ORIGINAL ARTICLE

Host–guest interactions between dapsone and β-cyclodextrin (part I): study of the inclusion compound by nuclear magnetic resonance techniques

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Abstract Dapsone (4,4′diaminodiphenylsulfone) is a very effective drug to treat leprosy and a broad range of infectious conditions such as *Pneumocystis carinii* pneumonia, toxoplasmosis and tuberculosis. However, the oral administration of this drug generally is related to serious side effects and treatment failures. It is believed that the inclusion compound of this drug and cyclodextrins would increase the wettability and the solubility of the encapsulated drug for a supported and gradual release, maximizing its biodisponibility over time. The encapsulation of dapsone in β-cyclodextrin was investigated by five nuclear magnetic resonance spectroscopy techniques. The data obtained for the complex in aqueous solution and in solid-state revealed a strong interaction between host and guest, showing that the drug molecule is deeply inserted in the CD cavity. The diffusion experiments (diffusion ordered spectroscopy) showed a high percentage of complexation (86 %) and that the drug molecule is preferentially interacting with the large side of the β-cyclodextrin cavity.

Keywords Dapsone · β-Cyclodextrin · T1 · ROESY · DOSY · CPMAS

Abbreviations

$\Delta\delta$	Chemical shift difference
δ	Chemical shift
At	Acquisition time
CD	Cyclodextrin
DPS	Dapsone
βCD	β-Cyclodextrin
D	Diffusion coefficient
D1	Acquisition delay
DOSY	Diffusion ordered spectroscopy
FD	Freeze-drying
FID	Free induction decay
Lb	Line broadening
NMR	Nuclear magnetic resonance
nt	Number of transients
PM	Physical mixture
PW	Pulse width
rOe	Rotating overhauser effect
ROESY	Rotating frame overhauser effect spectroscopy
CPMAS	Cross-polarization magic angle spinning

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Introduction

Patients suffering from AIDS are usually affected by opportunistic diseases such as *Pneumocystis carinii* pneumonia, toxoplasmosis and tuberculosis. One drug of choice to treat them is dapsone (DPS) (4,4′diaminodiphenylsulfone), which is cheap and easy to be administered [1]. Also, the oral administration of DPS is very effective to treat a broad range of inflammatory conditions [2]. However, the oral delivery of DPS generally leads to serious adverse effects, such as the side effects are anemia, stomach upset, nausea, leg or back pain, headache, dizziness or peripheral neuropathy and treatment failures [3]. Leprosy is another important treatment application of DPS although frequently induces hemolytic anemia and methemoglobinemia [4].

Cyclodextrins (CDs) are one of host molecules more extensively studied in supramolecular chemistry, because

of their advantages: they are biocompatible, produced by natural enzymatic degradation of starch, they are relatively cheap and non-toxic, allowing applications in drugs, foods and cosmetics [5]. CDs are cyclic oligomers of α-D-glucose connected through glycosidic α-1.4 bonds, crystalline, non-hygroscopic, and torus-like macrocycles, formed by the action of the enzyme CD glucosyltransferase (CGTase) on starch. Due to their intramolecular cavity they have the ability to include guest molecules altering their physical, chemical, biological and pharmacological properties through the formation of inclusion complexes [6, 7]. CD is often used to increase the aqueous solubility, stability and bioavailability of drugs [8]. The three most commonly used CD are αCD (six glucose units), βCD (seven glucose units), and γCD (eight glucose units) [9]. The size of the cavity of βCD is just right to interact with a great number of molecules and, therefore, this CD was used in this study. There are many techniques that can be applied to study interactions between hosts and CDs molecules but NMR is one of the most important because association in the CDs inclusion complex is due to non-covalent interactions. Also, generally CD applications involve the complex in a liquid solution which again reveals the importance of NMR spectroscopy in their study [10]. In this way, due to the DPS uses in many diseases and the properties of this carrier, it was decided to study the encapsulation of this drug in βCD with the goal of increasing the wettability and the solubility of the encapsulated drug for a supported and gradual release, maximizing its biodisponibility over time.

One of the main technique to characterize drug:CD is the nuclear magnetic resonance (NMR) spectroscopy due to its wide field of applications from structural elucidation of structures to investigations on intra/inter-molecular. The simplest experiment of NMR as an indicative of complexation is the observation of the difference in the proton chemical shifts between the free guest and host species and the suggested complex. There has been a long time since Demarco and Thakkar [11] started studies on CDs complexes by observing the chemical shifts changes of the protons H3 and H5 inside the cavity of αCD when in presence of aromatic molecules due to the anisotropic effect of the aromatic ring. When there is a host–guest interaction, it leads to a change in the δ of the hydrogens due the complexation. This is a first evidence of the guest inclusion in the CD cavity [10].

Other important experiments include: T_1 measurement, which is directly related to the relaxation phenomenon [12]; the DOSY experiment, where the molecular diffusion in solution is a phenomenon related to the molecular dynamics in biological and chemical systems [13]; the ROESY experiment, which is widely applied for structural elucidation of guest:CD inclusion complexes, which are done through the internuclear NOE enhancement measures

between the guest nuclei and the CD inner cavity nuclei H3, H5 and H6 [14, 15]; and ^{13}C-cross polarization magic angle spinning (CPMAS) NMR which is a powerful non-invasive approach to the molecular analysis of starch-related structures, and provides information on the molecular organization at shorter distance scales [16].

