

¹³C CPMAS AND ¹H NMR STUDY OF THE INCLUSION COMPLEXES OF β-CYCLODEXTRIN WITH CARVACROL, THYMOL AND EUGENOL PREPARED BY SUPERCRITICAL CARBON DIOXIDE

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β-cyclodextrin inclusion complexes with carvacrol, thymol and eugenol (components of essential oils of vegetable origin) were prepared by the supercritical carbon dioxide technique and their structural characterisation was made by means of ¹H NMR in aqueous solution and ¹³C CPMAS NMR in the solid state. Evidence of the formation of the inclusion complexes for all the examined systems was obtained by ¹H NMR in solution, while 2D-ROESY NMR experiments were used to investigate the geometry of inclusion. In addition, the dynamics of these inclusion complexes in the kilohertz timescale was investigated by analysis of the proton and carbon spin-lattice relaxation times in the rotating frame.

INTRODUCTION.

Several monoterpene hydrocarbons and their oxygenated derivatives, which are components of essential oils, have been shown to have bactericidal, microbicidal and fungicidal properties [1-4]. However, their very low solubility in water limits their applications and bioavailability. Furthermore, these essential oils are oxidized, decomposed, or evaporated when exposed to the air, light, or heat. One way to stabilise and use these substances is by inclusion in β-cyclodextrin that greatly reduces their volatility, oxidation, and heat-decomposition [5-9]. Moreover, a marked increase in solubility occurs in the guest molecules in water, so that their potential fields of application can be enlarged considerably. Here we report the results of a structural study of the inclusion complexes of βCD with carvacrol, thymol, and eugenol prepared by the supercritical carbon dioxide (SCF) technique [10, 11]. These complexes have been already prepared in water by co-precipitation of the host and guest molecules [12]. The greatest advantage of the SCF technique compared to the classical preparation method in water is that it can be scaled up for industrial applications, since the fact that the guest molecules are much more soluble in CO₂ than in water gives important yields in complex preparation. The structural characterization of the inclusion complexes was carried out by one- and two-dimensional ¹H NMR spectroscopy in aqueous solution and ¹³C CPMAS NMR spectroscopy in the solid state. Moreover, proton and carbon spin-lattice relaxation times in the rotating frame, $T_{1r}({}^1H)$ and $T_{1r}({}^{13}C)$, were analysed to obtain information on the dynamics in the kilohertz scale.

MATERIALS AND METHODS.

General. β CD (CAVAMAX[®]7 PHARMA) was obtained from Wacker – Chemie Italia SpA and used as received; CO₂ (purity 99%) was supplied by SIO (Società Italiana Ossigeno, Cagliari, Italy), while carvacrol, thymol and eugenol were purchased from the Sigma Chemical Co. and used without further purification. D₂O (99,9%) and chloroform-*d* (99,8%) were purchased respectively from Cambridge Isotope Laboratories and Euriso-top France.

SCF Apparatus. Inclusions of carvacrol, thymol and eugenol in β CD were performed in a SCF laboratory apparatus, equipped with a thermostatted 400 cm³ autoclave and supercritical CO₂ pressurized by a high pressure diaphragm pump (Lewa, model EL 1) with a maximum capacity of 6 kg/h, pumping liquid CO₂ at the chosen pressure. Temperatures and pressures along the extraction apparatus were respectively measured by thermocouple and Bourdon-tube test gauges. Pressure was regulated by high pressure valves under manual control [13]. The apparatus was used in the batch mode. β CD (about 20 g) with the guest molecules at a molar ratio of 1 : 2.5 were loaded into the autoclave, which was then thermostatted and pressurized with CO₂ at fixed pressure and contact time. Then a rapid drop in pressure allowed to vaporize CO₂ and separate the solid deprived of solvent. Although an excess of guest molecule was used in all the preparations by SFC, only an approximately equimolar guest-host ratio was achieved. The molar ratio in aqueous solution of the inclusion complex was determined via integration of the ¹H NMR signals of the host and the guest molecules (spectra shown in Fig. 1).

