$SCCO_2$-ASSISTED PREPARATION OF OMEPRAZOLE/CYCLODEXTRIN INCLUSION COMPLEXES. COMPARATIVE STUDY WITH CONVENTIONAL METHODS

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Supercritical carbon dioxide is a very attractive medium for pharmaceutical processing as an alternative to aqueous and organic solvents. Many drug formulations use cyclodextrins since their lipophilic cavities provide microenvironments where drug molecules can enter and form inclusion complexes, leading to an increase in the solubility and stability of the drug. Omeprazole shows low stability and poor solubility in water. The aim of this study was to test the viability of preparing omeprazole/cyclodextrins inclusion complexes using dense carbon dioxide in order to enhance these parameters. The CO_2-assisted method was compared with kneading (KN), a conventional inclusion complex formation method. In addition, data were compared with the physical mixture, omeprazole with lactose instead cyclodextrin, and pure omeprazole. As omeprazole is a very sensitive drug and because in our first experiments in $scCO_2$ at 40ºC, we observed omeprazole degradation, we decided to test the viability of performing the inclusion complexes at low temperature, near the critical temperature of CO_2 ($scCO_2$) and also at room temperature using liquid CO_2 (LIQ CO_2). All powders obtained were kept at dark conditions and were analysed by DSC, FTIR and dissolution studies in order to compare the processing methods.

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides obtained from enzymatic conversion of starch containing mainly six (βCD), seven (αCD) and eight (γCD)-linked α-D-glucopyranose units [1]. There are several methods for the synthesis of CD-guest complexes depending on the properties of the included compound, such as, kneading, neutralisation, grinding, co-precipitation, heating in a sealed container and freeze-drying [2]. In the last years supercritical fluid technology has been intensively investigated in the formation of inclusion complexes between cyclodextrin and drugs. There is a good recent review on the processing of cyclodextrins using dense gas technology [3].

Omeprazole (OME) is a well-known antiulcerative drug that has been extensively used to control acid disorders by inhibiting the acid gastric secretion by blocking the H^+/K^+ ATPase pump. OME formulations show many pharmaceutical drawbacks connected to the physicochemical instability to heat, light, and acidic media, even with coated formulations [2,4]. In addition, the low aqueous solubility of OME is responsible for low dissolution rates and hence low bioavailability [1].

The main goal of the complexation procedure with cyclodextrins (CDs) is to improve the biopharmaceutical properties of drugs with poor water solubility and stability. The increase

in solubility improves the release/dissolution and consequent biovailability of the drug. β-Cyclodextrin (*β*CD) and some of its derivatives have attracted most of the experimental work found in literature, leading to several industrial applications, as they show water solubility and the ability to accommodate a variety of molecules in their inner cavities [5,6].
Different preparation techniques: kneading and CO_2-assisted methods were tested and compared. The powders obtained were characterized by differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The dissolution studies were also suported by the USP 32 method [7].

MATERIALS AND METHODS

1. MATERIALS

β-cyclodextrin (*β*CD; Mw = 1135.00), hydroxypropyl-β-cyclodextrin (HP*β*CD; Mw = 1387.15) and dimethyl-β-cyclodextrin (DM*β*CD; Mw = 1331.36; DS= 1.8), lactose (LAC; Mw = 342.30) and Omeprazole, 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulfinyl-1*H*-benzimidazole (OME) (Mw = 345.42) was kindly supplied by MEDINFAR, Portugal. Hydroxypropylmethyl cellulose (HPMC) capsule nº3 shells were purchased from Qualicaps Europe (Spain). Phosphate buffer (pH 6.8) solutions were used as dissolution media [8]. Carbon dioxide was obtained from Air Liquide with purity better than 99.998%.

2. PREPARATION OF INCLUSION COMPLEXES IN THE SOLID STATE

Solid systems were prepared with equimolar ratios of OME and CD, according to previous phase solubility studies, using distinct methods: kneading and supercritical and liquid carbon dioxide. In addition physical mixtures of omeprazole with CD and with LAC instead of CD, and pure omeprazole were also prepared as control formulations. All the final products were then allowed to equilibrate at controlled temperature and humidity and protected from light.

