, P indicator are not visible in this photo.*

- pump flow rate up to 400mL/min;

The SFE system can simultaneously support two vessels, but, for this study, only one vessel at a time is used, since only one controlled release valve is available.

Figure 2.17 is a photo of the whole plant.

2.3.2 View cell plant

The view cell was used to observe the effect of carbon dioxide on the various materials and to carry out experiments, when stirring is necessary (Figure 2.18).

The view cell is specifically studied to see what happens inside the vessel, varying the pressure.

The equipment is composed by:

- a 138 cm^3 vessel with pressure-resistant sapphire glass, able to withstand high pressure;
- a thermostatic bath, for temperature control;
- a reciprocating pump, to pump the CO_2 up to the desired pressure;
- a cooling water system before the pump, set to 5°C, in order to be able to pump CO_2 as a liquid;

Element	Model	Manufacturer	Other information
Electric heater	E200	Lauda	
Valves	1.4401 NW1	Novaswiss	max 1000 bar
Cooler	RUK40S	Lauda	range: -100°C; +100°C
Pump	PGLP 220	Siemens LEWA GmbH	
Immersible electric stirrer	50088147	Variomag Maxi	water range: 0°C; +50°C Speed: 130-1000 rpm

Table 2.9: *Detail data about the equipment used.*

- sensors for temperature and pressure measurement inside the vessel;
- manual valves at the inlet and at the outlet of the vessel;
- piping and valves necessary to connect the storage of CO_2 to the vessel;
- electric magnetic stirrer, to stir the content inside the vessel; stirring speed may be controlled manually.

Details about this equipment are listed in Table 2.9. Some other important data are:

- pressure of CO_2 coming from the storage: 60 bar;
- range of pressure attainable in the vessel: 1 bar to 300 bar;
- range of temperature allowed in the bath: ambient temperature to 50°C;

2.4 Analytical instrumentation

For the analysis of the samples, the following instruments were available: a UV-visible spectrophotometer, an optic microscope and a scanning electronic microscope.

2.4.1 UV spectrophotometer

The drugs considered absorb light at UV frequencies. Therefore, UV absorption can be used to quantify the amount of drug present in a sample. The sample cannot be measured as it is, because it is a solid: a procedure retrieved from literature was applied.

Drug concentrations was determined using the Beer-Lambert law:

$$A = \log_{10}(I_0/I) = \epsilon \cdot c \cdot L, \tag{2.1}$$

where:

A is the absorbance, determined with the UV absorber;

I_0 is the intensity of the incident light;

I is the intensity of transmitted intensity;

ϵ is the absorbivity, determined with the calibration curve;

c is the concentration of the solution, that is the unknown;

L is the path length through the sample, that for the used cuvette is 1 cm.

First of all, a calibration curve has to be determined, in order to calculate ϵ. To do this, a mother solution was prepared with a known amount of drug dissolved in 10 mL of ethanol. The solvent was pure ethanol 99.8%, which is a good solvent for both caffeine and ibuprofen sodium salt. Moreover, it is a good solvent to be used with a UV absorber.

From mother solution four dilutions were prepared with 100 μL, 200 μL, 300 μL, 400 μL in 10 mL of ethanol. These four solutions were then analyzed with the UV spectrophotometer, at a suitable wave length, which was chosen doing a first wave scan with one of the solutions, in order to find out a convenient peak.

By plotting the measured absorbance versus concentration, a straight line was found from which the absorbivity ϵ was calculated.

The instrument used is a UV-visible recording spectrophotometer *Shimadzu UV-160 A (P/N 204-04550-01).*

Caffeine

For caffeine, mother solution has been prepared with 5 mg of caffeine in 10 mL of ethanol. The peak was selected at 272 nm (Figure 2.19) and an ϵ value of 43.77 was found ($R^2 = 0.99$) (Figure 2.20).

Ibuprofen sodium salt

For ibuprofen sodium salt, mother solution has been prepared with 2 mg of ibuprofen sodium salt in 10 mL of ethanol. The peak was selected at 223 nm (Figure 2.21) and a ϵ value of 88.75 was found ($R^2 = 0.99$) (Figure 2.22).

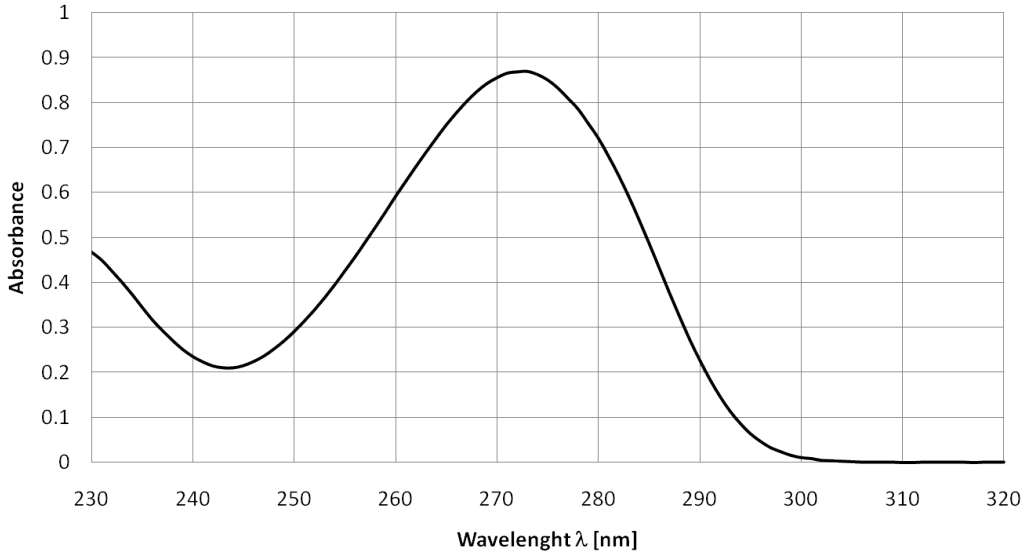

Figure 2.19: *Wavelength scan of caffeine. The peak is at λ=272 nm.*

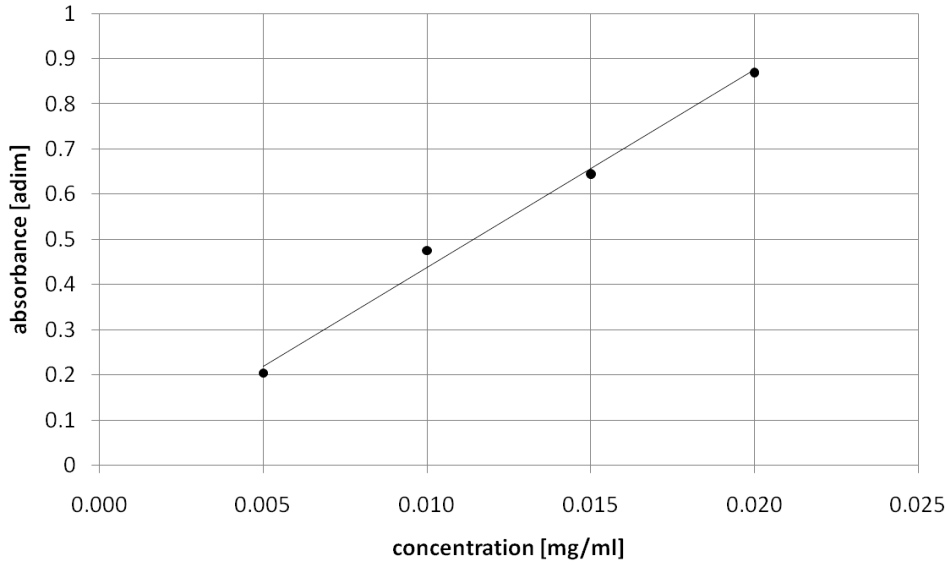

Figure 2.20: *Calibration curve for caffeine. λ=272 nm.*

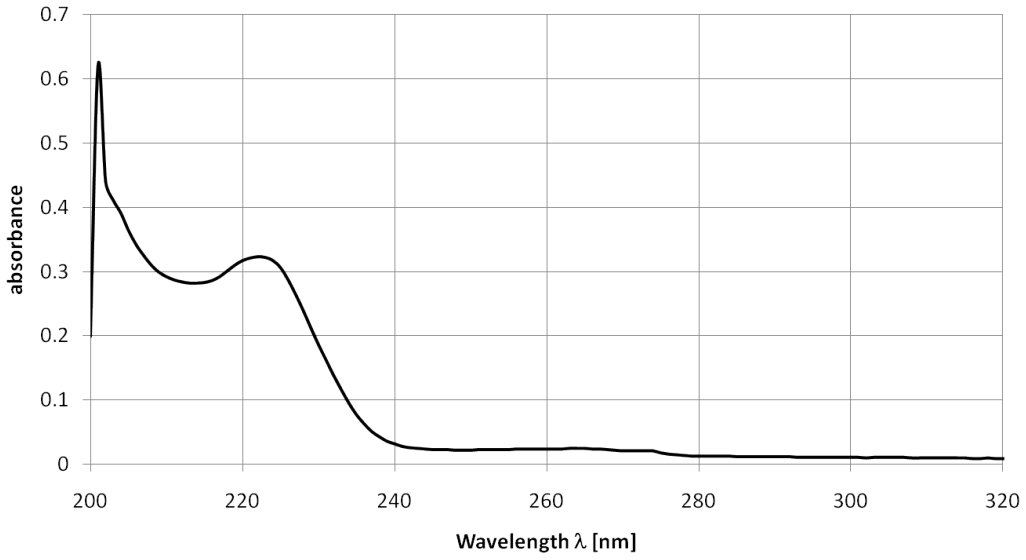

Figure 2.21: *Wavelength scan of ibuprofen sodium salt. λ=223 nm.*

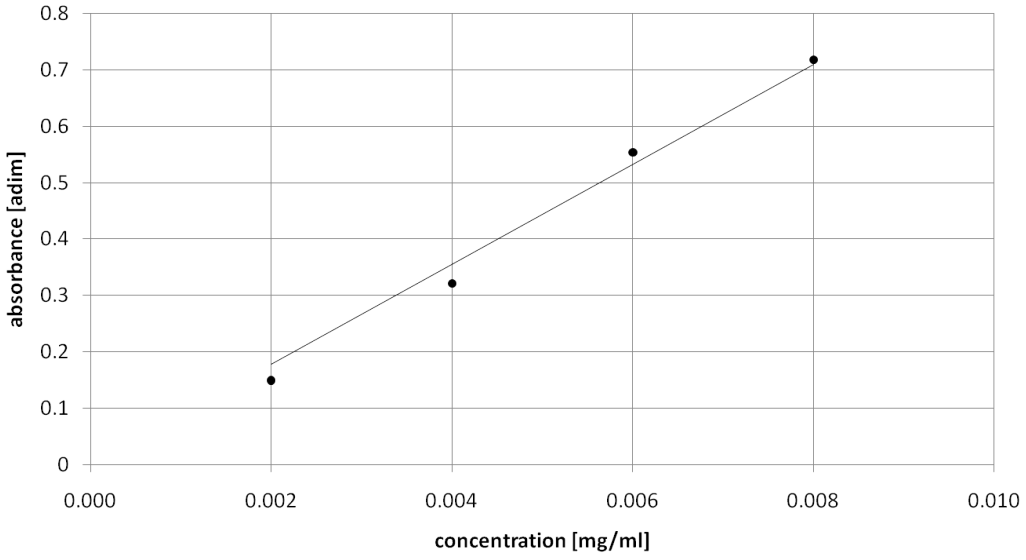

Figure 2.22: *Calibration curve for ibuprofen sodium salt. λ=223 nm.*

2.4.2 Optic microscope

All the samples have been analyzed with a optic microscope, which is useful to see the effects of the treatment with supercritical carbon dioxide: changes in the material characteristics, in the crystalline habitus or in the particle dimensions may be seen.

The microscope used was a *Reichert Austria, Nr 350117*. The enlargements available are: 25x, 1000x, 630x, 100x, 40x. Photos were taken using a *Samsung digital camera, model PL65*, equipped with a 5x zoom.

Particles dimension analysis was performed by the image processing program *ImageJ 1.44*.

2.4.3 Scanning electron microscope

Some of the most important samples were analyzed by a scanning electron microscope SEM. This device allows to see the surface of the particles in detail. The model of the instrument is *JSM Jeol 6490*.

Samples were sputtered with a 10 - 20 nm thick gold layer under a vacuum, by an Edwards *S150A Sputter Coater*.

Chapter 3

Experiments

3.1 Aim of the research

Supercritical carbon dioxide has been used so far in many research projects involving drugs and drug formulations, with the aim of modify their characteristics, in order to control their bioavailability and processability. The most commonly investigated features are:

- particles dimensions,
- crystallinity degree,
- crystalline habitus,
- possibility of complex formation with other materials.

In fact, the solubility rate of a drug may be enhanced decreasing either its crystallinity degree or its particle size.

Many researches have been focused on preparing drugs in their amorphous form. When prepared in their glassy, higher free-energy form, many poor soluble drugs exhibit significantly higher solubility and faster dissolution than their crystalline forms. Unfortunately, amorphous solids are inherently instable and tend to recrystallize. The current general strategy employs appropriate polymeric matrices as substrates, in order to stabilize the glassy drug and to inhibit crystallization. Other techniques to control drug behavior are micronization and complexation with macromolecules. The impact of several polymers on the crystallization tendency of amorphous pharmaceuticals has been well demonstrated by selecting a number of acceptable polymers.

