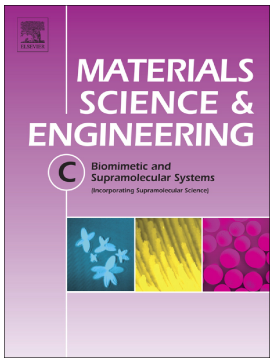
Accepted Manuscript



Succinyl-β-cyclodextrin: Influence of the substitution degree on albendazole inclusion complexes probed by NMR

J. Priotti, A. García, D. Leonardi, M.J. Ferreira, M.C. Lamas, T.G. Nunes

PII:	S0928-4931(17)32385-8
DOI:	doi:10.1016/j.msec.2018.07.013
Reference:	MSC 8725
To appear in:	*Materials Science & Engineering C*
Received date:	3 July 2017
Revised date:	29 June 2018
Accepted date:	3 July 2018

Please cite this article as: J. Priotti, A. García, D. Leonardi, M.J. Ferreira, M.C. Lamas, T.G. Nunes , Succinyl-β-cyclodextrin: Influence of the substitution degree on albendazole inclusion complexes probed by NMR. Msc (2018), doi:10.1016/j.msec.2018.07.013

Succinyl-β-cyclodextrin: influence of the substitution degree on albendazole inclusion complexes probed by NMR

J. Priotti[a], A. García[b], D. Leonardi[a,b], M. J. Ferreira[c], M. C. Lamas[a,b,*], T. G. Nunes[c,**]

[a]IQUIR-CONICET, Suipacha 570, 2000 Rosario, Argentina

[b]Área Técnica Farmacéutica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 570, 2000 Rosario, Argentina

[c]CQE, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

[a,*] CORRESPONDING AUTHOR.
E-mail address: mlamas@fbioyf.unr.edu.ar

[c,**] CORRESPONDING AUTHOR.
Tel. +351-21-8419043; Fax +351-21-8464455.
E-mail address: teresa.nunes@tecnico.ulisboa.pt

Abbreviations

ABZ, Albendazole; CD, Cyclodextrin; NMR, Nuclear Magnetic Resonance; ROESY, rotating-frame Overhauser spectroscopy; DS, degree of substitution; FWHM, full width at half height.

KEYWORDS: CYCLODEXTRIN; SUCCINYL SUBSTITUENT; ALBENDAZOLE; SOLUTION NMR; SOLID-STATE NMR

ABSTRACT

Succinyl-β-CD derivatives were obtained by green synthesis with degrees of substitution (DS) 1.3 and 2.9. The spray-drying technique was used to obtain albendazole (ABZ):succinyl-β-CD inclusion complexes. Phase solubility diagrams indicated that both succinyl-β-CD derivatives formed 1:1 molar ratio ABZ complexes, but the complex with DS 2.9 has a lower formation constant. The presence of stable inclusion complexes in aqueous solution was confirmed by NMR. For both complexes the aromatic moiety is encapsulated into the host cavity. In the solid-state, ^{13}C and ^{15}N NMR spectral differences between ABZ and ABZ included in spray-dried systems showed that strong structural changes occurred in the systems. At least two different ABZ amorphous species were identified based on DS. ABZ species were stable over more than six months based on spectral data. Finally, the influence of DS in the number and type of the inclusion complexes was elucidated.

1. Introduction

About 24% of the world's population suffers from soil-transmitted helminth infections, mainly disseminated in tropical and subtropical areas, particularly in sub-Saharan Africa, the Americas, China and East Asia [1]. Albendazole (ABZ) (methyl N-(6-propylsulfanyl-1H-benzimidazol-2-yl) carbamate), is one of the most effective broad-spectrum anthelmintic agents, also active against various protozoa [2]. In the solid-state, ABZ presents three tautomeric forms (Forms I, II and III) [3], I and II are enantiotropically related forms [4], which are desmotropes [5], as confirmed by several techniques including solid-state NMR [6] (see Scheme 1A). ABZ Form I, the commercially available one, is metastable at room temperature while Form II is stable. A method based on Raman spectroscopy to obtain the abundance of Form I in ABZ bulk drug has recently been proposed [7]. The physical stability of both forms is explained on the basis of the high-energy barrier necessary for interconversion [4].

Due to its low solubility in water (1 μg/mL) [8], ABZ has poor bioavailability and therefore an unpredictable therapeutic response. The bioavailability of poorly water-soluble drugs is a major concern in the pharmaceutical industry [6], and has lead to the development of several approaches to enhance the solubility and the dissolution rate of these type of molecules. In the case of ABZ, crystal engineering using either solvent recrystallization or spherical agglomeration has been reported. With hydroxypropylcellulose as a binder, a 5.60-fold improvement in dissolution efficiency was achieved when compared to raw ABZ [9]. Drug amorphization is another ongoing approach to improve solubility, as recently reviewed [10]. β-Cyclodextrin (CD) inclusion complexes have been reported to stabilize amorphous ABZ and consequently improve its solubility and dissolution rate in water [2, 11]. The complexes