2.1. PHYSICAL (PM) AND REFERENCE MIXTURES

OME:LAC, OME:*β*CD, OME:HP*β*CD and OME:DM*β*CD were prepared in a ceramic mortar by simply blending of OME, *β*CD, HP*β*CD and DM*β*CD were used in 1:1 stoichiometric ratio.

2.2 KNEADED BINARY SYSTEMS (KN)

*β*CD, HP*β*CD or DM*β*CD were wetted in a ceramic mortar with alkaline aqueous solution water/ammonia (pH about 10.3 until a paste was obtained (about 50% of the total weight of CD and OME). The required amount of OME was then slowly added and stirred, for about 45 min, until obtaining powder. In this process an appropriate quantity of alkaline aqueous solution was added in order to maintain a suitable consistency.

2.3 CO_2-ASSISTED METHOD (FSC)

The controlled particle deposition (CPD) process was performed in a 11 mL hig-pressure view cell, equipped with two sapphire windows that allow full observation of the inside. Powders of inclusion complexes CD + drug were prepared by mixing vigorously a mixture CD + drug (1:1) and 100 µL of deionized water at high-pressure followed by a rapid rate of depressurization. The cell was then immersed in a thermostatted water bath set to 32 ºC, with stability ± 0.01 ºC. Temperature control was made through a RTD probe contacting the cell, connected to a Hart Scientific PID controller. Carbon dioxide was added up to 25 MPa. The experiments with liquid CO_2 were carried out at 18 ºC. CO_2 was added till liquid CO_2 filled

the cell. Stirring was achieved by means of a Teflon coated magnetic bar. With stirring the cell obtained a milky-appearence. The experiments proceeded for 3 hours. Water was added to destabilize the water inside the CD cavity and improve inclusion [8].

3. CARACTERIZATION

3.1. THERMAL ANALYSIS

Differential scanning calorimetry (DSC) measurements were carried out in a Setaram (Model DSC 131) equipment. The thermal behaviour was studied by heating the samples (around 10 mg) in a sealed aluminium pan from ambient temperature to 300 ºC at 5 ºC/min under dry nitrogen atmosphere.

3.2. FOURIER TRANSFORM-INFRARED SPECTROSCOPY (FTIR)

Spectra were recorded using a Nicolet spectrometer, model Impact 400, USA, associated to a software Ominic 2.1 Nicolet Instrument Corp. The samples were prepared in KBr disks method and spectra acquisitions were performed directly in powder samples with the application of 256 scans, at a resolution of 16 cm^{-1} over the range 4000–400 cm^{-1}.

3.3. SCANNING ELECTRON MICROSCOPY (SEM)

The morphology of the raw materials (OME, βCD, HPβCD and DMβCD) and binary systems was characterized using scanning electron microscopy (SEM) in a Hitachi S-2400 equipment, with an accelerating voltage set to 15 kV. Samples were mounted on aluminium stubs using carbon tape and were gold coated.

3.4. DISSOLUTION STUDIES

The dissolution profiles of the inclusion complexes were performed using a dissolution apparatus (Sotax model AT7, Switzerland) following USP procedures [7]. The dissolution media consisted of 900 mL of phosphate buffer solution (pH 6.8), previously prepared and maintained at 37 ± 0.5 ºC during the experiments. The assay was performed according to the USP paddle method at 50 ± 2 rpm. The drug released was quantified spectrophotometrically in a UV/Vis spectrophotometer (U-200, Hitachi, Japan) set at 300 nm, at predetermined times (10, 20, 30, 60, 90, 180 min). The cumulative fraction of the drug released was calculated from the total amount of OME and plotted as a function of time (n = 3). An accurate amount of each powder mixture (control, PM, KN, $scCO_2$ and LIQ CO_2) was manually filled into the HPMC capsule body. For the dissolution studies under sink conditions one capsule was placed in each vessel. Data from OME release was analyzed using two dissolution parameters, the amount released at certain times (M_{tmin}) and the similarity factor (f_2) using the OME:LAC formulation as reference [9].

