Preparation and Characterization of Imazalil / b-Cyclodextrin Inclusion Complex by Supercritical Carbon Dioxide and ^{13}C Cp-Mas and ^{1}H NMR Spectroscopy: Preliminary Results

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ABSTRACT

Imazalil, a selected fungicide, was inclused into β-cyclodextrin using supercritical carbon dioxide. The best experimental conditions were determined to prepare the insertion complex, which was investigated by means of ^{1}H NMR in aqueous solution and ^{13}C CP-MAS NMR in the solid state. A comparative analysis of the spectra of the free βCD, IMZ and βCD-IMZ gave information on structural properties of the βCD-IMZ complex.

INTRODUCTION

Imazalil (IMZ), one of the few fungicides permitted in the post-harvest treatment of fruits [1-3], is poorly soluble in water (0.018g/100mL), that reduces notably its antifungal activity in the agricultural applications. To improve the solubility in water, IMZ was microencapsulated in β-cyclodextrin (βCD) by using supercritical carbon dioxide (SCF).

Generally, to prepare βCD-drug inclusion complexes several preparation methods in solution are available [4-6]. However, many of these methods are time-consuming and need multistage processing, involving evaporation of large volumes of solvent, which often occurs in the complex as a residue.

SCF approch permits to avoid such disadvantages and, in particular, to improve the purity of the βCD-IMZ complex, being moreover a well known environmental sustainable method [7-10].

Information on the stoichiometry and structure of this inclusion complex was obtained using ^{1}H NMR in aqueous solution and ^{13}C CP-MAS.

MATERIAL AND METHODS

Materials

β-CD (CAVAMAX ®7 PHARMA) was obtained from Wacker – Chemie Italia SpA and was used as received; CO_2 (purity 99 %) was supplied by SIO (Società Italiana Ossigeno, Cagliari, Italy), while IMZ (97%) was purchased from Dr. Ehrenstorfer (Augsburg, Germany) and used without further purification.

SCF Apparatus

Supercritical CO_2 inclusions were performed in a laboratory apparatus equipped with a 400 cm^3 autoclave, thermostated and supercritical CO_2 pressurized by a high pressure diaphragm pump (Lewa, model EL 1) with a maximum capacity of 6 kg/h, pumped liquid CO_2 at the desiderate pressure. Temperatures and pressures along the extraction apparatus were measured by thermocouple and Bourdon-tube test gauges, respectively. Pressure was regulated by high pressure valves under manual control [11].

The apparatus has been used in the batch mode. The autoclave was charged with about 20 g of βCD and with the guest compound at a molar ratio of 1:2.5. Then, thermostated and pressurized with CO_2 at the fixed constant value for the prefixed time of contact. Then a rapid drop of pressure permitted to vaporized CO_2 and to separate the solid deprived of solvent.

Process parameters of the SCF preparations

The following equilibrium is obtained when one drug molecule (D) forms a 1:1 complex with βCD:

$$\beta CD + D \Leftrightarrow \beta CD\text{-}D \qquad (K_{1:1})$$

where βCD-D is the insertion complex and $K_{1:1}$ is the apparent constant of the complex. The complexation efficiency (CE) can be defined as the [βCD-D]/[βCD] molar ratio in the solution. If the solution is saturated with the drug, the concentration of the free drug ([D]) will be equal to the intrinsic solubility of the drug (S_0).

NMR experiments.

^{1}H NMR spectra of the β-CD/IMZ complex in D_2O were recorded in 5 mm tubes on a Varian VXR-300 spectrometer at T = 300 K. Residual water signal was suppressed by presaturation with low irradiation power.

Cross-polarization magic-angle spinning (CPMAS) ^{13}C NMR spectra were recorded on a Varian 400 Unity Inova spectrometer at a resonance frequency of 100.57 MHz. A matched Hartmann-Hann condition was established at the spin-lock field of 38 KHz. The rotor was spun at a rate of 4 KHz and, working at room temperature, a contact time of 500 μs was applied to obtain polarization transfer. A recycle delay of 5 s and the 90 degree pulse of 5.8 μs were used. Chemical shifts of CPMAS spectra were originally obtained with respect to the methylene carbon resonance of solid adamantane, 38.3 ppm with respect to Me_4Si, measured before each measurement. Experiments were conducted on 160-180 mg samples of cyclodextrin packed into a 7 mm ZrO_2 rotor.