Complexation of drugs with specific carriers is important not only for pharmacokinetics, but also for processing reasons [Rowe et al., 2009]. The preparation of a

solid dispersion can be achieved using technologies such as hot-melt extrusion, spray drying and freeze drying [Ping, 2008].

This work has investigated the possibility of using starch as an excipient for drug impregnation or dispersion, with $scCO_2$ technologies. As explained later, two techniques have been used, depending on the specific characteristics of the used drugs:

1. for starch impregnation with drug, supercritical carbon dioxide was used as an extractor solvent to dissolve and carry the drug into the polymeric matrix;
2. for drug dispersion on the starch, supercritical carbon dioxide was exploited as a component able to melt the drug and allow its processing, in order to form a complex.

Two apparatuses were used, as explained in the next section. In both cases:

- no organic solvents are needed and problems related to further purification are avoided;
- high temperatures are not necessary, preventing the risk of thermal degradation.

Two drugs were taken into consideration:

1. caffeine,
2. ibuprofen sodium salt.

3.2 Experimental procedure

For impregnation experiments, the *Spe-ed SFE system* was used (see Section 2.3.1) with two different set-up, as explained below.

For solid dispersion, the view cell was used, since stirring was essential (see Section 2.3.2).

3.2.1 Impregnation

Different set-ups

In the first configuration (Figure 3.1), the drug is placed in a metallic beaker, located at the bottom of the 300 cm^3 reactor. Above it, a filter-bag with the starch is placed. The bag has the only purpose of keeping the starch entrained.

In the second configuration (Figure 3.2), the drug is placed on a metallic net and the starch is placed on another metallic net 7 cm above it. Vessel volume is 120 cm^3.

With the second configuration, carbon dioxide passes through the drug and then through the starch, enhancing mass transfer. It is believed that, in such a way, solubility equilibrium may be reached faster.

Operational procedure

For all the experimental runs, the following operating procedure was developed and followed:

- Check that the valve between pump and vessel is closed, and that the valve after the vessel is open.
- Turn on electricity.
- Open the pressurized air valve for the pump. The manometer should indicate 6 bar.
- Open the pressurized air valve for the automatic valve. Use the regulator to set the pressure at 3 bar.
- Insert the sample into the vessel and close it, paying attention to the seals.
- Close the oven, set the desired temperature and switch it on.
- Set the data for compression and decompression in the *LabVieW* screen.
- Before starting the experiment, wait until:
  - cooling water for carbon dioxide has reached -20°C;
  - the vessel has reached the set point temperature;
- Press *'run'* in the *LabWiev* screen to record temperature and pressure data.
- Open the valve between pump and vessel: pressure in the vessel will start to increase up to 60bar. Meanwhile, leave the valve after the vessel open. In this way, air inside the vessel will flow out and will be replaced by CO_2.
- After approximately 10 seconds, close the valve after the vessel.
- 60 bar is the pressure of carbon dioxide coming from the storage tank.
- Start pumping CO_2 to reach the desired pressure, opening the pump manual valve.

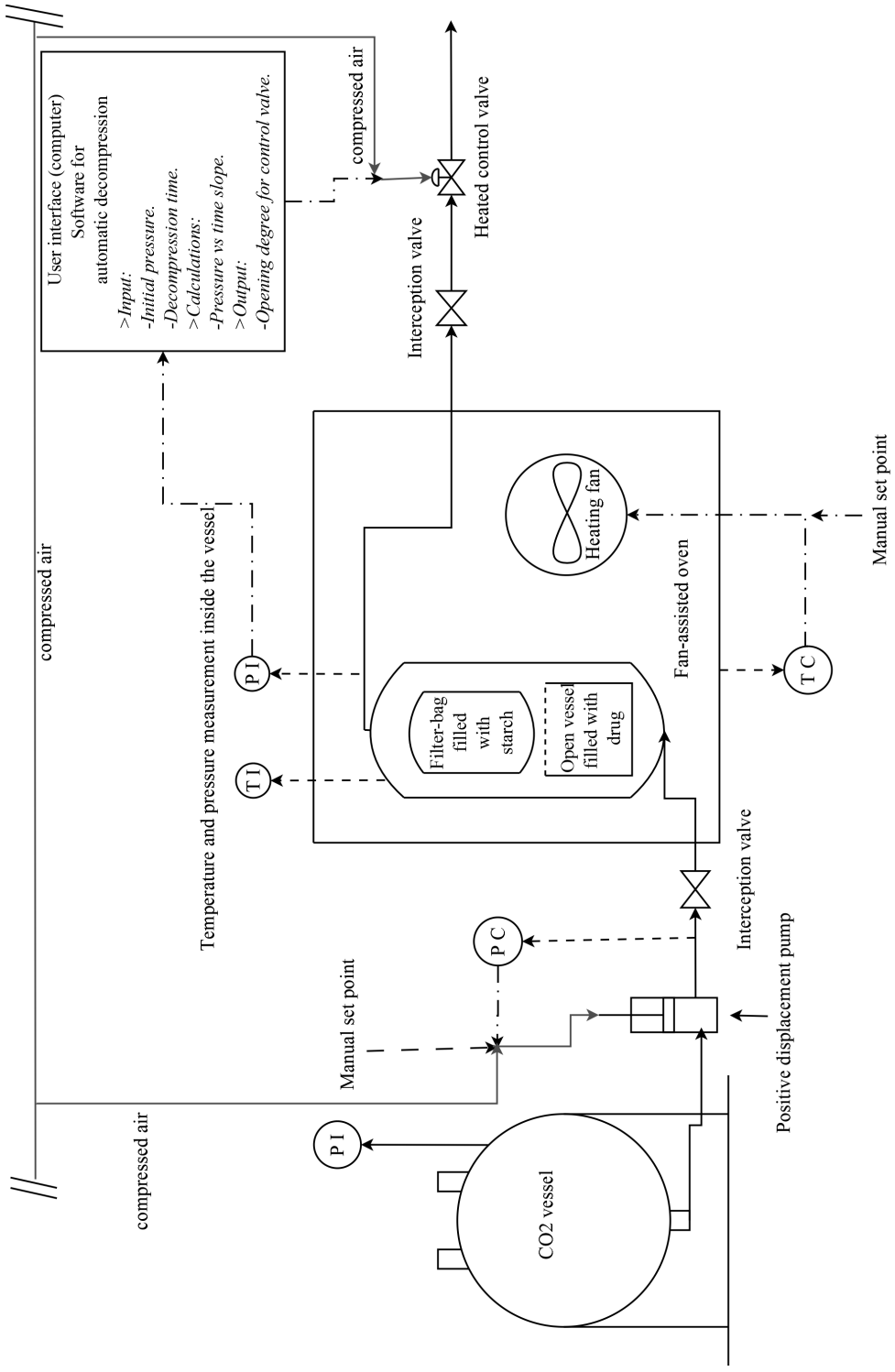

Figure 3.1: *The first of the two plant configurations used. Drug is in a metallic beaker, starch is in a filter bag above it.*

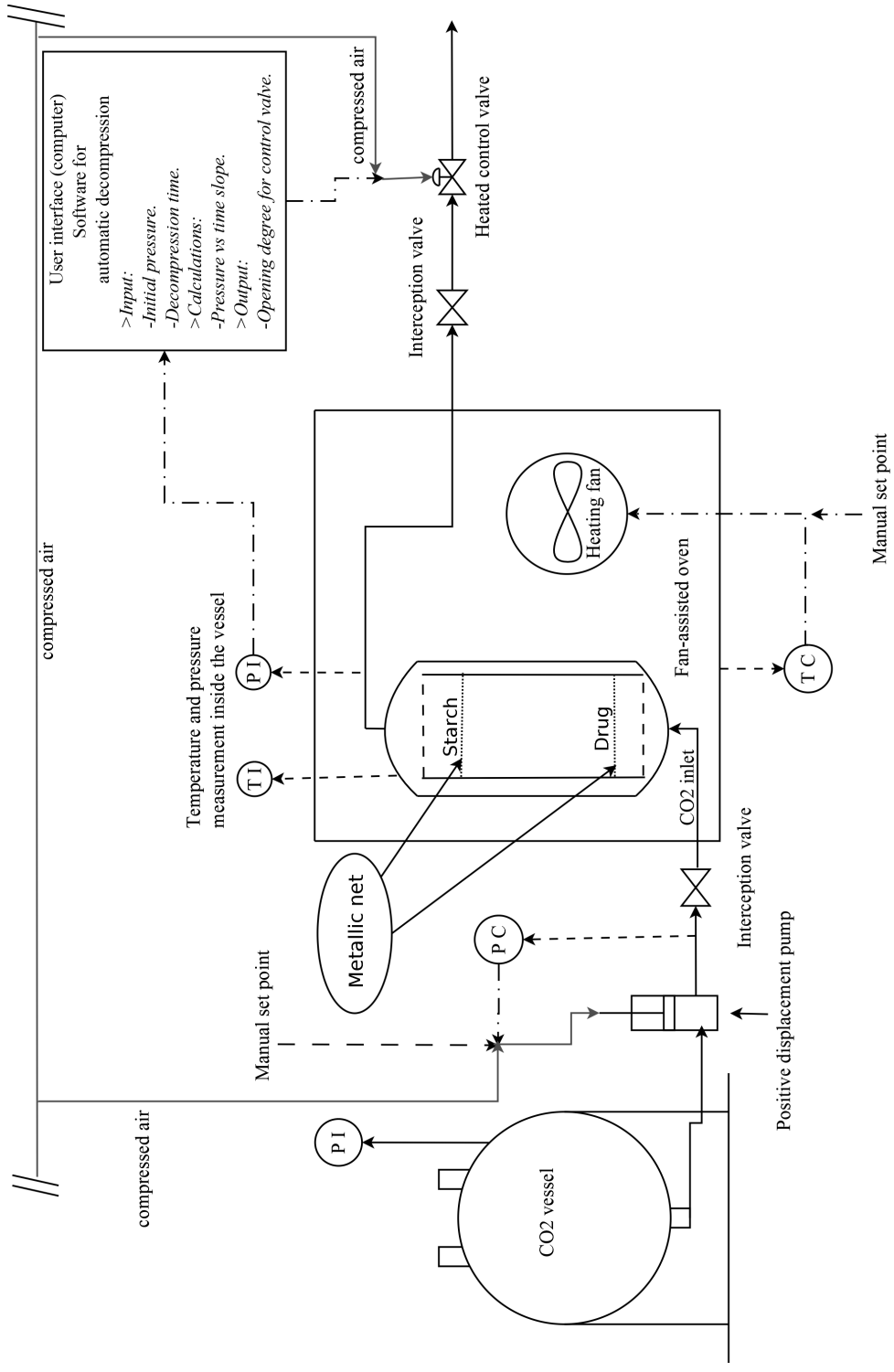

Figure 3.2: *The second of the two plant configurations used. Drug is on a metallic net, starch is on a metallic net above it. Carbon dioxide is let pass through them.*

- When desired T and P are reached, leave the plant in these conditions for the set time.
- After the set time, start the decompression procedure, as explained below.
- Close the valve between pump and vessel.
- Press *'Automatische drucksteuerung starten'* to start controlled decompression.
- Switch on the heater for the automatic valve (70°C), otherwise the valve freezes.
- Open the valve after the vessel. The automatic release valve should now be working.
- Wait until atmospheric pressure within the vessel is reached.
- Stop oven and valve heater.
- Press *'stop'* in the *LabView* screen and save data record.
- When temperature is acceptable, open the vessel and take out the sample.
- Let some CO_2 flow out to be sure that piping between pump and vessel is clean.
- Close interception valves for CO_2 and for pressurized air.
- Allow CO_2 to escape from the pump until 60bar are reached.
- For security reasons, leave the valve between pump and vessel closed and the valve after the vessel open.
- Turn off electricity.

In the procedure above, it is stated that a heating system is necessary for the automatic releasing valve. When the pressure of a gas is lowered by throttling, temperature will change. In most cases, it will decrease and this happens also with carbon dioxide at conditions of interest, so that the *Joule Thomson effect* has always to be considered while working with supercritical carbon dioxide. For this reason the valve must be heated, otherwise it will cool down and freeze. This problem is bigger with higher pressure, whereas, when decompressing from 60bar to ambient pressure, the related cooling makes no problem.

