INCLUSION OF MICONAZOLE INTO CYCLODEXTRINS BY MEANS OF SUPERCRITICAL CARBON DIOXIDE: INFLUENCE OF THE ADDITION OF AN ACIDIC TERNARY COMPOUND

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By means of supercritical carbon dioxide, inclusion complexes can be obtained at the solid state. In this paper, inclusion complexes formed between miconazole, an antifungal drug with a basic character and a poor aqueous solubility, and several cyclodextrins are studied. The experiments were performed with both miconazole base and nitrate. The physical mixture was processed with supercritical carbon dioxide at 125°C and 30 MPa during 60 minutes. The influence of the addition of an acidic ternary compound (citric acid, fumaric acid, maleic acid and malic acid) has been evaluated. The inclusion yields are measured by the differential solubility method and by an HPLC method. The results show that miconazole base gives better inclusion yields than miconazole nitrate. The use of both an hydroxypropyl cyclodextrin derivatives and an acidic ternary compound enhances the inclusion yield.

INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides produced by an enzymatic degradation of starch by a glucosyltransferase derived from Bacillus macerans [1]. The most common compounds are the a-, ß- and ?-cyclodextrins, containing 6, 7 and 8 D-glucopyranose units, respectively, bound by a(1-4)-glycosidic linkages. Their structures present a toroidal shape. Their apolar cavity is able to include some large organic molecules by non-covalent interaction forces (hydrogen bounds, Van der Waals forces). The complex formed by CDs and their derivatives may modify the stability, solubility and bioavailability of the guest.

Miconazole (1-2-((2,4 dichlorophenyl)-2-(2,4-dichlorophenyl)-methoxy)ethyl)-1-imidazole) is an antifungal drug wich presents a poor water solubility. Several CDs (β-CD, HP-βCD, SBE-7-β-CD, γ-CD, HP-γ-CD,…) have been used to complex miconazole into their cavity [2, 3, 4, 5]. The influence of a ternary acidic compound was studied in solution and the results show a synergic effect between the CDs and the acid on the miconazole solubility, this effect being independent of the pH value.

Since a few years, supercritical carbon dioxide (SC CO_2) has been successfully used by authors for the preparation of inclusion complexes with CDs. Van Hees et al. were able to prepare inclusion complexes between piroxicam, an anti-inflammatory non steroidal drug, and ß-CD by processing of a physical mixture with SC CO_2 (150°C, 45 MPa during 180 minutes) [6].

The aim of the present work is, firstly, to prepare inclusion complexes between miconazole and several CDs (ß-, HP-ß-, ?- and HP-?-CD) and secondly, to determine the influence of an acidic ternary compound on the inclusion yield.

EXPERIMENTALS

MATERIALS

Miconazole base (Eur. Ph. 4th Edition) has been obtained from Janssen Pharmaceutica (Beerse, Belgium). Miconazole Nitrate (Eur. Ph. 4th Edition) was purchased from Bufa (Uitgeest, Holland), ß-cyclodextrin (Eur. Ph. 4th Edition, 3.31% of H_2O) and HP-ß-cyclodextrin (3.22 % H_2O) are from Roquette (Lestrem, France). ?-cyclodextrin (4.25 % H_2O) and HP- ?-cyclodextrin (1.62 % H_2O) are from Wacker Chemie GmbH (München, Germany). CO_2 was of quality N48 from Air Liquide (Liège, Belgium). All other products were of analytical grade.

METHODS

Preparation of inclusion complexes

The inclusion experiments with supercritical carbon dioxide were realized with a SUPREX SF Extractor Autoprep 44 (Pittsburgh, PA, USA) (figure 1). A 1 ml vessel is filled with 400 or 600 mg of physical mixtures of miconazole-CD 1:1 (mol:mol) or miconazole-CD-acid 1:1:1 (mol:mol:mol). The content is pressurized (in +/- 1 minute) and left in a static mode during 60 minutes. At the end of the experiment, the vessel is depressurized within 15 seconds. The vessel content is emptied, ground and homogenized in a mortar.

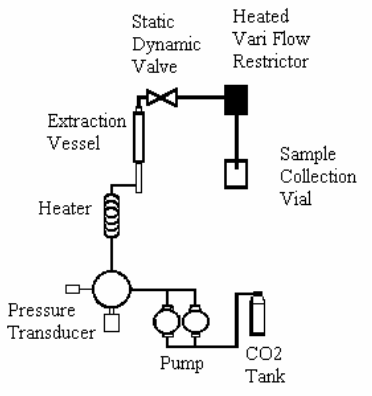

Figure 1 Schematic diagram of the equipment for the preparation of the inclusion complexes.