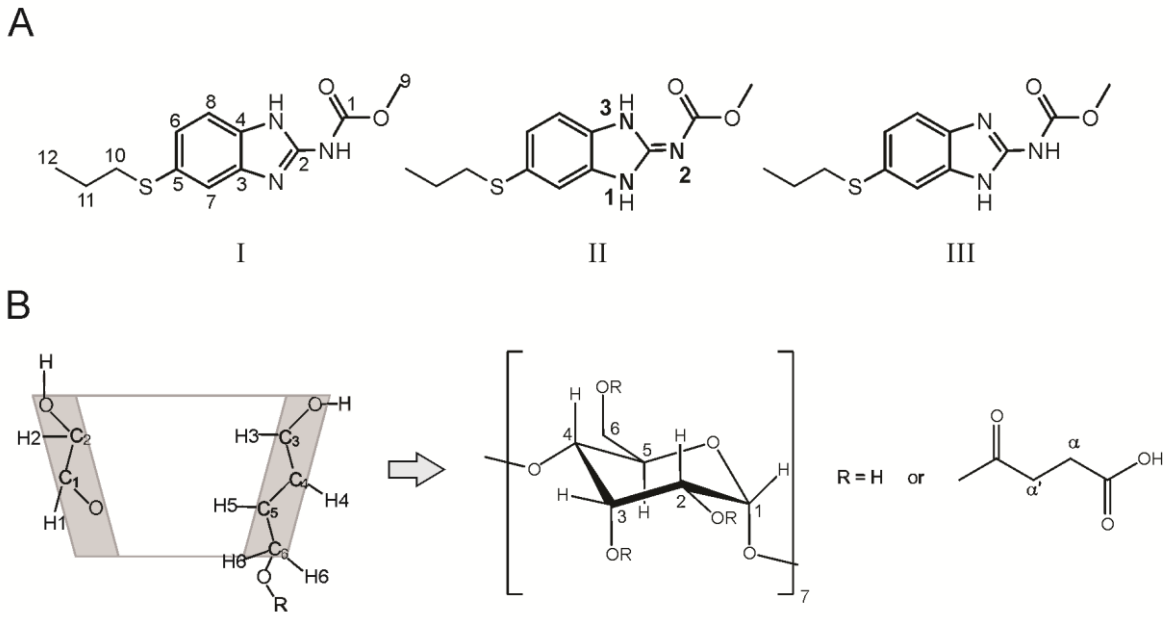
were obtained by the spray-drying technique which is widely used to prepare formulations of amorphous compounds [12].

Several β-CD derivatives have been used as hosts of poorly water-soluble drugs because β-CD has also low solubility. This CD, in particular, has a quite rigid structure due to the presence of a hydrogen-bonding network. At the wider edge, the secondary OH groups 3 and 2, in sequential glucose units (Scheme 1B), form intramolecular bonds [13]. At the narrow rim, the primary hydroxyl groups, located in the 6 position, are not involved in intramolecular hydrogen bonds and, by suitable rotation, may partly block the torus cavity [14]. The three types of hydroxyl groups have different reactivities [15]. The OH groups 6 (primary alcohols) are more nucleophilic than OH 2 and 3 (secondary alcohols). Therefore, selective functionalization of the primary hydroxyl groups has been mostly studied [15]. Design and evaluation of CD-based drug formulations have been reviewed and aspects like drug release enhancement [16, 17] and the ability to deliver a drug to a targeted site have been discussed [16].

Overall, numerous CD-derivatives, CD-polymers and CD-conjugates, classified as hydrophilic, hydrophobic or ionic derivatives have been evaluated for pharmaceutical applications [18]. Supramolecular nanoarchitectures may be formed by amphiphilic CD-derivatives in the presence of phospholipids, surfactants or oligonucleotides with potential biomedical applications [19].

Solid-state NMR studies on ABZ and several β-CD derivatives have been reported. A strong dependence of the number and type of ABZ species on the β-CD substituent has been revealed [2]. The solubility of ABZ was improved drastically by the complexation with citrate-β-CD in comparison with other native and substituted CDs, by the way dominant signals in solid-state NMR spectra were assigned to an inclusion complex [2, 11]. A succinyl-β-CD derivative (Scheme 1B) with DS 2.9 was also used to prepare ABZ:succinyl-

β-CD spray-dried samples which were studied by multiple techniques [20]. The data support the formation of an equimolar inclusion complex with the aromatic ring partially inserted in the CD cavity and the CH_3 group closer to the succinyl methylene groups [20]. However, DS is expected to influence guest-host interactions, as already reported on 2-hydroxyethyl-β-CD and 2-hydroxypropyl-β-CD used as hosts of poorly water-soluble drugs [21-23]. Aiming to improve ABZ solubility in water, the main goal of the present study was to evaluate ABZ:succinyl-β-CD spray-dried samples as a function of the average number of succinyl substituents. Therefore, we synthesized succinyl-β-CD derivatives with two different DS that were subsequently used to prepare ABZ:succinyl-β-CD spray-dried samples. Assuming that solubility enhancement may be related to the presence of inclusion complexes, NMR experiments in solution (ROESY) and solid-state (^{13}C and ^{15}N CP/MAS) were thoroughly performed to validate this hypothesis.



SCHEME 1. CHEMICAL STRUCTURES CORRESPONDING TO THREE POSSIBLE ABZ TAUTOMERS (A) AND SUCCINYL-β-CD (B). ATOM NUMBERINGS FOLLOWED IN NMR SPECTRAL ASSIGNMENT ARE ALSO SHOWN.

2. Materials and Methods

2.1. Materials

ABZ, succinic acid and sodium hypophosphite monohydrate were supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). β-CD was kindly donated by Ferromet S.A. (agent of Roquette in Argentina). All other chemicals used in this study were of analytical reagent grade.