Therefore, we characterized the inclusion complex of DPS and βCD by five NMR spectroscopy techniques. Information on interactions between host and guest were obtained by ^{1}H-NMR and ^{13}C-CPMAS; measurements of diffusion coefficients where studied from diffusion ordered spectroscopy (DOSY) experiments and from T1 relaxation times; structural information of the complex were from ROESY-1D (rotating frame overhauser effect spectroscopy).

Experimental

Materials and equipments

DPS was supplied by Ecofarma Farmácia Ltd.; βCD was a gift from ISP Technologies, Inc. and it was used as received; ethyl alcohol 99.5 % P.A. was purchased from LabSynth Ltd. Products for Laboratories; 99.9 % deuterium oxide was purchased from Cambridge Isotope Laboratories, Inc.; Freeze-dryer FTS systems; NMR spectrometer Bruker Avance II 300 MHz and Varian 500 MHz; rotary evaporator RE111, water bath 461 and vacuum pump Büchi Labortechnik AG.

Preparation of the inclusion complex

The inclusion complex DPS:βCD was prepared by adaptation of two methods: co-precipitation and lyophilization. βCD was dissolved in 70 mL of deionized water, followed by addition of 20 mL of DPS in ethanolic solution in a molar ratio 1:1. The mixture was stirred at room temperature for 24 h. After removal of ethanol in a rotatory evaporator at 40 °C, the suspension was frozen in liquid nitrogen and lyophilized for 48 h.

Preparation of physical mixture

The physical mixtures (PMs) were prepared using the molar ratio of βCD and DPS by simply mixing the two compounds for 2 min.

NMR spectroscopy experiments

All experiments with liquid samples were run on a Varian INOVA-500 spectrometer (BO = 11 T), operating at 500 MHz for ^{1}H, keeping the temperature at 297.6 ± 0.1 K. The chemical shifts were referenced against the HOD

resonance (δ = 4.67 ppm). The samples were prepared by dissolving of 2–4 mg of the complex in approximately 0.6 mL of D_2O. The data were acquired using standard Varian software and processed using the program VNMR of the equipment. The conditions to obtain the ^{1}H NMR spectra were PW = 6.1 s At = 3.3 s and D1 = 3.0 s. The accumulated number of transients (nt) was 32 scans and Lb = 0.2 Hz.

DOSY

The pulse sequence used for the DOSY experiments was gradient compensated stimulated echo with spin lock (DOSY GCSTESL). In all the analyzes 25 different pulsed gradient amplitudes were used, D1 = 6.1 s, At = 3.3 s, nt = 32, and Lb = 0.2 Hz.

T1 measurements

The samples were prepared as usual. For ^{1}H-NMR, a 90° pulse was typically of 15 μs, and the recycling time was set to 15 s. Longitudinal relaxation times were obtained by the conventional inversion-recovery method.

ROESY

ROESY experiments were carried out using the parameters At = 1.0 s; D1 = 3.0 s; nt = 128 scans; Lb = 1.0 Hz. It a sequence of 180°–90° sel.—spin lock-FID pulses was applied with a mixing time of 500 ms, FIDs acquired through the sequence of 90° sel.—spin lock-FID pulses. A modulator generated the selective pulses and automatically attenuated the power and duration of the pulse.

^{13}C-CPMAS

All ^{13}C-CPMAS spectra of lyophilized samples, PM, DPS and βCD were run on a Bruker 300 MHz, at 298 K and 10 kHz.

Results and discussion

^{1}H-NMR

In this experiment, the behavior of the chemical shifts of the protons inside the cavity was observed. Thakkar and Demarco [11] initiated NMR studies of CD complexes observing changes in the chemical displacements of the H3 and H5 protons inside the cavity of αCD in the presence of an aromatic substrate, due to the anisotropic effect of the aromatic ring. Since the NMR signals of the external H1, H2 and H4 protons did not show any variation, they

inferred that the guest molecule was inside the cavity. Thus, the chemical displacement of the CD signals induced by the presence of the guest is a first evidence of its inclusion in the CD cavity [10]. The protons of DPS and βCD and the NMR spectra of DPS, βCD and the inclusion complex DPS:βCD were illustrated in Fig. 1.

The chemical shifts of the main protons of each molecule and their shifts in the complex are summarized in Tables 1, 2.

According to Greatbanks and Pickford [17], when $\Delta\delta$ H3 > $\Delta\delta$ H5 the inclusion of the guest molecule inside the cavity is partial and when $\Delta\delta$ H3 ≤ $\Delta\delta$ H5 the guest molecule is included more deeply inside the cavity. As it is observed in Table 1 one can conclude that DPS is totally included in the CD cavity due to the high affinity between the rings of DPS with the hydrophobic environment of this cavity. Also, in Table 1, we observe that even though all protons have $\Delta\delta$ when DPS is included in the cavity of CD, the signals of the protons localized in the inner cavity of CD (H3 and H5) undergo the strongest shift [18]. One interesting characteristic that can be observed is that all the βCD protons had a variation on their chemical shifts. This fact can be explained by the strong intermolecular interaction of the drug and its high hydrophobicity, what leads the encapsulated and the free drug not only interact with the protons of the βCD cavity but also with its external protons. Also, as we have seen in other studies, the –SO_2 group of DPS interacts with the hydroxyl group of βCD (Table 3).