Assay of miconazole

Miconazole was assayed by an HPLC method. The HPLC system consists in a L-6000 Merck-Hitachi pressure pump, a L-7200 Merck-Hitachi autosampler, a L-7350 Merck-Hitachi column oven, a L-7400 Merck-Hitachi UV detector and a D-7000 interface. The system is

controlled by a computer running the “HPLC System Manager v 4.0” acquisition software developed by Merck-Hitachi.

Twenty-µl samples were injected on a Lichrocart column (125 x 4 mm i.d.) prepared with an octadecylsilane (C8) phase Lichrospher 60 RP-Select B 5 µm (Merck) and maintained at 30°C. The mobile phase consisted in a 70:30 (v/v) mixture of methanol (HPLC grade) and a 0,05 M acetate ammonium buffer pH 3.5. The flow rate was adjusted at 1.0 ml/min. All the samples were analyzed in duplicate. The detector was set at 230 nm. The present method was validated [7] and showed good linearity, reproducibility and accuracy between 5 and 80 µg/ml.
The limits of detection (LOD) and quantification (LOQ) were determinated and found to be 0.4 µg/ml for the LOD and 1.33 µg/ml for the LOQ.

Inclusion yield determination

The miconazole inclusion yield was measured using a differential solubility method [8]. It is given by the following equation:

$$\text{Inclusion yield} = \frac{[\text{total miconazole content}] - [\text{free miconazole content}]}{[\text{total miconazole content}]}$$

The total miconazole content is determined by dissolving an exactly known quantity of miconazole complex in the HPLC mobile phase. The free miconazole content is measured by dispersing an exactly known quantity of miconazole complex in acetonitrile. The suspension is sonicated (Transsonic 460) for five minutes, filtered through a Millex-GV 0.22 µm filter (Micropore) and analyzed by HPLC after appropriate dilution.

RESULTS

Previous works show that miconazole is stable and soluble in SC CO_2. In this work, with the aim to compare the ternary compound influence, the SC CO_2 processing conditions were fixed and so, the physical mixtures were processed with SC CO_2 at 30 MPa, 125°C during 60 minutes in all experiments.

Table 1 shows the results for the binary mixtures: inclusion yields with miconazole base are more important than those obtained with miconazole nitrate. Indeed, in these supercritical conditions, the solubility is higher for the miconazole base. The inclusion yields increase for both miconazole types from ß-CD to HP-?-CD. The larger cavity size and the hydroxypropyl substituent promote miconazole inclusion.

Table 1 miconazole inclusion yields in function of both miconazole and CD types (n = 3, all concentrations expressed in miconazole base)

CD type	Miconazole type	Total Miconazole Content +/- SD (%)	Free Miconazole Content +/- SD (%)	Inclusion yield (%)
ß-CD	NO_3	24.27 +/- 1.54	23.69 +/- 1.37	2.39
	Base	22.60 +/- 0.51	21.95 +/- 1.07	2.88
HP-ß-CD	NO_3	21.47 +/- 0.26	20.46 +/- 0.61	4.70
	Base	23.13 +/- 0.13	22.09 +/- 0.34	4.49
?-CD	NO_3	20.97 +/- 0.68	19.76 +/- 0.50	5.78
	Base	23.66 +/- 0.33	17.70 +/- 0.22	25.19
HP-?-CD	NO_3	19.03 +/- 0.23	14.55 +/- 0.46	23.54
	Base	18.88 +/- 1.15	11.93 +/- 0.65	36.81

As shown in table 2, citric acid increases the miconazole inclusion yield. This effect clearly appears when hydroxypropyl CD derivatives are used. Moreover, it is more pronounced for miconazole base than for miconazole nitrate, because the latter is protonated on the imidazole ring and is less reactive than miconazole base. For example, the miconazole base/HP-?-CD/citric acid combination gives an inclusion yield of about 80%, the addition of this acid having a positive influence.

Table 2 miconazole inclusion yields in presence of citric acid, in function of both miconazole and CD types (n = 3, all concentrations expressed in miconazole base)

CD type	Miconazole type	Total Miconazole Content +/- SD (%)	Free Miconazole Content +/-SD (%)	Inclusion yield (%)
ß-CD	NO_3	21.40 +/- 0.51	21.07 +/- 0.68	1.54
	Base	19.88 +/- 0.32	19.78 +/- 0.26	0.50
HP-ß-CD	NO_3	19.65 +/- 0.42	17.61 +/- 0.39	10.38
	Base	20.46 +/- 1.50	9.52 +/- 0.44	53.47
?-CD	NO_3	19.45 +/- 0.14	16.77 +/- 0.73	13.78
	Base	21.56 +/- 0.28	18.61 +/- 0.22	13.68
HP-?-CD	NO_3	17.03 +/- 0.22	13.32 +/- 0.34	21.79
	Base	17.77 +/- 0.26	3.94 +/- 0.02	77.83

In order to estimate the effect of the acidic compound conformation, fumaric acid, maleic acid and malic acid were used. The results are shown in table 3 for fumaric acid, in table 4 for maleic acid and in table 5 for malic acid. When the double bound conformation is CIS (maleic acid), the interactions between the three CD inclusion complexes components are higher than those when the conformation is TRANS (fumaric acid). Malic acid gives better results than those obtained with citric acid, maybe because the malic acid structure is less sterically hindered than the citric acid structure, so the interactions seem to occur more easily.