2.2. Methods

2.2.1. Synthesis of Succinyl-β-CD

Succinyl-β-CD derivatives with DS 1.3 and 2.9 were obtained by green synthesis as previously reported [20]. For this purpose, succinic acid (8.12 mmol) was dissolved in water (0.8 mL); sodium hypophosphite monohydrate (0.58 mmol) and β-CD (1.16 mmol) were subsequently added. DS 2.9 was obtained when the β-CD was previously dried at 60ºC for 72 h. DS 1.3 was obtained when the β-CD was not dried. The reaction mixture was refluxed at 118 ºC for 5 h. After that, absolute ethanol (20 mL) was added to precipitate the product. The precipitate was filtered and washed until the pH of the supernatant was neutral. Finally, the product was dried at 60 ºC for 24 h. The DS of the synthesized β-CD derivatives were obtained as described next. ^{1}H NMR spectra were recorded from succinyl-β-CD samples prepared at 24 mM in D_2O (see Fig. S1 in Supplementary material). In order to calculate each DS, the areas of the peaks assigned to the methylene group (H6a and H6b, Scheme 1) and to the anomeric hydrogen H1 were used in the following equation:

$$DS = \left(\frac{Area_{H6a} + Area_{H6b}}{2 x Area_{H1}} \right) x7 \qquad (1)$$

2.2.2. PHASE SOLUBILITY DIAGRAMS

Phase solubility studies were performed according to Higuchi and Connors [24]. Excess amount of ABZ was added to solutions in glass vials containing different concentrations of succinyl-β-CD (0-50 mM) with DS 1.3 or 2.9. Samples were shaken at 180 rpm, for 72 h at 25ºC. After that, samples were filtered through a 0.22 µm membrane filter and ABZ concentration was determined at 292 nm using UV spectrophotometry.

The formation constants of inclusion complexes (K_C) were calculated from the linear plot of the phase solubility diagram according to the equation:

$$K_c = Slope / S_0(1 - Slope) \qquad (2)$$

where S_0 is the intrinsic solubility of drug.

2.2.3. PREPARATION OF THE INCLUSION COMPLEXES AND PHYSICAL MIXTURES

Binary systems of ABZ with succinyl-β-CD derivatives (1:1 molar ratio) were prepared using the spray-drying technique, as previously described [11]. Briefly, ABZ (0.56 mol) was dissolved in acetic acid (10 mL) and succinyl-β-CD (0.56 mol) was dissolved in water (20 mL) and subsequently added. The resulting solution was spray-dried in a Mini Spray Dryer Büchi B-290 (Flawil, Switzerland).

Additionally, physical mixtures between CDs and ABZ were prepared in a mortar by mixing the drug and carrier for 10 minutes.

2.2.4. APPARENT SOLUBILITY

Apparent solubility values of ABZ loaded in the inclusion complexes with both succinyl-β-CD (DS 1.3 or 2.9) were obtained from equilibrium solubility studies.[20] Briefly, an excess of each sample was placed into sealed vials with 10 mL of bidistilled water (pH 6.3) and the

samples were shaken at 25 ºC for 72 h. Samples were filtered and ABZ concentration was analyzed by spectrophotometry as already described in section 2.2.2.

2.2.5. ^{1}H NMR IN SOLUTION

NMR SPECTRA WERE RECORDED ON A BRUKER AVANCE 300 SPECTROMETER (KARLSRUHE, GERMANY). ROESY SPECTRA WERE ACQUIRED FROM ABZ:SUCCINYL-β-CD SPRAY-DRIED SAMPLES (10 MG) SOLUBILIZED IN 0.1 N DCL (0.5 ML). THE CHEMICAL SHIFTS WERE REFERENCED TO THE INTERNAL REFERENCE OF D_2O (δ 4.7 PPM).

ROESY measurements were performed following these conditions: 32 scans, acquisition time 0.222 s, pulse delay 1.92 s and 512 data points.

2.2.6. ^{13}C AND ^{15}N SOLID-STATE NMR

Powdered samples of ABZ:succinyl-β-CD with DS 1.3 or 2.9 (~200 mg) were packed into 7 mm o.d. cylindrical zirconia rotors, equipped with Kel-F caps. ^{13}C cross polarization/magic angle spinning (CP/MAS) spectra were obtained at 75.485 MHz on a Tecmag Redstone/Bruker 300 WB spectrometer at a MAS rate of 3.5 kHz with 90º RF pulses of about 4 μs, contact time of 1 ms and a relaxation delay of 10 s. The contributions of the ^{13}C spinning side bands, particularly intense in the aromatic and carbonyl regions, were suppressed by running the spectra with the TOSS sequence [25]. ^{15}N were acquired at 30.415 MHz using the standard CP/MAS sequence with 90º RF pulse of 7.3 μs, 3 ms contact time, 5 s relaxation delay and a total of transients always higher than 30000.

^{13}C and ^{15}N chemical shifts were referenced with respect to external glycine (^{13}CO observed at 176.03 ppm and $^{15}NH_2$ at 32.4 ppm in the liquid $^{15}NH_3$ scale, respectively; the ^{15}N chemical shifts can be converted to the nitromethane scale by adding -380 ppm, because $^{15}NH_2$ is observed at -347.6 ppm in this scale). Data were always measured at about 20ºC.

Gaussian functions were chosen to iteratively deconvolute key signals in ^{13}C CP/MAS spectra by the least-squares method using the software Origin (Microcal Software, Inc., USA).

3. Results and discussion

3.1. Synthesis of β-CD derivatives, phase solubility diagrams and apparent solubility studies

High yield succinyl-β-CD derivatives were obtained by green synthesis with degrees of substitution (DS) 1.3 with a yield of 77.9% and 2.9 with a yield of 76.2%.