Diffusion ordered spectroscopy

DOSY is an important NMR technique that gives the diffusion coefficients of different molecules in only one experiment. The diffusion coefficients (D) supply important information on molecular organization and phase structure. This technique is non-invasive and can provide individual multi component translational D with good precision. The sequence used was gradient compensated stimulated echo spin lock (GCSTESL), which is adequate when there is no exchange effect and modest gradients are enough for the acquisition. After correction of the base line and selection of the points, the D were processed automatically and the value of each D was calculated as the average of all the listed coefficients. The guest population (p) involved in the complexation was calculated from the observed D of each component in the complexed and in the free form through Eqs. 1–3 [19, 20].

$$D_{observed} = p_{free} D_{free} + p_{complexed} D_{complexed} \qquad (1)$$

where

$$p_{free} + p_{complexed} = 1 \qquad (2)$$

Then
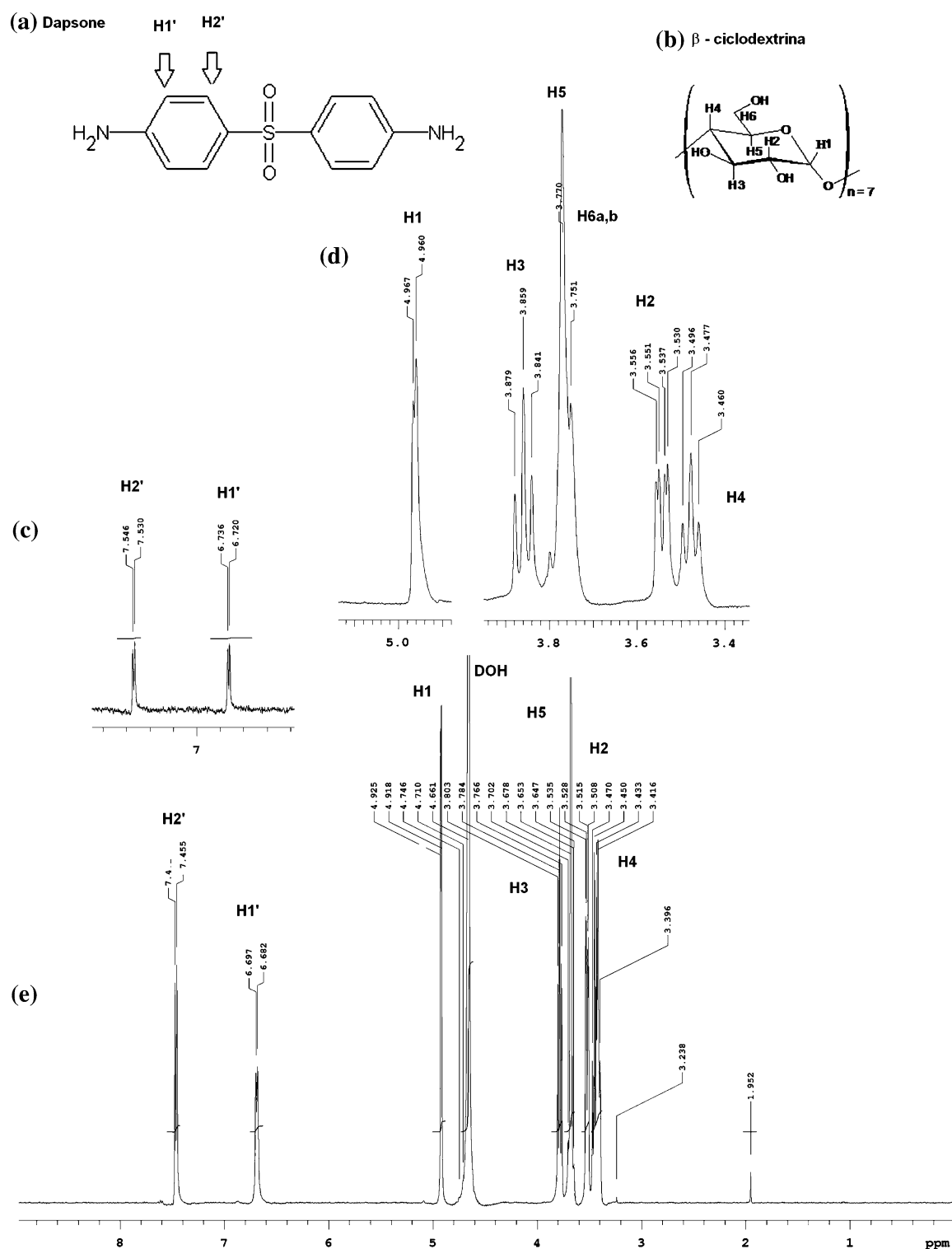
Fig. 1 The molecular structures of **a** DPS and **b** βCD; ^{1}H NMR partial spectrum of **c** DPS protons and **d** βCD protons at 500 MHz, in D_2O and 297.6 K; **e** the ^{1}H NMR spectra of βCD:DPS inclusion complex in aqueous medium at the same conditions

$$p_{\text{free}} = 1 - p_{\text{complexed}} \qquad (3)$$

D_{observed} is the DPS diffusion coefficient with βCD in the medium; D_{free} is the DPS diffusion coefficient in the

absence βCD; $p_{\text{complexed}}$ is the completely complexed DPS population and $D_{\text{complexed}}$ is the completely complexed DPS diffusion coefficient. Substituting Eq. 3 in 1, considering that the completely complexed DPS diffusion