RESULTS AND DISCUSSION

Process parameters of the SCF preparations

To improve the efficiency in the complex preparation of the βCD-IMZ insertion complex by SCF, we have tested the process thermodynamic parameters, such as temperature and pressure, in order to increase $K_{1:1}$ and S_0 in supercritical CO_2 medium. We have also monitored the influence of the contact time IMZ-βCD-CO_2 on the complexation efficiency, using different temperatures (55, 65, 75, 85 °C) at P = 150, 200 bar, different pressures (130, 150, 170, 200 bar) at T = 65 °C and different contact times (2, 4, 6 h) at T = 65 °C and P = 150 and 200 bar (Table I). The data reported in Table I show that the complexation efficiency increases as a function of the contact time in both the experiments at 150 bar and at 200 bar. As shown, a contact time of 6 hours is necessary to achieve the best distribution equilibrium between the supercritical phase and the solid phase. In addition, the complexation efficiency increases as the pressure increases (from 130 bar to 200 bar at 65 °C) due to an increases solubility of IMZ in supercritical CO_2, since the density and the solvent power of CO_2 increases as a function of the pressure.

Table I shows that the complexation efficiency increases as the temperature increases (from 55 to 85 °C) at 150 bar, while at 200 bar it decreases at the increasing of the temperature (from 65 to 85 °C). This result can be explained considering that the temperature has two opposite effects. In fact, on the one hand, the increase of temperature causes an increase of the vapour tension of IMZ, thus enhancing its solubility in supercritical CO_2, on the other hand, too high temperatures cause the decrease of density of supercritical CO_2 thus decreasing its solvent power [8]. It stands to reason that the crossing point (75 °C) is very

close to the temperature value (65 °C) where a reversal of behaviour of IMZ solubility in supercritical CO_2 was observed.

In the light of these findings, evidence is given that P = 200 bar, T = 65 °C and contact time = 6h are the best conditions to obtain a good efficiency in the complex formation (complexation efficiency = 1.0).

Although an excess of guest molecule was used in all the preparations by SCF only equimolar guest-host ratio was achieved.

^{13}C CP-MAS and ^{1}H NMR characterization

Evidence of the formation of the βCD-IMZ complex was given by ^{1}H NMR spectroscopy in aqueous solution. In Figure 1 a comparison between the ^{1}H NMR spectra of the free and complexed βCD is shown. It is easy to see that in the spectrum of the βCD-IMZ complex the IMZ signals also appear, despite the very low solubility of IMZ in water, clearly indicating that the formation of the inclusion complex occurred. The assignment of the resonances was made following previous NMR investigations [4]. As shown, the resonances of the βCD in the inclusion complex exhibit significant changes in chemical shifts and linewidths. In particular, relevant highfield shifts (about 0.2 ppm) were detected for H_3 and H_5 protons, which point into the hydrophobic cavity of the βCD, in agreement with the results obtained for the inclusion complex prepared in water [4].

A stoichiometry of approximately 1:1 was determined by the integration of the host and guest signals in the ^{1}H spectrum of the βCD-IMZ [4].

Figure 2 shows the solid-state ^{13}C CP-MAS NMR spectra of the free βCD and IMZ. The assignment of the resonances was made following previous NMR investigations [4] and for IMZ was obtained from the analysis of the coupled and decoupled spectra in $CDCl_3$ (see Table II).

As it can be observed, the spectrum of βCD has a relatively poor resolution, due to the partial hydration of the sample, however, multiple resonances appear mainly for the $C_{2,3,5}$, C_4 and C_6 atoms of the glucopyranose unit, that indicates the coexistence of different structural arrangements [12]. The spectrum of the inclusion complex show that all of the major signals of βCD shift and change in intensity compared to their free components, and a marked broadening of the signals of the guest molecule occurs.

It is note worthy that the parameters responsible for these changes are differently affected. In fact, each set of multiple resonances of carbons of the glucose units tends to converge to a single peak, thus suggesting that in the complex the glucose units adopts a more symmetrical conformation.

The analysis of the spin-lattice relaxation times in the rotating frame, $T_{1\rho}(^{1}H)$, and of the ^{129}Xe NMR spectra is now in progress to obtain information on the dynamics and the hydrophobic character of the βCD-IMZ complex .

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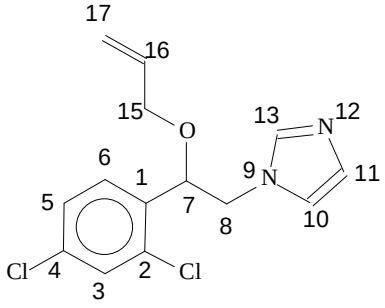
Table 1: Experimental parameters used to prepare βCD-IMZ inclusion complex: T, temperature (°C); P, pressure (bar); t, time of contact (h); R, starting stoichiometry ratio of free βCD and IMZ; CE, complexation efficiency of the complex, βCD-IMZ equivalents ratio.