RESULTS

Figure 1 shows the SEM images of the obtained powders corresponding to the physical mixture and kneading, $scCO_2$ and Liq CO_2 processes. An obvious conclusion is that CO_2 (liquid and supercritical) was probably able to decrease the melting point of the substituted cyclodextrins since the images show the cyclodextrin with a morphology consistent with previous melting or dissolution and precipitation. The SEM image of the HPβCD complex prepared in liquid CO_2, shows a completely different morphology with large particles of cyclodextrin loaded with sub-micron sized OME particles precipitated inside.

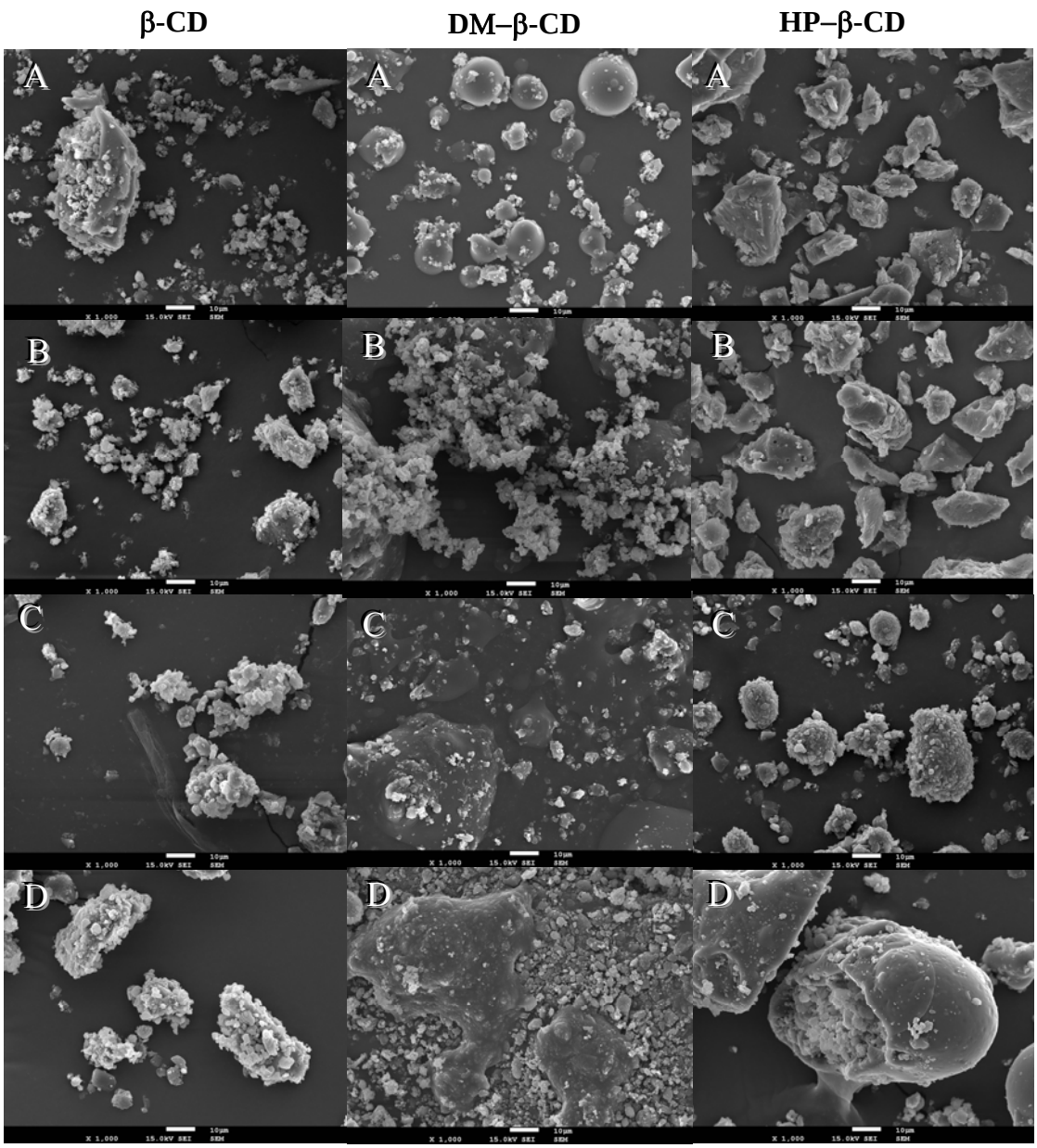

Figure 1: SEM images of the obtained powders: A) PM; B) KN; C) $scCO_2$; D) LIQ CO_2

The DSC curves of the binary system (OME:*β*CD, OME:DM*β*CD and OME:HP*β*CD) complexes reveal a complete different profile compared to CDs or OME alone.

