

# PREPARATION AND EVALUATION OF DRUG/? - CYCLODEXTRIN SOLID INCLUSION COMPLEXES BY SUPERCRITICAL FLUID TECHNOLOGY

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Modern drugs are often lipophilic compounds. To act on the target structures, the drugs must be dissolved in physiological fluids and absorbed through entrance ports. Due to costs, convenience and compliance, oral application of solid forms is the preferential way. Since the bioavailability of orally applied drugs depends on the velocity of dissolution rate and absorption, methods to improve the dissolution are required. The complexation of drugs with cyclodextrins is known to improve the solubility, permeability and bioavailability. In this study, a Controlled Particle Deposition (CPD) method has been developed for the preparation of inclusion complexes ( $\beta$ -cyclodextrin) with non-polar drugs (ibuprofen) using supercritical carbon dioxide (scCO<sub>2</sub>) as an alternative to solvents. The complexes were prepared in an incubation vessel with separate chambers for the drug and the complexing agent. The resulting product was compared to complexes obtained by freeze-drying or coprecipitation. Differential scanning calorimetric (DSC) showed almost complete inclusion of ibuprofen in the scCO<sub>2</sub>-product and in the freeze-dried product but significant amounts of not complexed ibuprofen in the coprecipitated material. HPLC-analysis of the ibuprofen content in the complexes confirmed the almost complete inclusion of ibuprofen in  $\beta$ -cyclodextrin in the scCO<sub>2</sub>- and freeze-dried complex. In contrast, the coprecipitated complex appeared like a physical mixture with about 35 % of the total ibuprofen content outside the  $\beta$ -cyclodextrin. The inclusion rates obtained, however, were about 1:11 (mol) in the scCO<sub>2</sub>-complex but about 1:1 in the freeze-dried complex.

Taking together, the method developed for Controlled Particle Deposition is very useful for the production of highly pure  $\beta$ -cyclodextrin complexes, avoiding partitions of uncomplexed material. The efficacy of the process must still be improved.

## INTRODUCTION

According to the US Food and Drug Administration's (FDA's) Biopharmaceutics Classification System (BCS) (Table 1), drug products are classified into four groups based on the ability of a given drug substance to permeate biological membranes and its aqueous solubility [1]. Unfortunately, most new drugs exhibit poor solubility in water, or in other words, class II or class IV compounds according to the BCS and their absorption in the gastrointestinal tract are limited by their dissolution rate.

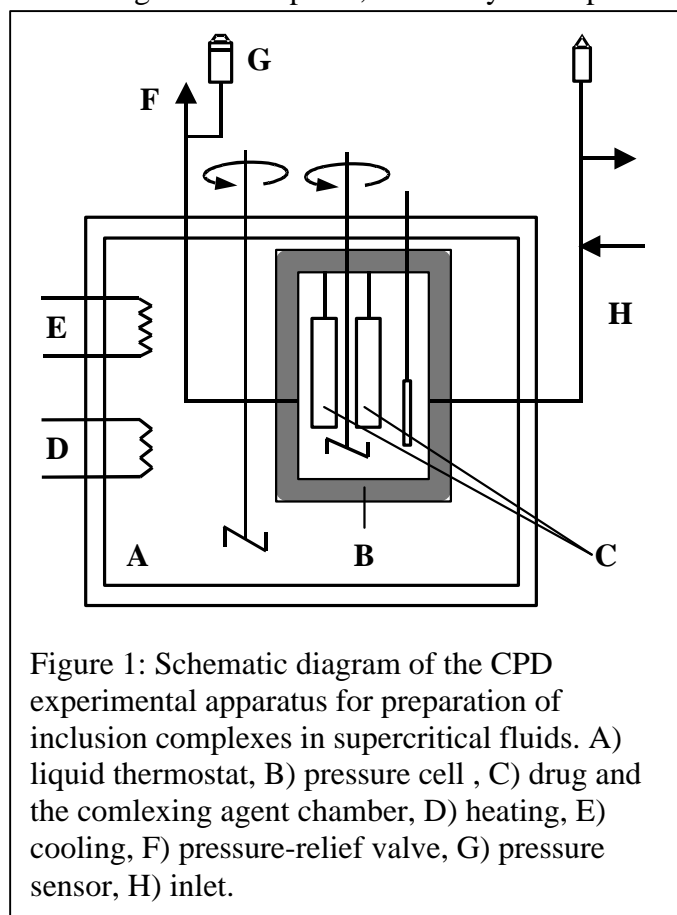
Table 1: The Biopharmaceutical Classification System

Class	Solubility	Permeability
I	High	High
II	Poor	High
III	High	Poor
IV	Poor	Poor

relatively hydrophobic central cavity and hydrophilic outer surface. The most common cyclodextrins are  $\alpha$ -cyclodextrin,  $\beta$ -cyclodextrin, and  $\gamma$ -cyclodextrin, formed by six, seven, and eight glucose units. Cyclodextrins are able to form inclusion complexes with many drugs by taking up the drug molecule or a hydrophobic moiety of the molecule into the cavity [2]. Through complexation with cyclodextrins, it is possible to move class II and even class IV drugs into class I (Table 1).