T/°C	P/bar	t/h	R	CE
65	130	6	2.5	0.73
65	150	6	2.6	0.80
65	170	6	2.6	0.87
65	200	6	2.6	0.96
55	150	6	2.7	0.74
65	150	6	2.6	0.80
75	150	6	2.7	0.88
75	150	6	2.6	0.86
75	150	6	2.8	0.84
85	150	6	2.6	0.92
65	200	6	2.6	0.96
75	200	6	2.6	0.80
85	200	6	2.5	0.70
85	200	6	2.5	0.69
65	150	2	2.7	0.67
65	150	4	2.7	0.74
65	150	6	2.6	0.80
65	200	2	2.7	0.83
65	200	4	2.6	0.90
65	200	6	2.6	0.96

Table 1: ^{13}C chemical shifts, δ (ppm), of βCD, IMZ and βCD-IMZ.

	Carbon type	d (ppm)	
		Free	**insertion complex**
b**CD**	C_1	103.8 103.0	104.1 103.0
	C_4	84.9 84.0 82.8 82.0 79.0	84.3 83.1 82.1 80.9 79.1
	$C_2/C_3/C_5$	76.8 75.5 74.3 73.5 72.5	76.8 73.9
	C_6	64.4 62.5 60.5	64.3 62.4 61.0
IMZ	N-CH=N Cl-C (ar) -C_3H= (ar) -$C_{5,6}$H= (ar) N-C_{11}H= N-C_{10}H= O-CH- O-CH_2- N-CH_2-	139.1 136.5 131.2 129.4 125.7 120.4 76.1 71.7 52.8	138.5 135.8 130.0 128.7 124.7 119.6 nd nd 52.5