Table 1 ^{1}H NMR chemical shifts of βCD protons in D$_2$O and their change due to the presence of DPS ($\Delta\delta = \delta_{\text{complexed}} - \delta_{\text{free}}$) obtained from the inclusion complex ^{1}H NMR spectra and the βCD ^{1}H NMR spectra alone

H	$\delta_{\beta CD}$	$\delta_{DPS:\beta CD}$	$\Delta(\delta_{DPS:\beta CD} - \delta_{\beta CD})$
H1	4.961	4.922	−0.039
H2	3.551	3.512	−0.039
H3	3.856	3.784	−0.072
H4	3.475	3.416	−0.054
H5	3.757	3.678	−0.079

Table 2 ^{1}H NMR chemical shifts of DPS protons in D$_2$O and their change due to the presence of βCD ($\Delta\delta = \delta_{\text{complexed}} - \delta_{\text{free}}$) obtained from the inclusion complex ^{1}H NMR spectra and the DPS ^{1}H NMR spectra alone

H	δ_{DPS}	$\delta_{DPS:\beta CD}$	$\Delta(\delta_{DPS:\beta CD} - \delta_{DPS})$
H1′	6.731	6.690	−0.039
H2′	7.519	7.458	−0.061

Table 3 Free and complexed DPS and βCD diffusion coefficients, and percent values of the DPS population complexed with βCD obtained from the DOSY experiment using the pulse sequence GCSTESL and performed at 500 MHz, 297.6 K in D$_2$O

Sample	Molecule	D ($\times 10^{-10}$ m^2/s)	$p_{\text{complexed}}$
βCD	βCD	3.2 ± 0.3	84.6 %
	DOH	14.8 ± 0.3	
DPS	DPS	5.2 ± 0.3	
	DOH	17.2 ± 0.6	
DPS:βCD	DPS	3.0 ± 0.1	
	βCD	2.6 ± 0.1	
	DOH	17.1 ± 0.3	

coefficient is approximately the same as the completely complexed coefficient of βCD and assuming that the observed βCD diffusion coefficient is almost the same as when it is in its free form.

$$D_{\beta CD \text{ complexed}} \approx D_{\beta CD \text{ observed free}} \approx D_{\beta CD \text{ free}} \quad (4)$$

Substituting Eq. 4 in 1:

$$p_{\text{complexed}} = \frac{(D_{\text{free}} - D_{\text{complexed}})}{(D_{\text{free}} - D_{\beta CD \text{ observed}})} \quad (5)$$

The diffusion coefficients and percentages of the DPS population complexed with βCD are given in Table 5 and the NMR spectra are shown in Fig. 5. It can be observed that the values of the D for HOD, βCD and the DPS population are quite distinct and their magnitudes are coherent, since D is directly related with the molecular weight of each molecule. Moreover, one can observe that

the D of DPS in presence of βCD decrease approximately to the same value as that of βCD alone indicating that the drug is located inside the βCD cavity. In Fig. 2, it is observed a bi-dimensional plot of the data obtained from DOSY experiment from DPS, βCD and the DPS:βCD inclusion complex. The plot of each data has as axes the chemical shifts and the D values. Analyzing this figure, we can observe that the D of the DPS protons is differently located in the 2D-plot when βCD is present.

T1 measurement

The application of the B1 field at the resonance frequency results in a lowering of the magnetization in the z direction owing to the energy absorption and the conversion of some +1/2 spins into −1/2 spins. This relaxation returns the system to equilibrium with time constant T1, called spin–lattice or longitudinal relaxation. The major source of these magnetic fields is the magnetic nuclei in motion which depends on the molecular surroundings. Therefore, longitudinal relaxation times (T1) give information about the nucleus mobility in solution and the interaction between host and guest molecules [21].

The spin–lattice T1 were measured for free and complexed DPS and βCD hydrogens. The values for each sample are summarized in Tables 4, 5. One can observe that all T1 values decrease in the inclusion compounds approximately to the same value as that of βCD alone, same observed by DOSY experiment, indicating that the drug is located inside the βCD cavity, and so, it becomes with the same behavior as the host. Grillo et al. [22] showed that T1 decreases of the host and guest are strong evidence of the interaction between both molecules.

ROESY-1D

The ROESY experiment in its 1D version with selective pulses leads to a direct, fast and easily quantified analysis [23]. The ROESY spectrum provided information about which βCD proton has an intermolecular correlation when the DPS H1′ and H2′ protons were selectively irradiated. The spectra are in Figs. 3, 4. Analyzing both figures, we observe one can conclude that the H1′ proton is deeply located in the cavity of CD because when it was irradiated, the experiment generated a strong signal in all CD protons. These results are also indicative of a high association constant between the DPS and the βCD [24].