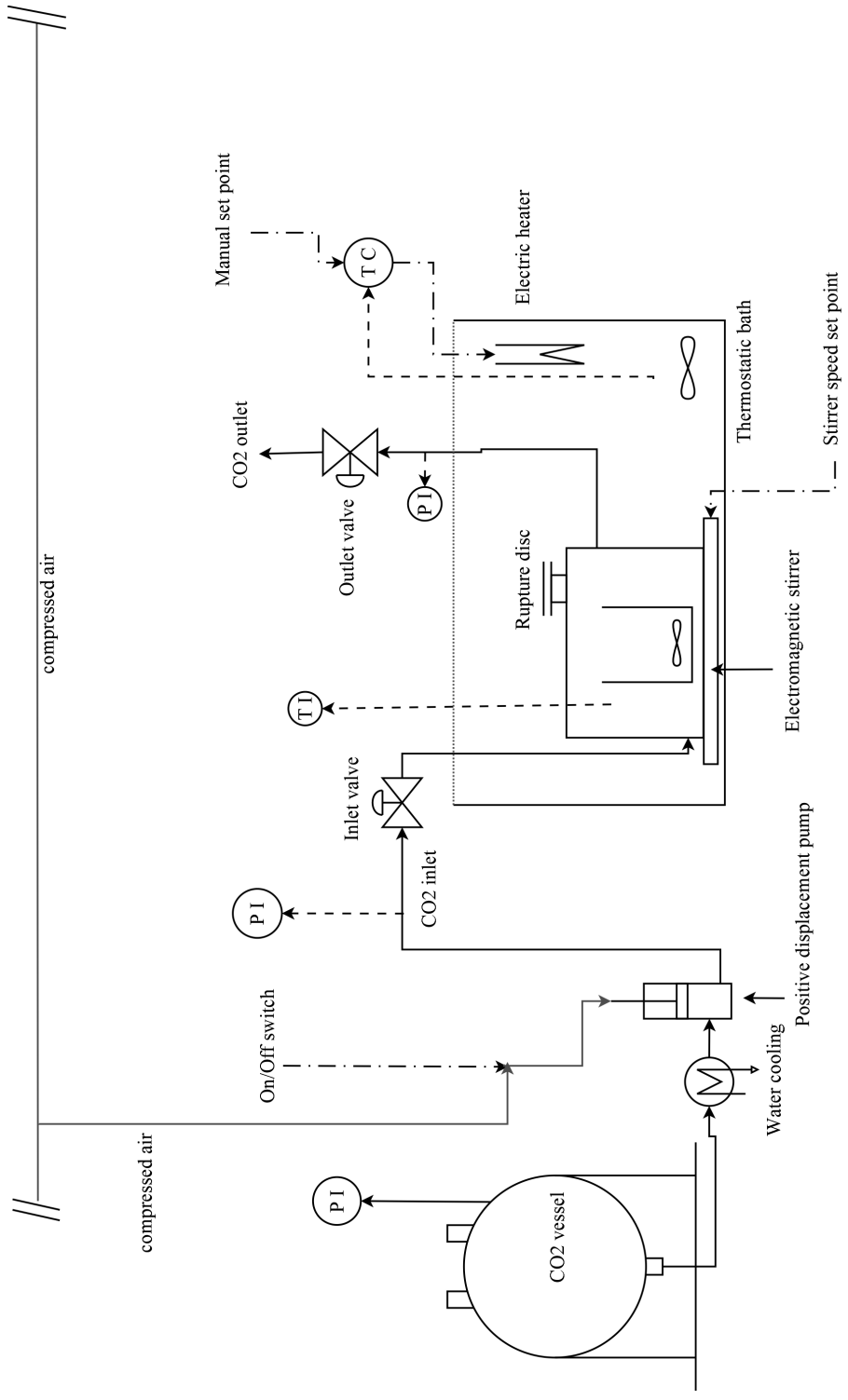

Figure 3.3: *Plant scheme for the view cell.*

3.2.2 Solid dispersion

Experimental set-up

For the view cell, the vessel is placed inside a thermostatic bath to have the possibility to see what happens inside the vessel during the run. A digital camera can also be placed in front of the vessel, to record a video or take photos. A light is placed behind the vessel, to light up the inside. This plant, a scheme of witch is reported in Figure 3.3, can be used for batch experiments and the sample may be stirred or not, depending on demand.

Operational procedure

The following operational procedure has been developed and followed:

- Check that the valve between pump and vessel is closed and the valve after the vessel is open.
- Open the valve to let carbon dioxide reach the pump.
- Start the cooling system.
- Check valves around the pump: the vent valve should be closed and the manometer connected to the pump should indicate 60 bar.
- Wait until the system is cooled down, before starting the pump.
- Turn on electricity.
- Put the sample in a glass beaker, together with a magnet.
- Put the beaker inside the vessel and close it.
- Open the inlet valve, to let some carbon dioxide flow through the vessel.
- Close inlet and outlet valve, leaving inside the vessel a pressure a little higher than ambient pressure. This is useful to detect sealing problems, before water can penetrate inside the vessel.
- Pour water in the thermostatic bath, until the vessel is completely covered.
- Start heating system at the set-point temperature. The heater has a minimum water level control: it is not possible to start it, if the amount of water is not sufficient.
- Wait until the set-point temperature is reached.

- Open the CO_2 inlet valve: pressure will increase to 60 bar, which is the pressure of carbon dioxide coming from the storage vessel.
- Press the *on* button to start the pump.
- When the desired pressure is reached, close the inlet valve and then stop the pump, by pressing the *off* button.
- If stirring is needed, check if the stirrer is working.
- Leave the plant in these conditions for the set time.
- After the set time, start to decompress by opening the valve after the vessel. Note that an automatic valve is not available, so that decompression process has to be controlled manually.
- Wait until atmospheric pressure is reached.
- Drain the thermostatic bath.
- Open the vessel, paying attention to avoid water spills in the sample.
- Turn off electricity.
- Clean the vessel.
- Let some CO_2 flow out to be sure that piping between pump and vessel is clean.
- Close interception valves for CO_2.
- Allow CO_2 to vent from the pump until ambient pressure is reached.
- Stop cooling system.
- For safety reasons, leave the valve between pump and vessel closed and the valve after the vessel open.

3.3 Preliminary observations

3.3.1 Drugs behavior with supercritical carbon dioxide

Caffeine is known to be soluble in carbon dioxide and relevant data have already been reported in Chapter 2, Table 2.6.

For ibuprofen sodium salt, however, no data are available and really few literature is published. This molecule is somewhat similar to ibuprofen, for which information has been reported in Chapter 2.

None of these two drugs were ever used before for impregnation experiments, though there is enough literature about impregnation of ibuprofen in different polymers, using $scCO_2$.

Ibuprofen sodium salt

In literature there are no data about the system CO_2 - ibuprofen sodium salt, but in one article [Martin et al., 2009] it is written that solubility of ibuprofen sodium salt in $scCO_2$ is very low, as it should be expected, noting its chemical formula. Therefore, it is not possible to process it with RESS (Rapid Expansion of a Supercritical Solution). In the article by Martin ibuprofen sodium salt is treated with a SAS technology (Supercritical antisolvent solution), where carbon dioxide acts as antisolvent.

Despite this information, experiments for impregnation of this drug have been carried out. In fact, as explained in Chapter 1, a molecule may be impregnated in a polymer, even if its solubility in carbon dioxide is not high. The matrix is the real key variable for impregnation.

Of course, knowing that this drug is not much soluble and that starch is not particularly affected by CO_2, good results were not expected.

First of all, we have studied the ibuprofen sodium salt behavior with carbon dioxide. Ibuprofen sodium salt was placed in a glass beaker in the view cell, with the magnetic stirrer. The system was pressurized and the effect was observed.

The following temperature and pressure ranges were used in the constant volume view cell:

Temperature range	Pressure range
29°C - 31°C	1 bar - 100 bar
33°C - 36°C	1 bar - 200 bar
39°C - 41°C	1 bar - 71 bar

Due to the volume constancy, when pressure is increased, temperature will always slightly change as reported, even with a thermostatic bath.

Unfortunately, solubility measurements were not practicable with this equipment. Nevertheless, something interesting was noted in the view cell.

- At temperatures around 30°C, with carbon dioxide near to its critical point, three phases were detected (Figure 3.4):

  1. a solid phase, mainly composed of drug;
  2. a liquid phase, mainly carbon dioxide, increasing in volume as pressure increases;
  3. a gas phase, mainly carbon dioxide, decreasing in volume as pressure increases.

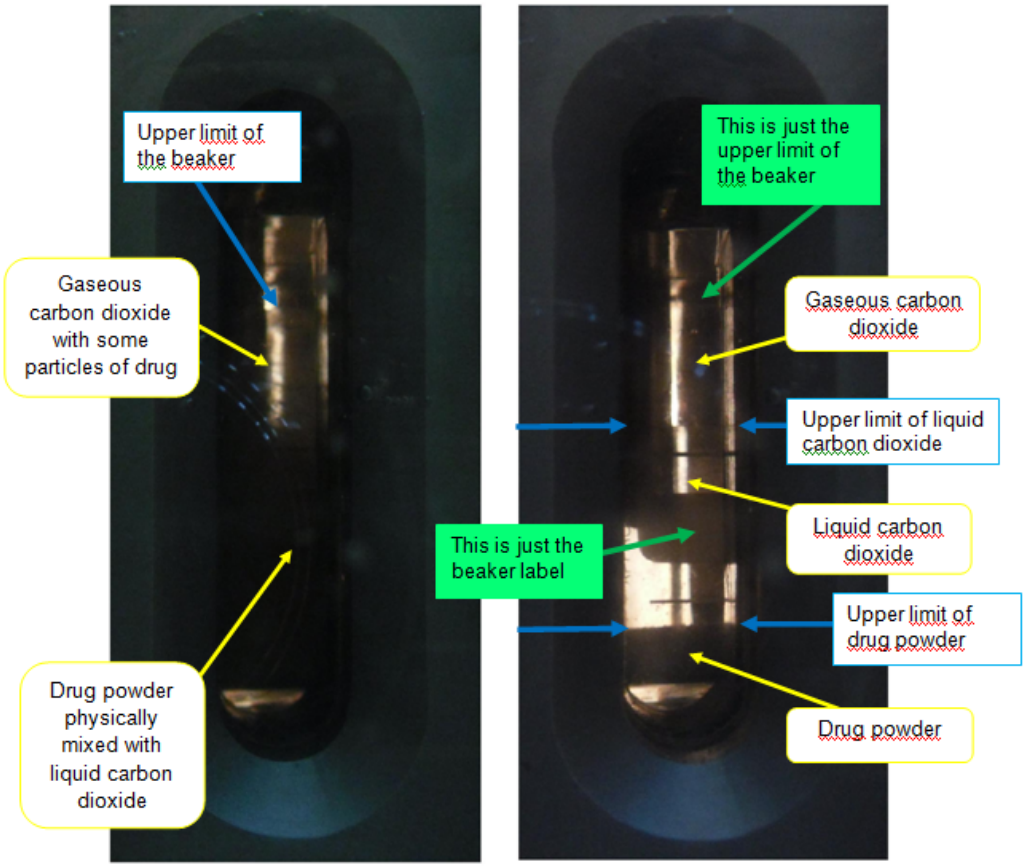

Figure 3.4: *View cell with 1 g ibuprofen sodium salt, $T = 29°C$, $P = 60$bar. On the left side, the stirrer is working: different phases are not detectable, because of the stirring. On the right side, the stirrer is stopped and different phases are recognizible.*

Figure 3.5: *Ibuprofen sodium salt melted, after pressurization with carbon dioxide at 41°C and 70 bar. The drug is still liquid, at ambient condition. First picture on the left: melt drug inside the view cell, under pressure. Picture in the middle: melt drug in the beaker, seen from the side. On the right: same beaker seen from above.*

Drug remained a solid power, in a separated phase. To verify the phase characteristics of the drug, the stirrer is useful. Activating and then stopping the stirrer, the powder was seen to swirl in liquid carbon dioxide and then settle down.

- At temperatures around 35°C, the same phenomenon as at 30°C was seen, but at this temperature carbon dioxide was supercritical, therefore only two phases are visible:

  1. a supercritical phase, mainly carbon dioxide;
  2. a solid phase, mainly ibuprofen salt.

- In the third experiment, while increasing pressure, ibuprofen salt melts at 41°C and 70 bar (Figure 3.5).

Note that at 36°C no melting point was detected up to 250 bar, but at 40°C a melting point depression was observed, around 70 bar. Due to the lack of precision of the instrumentation, it was not possible to identify more accurately this point.

Even if unforeseeable, this phenomenon is not surprising, because also ibuprofen shows a melting point depression, as noted in Chapter 2. However, for ibuprofen sodium salt, depression is larger, because at ambient pressure the melting point is at around 200°C, whereas under CO_2 at 70 bar, it gets down to 40°C. For ibuprofen, instead, the normal melting point is at 78°C and it is lowered close to 50°C in literature, but no specific data are reported [Suleiman et al., 2005].

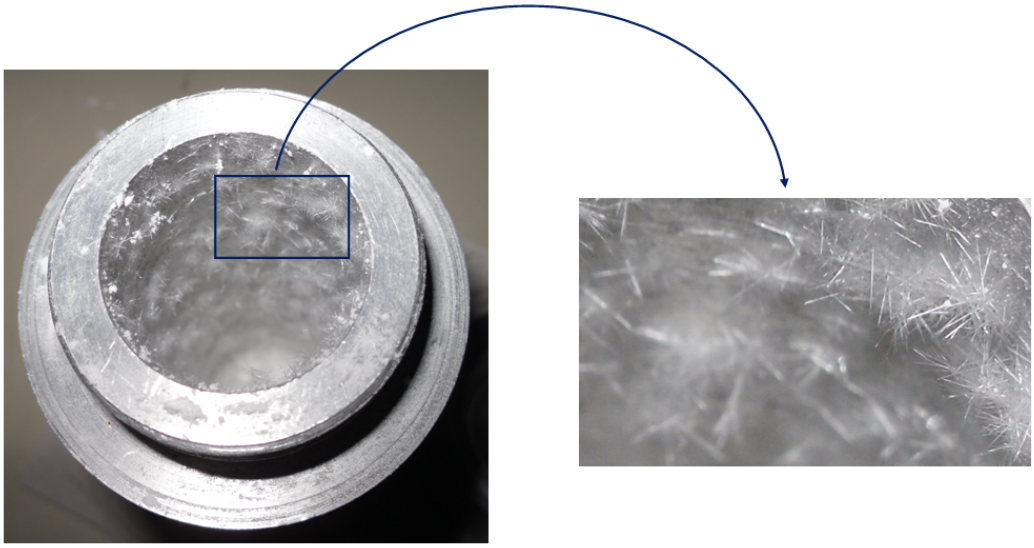

Figure 3.6: *Needles of crystalline caffeine, formed in the vessel after pressurization with carbon dioxide at 300 bar and 60°C. On the right, a zoom on the needles is provided.*

This particular characteristic of ibuprofen sodium salt will be exploited by developing a solid dispersion technique, even though this was not scheduled at the beginning. Refer to Section 3.4.2 for details.