NMR experiments. The ¹H NMR spectra in D₂O were recorded at $T = 25^{\circ}\text{C}$ on a Varian 400 Unity Inova spectrometer. All the ¹H NMR spectra were recorded in 5 mm tubes, using a 9.1 μs pulse (90°), a 23.7 s repetition time and a spectral width of 4000 Hz. The residual water signal was suppressed by presaturation with low irradiation power. 2D rotating frame nuclear Overhauser enhancement spectroscopy (ROESY) NMR spectra were recorded with mixing times of 50, 250 and 500 ms. In all cases, a 4000 x 4000 Hz window at carrier frequency of 4.74 ppm was obtained acquiring between 128 and 256 transients with 2k data points along t_2 axis. Prior to two-dimensional Fourier transform, free induction decays were multiplied by a shifted square sine-bell function. Phase sensitive detection was achieved by the hypercomplex method. Cross-polarization magic-angle spinning (CPMAS) ¹³C NMR spectra were recorded at room temperature on a Varian 400 Unity Inova spectrometer at the resonance frequency of 100.57 MHz. Experiments were conducted in 7 mm ZrO₂ rotor that was spun at a rate of 4 kHz. A matched Hartmann-Hahn condition was established at the spin-lock field of 43 kHz, and a contact time of 500 μs was applied to obtain polarization transfer. A 90-degree pulse of 5.8 μs and a recycle delay of 5 s were used. Chemical shifts of CPMAS spectra were obtained with respect to the methylene carbon resonance of solid hexamethylbenzene (17.3 ppm downfield from Me₄Si) determined before each measurement.

CONCLUSION.

Preparation of β CD-flavour molecules by SCF. In order to prepare the β CD inclusion complexes by SCF with the highest host-guest molar ratio, the experimental conditions were optimised by testing several values of the process thermodynamic parameters, such as temperature, pressure, and β CD/guest molecule/CO₂ contact time. The following best experimental conditions were found: a temperature of 50°C, a pressure of 80 bar (which

corresponds to conditions of highest solubility of essential oils in supercritical CO₂) and a contact time of 6 hours [14]. ¹H NMR. Fig. 1 shows the ¹H NMR spectra in D₂O of the inclusion complexes, and for comparison the spectrum of the free βCD. Despite the very low solubility of the free guest molecules in water, their proton resonances are easily observed in all the spectra of the inclusion complexes, providing direct evidence for complex formation. With the exception of eugenol, the proton resonances of the host and the guest compounds were assigned according to previous NMR investigations [12]. Significant variations in the chemical shifts are observed for the aromatic protons of the guest molecules and mainly for the H-3 and H-5 resonances of the glucose units of βCD which lie inside the hydrophobic cavity, indicating that these protons are directly involved in host guest interactions. Information on the geometry of the inclusion complexes in aqueous solution was obtained via rotating frame nuclear Overhauser enhancement spectroscopy (ROESY). These experiments allow to detect intermolecular dipolar interactions between host and guest protons, and therefore were used to determine qualitatively the orientation and penetration of the guest molecule inside the cavity of the cyclodextrin. 2D-ROESY spectra of all inclusion complexes in D₂O solution showed several cross-peaks involving the H-3 and H-5 protons inside the βCD cavity and a number of protons of the guest molecules. Based on these results, it can be stated that in all three complexes the aromatic rings of the guest molecule are fully immersed in the hydrophobic cavity of βCD. The geometry of inclusion deduced from the ROESY data is shown in Scheme 1. ¹³C CPMAS NMR spectra. Fig. 2 shows the ¹³C CPMAS NMR spectra of the βCD-inclusion complexes together with those of free βCD and thymol, while the spectra of free carvacrol and eugenol have not been recorded because they are liquid. As can be observed, in the spectra of βCD-carvacrol and βCD-eugenol, the signals of the guest molecules were not detected probably because they have high molecular mobility inside the βCD cavity. The βCD resonances were assigned following a previous NMR investigation [15], while the resonances of thymol were attributed from the analysis of the coupled and decoupled ¹³C NMR spectra in chloroform-*d* (see Table 1). It is worth noting that the spectrum of the free βCD has a relatively poor resolution due to the presence of multiple resonances for the carbon atoms of the glucose units (C-2, C-3, C-4, C-5, and C-6), which can be ascribed to the coexistence of different structural arrangements [15] induced by partial hydration of the sample [16]. Such a set of arrangements is also observed in the inclusion complexes, clearly suggesting that only weak interactions are established between the βCD and the guest molecules. The analysis of the proton and carbon spin-lattice relaxation times in the rotating frame, $T_{1r}({}^1H)$ and $T_{1r}({}^{13}C)$, that give information on the dynamics of the βCD complexes in the kilohertz range indicate that the guest molecules within the cyclodextrin cavity retain a mobility similar to that of the free molecules.