^{13}C cross polarization magic angle spinning NMR

The inclusion complex of DPS:βCD were also investigated by solid-state ^{13}C-CPMAS NMR spectroscopy. This

Fig. 2 Bi-dimensional plot of DOSY/NMR spectra of **a** DPS; **b** βCD; **c** DPS:βCD inclusion complex obtained from the pulse sequence GCSTESL with 25 different pulsed gradient amplitudes, D1 = 6.1 s, At = 3.3 s, nt = 32, and Lb = 0.2 Hz (500 MHz; D_2O; 297.6 K)
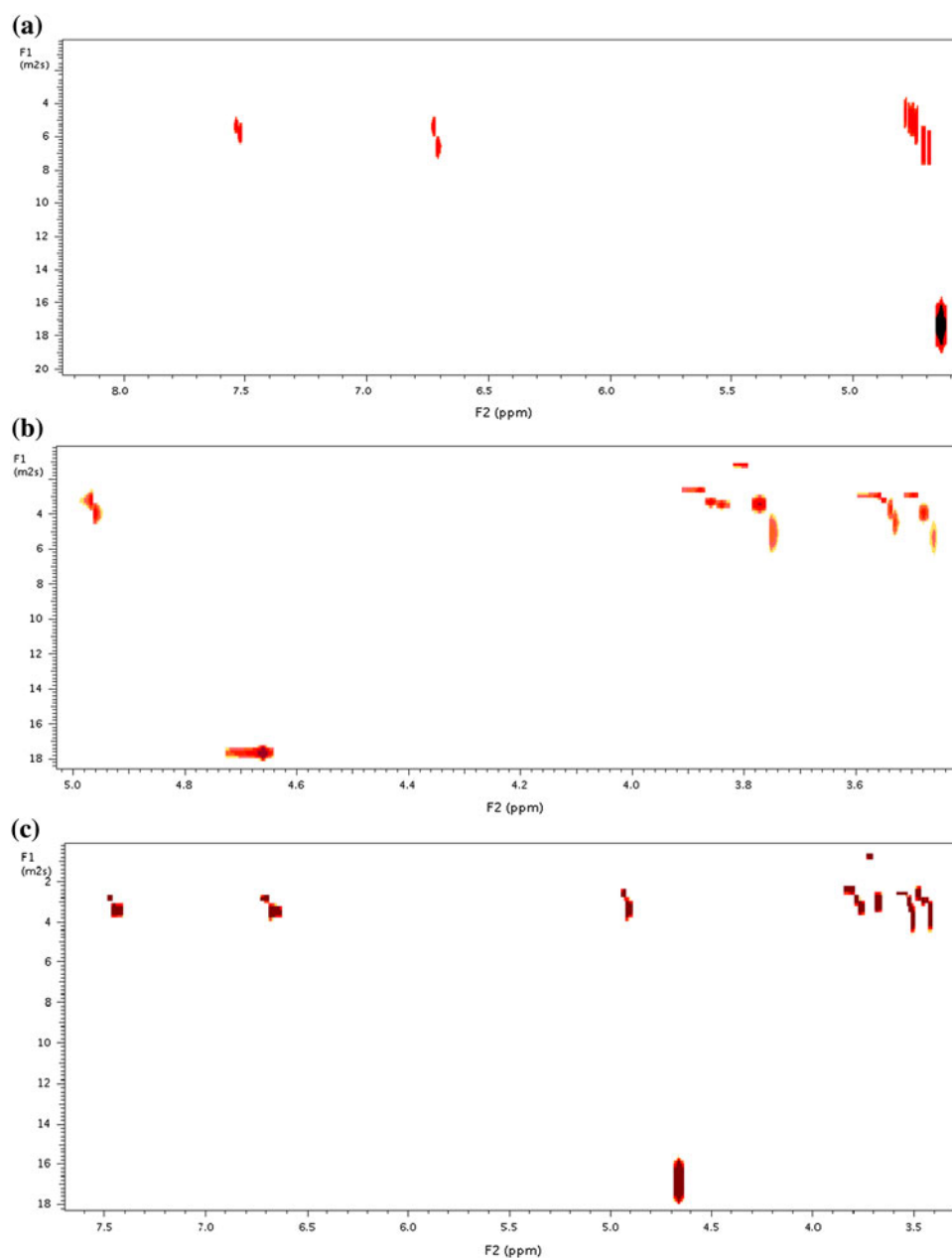
Table 4 Values of longitudinal relaxation times (T1, s) of DPS hydrogens isolated and in presence of βCD in the inclusion complex obtained by the conventional inversion-recovery method

Proton	T1$_{DPS}$ (s)	T1$_{DPS:\beta CD}$ (s)
H1$'$	2.78 ± 0.54	1.02 ± 0.16
H2$'$	3.11 ± 0.17	1.73 ± 0.22

Table 5 Values of longitudinal relaxation times (T1, s) of βCD hydrogens isolated and in presence of DPS in the inclusion complex obtained by the conventional inversion-recovery method

| Proton	T1$_{\beta CD}$ (s)	T1$_{DPS:\beta CD}$ (s)
H1	1.2 ± 0.1	0.73 ± 0.07
H3	1.56 ± 0.13	1.48 ± 0.07
H5	0.63 ± 0.03	0.39 ± 0.03
H4	0.91 ± 0.06	0.81 ± 0.04

technique allows distinguishing between the free host and the inclusion complex, since the chemical environment between these two species is different and as result the ^{13}C chemical shift patterns are different [25]. Therefore, one can obtain the spectra of DPS, crystalline and lyophilized free βCD, their PM and their lyophilized complex.