The correlation between the ABZ solubility (guest) and the succinyl-β-CD derivatives concentration was confirmed by phase solubility studies described as follows.

Phase solubility diagrams revealed that the amount of solubilized ABZ varies linearly with the concentration of succinyl-β-CD both with DS 1.3 or 2.9 (Fig. 1). These data are typical of A_L-type systems [24]. Therefore, pHASE SOLUBILITY studies indicated that succinyl-β-CD derivatives with DS 1.3 or 2.9 formed 1:1 inclusion complex with ABZ.

Table 1 shows the formation constants obtained from β-CD and succinyl-β-CD with two different DS.

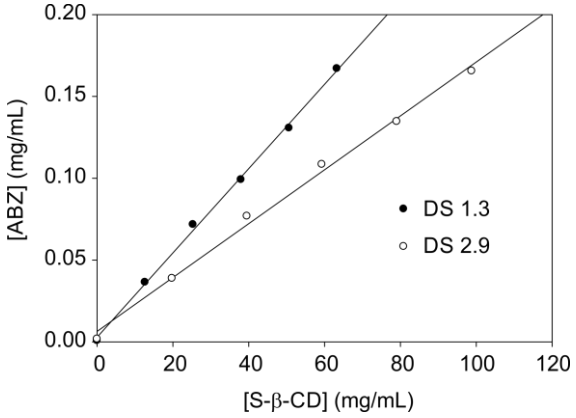


FIG. 1. PHASE SOLUBILITY DIAGRAMS OF ABZ IN THE PRESENCE OF succinyl-β-CD (S-β-CD) both with DS 1.3 or 2.9.

TABLE 1

Formation constants (K_c, M^{-1}) obtained for ABZ:β-CD complexes with the β-CD and succinyl-β-CD both with DS 1.3 or 2.9.

	β-CD	**succinyl-β-CD**	
DS	-	1.3	2.9
***K*$_c$**	68[(a)]	1164	515

[(a)][11]

THE STABILITY OF THE ABZ:SUCCINYL-β-CD INCLUSION COMPLEX WAS ABOUT 2-FOLD HIGHER USING A β-CD DERIVATIVE WITH A DS 1.3 THAN WITH THREE SUBSTITUENT GROUPS PER MOLECULE (DS 2.9).

THE APPARENT SOLUBILITY OF THE ABZ LOADED IN THE INCLUSION COMPLEX PREPARED WITH A DERIVATIVE WITH A DS 1.3 WAS 0.095 ± 0.001 MG/ML.

NOTICEABLY, A LOWER DS PRODUCE A β-CD DERIVATIVE WITH HIGHER COMPLEXING ABILITY, PERHAPS BECAUSE THERE IS NO INTERACTION OR STERIC HINDRANCE BETWEEN THE DERIVATIVE AND THE ABZ. THE DECREASE IN THE COMPLEXING ABILITY OF SUCCINYL-β-CD DERIVATIVE WITH DS 2.9 MAY BE DUE TO

A STERIC HINDRANCE OF SEVERAL SUCCINYL GROUPS, WHICH IS NOT BALANCED BY AN INCREASE IN THE HYDROPHOBICITY OF THE SUBSTITUENT.

OTHERWISE, THE APPARENT SOLUBILITY OF ABZ LOADED IN THE INCLUSION COMPLEX PREPARED WITH A DERIVATIVE WITH A DS 2.9 WAS 0.143 ± 0.003 MG/ML. THUS, THE BETTER COMPLEXING ABILITY IT IS NOT DIRECTLY PROPORTIONAL TO A HIGHER APPARENT SOLUBILITY OF ABZ. Spray-drying technique increased free ABZ solubility three times (see Supplementary data). Therefore, these results were achieved by the combination of the spray drying technique and the effect of the inclusion complex formation with succinyl-β-CD derivatives.

3.2. ^{1}H NMR IN SOLUTION

A succinyl-β-CD derivative with DS 2.9 and the ABZ:succinyl-β-CD spray-dried sample were previously studied using multiple solution NMR techniques and a full assignment was reported on ABZ and succinyl-β-CD NMR signals [20]. The rotating-frame Overhauser spectroscopy (ROESY) was selected to obtain evidences on the eventual presence and type of inclusion complexes. Such experiments allow the probing of inter- and intra-molecular interactions because the observation of cross-peaks indicates that the distances between the hydrogen nuclei from probed molecules are less than 0.4 nm [26]. The ROESY spectrum of ABZ:succinyl-β-CD system with DS 1.3 was obtained (Fig. 2). The spectrum shows correlation signals between the three aromatic protons of ABZ (‘6’, ‘8’ and ‘7’), internal protons (H3 and H5) and external proton (H6) of the succinyl-β-CD. However, the intensities of cross-peaks assigned to ‘6’ and ‘7’ protons are weaker than the ones associated with ‘8’ protons. This observation is consistent with ABZ aromatic ring being only partially contained within the cyclodextrin cavity. Moreover, Hα-α’ succinyl protons exhibit correlation with ‘12’ and ‘11’ protons of ABZ, suggesting that propylthio group could be located in the narrow side of succinyl-β-CD. Also, cross-peaks between Hα-α’ and internal

protons of the succinyl-β-CD would indicate that the succinyl group is close to unsubstituted glucopyranose units.