There are several methods for the preparation of a drug/CD inclusion complex such as, kneading, grinding, coprecipitation and freeze drying. Some of these methods use organic solvents as media, which can be found as residual in the product.

In this study, a Controlled Particle Deposition method has been developed for the preparation of inclusion complexes ( $\beta$ -cyclodextrin) with non-polar drugs (ibuprofen) using supercritical carbon dioxide ( $scCO_2$ ) without co-solvent as an alternative to aqueous and organic solvent. The key idea behind CPD is to dissolve the solute of interest in supercritical carbon dioxide, followed by permeation of the binary mixture into the pores and precipitation of the drug inside the pores, caused by a fast pressure drop.



Various methods have been used to increase the dissolution rate including micronisation, modification of the physicochemical properties of the drug, and complexation with cyclodextrins and their derivatives.

Cyclodextrins (CDs) are cyclic oligosaccharides of D-glucopyranose units  $\alpha$ -(1, 4) linked in a ring formation, containing a

## 1- MATERIALS AND METHODS

### 1.1 Materials

Ibuprofen 50 was generously supplied from Knoll Pharmaceuticals (UK).  $\beta$ -Cyclodextrin has been supplied by Wacker-Chemie GmbH (Germany), and carbon dioxide by Linde AG (Germany). All other materials and solvents were of the purest grade available.

### 1.2 Preparation of ibuprofen/ $\beta$ -CD inclusion complexes with CPD method

The experimental apparatus for preparing the complexes is illustrated in Figure 1. This apparatus allows investigations in a temperature range from 5 °C to 80 °C and pressure up to 60 MPa [3]. The inclusion experiments were carried out using a static incubation technique. In all

experiments, the drug (ibuprofen) and the carrier ( $\beta$ -cyclodextrin) were filled into separate cartridges inside the high-pressure cell. The system was then immersed in a constant temperature water bath. Prior to the inclusion experiments, the system was purged with low  $\text{CO}_2$  pressure in order to remove moisture and air. Thereafter, the required amount of liquid  $\text{CO}_2$  was condensed into the high-pressure cell and heated to the desired temperature. The temperature inside the high-pressure cell was measured with a calibrated Pt-100 thermometer within the total limit of  $\pm 30$  mK. A piezo-resistive pressure gauge was used to determine the system pressure. As soon as the selected pressure in the high pressure cell was reached, the time of contact was fixed to either 24 or 48 hours. At the end of the experiments, depressurization occurred within 15 minutes.

### *1.3 Preparation of ibuprofen/ $\beta$ -CD inclusion complexes with the coprecipitation method*

$\beta$ -Cyclodextrin in aqueous solution was mixed with a solution of ibuprofen in diethyl ether with a molar ratio of 3:1 and stirred for two weeks at 25 °C. At the end of the incubation period, the suspension was kept at 0 °C for 24 hours and finally the microcrystalline precipitate was filtered, washed with water and dried at 50 °C according to Kurozumi et al [4].

### *1.4 Preparation of ibuprofen/ $\beta$ -CD inclusion complexes with freeze-drying method*

$\beta$ -Cyclodextrin and ibuprofen were dissolved with molar ratio of 1:1 in aqueous ammonium solution. The solution was stirred for 15 min at 25 °C and thereafter frozen and lyophilised (Lyovac GT 2), according to Kurozumi et al [4].

### *1.2 Characterisation of the thermal behavior of the inclusion complexes*

Differential Scanning calorimetry (DSC) measurements were performed using a Mettler TA 8000 with a TAS 811 System and a DSC 820 measuring cell (Mettler Toledo, Germany). The samples (4-8 mg per run) were placed in perforated 40  $\mu\text{l}$  aluminium standard pans. The heating sequences were carried out within a temperature range from 25 to 200 °C, at heating rate of 10 °C/min, purging continuously with 20 ml/min nitrogen gas.

### *1.6 Determination of drug content*

The drug content of the complexes was analysed by HPLC-UV. The chromatographic experiments were performed with a Shimadzu LC-6A HPLC system, UV detector Shimadzu SPD-6A at 230 nm, integrator Shimadzu C-R6A (Shimadzu Europa, Germany), autosampler AS-200A (Merck/Hitachi, Germany), using a column (125 x 4) packed with Nucleosil 100-5 C18 (Macherey-Nagel, Germany) and mobile phase of acetonitrile/ 20 mM  $\text{K}_2\text{HPO}_4$  PH 2.5 (50:50, v/v) at the flow rate 1.5 ml/min. Injection volume was 20  $\mu\text{l}$ .