Fig. 3 ROESY-1D spectrum of DPS:βCD inclusion complex obtained when the H1′ proton at 6,731 ppm of DPS was selectively irradiated, showing the NOE effects in the βCD hydrogens signals with mixing time of 0.5 s (500 MHz; D$_2$O)
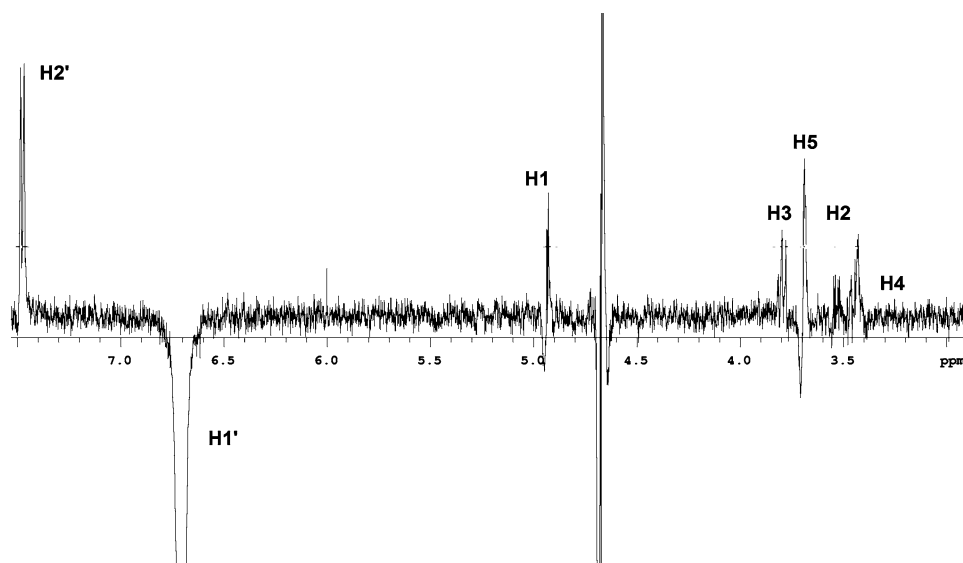
Fig. 4 ROESY-1D spectrum of DPS:βCD inclusion complex obtained when the H2′ proton at 7,519 ppm of DPS was selectively irradiated, showing the NOE effects in the βCD hydrogens signals with mixing time of 0.5 s (500 MHz; D$_2$O)
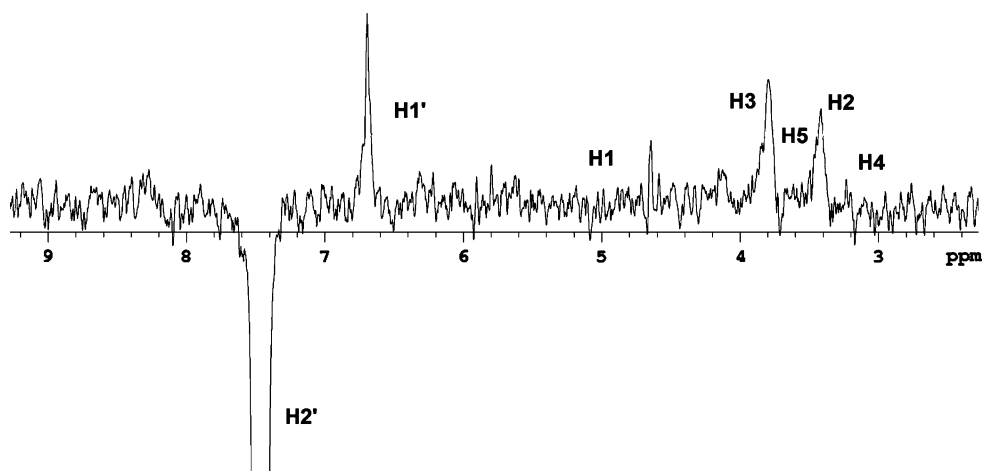
The use of ^{13}C-CP/MAS NMR in CD characterization provides information on the molecular organization at shorter distance scales [26]. The comparison of the lyophilized and non-lyophilized βCD spectra is necessary because some changes in their pattern can occur when one compares the spectrum of the isolated molecules with the inclusion compound due to the freeze-drying process, which leads to amorphization of its structure, with no indication of the host–guest interaction. The carbon resonances of the spectra of βCD were assigned as previous reported [18]. Figure 5 shows the attributions of each carbon in the molecule of DPS and the ^{13}C-CP/MAS NMR spectra of DPS, non freeze-dried and freeze-dried βCD, the PM and the suggested inclusion compound of DPS:βCD (Fig. 6).