To complete the investigation, also higher temperatures and pressures were then considered in the static plant:

Temperature range	Pressure range
40°C ± 3°C	1 bar - 300 bar
60°C ± 3°C	1 bar - 300 bar

As expected, melting point depression was also seen in these last two cases. Moreover, it was noted that, increasing pressure, a liquid foam is formed. A relevant amount of carbon dioxide seems to dissolve in the liquid drug.

Caffeine

For caffeine, the following temperatures and pressures were investigated:

Temperature range	Pressure range
40°C ± 3°C	1 bar - 300 bar
60°C ± 3°C	1 bar - 300 bar

In this case, a visible evidence of the solubility values found in literature was possible in some way. Caffeine, after pressurization and consequent depressurization,

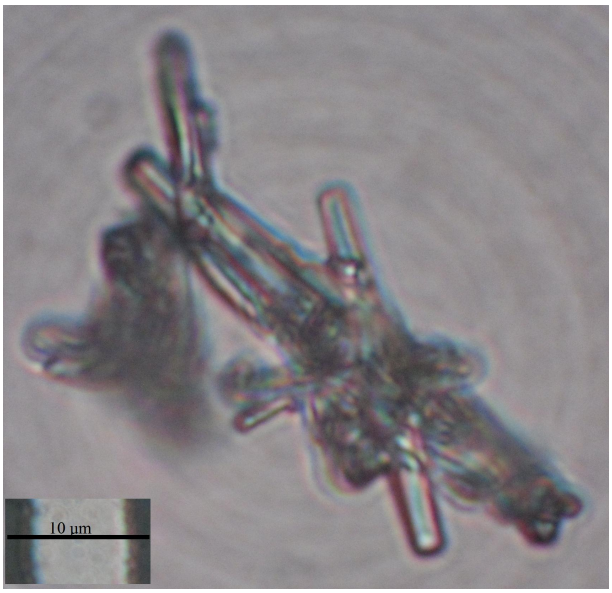

Figure 3.7: *A crystal of caffeine, as seen at the optic microscope, with paraffin oil. This is the caffeine as purchased.*

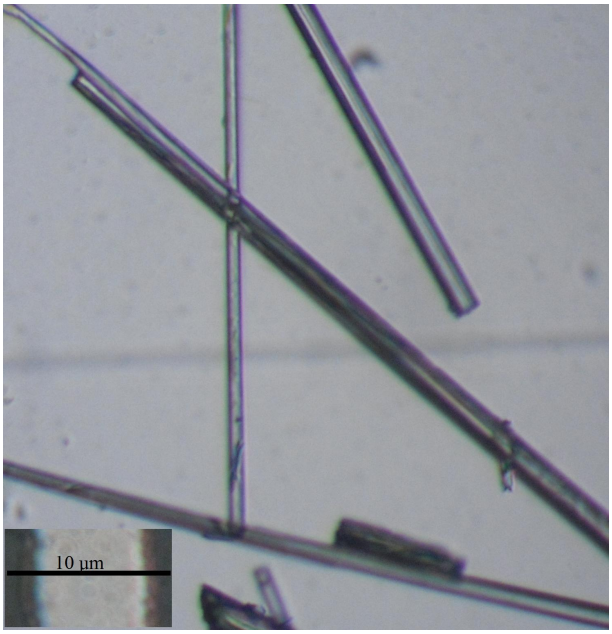

Figure 3.8: *A crystal of caffeine, as seen at the optic microscope, with paraffin oil. This is the shape gained after treatment with supercritical carbon dioxide, at 60°C and 300 bar, with slow decompression.*

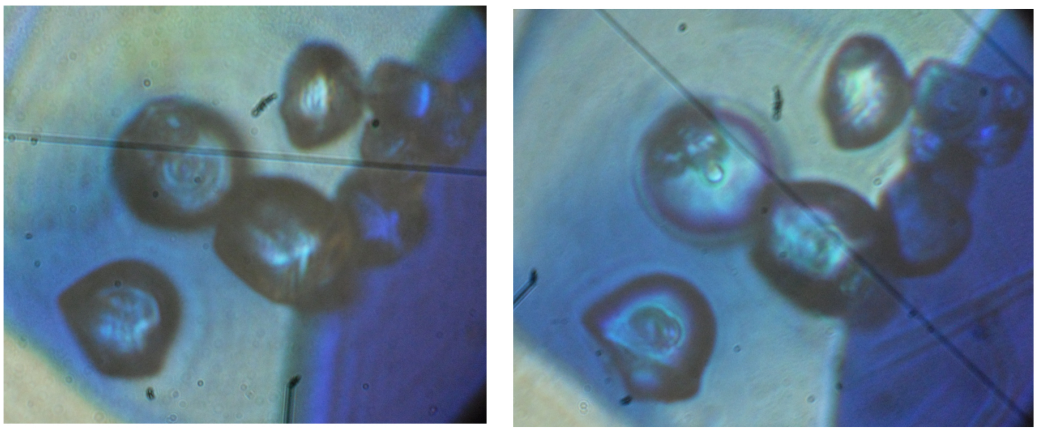

Figure 3.9: *Particles of tapioca starch, photographed before and after treatment with carbon dioxide, to verify the presence of any difference. On the left, starch particles before placing them in the vessel. On the right, the same starch particles after being placed in the view cell for 4 hours, at 40° C and 100 bar. The blue color in the photo is done with a pen on a glass slide. In this way, the same granules are detectable before and after the experiment, using the microscope.*

was spread all around the walls of the vessel, a sign that it is considerably dissolving in carbon dioxide. This phenomenon is almost negligible at 100 and 150 bar, but it becomes relevant at higher pressures. Also temperature plays a key role: the higher the temperature, the higher the solubility.

Furthermore, shape of crystals change because of this $scCO_2$ treatment. Dissolved caffeine, precipitating, tends to form long needles, which are longer and neat if decompression time is longer, because more time for recrystallization is left.

Also, with higher pressure and temperature these needles are longer, because crystals have more caffeine at disposal for their growth.

Figure 3.6 is a picture of the vessel with caffeine crystals made. In this specific case really long needles were produced. Caffeine was then studied at the optic microscope. Figure 3.7 shows the shape of caffeine as purchased, whereas Figure 3.8 shows needles of caffeine, obtained after treatment with supercritical carbon dioxide.

3.3.2 Starch behavior with supercritical carbon dioxide

According to literature, starch is not particularly affected by the presence of carbon dioxide. Moreover, it probably does not undergo permanent changes because of carbon dioxide under pressure. The amount of carbon dioxide absorbed by starch was not measurable with the technique available, but some visual experiments were arranged.

In particular, some tapioca starch particles were traced and pictures were taken before and after pressurization. This experiment was done placing starch particles in the view cell, at 40°C and 100 bar, for 4 hours. The pressure was chosen in relation to the remarks made in Section 2.2.1. This experiment was aimed to detect any macroscopic difference in the starch, due to the pressurization and depressurization with carbon dioxide. A picture of the same particles was taken before placing them in the view cell and after the treatment (Figure 3.9). The blue color in the photo was done with a pen on a glass slide. In this way, the same granules could be detected before and after the experiment, using a microscope. No visible difference was found in the photographed granules, the slight difference in the light being due to a slight difference in the focus of the microscope.

No macroscopic differences were detected inside the view cell during the experiment. Nevertheless, little absorption of carbon dioxide might happen, which however was not possible to measure. In any case, this should happen, as it is suggested in literature (see Chapter 2).

To verify the presence of lasting effects in starch granules, due to supercritical carbon dioxide, a statistical analysis of particles dimensions before and after treatment with $scCO_2$ was done. The following procedure was followed:

- take a sample of starch particles;
- using the microscope, take photos of a considerable amount of starch particles;
- place the sample in the beaker, in the view cell;
- pressurize the system for 4 hours, T = 40°C, P = 100bar;
- depressurize the system and take the beaker out;
- using the microscope, take photos of a considerable amount of starch particles.

The number of photographed particles should be enough to be considered statistically relevant. More than 100 granules were photographed.

Pictures were then analyzed using ImageJ. The area was measured for all the particles, then an average diameter was calculated, approximating particles as spherical. This experiment was made for corn starch.

For both samples, before and after treatment, the mean of the statistical distribution is 0.046 mm, a value different from the one found in literature. This could be ascribed to the particular technique used, that is not reliable, if absolute values are sought. However, it is reliable for comparison purposes. The results are summarized in the following table:

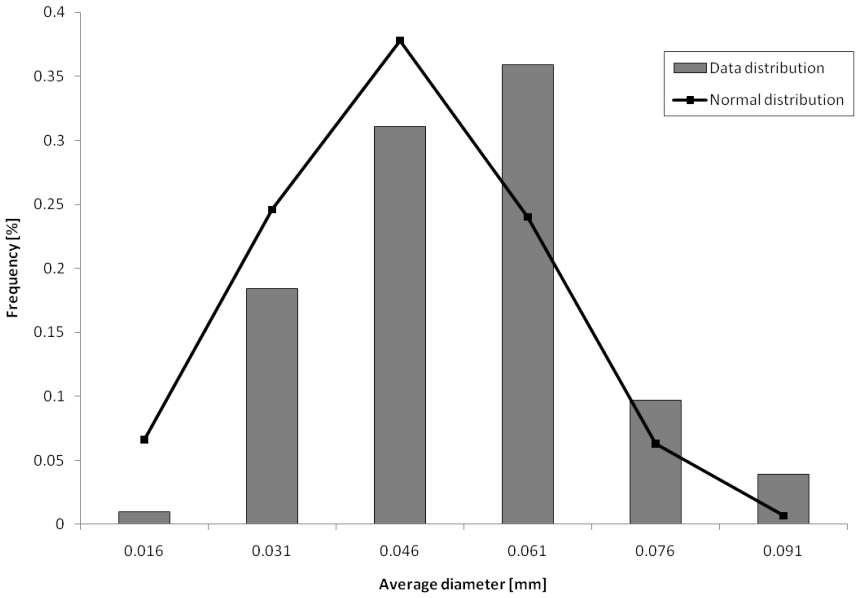

Figure 3.10: *Statistical distribution of particles diameter, for corn starch as purchased.*

	Starch before treatment	Starch after treatment
Mean	0.046 mm	0.046 mm
Standard deviation	0.016 mm	0.015 mm

Since mean and standard deviation are the same in both cases, it can be concluded that no permanent difference in particle size occurs because of carbon dioxide treatment. If swelling during pressurization happened, it would be a reversible phenomenon. The two distributions are detailed in Figure 3.10 and Figure 3.11.

These experiments answer also the problem discussed in Chapter 1, that is the final aspect of the polymer after treatment. It was stated that, depending on the rate of decompression, residual CO_2 may remain trapped inside the polymer, causing foam formation. In this case, depressurization has to be controlled in order to achieve the desirable physical characteristic for the polymer.

In case of starch, however, foaming did not occur. For this reason, time of decompression should not be an important variable, as long as the polymer is concerned.

3.4 Operational parameters

3.4.1 Impregnation experiments

For impregnation process, following operating variables were considered:

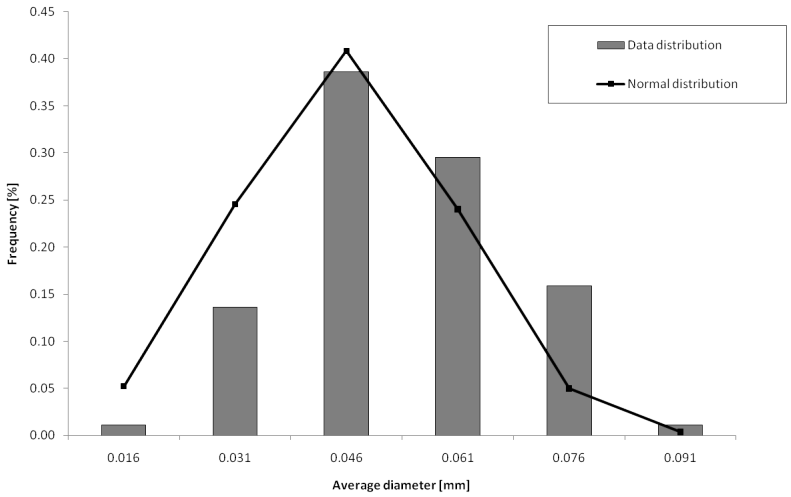

Figure 3.11: *Statistical distribution of particle diameter, for corn starch after being exposed to supercritical carbon dioxide.*

Temperature is considered as an important variable, because it directly influences solubility of drug in carbon dioxide. It also affects viscosity and diffusivity, important for impregnation kinetics.

Pressure plays a crucial role, as it changes the drug solubility in carbon dioxide and the solubility of carbon dioxide in starch. As temperature, it affects viscosity and diffusivity.

Time of impregnation is important if the kinetics is not really fast. In the available plant, stirring is not possible. Therefore, diffusion kinetics cannot be accelerated by convection.