In summary, these data support the formation of an inclusion complex (equimolar, according to the phase solubility studies described in section 3.1) with the aromatic ring of ABZ partially included in the CD cavity and CH_3 closer to the succinyl methylene groups. The ROESY data showed here for the system with DS 1.3 are consistent with the presence of an inclusion complex similar to the species reported on the succinyl-β-CD derivative with DS 2.9 [20]. Therefore, in aqueous solution, similar structural evidences were obtained for ABZ:succinyl-β-CD complexes regardless the β-CD degree of substitution (DS 1.3 or 2.9).

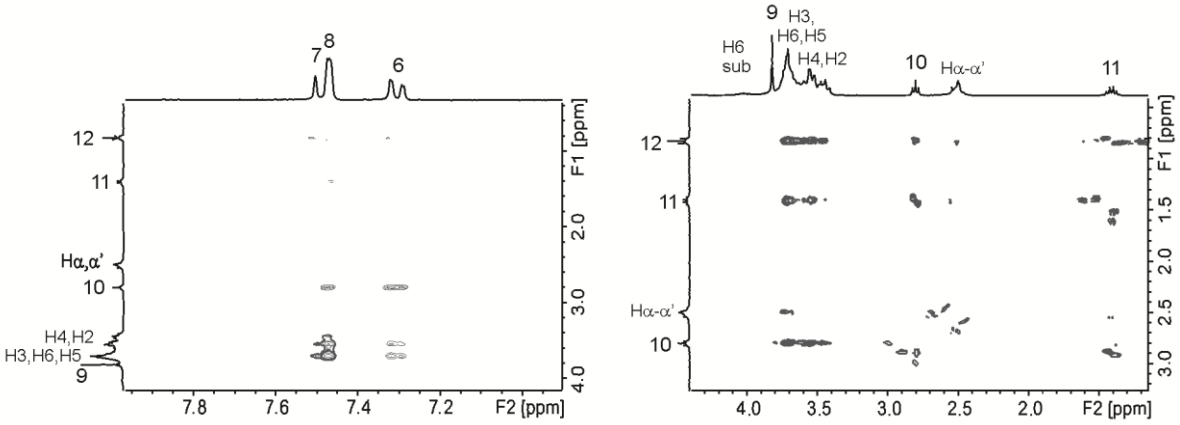


Fig. 2. Plot of two dimensional ROESY spectrum of ABZ:succinyl-β-CD system with DS 1.3.

3.3. Solid-state NMR

3.3.1. ^{13}C NMR of succinyl-β-CD

β-CD succinyl substitution was studied by ^{13}C solid-state NMR to complement ^{1}H NMR data in solution. Fig. 3 displays ^{13}C CP/MAS spectra obtained from succinyl-β-CD with DS 1.3 and 2.9 and Table 2 presents the corresponding chemical shifts. These data show that C2 and C3 did not present any resonances other than those originating from β-CD. Therefore, succinyl substitution occurred mainly at the OH in C6, confirming this as the most reactive group. This is to be expected since in linear chain polysaccharide of β(1→4) linked D-glucose units, like cellulose, this hydroxyl group is the most acidic and frequently the only group involved in esterification, carboxylation and polymer grafting [27].

The observation of β-CD methylene signals from substituted (C6' at 64.54 ppm) and raw molecules (C6 at 60.57 ppm) allow to selectively estimate the corresponding degree of substitution, as shown here for the succinyl-β-CD with the highest DS. The experimental curve was deconvoluted using two Gaussian functions (inset in Fig. 3) and the areas were obtained for C6 and C6', the latter in substituted β-CD molecules: 1.8 and 2.5, respectively. Hence, 58% is from C6' and, since each β-CD molecule has 7 D-glucopyranose units, a rough estimate of DS gives 4 per β-CD molecule. This value is higher than the one previously obtained [20] because it includes the contribution of part of the C2, 3, 5 peak which overlaps with C6'. Therefore, 2.9 was considered the correct DS value.