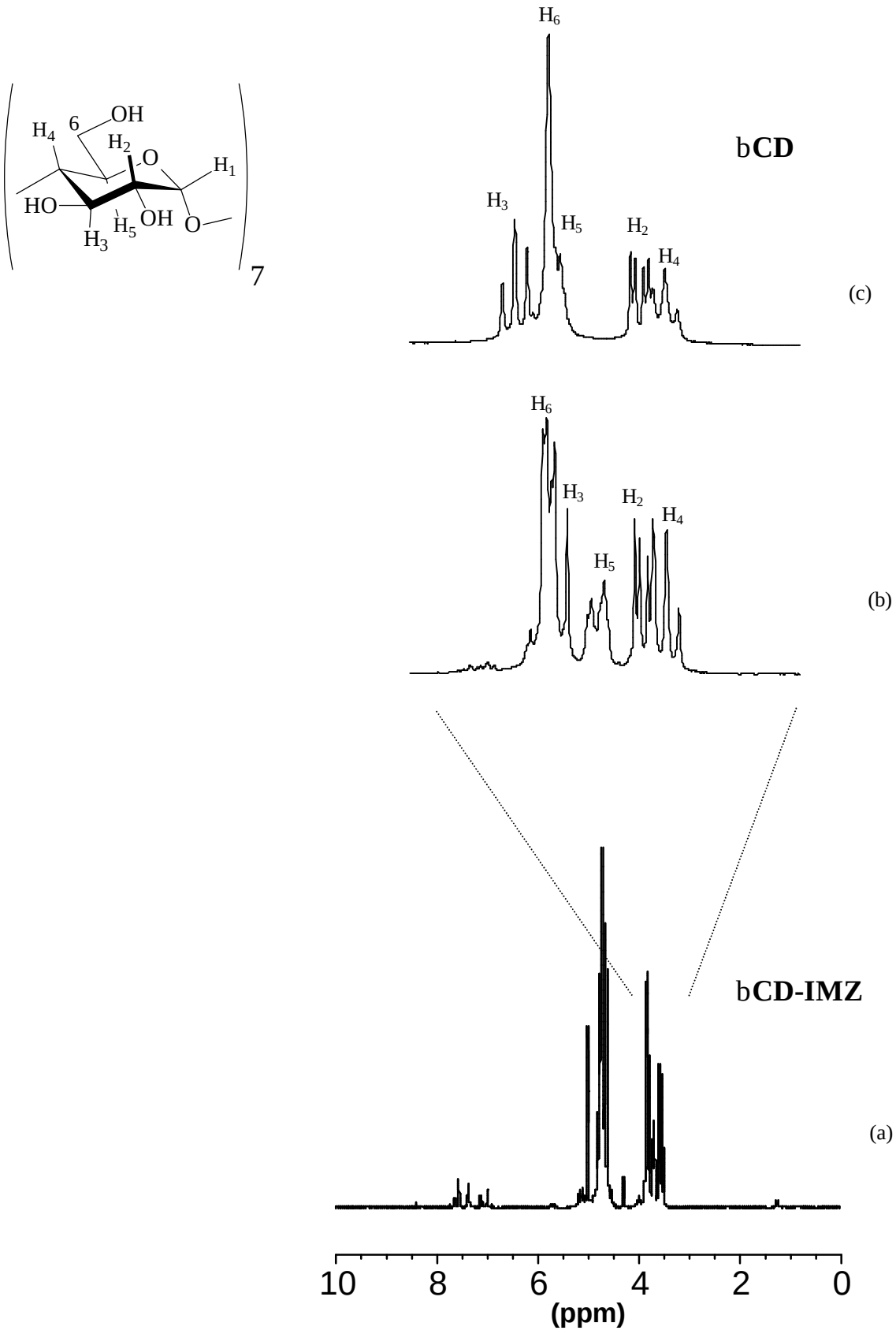


Figure 1: H NMR spectra of βCD-IMZ (a, b) and βCD (c) in aqueous solution.

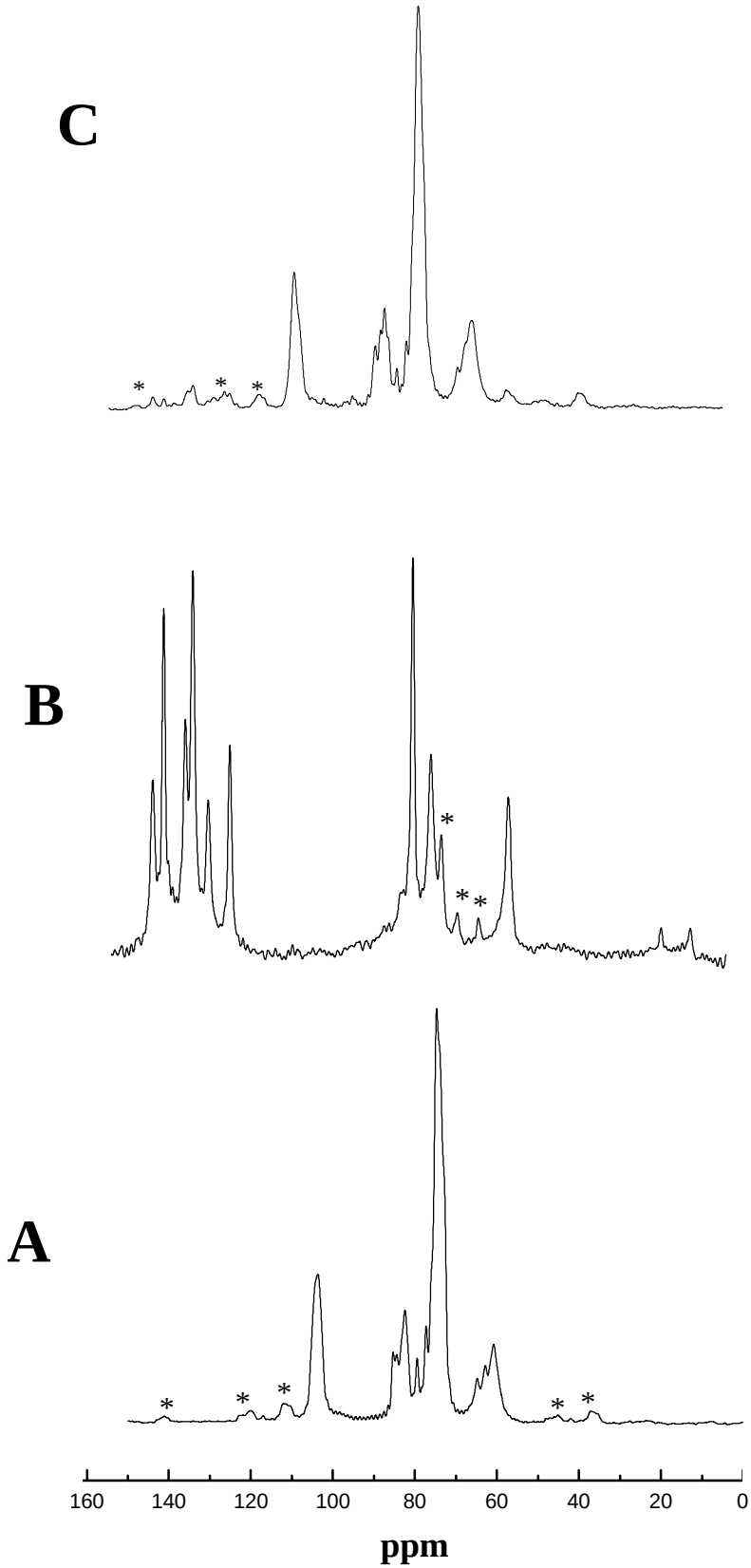


Figure 2: ^{13}C CP-MAS NMR spectra of βCD (A), IMZ (B) and βCD-IMZ (C).
* Spinning sidebands.