Time of decompression , which can probably affect the formed complex. It is desirable to use shorter times for decompression, in order to reduce the total operating time. Moreover, considering that both drugs are crystalline, a shorter decompression time could also help to reduce cristallinity degree.

Amount of drug in the vessel is finally considered. Most of the experiments are done with an excess amount of drug, comparing it to the value determined by solubility.

As two different configurations were used, we recall that. The second configuration, only for caffeine impregnation, was developed as an improvement, comparing to the

first configuration. This was done, in order to verify if the used configuration had a decisive influence on impregnation results.

Some other variables were neglected, because of technical reasons or because they are considered not important. Among them:

- compression velocity, which is not manageable in the plant,
- mixing, which is not possible in the apparatus used for impregnation.

The minimum amount of drug to be used was calculated as detailed in the following. Solubility is defined by:

$$Solubility = \frac{moles\ of\ drug}{moles\ of\ drug + moles\ of\ CO_2}. \tag{3.1}$$

The quantity of soluble drug is therefore:

$$Mass\ of\ drug = \frac{Solubility}{1 - Solubility} \times Moles\ of\ CO_2 \times MW\ of\ drug, \tag{3.2}$$

where MW is the molecular weight. Moles of carbon dioxide are determined as:

$$Moles\ of\ CO_2 = Molar\ density\ of\ CO_2 \times V, \tag{3.3}$$

where V is the volume of the vessel. Data for molar density are in Figure 3.12 [NIST, 2011]. The amounts of caffeine for a 300 cm^3 vessel (first configuration) and for a 120 cm^3 vessel (second configuration) are in Figure 3.13 and Figure 3.14.

Moles of carbon dioxide and solubility depend on temperature and pressure. Values are available in literature for caffeine and carbon dioxide. However, for ibuprofen sodium salt, solubility is not available. In this case, the following reasoning is proposed:

- ibuprofen data are known,
- ibuprofen sodium salt should be less soluble than ibuprofen, according to literature,
- the amount of drug determined using ibuprofen data is for sure more than the amount actually soluble.

This idea was checked during the experiments: as long as the drug was left at the bottom of the vessel after the experiment, its amount was in excess. To use always the same amount of drug and to be sure that it was always in excess, an amount higher than the solubility at 300 bar and 60°C was adopted for caffeine. In particular, 4 g were used for the 300 cm^3 vessel and 0.5 g for the 120 cm^3 vessel. For

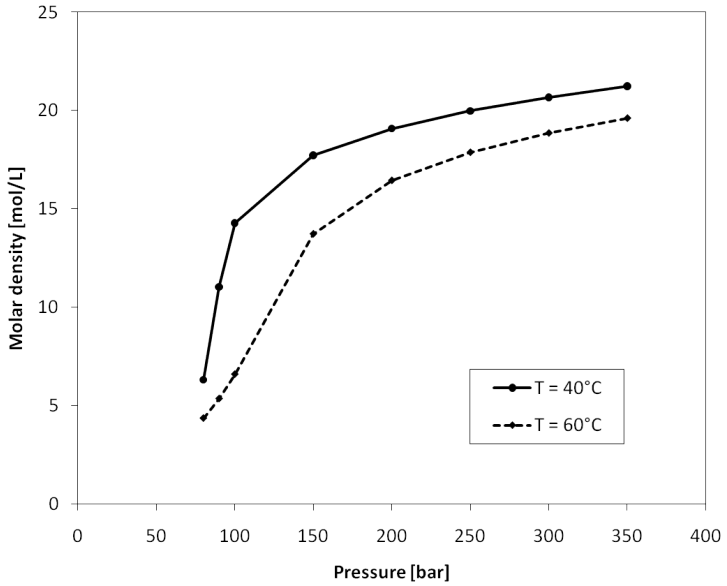

Figure 3.12: *Molar density of carbon dioxide, depending on temperature and pressure. This data are necessary to calculate the amount of drug soluble in the vessel.*

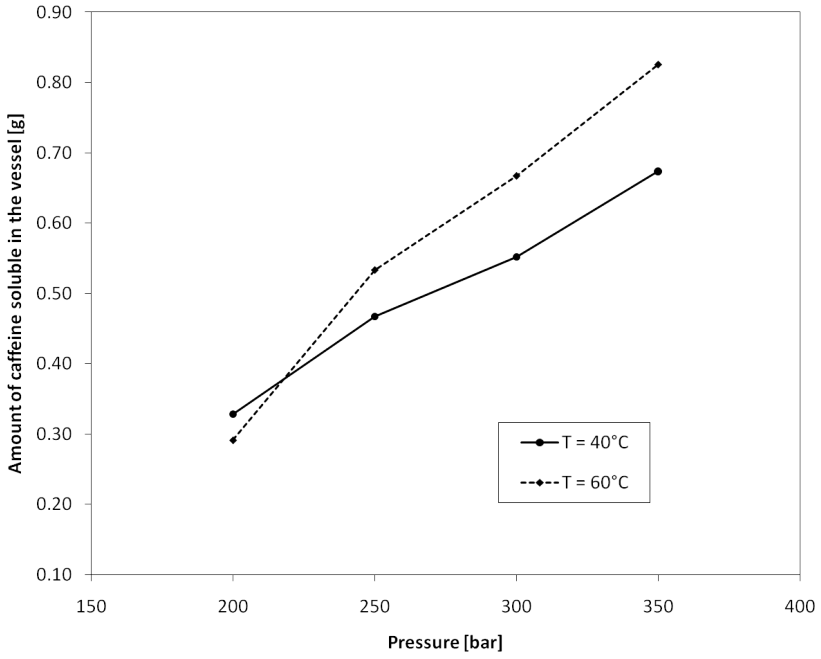

Figure 3.13: *Quantity of caffeine soluble in a vessel of 300 cm^3.*

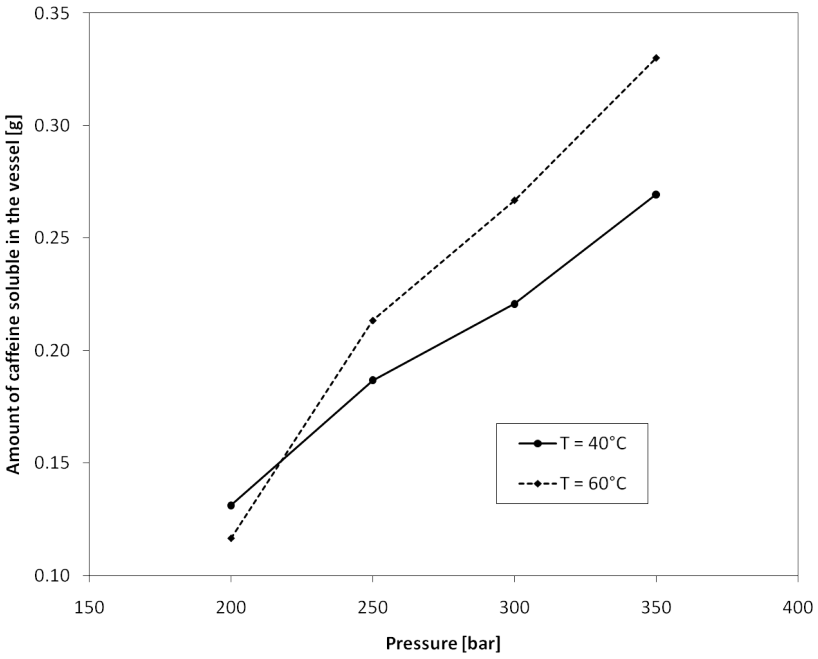

Figure 3.14: *Quantity of caffeine soluble in a vessel of 120 cm^3.*

the biggest vessel, 1 g could be enough, but since those were the first experiments made, we preferred to use more, to avoid uncertainty related to the solubility data.

As long as ibuprofen is concerned, data only up to 200 bar are available. These are the amounts of ibuprofen that could be soluble in a vessel of 300 cm^3:

- 4.05 g at 150 bar and 40°C;
- 8.08 g at 200 bar and 40°C.

For higher pressure and temperatures, values may be extrapolated. However, this has no sense for the purpose of these experiments. As it can be seen, ibuprofen solubility is very high, but the solubility of ibuprofen sodium salt seems to be definitely lower, since most of the used amount remained always at the bottom of the vessel. Moreover, there was no visible trace of drug on the walls of the vessel, as it happens with caffeine.

3.4.2 Solid dispersion

For solid dispersion, the following operating parameters are considered as relevant:

Temperature control is necessary to have a melt phase. It also affects viscosity.

Pressure is fundamental for viscosity control, that depends on carbon dioxide properties at the set T and P. Moreover, it determines melting point depression.

Mass ratio between drug and starch is an important parameter for processability of the compound. It also determines the actual composition of the complex, at the end of the treatment.

Total mass of compound in the vessel has to be considered for mixing properties. The right amount of compound inside the vessel has to be used, in order to have a good mixing.

Mixing determines homogeneity or not of the resulting complex.

Experimental evidence showed that total solid suspension is necessary to achieve sample homogeneity. Solid suspension is related to mass and mixer speed by the Zwietering correlation [Kerc et al., 1999]:

$$N_s = S \cdot \nu^{0.1} \left[\frac{g\,(\rho_S - \rho_L)}{\rho_L} \right]^{0.45} X^{0.13} \frac{d_p^{0.2}}{D^{0.85}}, \tag{3.4}$$

where:

N_s is the minimum mixer speed;

S is the Zwietering constant;

ν is cinematic viscosity;

g is gravitational acceleration;

ρ_S is solid density;

ρ_L is liquid density;

X is the percentage ratio between solid mass and liquid mass;

d_p is particles mean diameter;

D is the mixer diameter.

This means that the higher the solid amount, the faster must be the mixer. Moreover, characteristics such as viscosity and particles dimension are important to determine solid suspension.

Time of treatment was set to 1 hour, that is high enough to be sure that equilibrium was reached. This value was decided thinking that:

- ibuprofen sodium salt melts instantaneously, when the appropriate temperature and pressure is reached;

- 10 minutes after compression is completed, the system has always already reached the minimum value for viscosity. This is understandable, when looking at the mixing dynamics inside the vessel;
- thanks to the mixing, the system is homogeneous well before an hour has passed.

The uniformity of the compound formed was controlled at the microscope. Probably, less than an hour was sufficient, but this parameter was not studied. Another neglected parameter was the speed of the stirrer, because it was not possible to measure the actual speed value. Moreover, this parameter is strictly dependent on the specific configuration: changing stirring magnet or beaker, results will change. What was however considered is the presence or absence of mixing.

Chapter 4

Results and discussion

For each experiment, the sample processed have been observed at the microscope and analyzed with a UV absorber, as explained in chapter 2.

The results found are now explained and discussed.

4.1 Impregnation

4.1.1 Impregnation of ibuprofen sodium salt

Influence of temperature and pressure

Influence of temperature and pressure is considered first. Experiments are done with 10 g of ibuprofen salt and 10 g of starch in the static vessel. The drug amount is in excess, comparing to solubility. The impregnation time is set to four hours, followed by one hour of decompression. Impregnation is done on tapioca starch, using the first configuration.

Results for ibuprofen sodium salt are shown in Figure 4.1.

It is clear that impregnation does not achieve high values, as it was expected in Chapter 2. This is reasonably due both to the ibuprofen sodium salt and to the starch. The reasons for this statement will be explained after showing results of caffeine impregnation. The impregnated amount is around 0.1% in weight. A slight effect with temperature and pressure was found. In particular, impregnation seems to be higher at higher temperatures and higher pressures. Pressure effect seems to be more important than temperature one.

The best conditions examined are at 300 bar and 60°C. However, the difference with other points is scant. In Chapter 2, considerations about solubility of carbon dioxide in starch showed that lower pressure could allow better impregnation than higher ones. Analyzing these results, however, the influence of drug solubility seems to be the key point. Another possibility to be considered is that starch could be

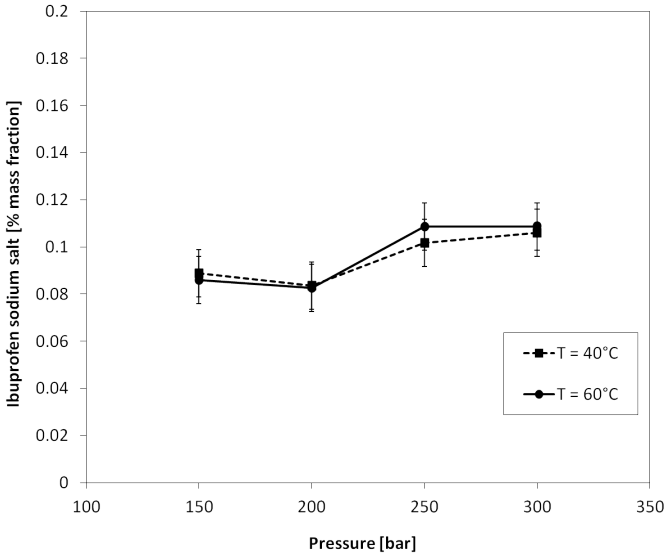

Figure 4.1: *Influence of temperature and pressure on impregnation results, for ibuprofen sodium salt. Lines connecting experimental points are used only to help reading data. Errors associated with experimental points are due to variability found repeating experiments. These runs have been performed with tapioca starch, in the plant with the first configuration.*

more swellable at higher pressures, despite what is found in literature. It is useful to remind that literature is contrasting on this subject.

In Chapter 1, it was stated that even though a substance is not much soluble in carbon dioxide, impregnation achieved could be high, if a suitable matrix is used. According to this idea, the main problem for low impregnation level could be starch.