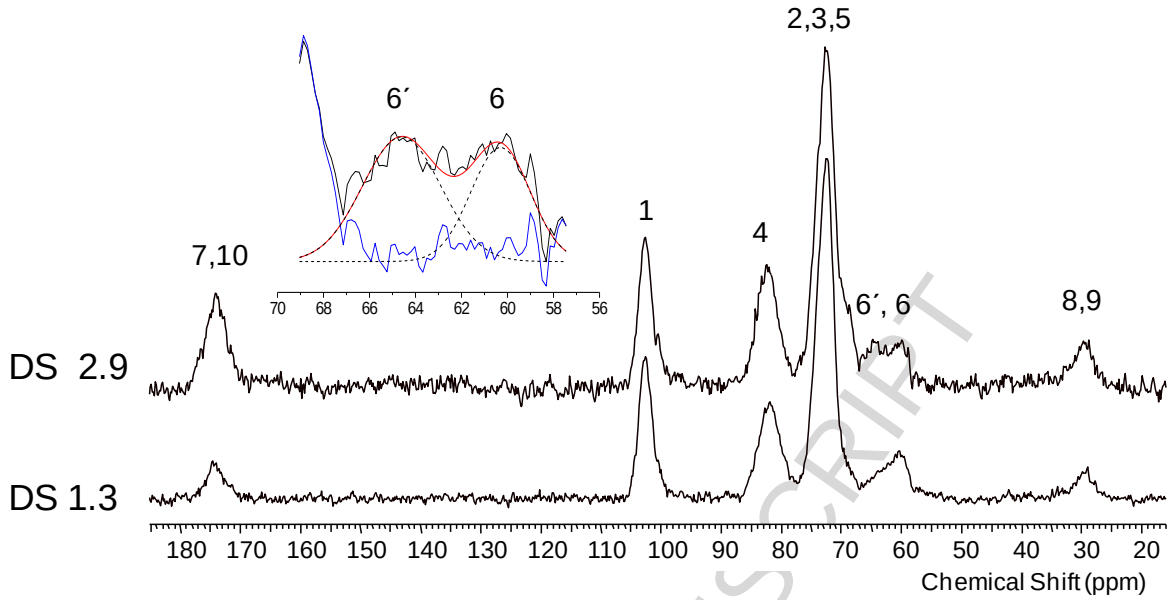


Fig. 3. ^{13}C CP/MAS spectra obtained from succinyl-β-CD with DS 1.3 and 2.9. C6 and C6' are from β-CD and succinyl-β-CD, respectively. The inset displays a sub-spectrum magnification of ABZ:succinyl-β-CD with DS 2.9 which was deconvoluted using two Gaussian functions (dashed lines); blue line shows the fitting residues. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 2
^{13}C chemical shifts obtained from succinyl-β-CD with two different DS (1.3 or 2.9). C6 and C6' are from β-CD and succinyl-β-CD, respectively.

	Chemical shift / ppm						
DS	C1	C2,3,5	C4	C6	C6'	C8,9	C7,10
1.3	102.77	72.67	82.19	60.22	-[a]	29.60	174.56
2.9	102.6, 100.7[b]	72.67, 69.04[b]	82.53	60.57	64.54	29.43	174.21

[a]not resolved. [b]shoulder, tentatively assigned.

3.3.2. ^{13}C and ^{15}N NMR of ABZ and ABZ:succinyl-β-CD spray-dried samples. ^{13}C NMR of ABZ:succinyl-β-CD physical mixtures

Solid-state NMR studies of ABZ:succinyl-β-CD systems are expected to reveal more than one ABZ species, owing to the structural complexity of pure ABZ. Single-crystal X-ray structure was reported on ABZ Form II and tautomer II (Scheme 1) was the tautomeric structure identified, which is best described by tautomer II assuming 50% occupancy of C5 and C6 sites for the propylthioside chain [2]. The single-crystal structure of ABZ Form I, the commercialized ABZ, remains unknown. Recently, a study on ABZ Forms I and II desmotropes using advanced solid-state NMR, powder X-ray diffraction, thermal analysis, and Fourier transform infrared spectroscopy was reported [6]. A complete characterization of both forms has been performed enabling unambiguous identification of the tautomeric species in each desmotrope [6]: based on electronic structure analyses of several benzimidazole carbamate derivatives [3], Forms I and II should correspond to tautomers I and II in Scheme 1, respectively. Moreover, it has also been shown that Forms I and II may be represented as dimeric structures [6].

Multinuclear solid-state NMR studies were previously reported on ABZ Form I (commercially available crystalline powder) but the tautomers that best describe its structure were not unequivocally identified [2]. The full assignment of carbon resonances is in agreement with the presence of three molecules in the crystallographic asymmetric unit [2]. These data has suggested that the propylthioside chain could be located at two different positions in Form I (as in Form II [2]). However, it has remained unclear if Form I is more correctly described by tautomer II (like Form II) or simultaneously by the three tautomers (Scheme 1). The present study aims to bring clarification into this issue. Simulations of the ^{13}C spectra of the three tautomers shown in Scheme 1 were now performed to assist identifying which tautomeric forms are present in the ABZ sample under study (the experimental ^{13}C spectrum is shown in Fig. 4). These simulations gave identical chemical shifts for the propylthio group in the three tautomers (Table 3). However, it must be pointed

out here that such predicted data are only based on substituent effects and do not take into account either conformational effects or intra- and inter-molecular interactions like hydrogen bonds [28]. Hence, the multiple resonances recorded from C10, C11 and C12 in raw ABZ propylthio group can only originate from conformational effects and, therefore, from different molecular packings or more than one molecule in the asymmetric unit cell. Namely, the orientation dependence of aromatic rings must be considered. The influence of the magnetic anisotropy of aromatic rings when submitted to an external magnetic field B_0 is well known by NMR users. Such rings induce a magnetic field that, depending on the ring orientation towards B_0 and on the nucleus position, decrease or increase the chemical shift due to aromatic ring-current shielding or deshielding, respectively [29]. The lowest chemical shift is expected to be obtained when the ring plane is oriented perpendicularly to B_0 and the nucleus lies near the perpendicular of that plane. Therefore, the three signals obtained from C12 (14.81, 13.95 and 11.70 ppm) must reveal different orientations of the propylthio groups towards ABZ aromatic rings. Also, a single narrow resonance was obtained for C6. Therefore, C6 and C12 NMR data show that, unlike in the crystal structure of Form II, there is no evidence for disorder of the propylthio side chain. In addition, 50% substituent occupancy of C5 and C6 positions should not be considered [4] because, a signal splitting or broadening would be recorded at least from C5 and C6 nuclei. Moreover, by comparing experimental and simulated data, it may be noticed that some C8 and C9 resonances are close to values for tautomers I and III, except for C8 at 107.88 ppm (Table 3). However, C3 and C4 data are similar to the tautomer II simulated results. C1 signal appears at 160.81 ppm, shifted downfield in comparison with simulated data (at about 153 and 155 ppm) most probably due to the involvement of the carbonyl oxygen in hydrogen bonding. In summary, experimental and simulated NMR data present evidences for the presence of more than one

tautomer in ABZ Form I; its structure is better represented as a mixture of tautomers I, II and III (Scheme 1).