DSC runs are reported in Figure 2. For OME (a), a single sharp endothermic effect corresponding to the melting of the drug was observed at 158.8 ºC, with a peak at 160.2 ºC. This effect was immediately followed by an exothermic peak related to the decomposition of the drug at 162.8 ºC: peak at 172.3ºC [3].

The endothermal effect of CDs was observed in the range of 50–150 °C, with different peak values depending on the cyclodextrin, which can be attributed to the partial dehydration process of the CD cavities [10].

The shape of the CDs endotherm has changed in all powders containing complexes, which indicates the existence of the OME inside the cavity of the CDs. This is due to the dehydration of their cavity through the substitution of the water molecules by the OME [10].

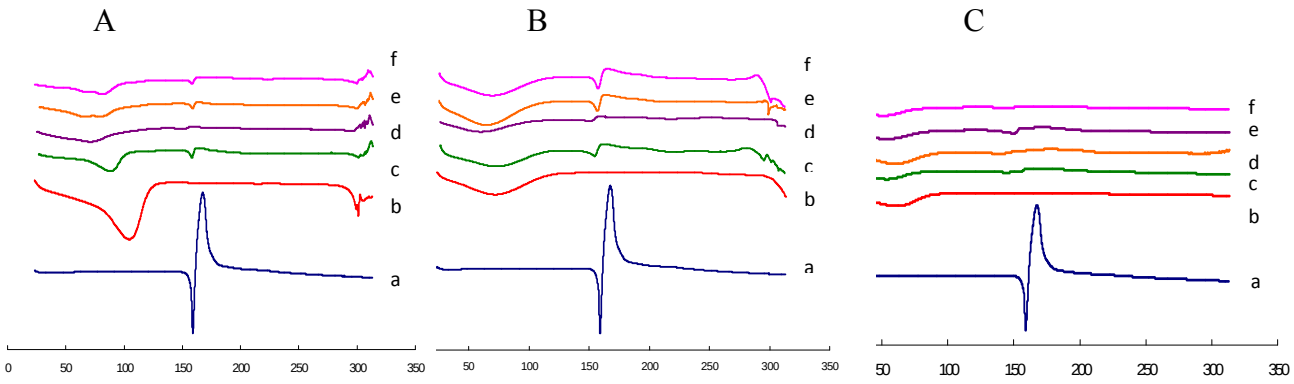

Figure 2. DSC curves of the OME:CD samples obtained: a) Omeprazole, b) Cyclodextrin (A-βCD, B-DMβCD, C- HPβCD); c) PM d) KN e) OME:CD $scCO_2$; f) OME: CD LIQ CO_2.

Figure 3 shows the FITR spectra for the prepared complexes. As it can be seen there are marked structural differences between pure components and the association complexes, which accounts the functional groups of OME involved in the complexation as shown for the functional groups C=C-N and S-C=N stretching link vibrations (at 1629.63 cm^{-1}) and Ar-C-OCH_3 vibration (at 1207.41 cm^{-1}).