### *1.7 Preparation of the samples [5, 6]*

The “n-hexane wash” method was used to determine the amount of non-complexed drug. This method is based on the fact that the  $\beta$ -CD and its complexes are completely insoluble in n-hexane but the free, not complexed drug is soluble in n-hexane.

The drug content in the complexes was determined by dissolving the washed complex with a small amount of dimethylsulfoxide and filled up with acetonitrile. After 12 hours, the  $\beta$ -CD has sedimented and the supernatant was filtered. The concentration of ibuprofen in the wash hexane and in the  $\beta$ -CD complexes was analysed with HPLC techniques described above.

## 2- RESULTS AND DISCUSSION

It is well known that compressed fluids are able to decrease the melting point of organic substances. As reported by Charoenchaitrakool et al, ibuprofen exhibits melting point depression when contacted with sc-CO<sub>2</sub>. The melting point of ibuprofen decreased to 45 °C when contacted with CO<sub>2</sub> at 18 MPa [7]. Therefore our experiments were performed at 35 °C and 40 °C and pressures between 24 MPa and 28 MPa to ensure that only a solid-fluid two-phase equilibrium (S<sub>2</sub>=G) exists for each pressure.

The thermal behaviour of β-CD inclusion compounds was studied with DSC to confirm the formation of a solid complex by the disappearance of the endothermic melting peak of crystalline ibuprofen (Figure 2). Pure ibuprofen exhibits an endothermic melting peak at 77 °C. The DSC thermograph of β-CD shows a broad endothermic peak around 115 °C, corresponding to the release of water. In the thermal curve of the physical mixture, the characteristic endothermic melting peak of ibuprofen and the broad peak corresponding to the dehydration of β-CD were absorbed, making an inclusion of ibuprofen in the β-CD unlikely. The thermograph of the ibuprofen/β-CD complex prepared by coprecipitation displayed a melting peak at 77 °C which can still reflect the presence of drug crystals not included in the carrier in the preparation and a broad peak around 115 °C due to dehydration of the complexes. In contrast, the complete disappearance of the endothermic peak of free ibuprofen was observed for the CPD and freeze-drying method and attributed to a complete encapsulation of the drug in the β-CD [4, 8].

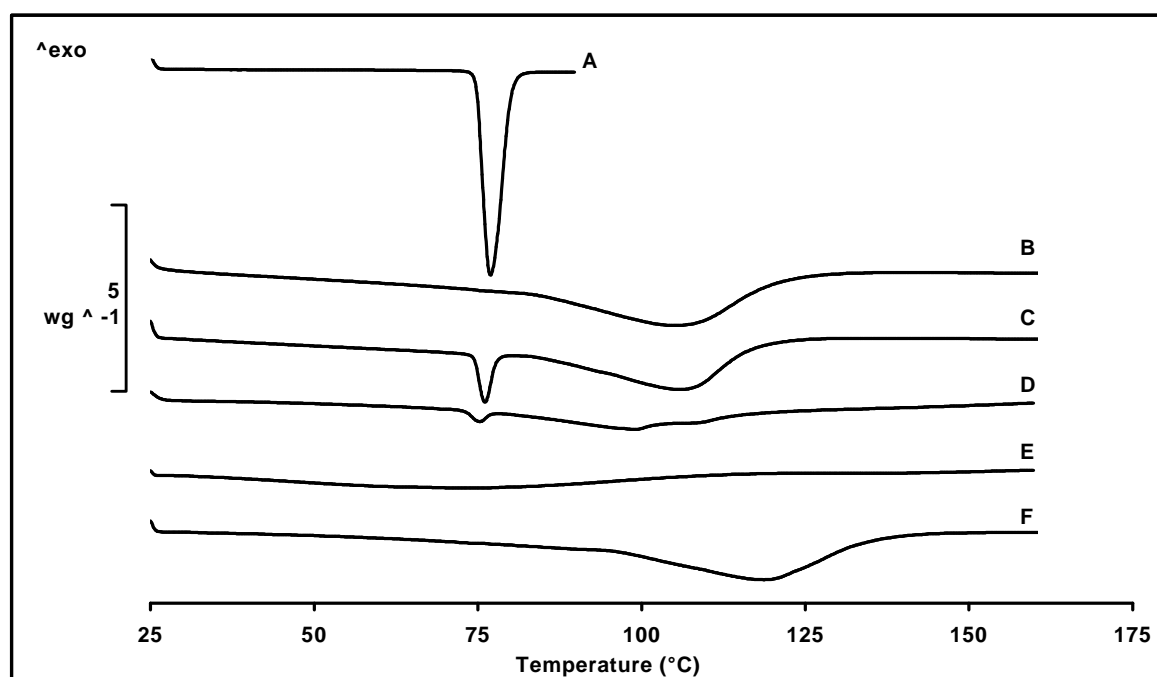


Figure 2: DSC thermographs: (A) pure ibuprofen, (B) pure β-CD, (C) ibuprofen/β-CD physical mixture, (D) ibuprofen/β-CD coprecipitation complex, (E) ibuprofen/β-CD freeze-dried complex, (F) ibuprofen/β-CD CPD complex performed at 24.4 MPa, and 39.5°C.