^{13}C CP/MAS spectra were recorded from physical mixtures and spray-dried samples of ABZ:succinyl-β-CD systems with DS 1.3 and 2.9. The spectra of physical mixtures are mere superpositions of raw ABZ and succinyl-β-CD signals (see Fig. S2 in Supplementary material). However, strong evidences were obtained for the occurrence of ABZ complexes as far as spray-dried samples are concerned. Fig. 4 shows that ABZ signals in the ^{13}C spectra of ABZ:succinyl-β-CD spray-dried systems are all much broader than the resonances obtained from raw ABZ. This fact proves that, under the NMR detection limit, only amorphous ABZ is present in these systems regardless the DS of the β-CD derivative. The spray-drying technique generated an amorphous state which is not directly associated with the interaction of ABZ and β-CD derivatives (see Supplementary data). Two different ABZ amorphous species were identified, which are designated here as tautomers A and B. The major species at DS 1.3 is tautomer A but when increasing DS to 2.9 another ABZ species is detected (tautomer B) along with tautomer A.

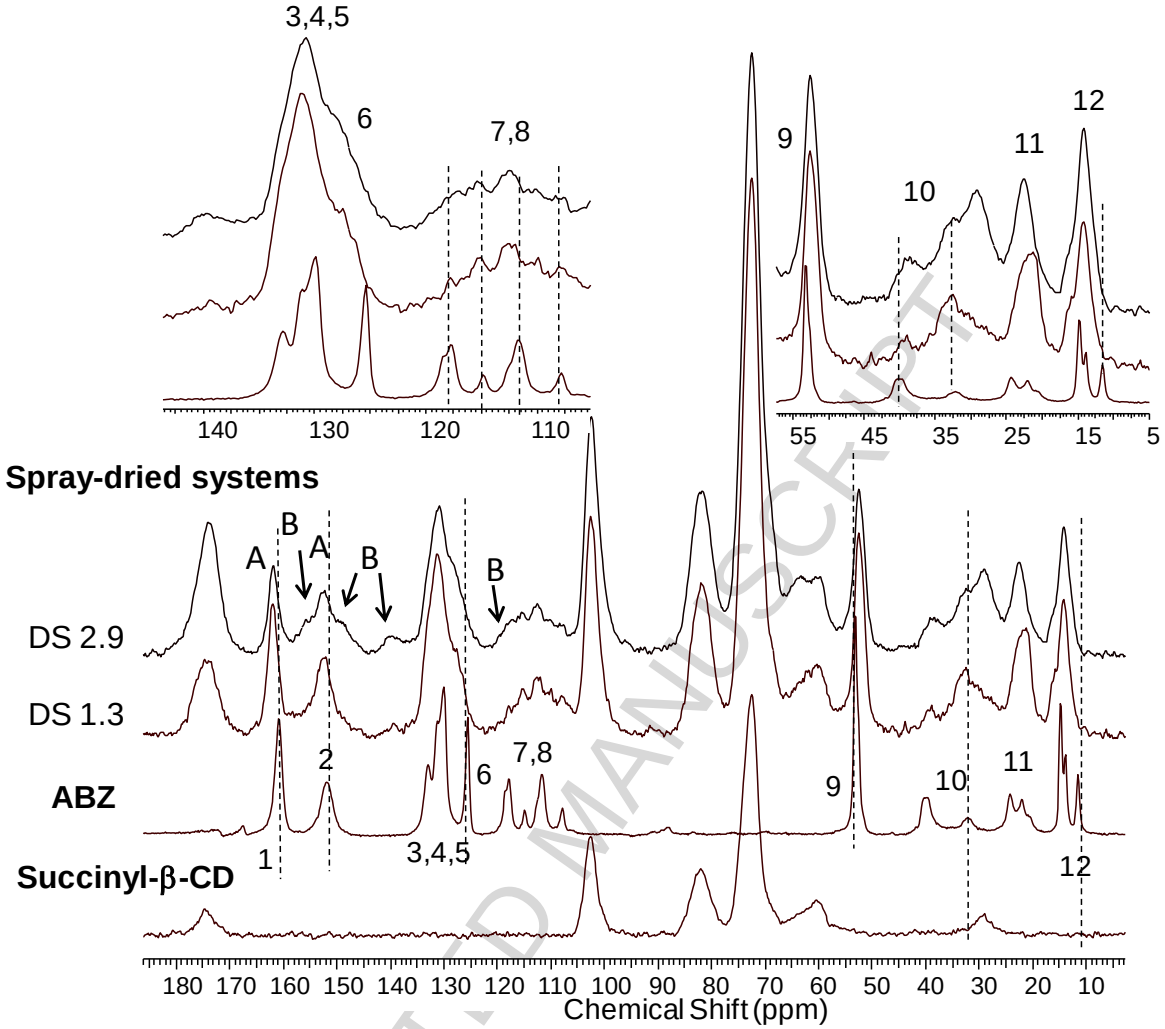


Fig. 4. ^{13}C CP/MAS spectra obtained from succinyl-β-CD (DS 1.3), ABZ and spray-dried ABZ:succinyl-β-CD samples with DS 1.3 and 2.9. The insets show sub-spectra magnifications of ABZ and ABZ:succinyl-β-CD with DS 1.3 and 2.9. The arrows indicate some tautomer B signals only recorded from ABZ:succinyl-β-CD with DS 2.9. In addition, part of tautomer A resonances are labelled. The lines are just guides to the eye.