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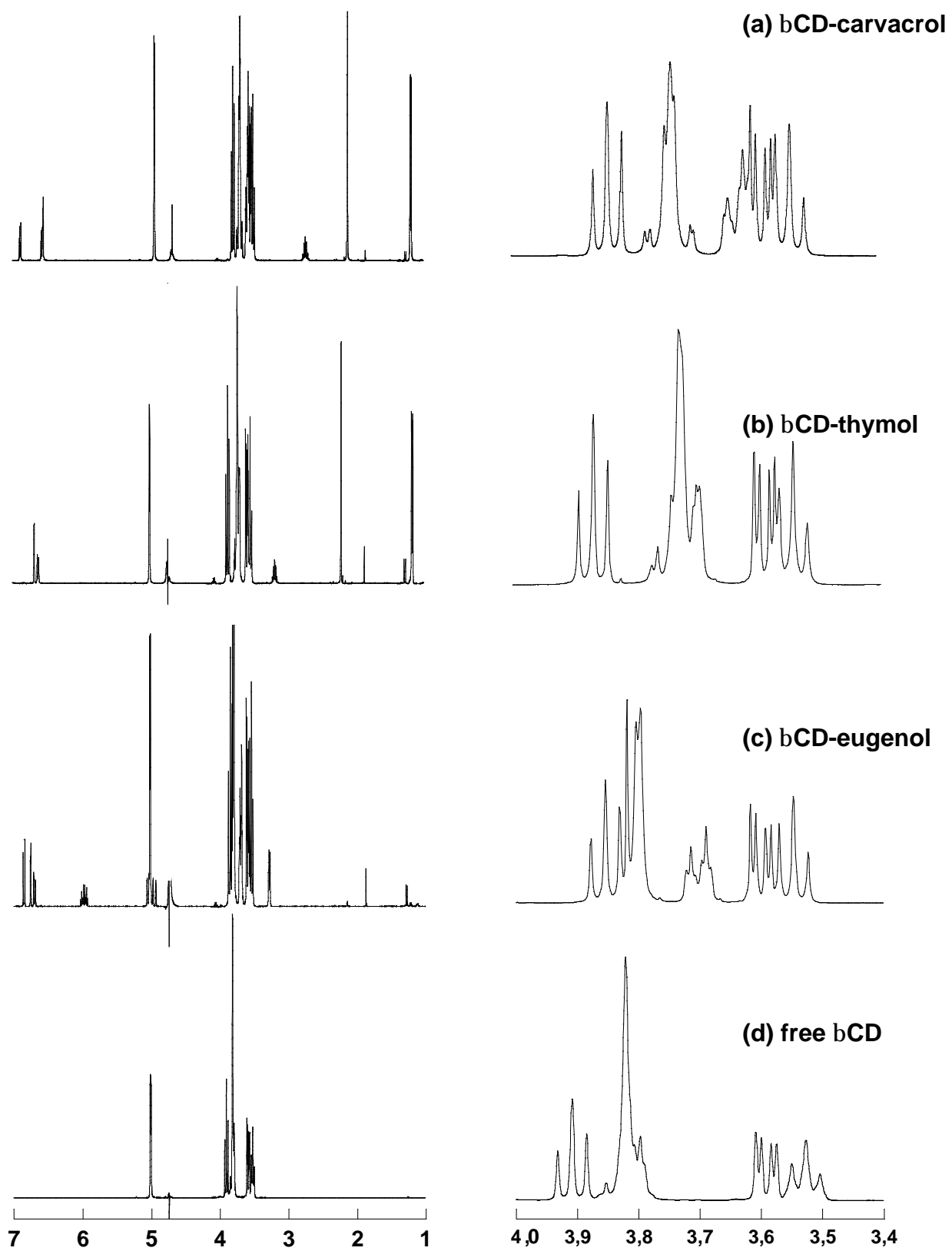


Figure 1. ^1H NMR spectra in D_2O .