Table 3 miconazole inclusion yields in presence of fumaric acid, in function of both miconazole and CD types (n = 3, all concentrations expressed in miconazole base)

CD type	Miconazole type	Total Miconazole Content +/- SD (%)	Free Miconazole Content +/- SD (%)	Inclusion yield (%)
ß-CD	NO_3	22.19 +/- 0.11	22.06 +/- 0.17	0.06
	Base	22.69 +/- 0.10	22.74 +/- 0.05	8.59
HP-ß-CD	NO_3	20.33 +/- 0.20	17.74 +/- 0.23	12.74
	Base	20.36 +/- 0.19	18.54 +/- 0.14	8.94
?-CD	NO_3	21.20 +/- 1.61	20.08 +/- 1.13	5.28
	Base	20.88 +/- 0.21	19.00 +/- 0.32	9.00
HP-?-CD	NO_3	18.17 +/- 0.30	14.28 +/- 0.33	21.41
	Base	17.91 +/- 0.43	12.19 +/- 0.13	31.94

Table 4 miconazole inclusion yields in presence of maleic acid, in function of both miconazole and CD types (n = 3, all concentrations expressed in miconazole base)

CD type	Miconazole type	Total Miconazole Content +/- SD (%)	Free Miconazole Content +/- SD(%)	Inclusion yield (%)
ß-CD	NO_3	21.75 +/- 0.40	21.89 +/- 1.51	-0.06
	Base	20.88 +/- 0.61	18.61 +/- 0.84	10.87
HP-ß-CD	NO_3	19.93 +/- 0.43	14.47 +/- 1.51	27.39
	Base	18.06 +/- 0.58	9.64 +/- 0.61	46.62
?-CD	NO_3	19.83 +/- 0.23	17.54 +/- 0.55	10.55
	Base	17.94 +/- 0.70	12.84 +/- 0.28	28.43
HP-?-CD	NO_3	17.95 +/- 0.62	11.99 +/- 0.44	33.20
	Base	17.09 +/- 0.23	7.14 +/- 0.05	58.22

Table 5 miconazole inclusion yields in presence of malic acid, in function of both miconazole and CD types (n = 3, all concentrations expressed in miconazole base)

CD type	Miconazole type	Total Miconazole Content +/- SD (%)	Free Miconazole Content +/- SD(%)	Inclusion yield (%)
HP-ß-CD	NO_3	20.77 +/- 0.27	18.23 +/- 0.77	12.23
	Base	20.88 +/- 0.67	10.27 +/- 0.14	50.81
HP-?-CD	NO_3	19.59 +/- 0.09	15.27 +/- 0.50	22.05
	Base	19.86 +/- 0.42	2.32 +/- 0.01	88.32

In figure 2, all the results are summarized with the aim to appreciate more easily the influence of the mixture composition on the miconazole inclusion yield.

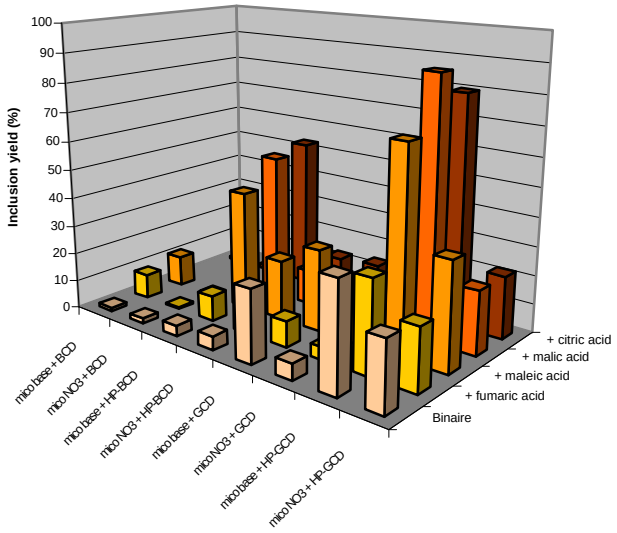

Figure 2 Miconazole inclusion yields in function of miconazole, CD and acid types

CONCLUSIONS

These experiments confirm the ability of SC CO_2 to promote the formation of inclusion complexes and the possibility of an acidic ternary compound to influence favourably the inclusion yield. The acidic compund acts by formation of hydrogen bounds between the CD and the miconazole, this kind of interaction being previously shown by a NMR method for a ketoconazole-ß-CD-tartaric acid complex [9].

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