4.1.2 Impregnation of caffeine

After having found low results with ibuprofen sodium salt, experiments with caffeine were considered.

Caffeine is quite soluble in carbon dioxide. Therefore, if starch allows impregnation, results should be better.

Influence of temperature and pressure

As with ibuprofen sodium salt, temperature and pressure effects are first considered. Experiments were done with 4 g of caffeine and 4 g of starch. First configuration was used, with 4 hours impregnation and one hour decompression. Amount of starch was chosen to be equal to the amount of drug. The drug amount is more that according to solubility.

Results are shown in Figure 4.2.

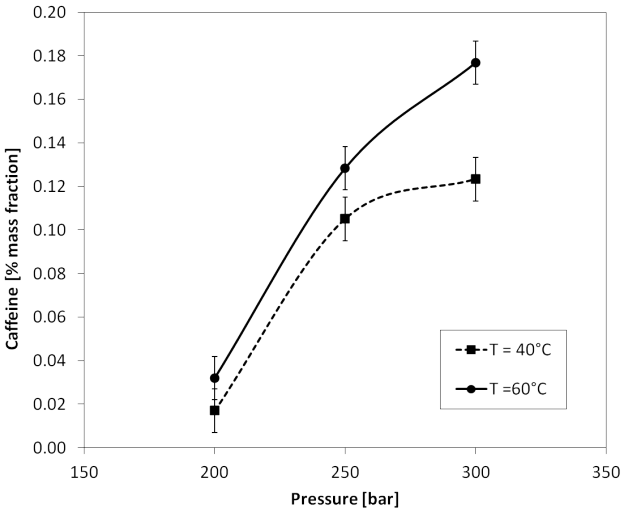

Figure 4.2: *Influence of temperature and pressure on impregnation results, for caffeine. Lines connecting experimental points are used only to help reading data. Errors associated with experimental points are due to variability found repeating experiments.*

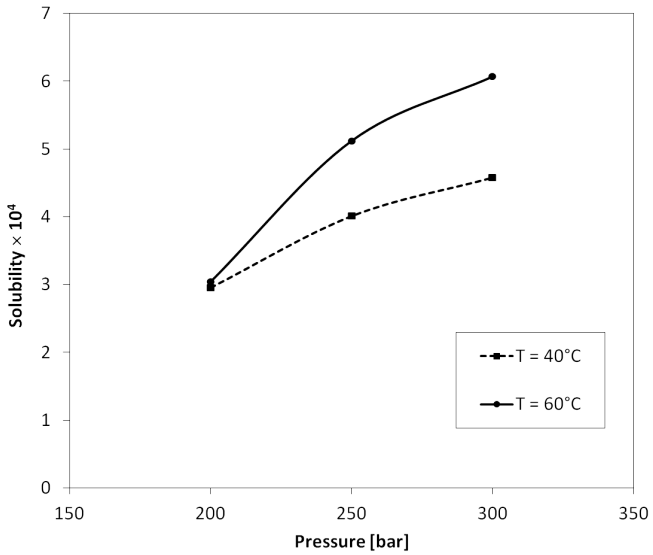

Figure 4.3: *Solubility of caffeine in carbon dioxide, at different temperatures and pressures. This is the plot of data already presented in Table 2.6. It is here presented again to compare it with impregnation results.*

Unlike ibuprofen salt, the influence of temperature and pressure for caffeine impregnation is more pronounced. Higher pressure and higher temperature correspond to higher impregnation level. Impregnation curves resemble solubility curves (see Figure 4.3). Solubility is therefore identified as the essential variable. Best point is found at 300 bar and 60°C. Figure 4.4 shows the correlation between caffeine solubility and impregnation. The presence of a correlation is clear and can be justified considering impregnation as strictly dependent on solubility.

Again, drug solubility appears to be more important than starch behavior with carbon dioxide. However, also in this case unsatisfactory results were obtained. To understand if better ones can be achieved in some way using starch, other experiments were carried out by changing the experimental set up.

Influence of plant configuration

Different plant configurations were considered to enhance impregnation. As already discussed in Chapter 3, two configurations were developed and the second one is believed to give better results because it enhances the contact between drug and carbon dioxide, and between carbon dioxide and starch is enhanced.

Results using the second configuration are actually better (Figure 4.5). For this reason, following experiments have been done in this way.

Experiments were performed with 0.5 g caffeine and 1 g starch, because the vessel dimensions are smaller. The relative amount of caffeine used is lower, because in the first experiments it was noted that a relevant amount of caffeine was left unchanged inside the vessel. Nevertheless, this amount is still in excess as evident looking inside the vessel after the experiment.

Even with the new configuration, the best result is a load of 0.25% of caffeine by weight. This result enforced the idea that the limiting factor is actually starch.

In fact, a parallel research tried to impregnate lavender oil in the same starch. Best point found is at 40°C and 100 bar. Impregnation achieved was 0.5% in weight [Gamse, 2010].

Different types of starch

Then impregnation was attempted with another type of starch. As already discussed in Chapter 2, tapioca starch has no pores, according to literature. So, a porous starch was considered, because porosity may help impregnation, letting carbon dioxide enter the starch core.

Repeating on corn starch the same experiment already done at 300 bar and 60°C, it was verified that the impregnated amount is actually higher (Figure 4.6), being as high as 0.46% by weight. Even though corn starch is slighlty better for

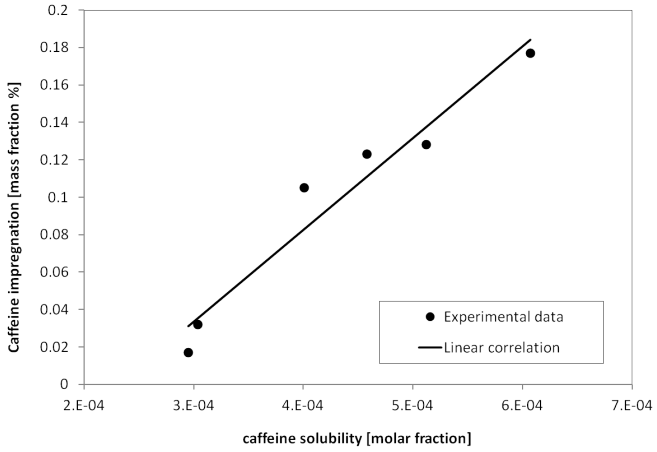

Figure 4.4: *Linear correlation between caffeine solubility and impregnation in the starch matrix. The impregnation data used here are the same presented in Figure 4.2. The correlation line is described by:* $y = 491 \cdot x - 0.11$

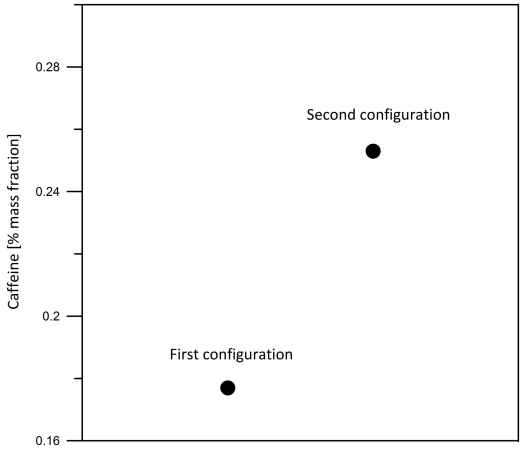

Figure 4.5: *Comparing results obtained with the first and second configuration. Points are relative to experiments at 300 bar and 60° C, four hours impregnation and one hour decompression.*

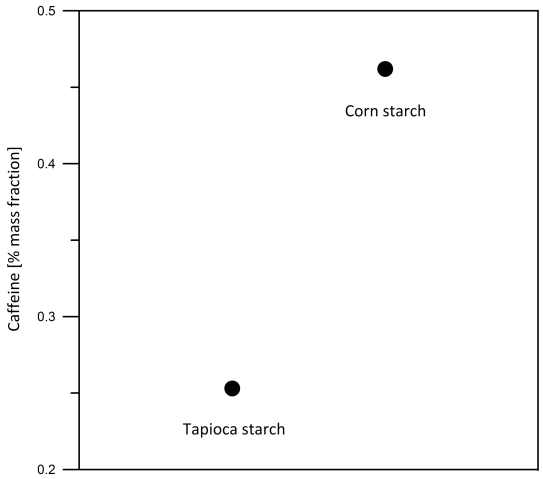

Figure 4.6: *Results obtained with different types of starch (tapioca and corn starch). Experiments are made with the new configuration, with 0.5 g caffeine and 1 g starch at 300 bar and 60°C. Four hours of impregnation are followed by one hour decompression.*

impregnation, results are still notably low. This conclusion is done by comparison with literature data, presented in Chapter 2.

In addition to starch swellability, another characteristic that has been considered to explain this phenomenon, is the compatibility between matrix and drug. This is important also to foresee if the complex formed is stable or not. To help stability, starch and caffeine should be able to form some secondary bonds between each other. Considering chemical formulas, they are likely to form Van der Waals and some dipole-dipole bonds. However, the relative strength of these bonds is not known, comparing to the strength of bonds between molecules of the same species.

Poor swellability by carbon dioxide and probably low compatibility between drug and starch are suggested as main causes for poor impregnation.

Influence of decompression time

For caffeine, also decompression time was studied. It was noticed that with a short decompression time better results are achieved. This was verified both with tapioca and corn starch, at the best point, and is probably occurring because, with shorter time, caffeine is actually entrapped inside starch. Whereas, after impregnation, caffeine may also be partially extracted if decompression is long.

Influence of decompression time seems to be higher with corn starch than with tapioca starch (see Figure 4.7 and Figure 4.8). However, the difference found between these two starches could also be due to relative errors in both experiments. This

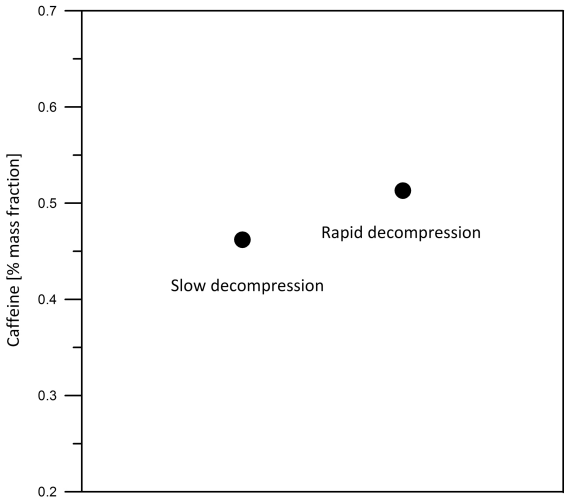

Figure 4.7: *Effect of different decompression times on corn starch impregnation. Rapid decompression is completed in 10 minutes. Slow decompression is done in one hour. Experiments are done at 300 bar, 60°C, with the new configuration.*

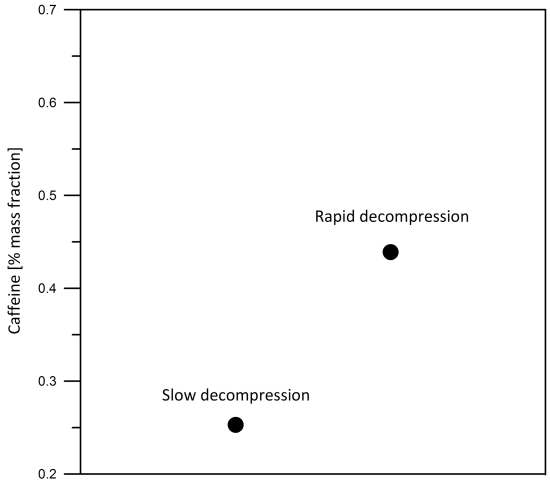

Figure 4.8: *Effect of different decompression times on tapioca starch impregnation. Rapid decompression is completed in 10 minutes. Slow decompression is done in one hour. Experiments are at 300 bar, 60°C, with the new configuration.*

is also a possibility, because the result obtained is not easily explicable. In fact, a high variation was not expected and results found for corn starch are considered reasonable.

Rapid decompression was completed in 10 minutes, slow decompression is in one hour.

Influence of impregnation time

Impregnation time was another parameter tested. In most of the experiments, the sample was impregnated for four hours. This is considered to be a quite long time, that should allow a good impregnation. In literature, experiments with ibuprofen required four hours, too.

In Figure 4.9 results and regression equation are shown. For caffeine, with increasing time, the impregnated mass fraction increases, as it is expected. After four hours, a plateau is almost reached. This means that maximum impregnation is almost reached after four hours. Increasing time again, impregnation value still increases, but more slowly. Value found after 15 hours was 0.55% by weigth.

This behavior may be represented by the following equation:

$$X = X_{max} \cdot \frac{t}{\alpha + t} = 0.59 \cdot \frac{t}{0.99 + t}, \tag{4.1}$$

where:

X is caffeine mass fraction inside the starch matrix;

X_{max} is the maximum impregnation value;

t is time in hours;

α is a empirical coefficient.

The values for α and X_max are found by minimizing the error between experimental values and calculated values.

Influence of drug amount

Experiments up to now always used an excess amount of drug inside the vessel, comparing to solubility. Even though values are already low, some experiments were carried out with less initial caffeine, in order to study the effect of this variable (Figure 4.10). Despite all the amounts are higher than the soluble quantity (according to literature data found), impregnation level still increases with the loaded amount. Apparently, an excess of drug is useful to achieve better results, but when this excess is too high, the curve reaches a plateau.