The effect of temperature, pressure, and the time of exposure on the inclusion yield is reported in Table 2. Our study demonstrated that the time of exposure had a positive but rather small influence, giving a better inclusion. The effect of the temperature was, however, much more prominent, while the pressure seems to have a rather small influence on the inclusion yields, our results corroborated some results of previous studies [7, 9].

Table 2: Effect of temperature, pressure, and exposure time on ibuprofen content in  $\beta$ -CD

	Temperature (°C)	Pressure (MPa)	Time of exposure (h)	Inclusion % (mol/mol)
a	34.5	25	48	14.34
	39.5	25	48	46.30
b	39.5	24.7	48	47.27
	39.5	27.8	48	51.48
c	39.5	24.7	24	42.50
	39.5	24.7	48	47.27

The complexed and non-complexed ibuprofen content was compared in CPD, freeze-dried and coprecipitated methods (Figure 3). The non-complexed part of ibuprofen was smaller for CPD and freeze-dried than for coprecipitation. In the last method the complex appeared like a physical mixture with about 35 % of the total ibuprofen content outside the  $\beta$ -cyclodextrin.

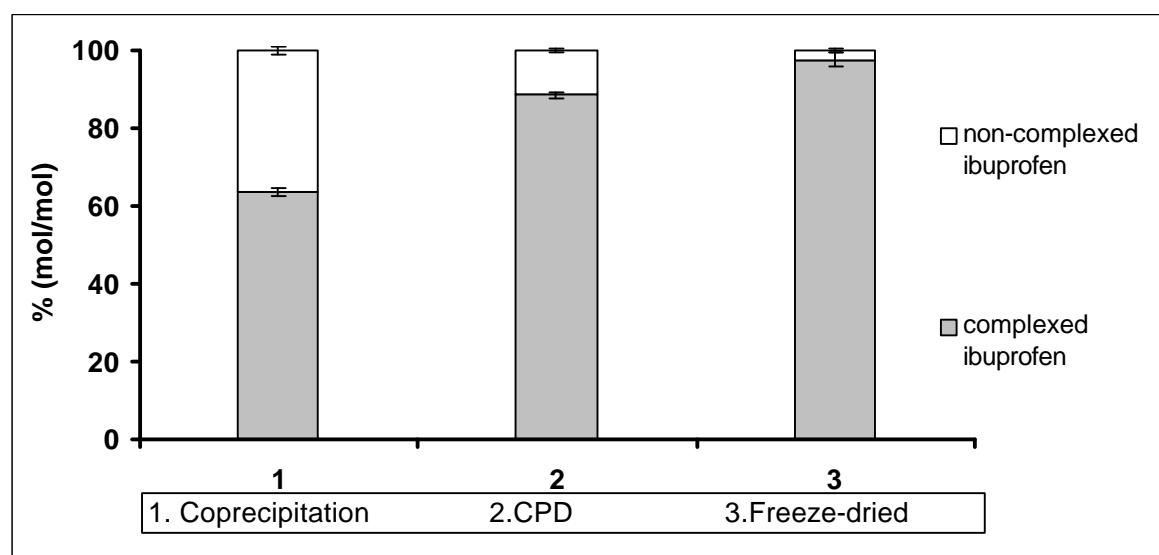


Figure 3: Complexed and non complexed ibuprofen content in ibuprofen/ $\beta$ -CD coprecipitation, CPD complex performed at 24.4 MPa, and 39.5°C, and freeze-dried methods.

## CONCLUSION

Using scCO<sub>2</sub>, an effective inclusion of a model drug – ibuprofen – was achieved by the CPD-method, avoiding the use of organic solvents or toxic additives (e.g. NH<sub>3</sub> in freeze drying) for the complexation. The CPD, in contrast to some conventional preparation procedures, gives an almost complete inclusion of the drug in the carrier without the appearance of a physical mixture. The CPD-method is, therefore, suitable for the production of β-cyclodextrin complexes to enhance the solubility and thereby the bioavailability of poorly soluble drugs. The method must, however, be further optimised and usefulness tested for other drugs too.

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