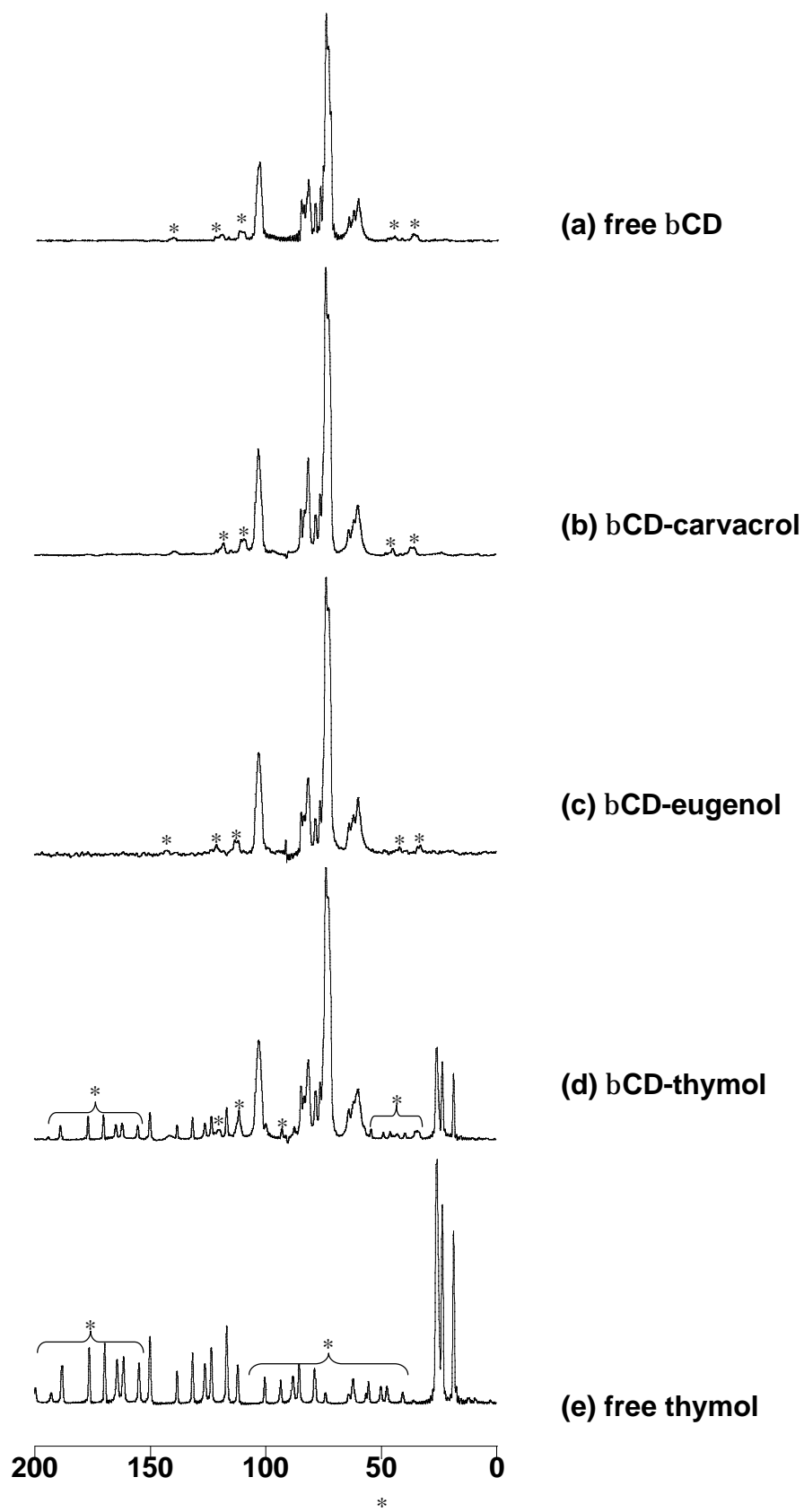
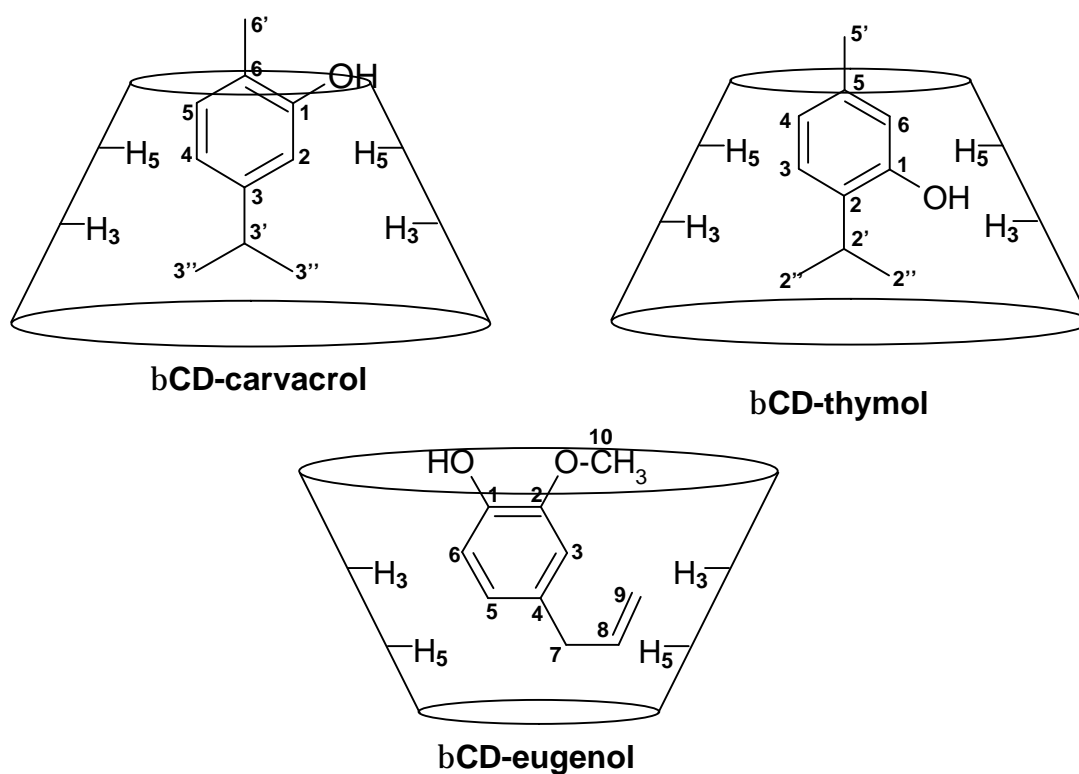


Figure 2 ^{13}C CPMAS NMR spectra. The asterisk indicates a spinning side-band

Table 1. ^{13}C CPMAS Chemical Shifts, d , of Free bCD, Thymol and bCD Complexes.

bCD					Thymol	
C-type	d /ppm				C-type	d^a /ppm
	free	bCD-thymol	bCD-carvacrol	bCD-eugenol		
C-1	103.1	103.3	103.5	103.3	C-5'	18.8
C-4	84.9	84.7	84.8	84.6	C-2''	23.7
	84.0	83.5	83.4	83.5	C-2'	26.1
	82.0	81.7	81.7	81.7	C-6	116.9
	79.0	78.5	78.5	78.6	C-4	123.6
	76.8	76.5	76.6	76.5	C-3	126.4
C-2/C-3/C-5	75.5				C-2	131.7
	74.3	74.0	74.1	74.0	C-5	138.4
	73.5	73.1	73.1	73.0	C-1	150.3
C-6	64.4	64.0	64.1	63.9		
	62.5	62.2	62.0	62.1		
	60.5	60.1	60.3	60.1		

^a Chemical shift values are referred to free and complexed thymol.



Scheme 1. Structures of the inclusion complexes.