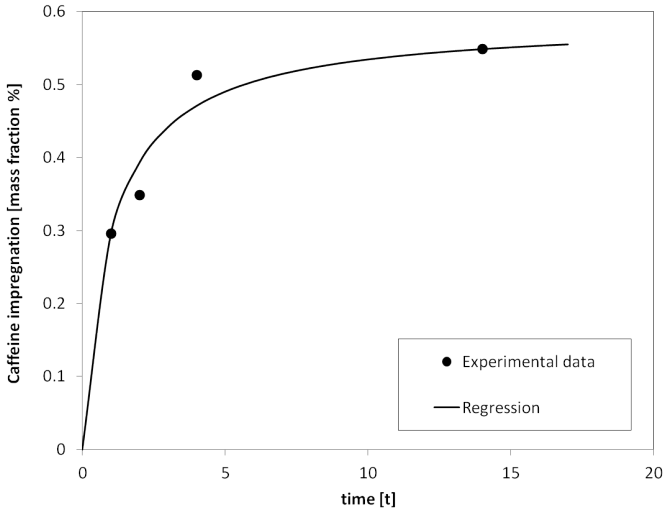

Figure 4.9: *Influence of time on caffeine impregnation. Regression curve according to equation* (4.1)*. Increasing time, impregnated mass fraction increases. After some hours, a plateau is almost reached. Value found after 15 hours is 0.55% by weigth. Experiments are performed with the second configuration, at 300 bar and 60°C, with corn starch and rapid decompression.*

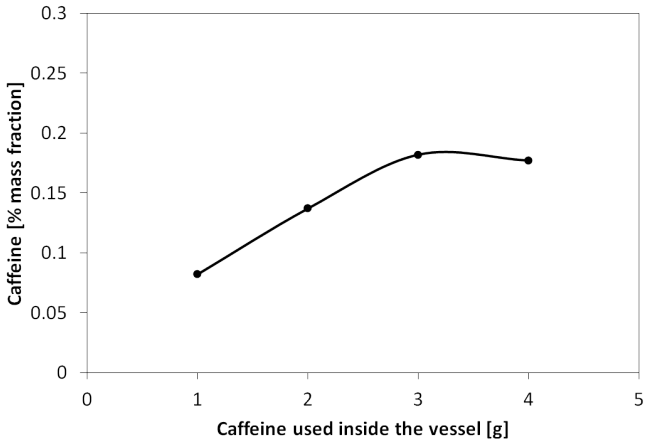

Figure 4.10: *Influence of initial caffeine quantity inside the vessel during experiments. Experiments are done with the first configuration, all with 4 g of tapioca starch. Parameters are: 300 bar, 60°C, one hour decompression.*

4.2 Solid dispersion

4.2.1 Solid dispersion of caffeine

The system developed for solid dispersion is not applicable to caffeine, because CO_2 is not able to melt caffeine in the pressure - temperature interval considered. Some experiments were done the same and samples were analyzed at the microscope. After experiments, starch and caffeine resulted to be still separate. The conclusion is that it is not possible to combine caffeine and starch with this technique.

4.2.2 Solid dispersion of ibuprofen sodium salt

Ibuprofen sodium salt melts at 40°C and 70 bar under CO_2 pressure(see Chapter 3). This characteristic has been exploited to prepare a solid dispersion. Moreover, when processed alone, it remains melted also after decompression, but the reason for this behavior is not clear.

Influence of temperature and pressure

As already stated, temperature cannot be less than 40°C, because no melting occurs. The interesting part of this technology is that the drug can be melted at rather low temperature and without solvents. Therefore, temperatures higher than 40°C have not been considered, because they are not necessary.

As far as pressure is concerned, it must be taken into account that:

- at 100 and 150 bar the drug viscosity is too high and stirring is not possible. So, the drug cannot be dispersed in the medium and the result is one single block, formed by melted drug and starch. This viscous mass slowly solidifies after removing from the vessel. Since stirring is not possible, the product characteristics are not uniform.

- at 200 bar and 250 bar starch and drug can be totally suspended by the stirrer. This is justified by viscosity decrease, due to higher carbon dioxide concentration, dissolved in the drug melt. Both pressures allow good mixing.

As optimum pressure 200 bar was selected, because lower pressure is not enough, whereas higher pressure, namely 250 bar, is unnecessary as it leads to similar results.

Influence of mixing and total solid mass

This technique allows to produce a fine powder, where drug is dispersed on the starch surface. Experimental evidence shows that without mixing it is not possible to achieve this result, because a uniform powder is not produced. This means that

if mixing is not present or is not enough, the final form will present clusters of drug that is not dispersed and starch particles that are still not covered with drug. When the mixture conditions are not uniform, starch and drug still separated are visible by means of a optic microscope. Therefore, a static vessel cannot be used for this purpose.

To perform these experiments, the system was optimized with the available apparatus. From experiments, it has been seen that mixing optimization is important. To produce a product with uniform properties, all particles have to be suspended in the supercritical medium. This means that either mixer speed or solid concentration, or both have to be optimized. Relation between minimum mixer speed and solid mass is well described by Zwietering correlation (see Chapter 3).

The stirrer speed adjustment in this vessel is practically difficult, therefore the total solid mass has been optimized, in order to be able to suspend it all with the available stirrer.

To perform these experiments, a glass beaker was placed inside the reactor. The glass beaker is necessary to contain the compound and to permit mixing. The stirrer is placed inside the glass beaker (for other details, see Chapter 3). Of course, the beaker used for these experiments has no wall baffles and the main vortex inside the beaker cannot be broken. Therefore the stirring speed cannot be too high or the total solid mass cannot be too low, otherwise, flooding happens and the material goes out of the beaker. It has been seen that:

- If total mass is 1 g, mixing speed must be lowered. If highest speed is used, too high vortex forms and the material overflows the beaker.
- If total mass is more than 3 g, total suspension is not possible and the resulting compound is not uniform.

Influence of mass ratio between drug and starch

Experiments showed that the ratio between drug and starch is decisive. Table 4.1 explains what was found. The drug mass inside the vessel is kept constant in all these experiments.

Tapioca starch is less cohesive, comparing to corn starch (see Chapter 2). Thanks to its free-flowing characteristic, good mixing can be achieved over a wider range of *drug:starch* ratio. This is clear, comparing experiments number 4 and 8. In these experiments, stirring is easy with tapioca starch, because its viscosity is acceptable. On the contrary, with corn starch stirring is really poor. According to Zwietering correlation, this is justifiable considering that solid suspension depends on viscosity and solid concentration. This means that the stirrer is not able to suspend such an amount of corn starch compound, because it is more viscous than tapioca starch.

N°	**Starch type**	**Starch mass** [g]	**IB_s mass** [g]	**Mixing observations**
1	Tapioca	0.5	1	stirrer is stuck and does not move
2	Tapioca	0.75	1	stirrer is stuck and does not move
3	Tapioca	1	1	good mixing, total solid suspension
4	Tapioca	2	1	good mixing, total solid suspension
5	Corn	0.5	1	stirrer is stuck and does not move
6	Corn	0.75	1	stirrer is stuck and does not move
7	Corn	1	1	good mixing, total solid suspension
8	Corn	2	1	poor mixing, partial solid suspension

Table 4.1: *Drug/starch ratios considered for experiments and results observation. Both corn and tapioca starch were used. Temperature is 40°C and pressure is 200 bar. IB_s stands for ibuprofen sodium salt.*

Obviously, solid dispersion n° 8 is not uniform and this has been confirmed by microscope and UV measurements, that give different results for different samples.

When the drug amount is higher than starch amount, the drug melt phase is predominant. Drug alone, when melt, is highly viscous and a sufficient starch ratio is essential to allow mixing. To have a working system, starch has to be added at least in a 1:1 ratio. The use of less starch leads to a drug matrix with starch particles inside, that is not homogeneous, because stirring is not feasible.

It has to be pointed out that such considerations are limited for the specific plant used. Using a different vessel geometry, results may be different, when stirring ability is considered. However, as long as the drug:starch ratio is concerned, a higher drug ratio will hardly give a dry powder as result, because the melt phase will be predominant.

Successful experiments: results.

As stated before, successful experiments are those made at 200 and 250 bar, 40°C, and different *drug:starch* ratio. Namely, we will now discuss the following runs:

1. ibuprofen sodium salt: tapioca starch = 1:1 at 250 bar;
2. ibuprofen sodium salt: tapioca starch = 1:1 at 200 bar;
3. ibuprofen sodium salt: tapioca starch = 1:2 at 200 bar;
4. ibuprofen sodium salt: corn starch = 1:1 at 200 bar.

For each one of these, the final composition is reported in Table 4.2. As it is clear, the final composition is different from the initial one. This is due to losses during experiments, that is to say, drug is dragged out of the vessel by carbon dioxide, during decompression. Ibuprofen sodium salt dragged out is not necessarily dissolved in carbon dioxide. It is more likely the spray of a suspension.

Compounds prepared with corn starch have a slightly lower drug mass fraction. This could be because corn starch is more cohesive and particles tend to form clusters, instead that flowing separately. In this way, it is more difficult to have all particles covered by drug all around their surface. For this reason, a higher amount of drug may remain unprocessed and go out with carbon dioxide during decompression. Difference, however, is not so high.

Anyway, these experiments show that it is possible to produce a uniform compound with a drug mass fraction up to 35%. If the right starch is used, that is a free flowing starch, the drug amount dispersed may be reduced to a low value. This can be done without any problem and the result will be uniform.

To conclude, some photos done with a scanning electron microscope are presented.

N°	Starch type	Material ratio IB_s:starch	Pressure bar	IB_s mass fraction % g/g
1	Tapioca	1:1	250	32.2
2	Tapioca	1:1	200	34.8
3	Tapioca	1:2	200	15.5
4	Corn	1:1	200	29.4

Table 4.2: *Composition of successful samples, determined with UV spectrophotometer analysis. Final composition is different from initial composition. IB_s stands for ibuprofen sodium salt.*

Figure 4.11 shows features of ibuprofen sodium salt as purchased and after being melted in carbon dioxide. Before treatment, two different crystal structures are visible, one developing more on one direction, the other forming flat shapes, as evidenced in the figure. After treatment, flat shaped crystals are predominant.

Then, we focus on the pictures of the solid dispersions produced.

Figure 4.12 shows solid dispersion number 2, prepared with 1 g ibuprofen sodium salt and 1 g tapioca starch, containing 34.8% of drug. Starch particles covered with drug are visible. The system seems uniform, since all particles are covered with drug and no clusters with just starch or drug are found with the electron microscope.

Figure 4.13 is the result found preparing a solid dispersion with 1 g ibuprofen sodium salt and 2 g tapioca starch (number 3). The presence of a lower amount of drug is visible, because starch is not completely covered as in the previous experiment.

Figure 4.14, instead, shows a solid dispersion prepared with corn starch (number 4). Corn starch tends to form clusters more than tapioca starch and this appears clearly at the microscope.

At least two different crystal shapes are recognizable in all these photos. This means that slightly different operational conditions may lead to different crystal polymorphs. This is not surprising, because it is already known that ibuprofen sodium salt has at least three different polymorphic forms [Martin et al., 2009].

Moreover, it has to be noted that crystals are smaller in solid dispersion, than in untreated ibuprofen sodium. This is really important, because smaller particle size usually leads to higher bioavailability and better drug release control.

Finally, even though the measurements done can be regarded only as preliminary, this technique is most likely able to decrease crystallinity, enhancing drug bioavailability: a promising result to start with a dedicated research project.