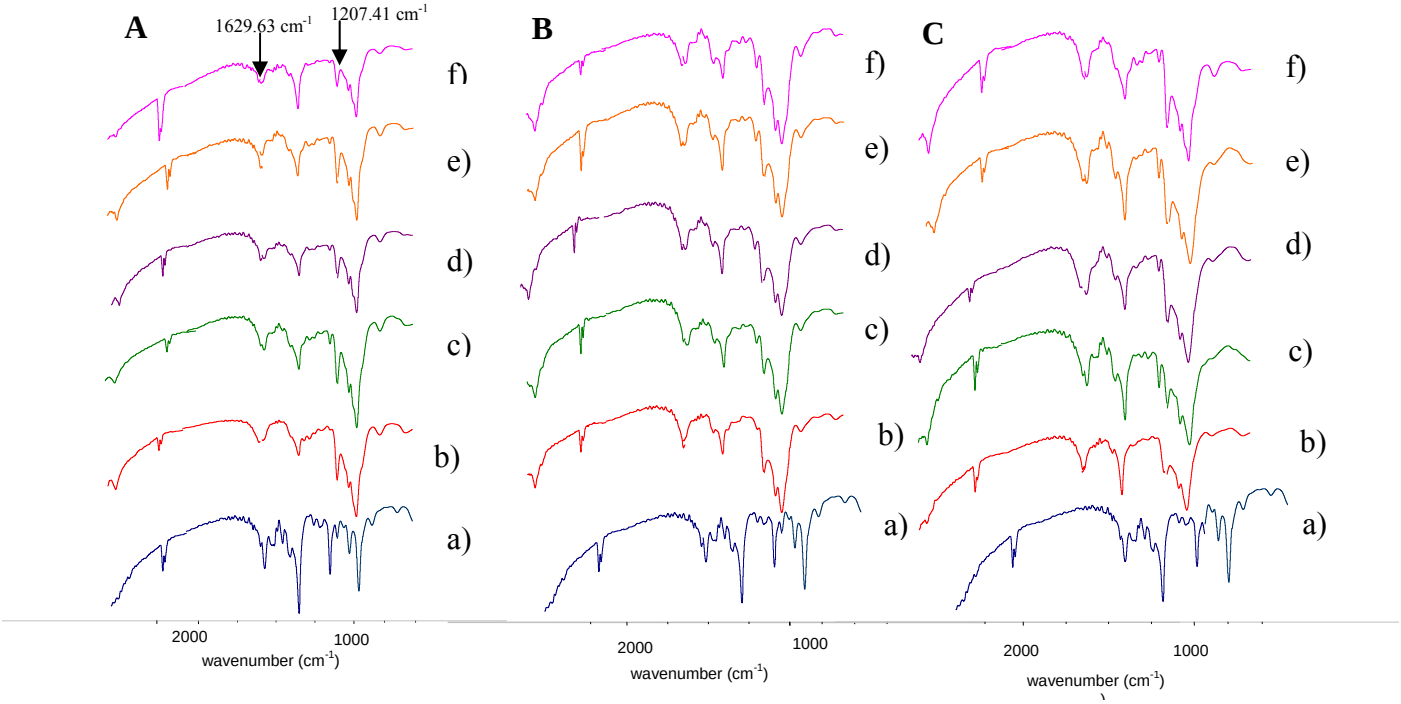

Figure 3: FTIR spectra of the drug, cylodextrin and binary mixtures: a) Omeprazole; b) Cyclodextrin (A-βCD, B-DMβCD, C-HPβCD); c) PM d) KN e) OME:CD $scCO_2$; f) OME: CD LIQ CO_2.

In addition a change on the resonance band at 1077 cm^{-1} assesses the interaction between CD and the guest molecule (OME) in the solid state [4]. The intensity of the absorption band at 1625 cm^{-1} decreased on binary systems when compared to the spectra recorded for the OME. Figure 4 shows, as an example, the dissolution profile for the βCD systems, where higher rates of OME release from the complexes prepared at high-pressure CO_2 were obtained. The OME:DMβCD LIQ CO_2 was the complexe that showed the best rate of release from all the complexes prepared.

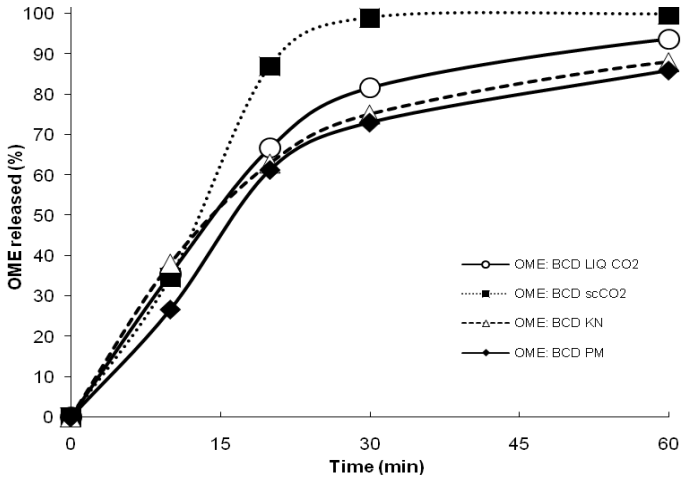

Figure 4: Dissolution profile for the βCD systems prepared by the different methods.