Chemical shifts of each carbon of βCD, DPS, PMs and complex are in Tables 6, 7, and 8.

Upon analysis of these spectra and tables, one can observe that the $\Delta\delta$ when DPS is complexed with βCD is

larger than in the PM. Also, peak broadening occurs in the inclusion complex spectrum. Both facts suggest that DPS is encapsulated into the CD cavity. Moreover, it is evident that the PM spectrum is a combination of the spectrum of βCD and DPS and no interaction occurs.

Therefore, in view of all results exposed above, we can suppose that the inclusion compound DPS:βCD present the following structure, where the aromatic ring of DPS is located inside the βCD cavity.
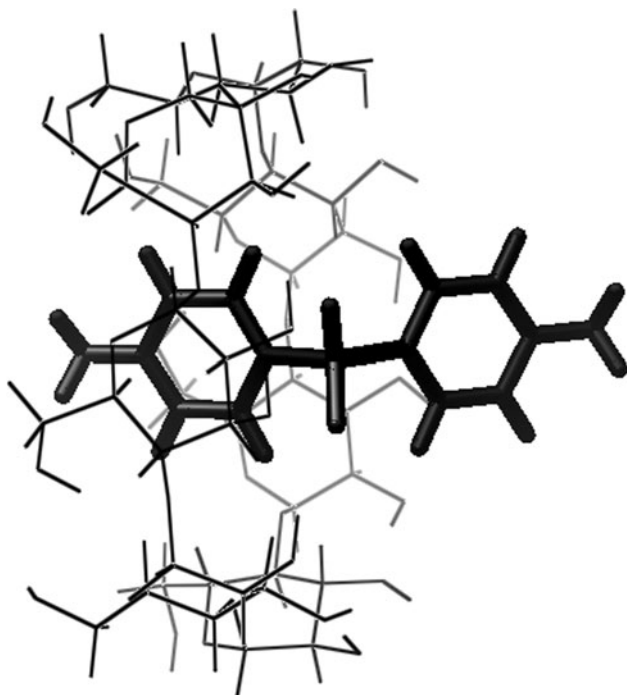
Conclusions

In summary, NMR spectroscopy was applied to characterize all the substances and the complex between DPS and βCD. First, a suggested inclusion compound of DPS:βCD was prepared successfully by a modified co-precipitation/ freeze-drying method in a 1:1 molar ratio. Also, its PM was prepared in the same molar ratio in such a way so that one

Fig. 5 a Attributions of each carbon in the molecule of DPS. **b** ^{13}C-CP/MAS NMR spectra of DPS. **c** ^{13}C-CP/MAS NMR spectra of nature βCD (non freeze-dried) and **e** freeze-dried βCD (10 kHz, 298 K) and **d** ^{13}C-CP/MAS NMR spectra of PM and **f** inclusion compound of DPS:βCD (10 kHz, 298 K)
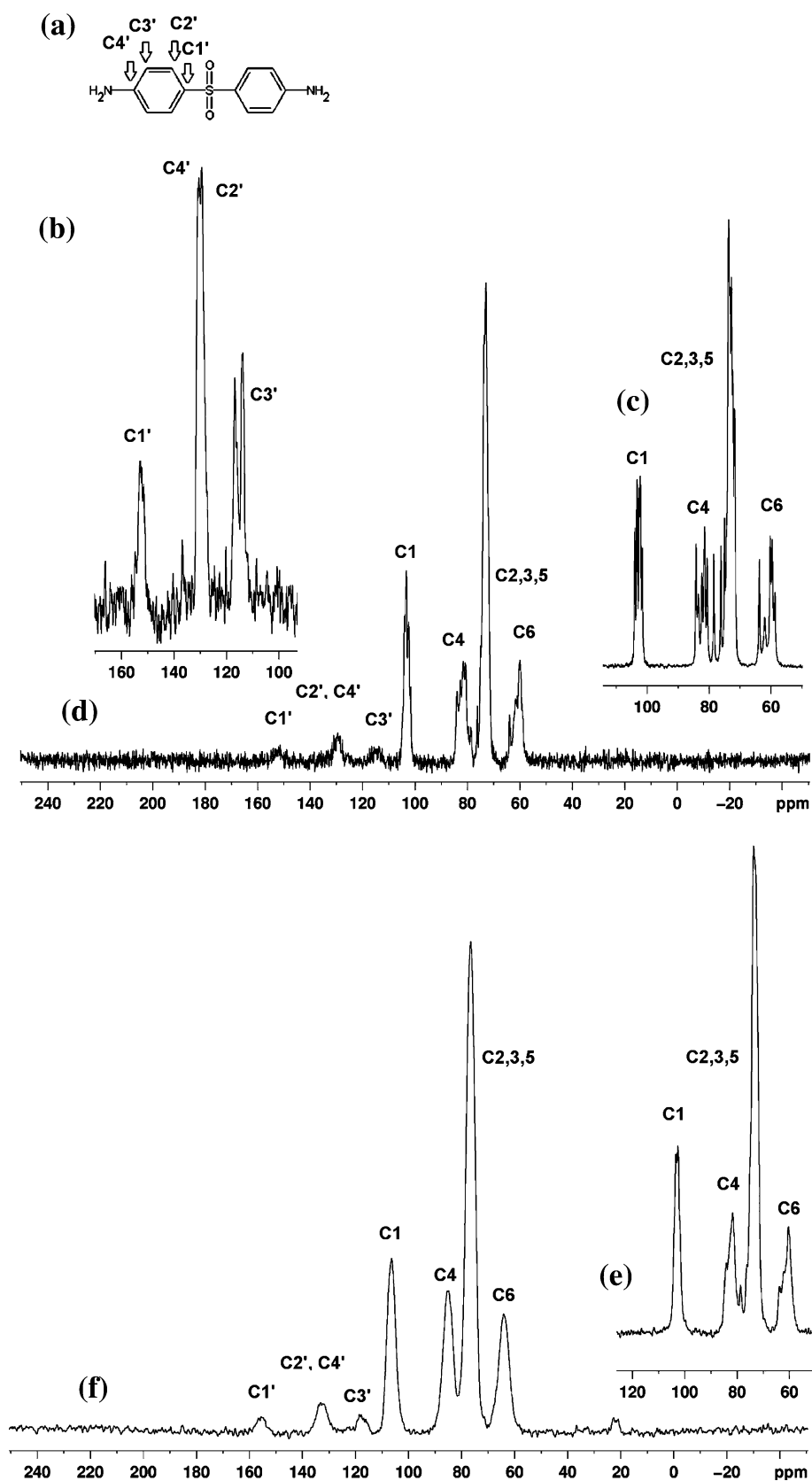
Fig. 6 Suggested structure of the inclusion compound DPS:βCD