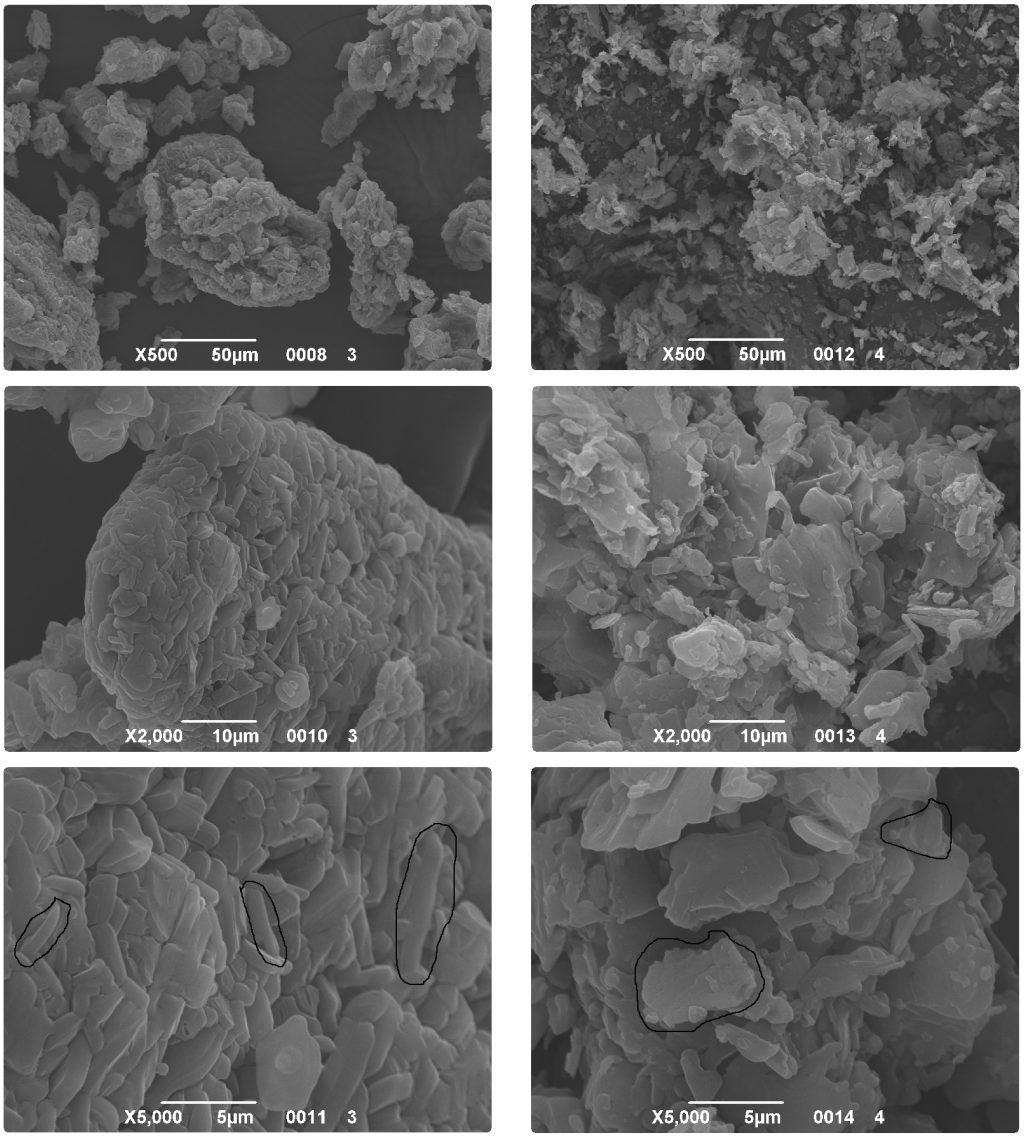

Figure 4.11: *Photos of ibuprofen sodium salt, taken with a scanning electron microscope. Enlargement is written in the picture, together with the scale. On the left, ibuprofen salt as purchased is presented, with different enlargements: 500 x, 2000 x and 5000 x. On the right, ibuprofen salt after melting in carbon dioxide is shown. Same enlargements are used: 500 x, 2000 x and 5000 x. The different types of crystals are evidenced in the pictures at the bottom: on the left, some unidirectional crystals; on the right, some flat crystals.*

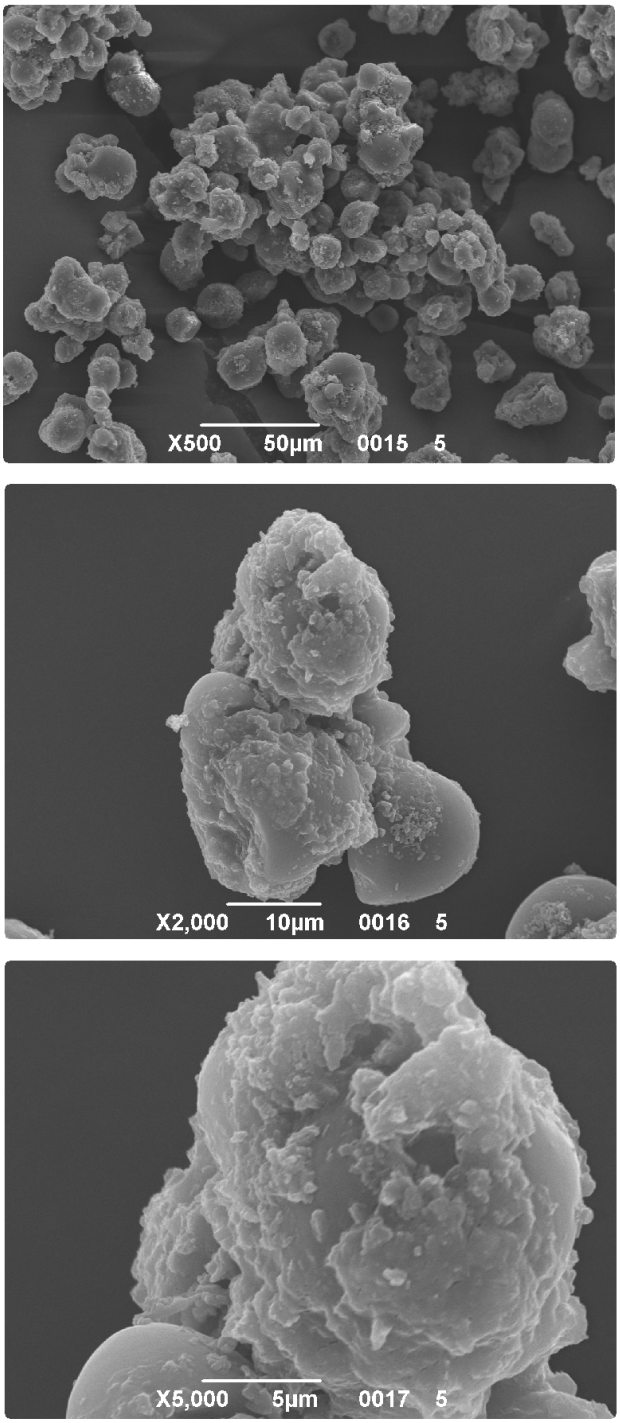

Figure 4.12: *Solid dispersion number 2, prepared with 1 g ibuprofen salt and 1 g tapioca. Photos taken with a scanning electron microscope with different enlargements, from top to bottom: 500 x, 2000 x, 5000 x.*

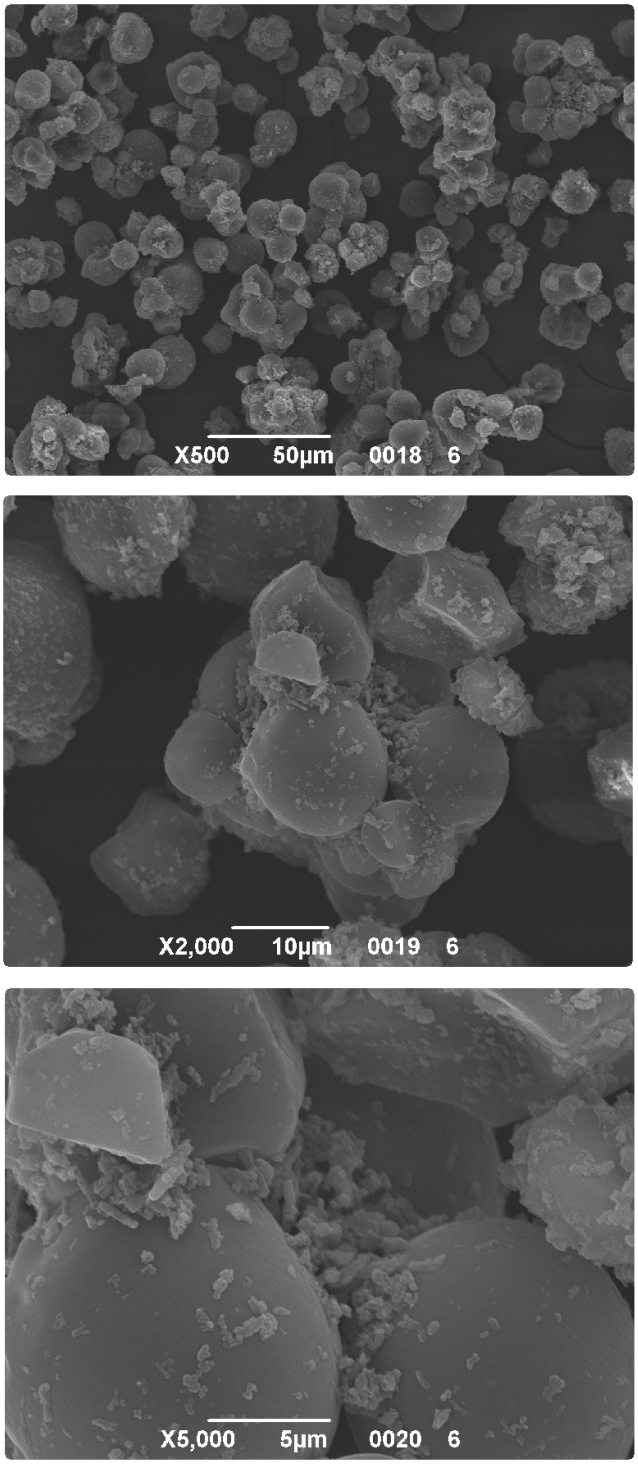

Figure 4.13: *Solid dispersion number 3, prepared with 1 g ibuprofen salt and 2 g tapioca. Photos taken with a scanning electron microscope with different enlargements, from top to bottom: 500 x, 2000 x, 5000 x.*

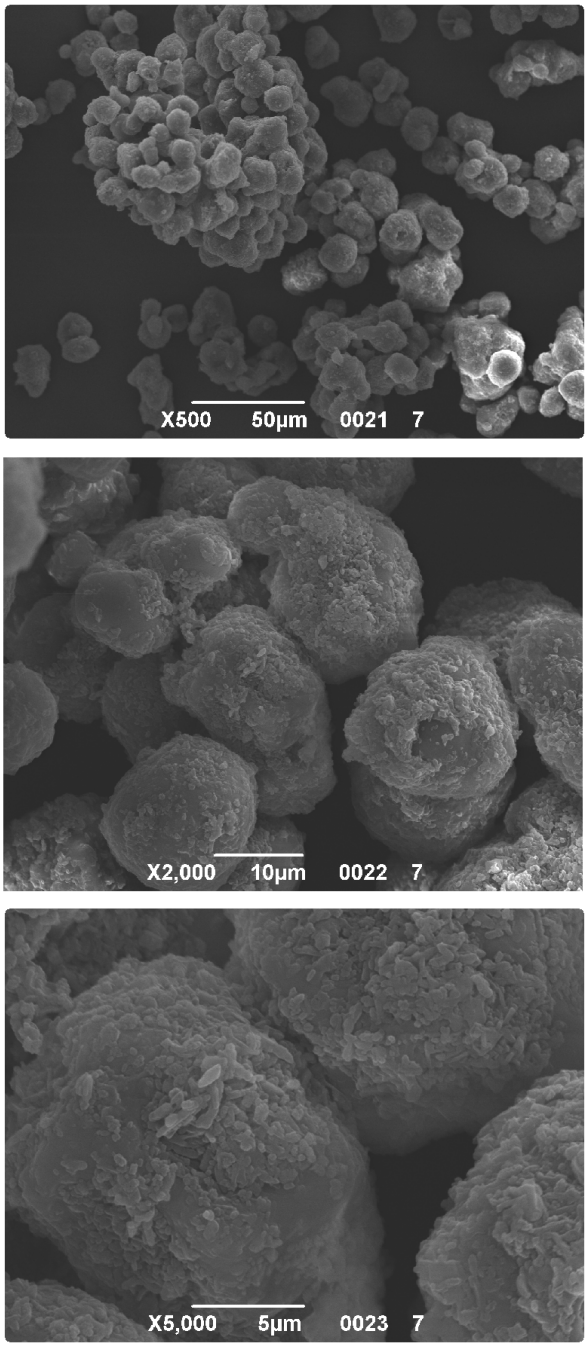

Figure 4.14: *Solid dispersion number 4, prepared with 1 g ibuprofen salt and 1 g corn starch. Photos taken with a scanning electron microscope with different enlargements, from top to bottom: 500 x, 2000 x, 5000 x.*

Conclusions

The aim of this thesis was to understand if starch is a suitable matrix for supercritical carbon dioxide impregnation of drugs.

Ibuprofen sodium salt and caffeine have been investigated as model drugs. Two starch types have been studied: tapioca and corn starch. Experiments have been carried out in a laboratory plant (vessels of 120 and 300 cm^3). Different operating variables have been considered for optimization, the most important are temperature, pressure and time of impregnation.

Caffeine is soluble in carbon dioxide and data have been retrieved from literature. For ibuprofen sodium salt, however, no data were available in literature. Experiments done for ibuprofen sodium salt revealed that:

- solubility in supercritical carbon dioxide is low, but specific data could not be collected with the available instrumentation;
- a melting point depression happens at 40°C and 70 bar.

The discovery of a melting point depression gave the idea for developing a solid dispersion technique, prepared with ibuprofen sodium salt and starch. This is considered as a technique different from impregnation, that could allow to treat pharmaceuticals and prepare dispersions, without using high temperature or organic solvents.

As long as starch impregnation is concerned, it is possible to draw the following conclusions:

- it is not possible to achieve satisfactory impregnation, for both the starches and drugs considered;
- impregnation is low, irrespective of drug solubility in carbon dioxide;
- a reason for these results is the low solubility of carbon dioxide inside the starch matrix: this means that swelling of starch matrix is not enough to allow good impregnation.

A meaningful result is that the best point found is 0.55% in weight, for caffeine impregnation at 60°C and 300 bar, after 14 hours of impregnation.

On the other hand, solid dispersion experiments gave good and promising results:

- operating variables have been optimized, in order to obtain a uniform dispersion;
- the optimized solid dispersion is composed by free flowing starch particles, covered with ibuprofen sodium salt;
- different mass fraction of drug in the pharmaceutical form have been achieved, up to 35%.

Further developments should consider the study of *in vitro* solubility kinetics for this compound. In fact, comparing with literature in this field, it is believed that this compound can improve drug releasing control and allow better bioavailability.

The presently proposed technique could be successfully extended to the process of other drugs, that present a melting point depression, when exposed to supercritical carbon dioxide.

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