Data from release/dissolution of OME are presented in Tables 1, 2, 3. There is a relevant difference on the dissolution values for the first sampling time (10 min) that may be justified by the difficulties on the capsules disintegration (lower values) and by the existence of inclusion complexes (higher values).

Table 1: Results of dissolution βCD

	M_{tmin} (%) [a]			Similarity factor
	10	30	60	f_2 (%)
OME:LAC	15.50 ± 9.97	90.49 ± 3.02	98.33 ± 1.97	-
LIQ CO_2	35.45 ± 8.87	81.62 ± 18.27	93.74 ± 11.30	51.34
$scCO_2$	34.73 ± 6.32	99.12 ± 2.13	100.44 ± 0.14	48.35
PM	26.73 ± 13.74	73.04 ± 12.08	85.94 ± 8.69	48.32
KN	38.12 ± 8.34	75.10 ± 8.38	88.05 ± 2.19	43.40

a) mean ± sd (n = 3)

Table 2: Results of dissolution DMβCD

	M_{tmin} (%) [a]			Similarity factor
	10	30	60	f_2 (%)
OME:LAC	15.50 ± 9.97	90.49 ± 3.02	98.33 ± 1.97	-
LIQ CO_2	43.34 ± 1.19	100.75 ± 5.07	100.94 ± 2.10	36.79
$scCO_2$	13.28 ± 10.53	77.82 ± 0.19	85.60 ± 0.08	47.53
PM	6.57 ± 4.89	85.22 ± 9.88	100.24 ± 0.29	53.04
KN	3.36 ± 2.59	100.36 ± 0.44	100.55 ± 0.18	57.49

a) mean ± sd (n = 3)

Table 3: Results of dissolution HPβCD

	M_{tmin} (%) [a]			Similarity factor
	10	30	60	f_2 (%)
OME:LAC	15.50 ± 9.97	90.49 ± 3.02	98.33 ± 1.97	-
LIQ CO_2	1.83 ± 0.68	56.89 ± 4.22	98.33 ± 6.08	29.11
$scCO_2$	3.31 ± 4.29	75.92 ± 14.05	77.29 ± 2.89	44.75
PM	24.97 ± 12.20	100.36 ± 0.24	100.45 ± 0.38	52.50
KN	6.46 ± 1.80	67.60 ± 6.22	88.74 ± 5.28	39.65

a) mean ± sd (n = 3)

Dissolution data obtained at 30 and 60 minutes for PM and KN are lower than expected, possibly due to drug degradation during the preparation procedure. Also a complete dissolution of OME was obtained for some of the samples prepared using high pressure technology (OME:βCD $scCO_2$, OME:DMβCD LIQ CO_2, OME:HPβCD LIQ CO_2). Compared to the kneading tecnique, all the samples prepared in liquid CO_2 showed higher dissolution rates within approximately 60 min.
The preparation of βCD and HPβCD complexes using the kneading technique shows f_2 values lower than those of the other preparations, which is probably due to degradation of OME promoted by the mixture preparation in a ceramic mortar. Also the OME:βCD $scCO_2$ and OME:DMβCD $scCO_2$ mixtures show enhancement of OME dissolution rate with respect to OME alone, and have good f_2 values.

CONCLUSIONS

The results obtained by DSC, FTIR and SEM have been used to study the inclusion complexes formed between OME and CDs (βCD, HPβCD or DMβCD) in solid form. The interactions between OME and CD strongly suggest the formation of inclusion complexes, which can lead to the improvement of the physico-chemical properties (solubility and consequently bioavailability) of the guest molecule (OME). Dense CO_2 seems to be a promissing medium to develop inclusion complexes of very sensitive drugs.

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