Table 6 ^{13}C-CP/MAS NMR chemical shifts of βCD non-freeze dried and their changes in the presence of DPS ($\Delta\delta = \delta_{PM} - \delta_{free}$)

C	$\delta_{\beta CD}$	$\delta_{DPS:\beta CD - PM}$	$\Delta(\delta_{DPS:\beta CD} - \delta_{\beta CD})$
C1	102.5	102.3	0.2
C2,3,5	73.6	73.6	0
C4	81.6	81.5	0.1
C6	63.7	63.8	−0.1

Table 7 ^{13}C-CP/MAS NMR chemical shifts of βCD freeze dried and their changes in the presence of DPS ($\Delta\delta = \delta_{complexed} - \delta_{free}$)

C	$\delta_{\beta CD - FD}$	$\delta_{DPS:\beta CD}$	$\Delta(\delta_{DPS:\beta CD} - \delta_{\beta CD - FD})$
C1	102.5	106.4	4.4
C2,3,5	73.7	76.5	2.8
C4	81.4	84.9	3.5
C6	63.8	64.1	0.3

Table 8 ^{13}C-CP/MAS NMR chemical shifts of DPS and their changes in the presence of βCD in the PM ($\Delta\delta = \delta_{PM} - \delta_{free}$) and in the inclusion complex ($\Delta\delta = \delta_{complexed} - \delta_{free}$)

C	δ_{DPS}	$\delta_{DPS:\beta CD - PM}$	$\Delta(\delta_{DPS:\beta CD - PM} - \delta_{\beta CD})$	$\delta_{DPS:\beta CD}$	$\Delta(\delta_{DPS:\beta CD} - \delta_{\beta CD - FD})$
C1′	152.8	153	0.2	155.5	2.7
C2′,4′	130	130.2	0.2	133.1	3.1
C3′	115.5	115.2	−0.3	118.6	3.1

could compare the interaction between both molecules by the two methods. The data obtained by the NMR techniques clearly indicate the formation of the inclusion compound. In simplest experiment, ^{1}H-NMR, the presence of both molecules induces strong chemical shifts in the protons H3 and H5. Also, according to Greatbanks and Pickford [17], as $\Delta\delta$ H3 $\leq \Delta\delta$ H5 the DPS molecule is included more deeply inside the cavity. In solution, the techniques of DOSY and T1 indicates a high association constant between host:guest, because the D and the T1 of DPS became similar in magnitude to those of D and T1 of CD. Moreover, DOSY provide a percentage of association of 84.6 %, indicating the only approximately 15.4 % of DPS molecules are not interacting with βCD. The ROESY results confirm how the drug molecule is spatially located inside the βCD cavity, i.e., the aromatic ring is located inside the cavity with its protons H1′ and H2′ interacting with the CD protons, because in the ROESY spectra is observed that the proton from the aromatic region of DPS present correlations with the protons from the βCD cavity.

The results of ^{13}C-CPMAS also confirm that there are interactions between the two components in the solid-state, not only by the induced chemical shifts observed, but also by the differences presented in the spectrum of the inclusion complex and the PM, in which no chemical shifts occurred. In the second part of this work, the DAP:βCD inclusion compound was characterized by other important techniques, infrared spectroscopy, differential scanning calorimetry, thermal gravimetric analysis and X-ray diffractometry. Also, the stoichiometry of this complex was determined by a job plot. The stability constant and the thermodynamics properties were obtained by fluorescence spectroscopy.

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