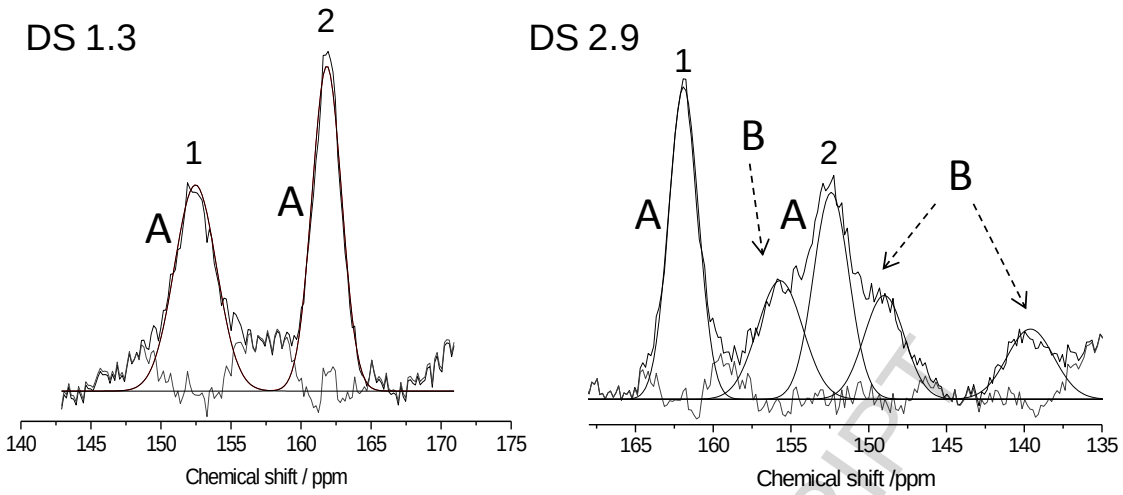


Fig. 5. ^{13}C CP/MAS sub-spectra obtained from ABZ:succinyl-β-CD systems with DS 1.3 and 2.9. The experimental curves were deconvoluted using two or five Gaussian functions, respectively (grey lines show the fitting residues). The arrows indicate the curves with δ (ppm), FWHM (Hz) and integral (arb. units), assigned to tautomer B: 155.87±0.96, 279±135, 0.92±0.35; 148.98±0.76, 211±105, 0.65±0.27 and 139.6±1.2, 226±75, 0.52±0.29. In addition, some tautomer A resonances are labelled. Other data are shown in Table 3.

Some relevant signals both from A and B tautomers are indicated in Fig. 4. In addition, Fig. 5 shows magnifications of sub-spectra of ABZ:succinyl-β-CD with DS 1.3 (140-175 ppm) and 2.9 (170- 135 ppm), and the Gaussian curves used to deconvolute the experimental curves. The parameters obtained to fit C1 and C2 signals (chemical shift, full width at half height (FWHM), and integral) from both tautomers are shown in Table 3; those used to fit the peaks assigned to tautomer B are presented in the Fig. 5 legend.

Table 3 also presents previously reported data on raw ABZ for comparison with the chemical shifts of the ABZ tautomers A and B [2]. The correlation of NMR data with solubility studies enables concluding that tautomer A is the ABZ species assigned to the inclusion complex with higher K_c (1164 M^{-1}, Table 1). Conversely, tautomer B is involved in much less stable complexes because, when both tautomers A and B are present as in spray-dried systems with DS 2.9, the K_c drops to 515 M^{-1} (Table 1) [20].

Based on ^{13}C data, tentative structural elucidations of the ABZ tautomeric forms present in ABZ:succinyl-β-CD systems were performed.

Firstly, ^{13}C data from ABZ:succinyl-β-CD systems and raw ABZ were compared. The most relevant spectral differences are:

a) signals from ABZ in spray-dried systems are broader (regardless DS) indicating the presence of amorphous ABZ in agreement with lack of order at short distance, as already pointed out; b) there are strong relative intensity changes of C7 and C8 signals, in particular for DS 2.9; c) the low-magnetic field shift (1.20 ppm) of C1 in tautomer A, which is consistent with the carbamate group in this tautomer being involved in stronger hydrogen bonds (shorter OH distance); d) a decrease in the number of C10, C11 and C12 resonances (for example, C12 signal at 11.70 ppm is missing and this indicates a structural change of the propylthio moiety in the presence of succynil-β-CD; in pure ABZ, that signal is consistent with C12 positioned above the aromatic ring but such twisted structure does not favor the formation of an inclusion complex); e) C6 resonance of both tautomeric forms is shifted to higher frequency by at least 2 ppm, referenced to raw ABZ.

^{13}C results were then compared with NMR data previously reported on ABZ desmotropes [6], on which basis tautomer A appears to be more similar to Form II than to Form I (Scheme 1). Namely, C1 and C9 were recorded at 162 ppm and 52 ppm, respectively, for Form II as compared with Form I (160 ppm and 53 ppm) [6]. A complete list of chemical shifts was not provided [6] but, based on the presented figures, it is clear that C10 and C12 resonances (at about 40 and 11 ppm, respectively) are missing in Form II spectrum [6]. Hence, these two resonances are from a propylthio moiety only present in Form I and may be used to distinguish it. However, the spectra of ABZ:succinyl-β-CD systems present a resonance at 40 ppm (more intense at DS 2.9) but not at 11 ppm. Although being not reasonable to fully assign tautomer A to a particular ABZ desmotrope, Form II (tautomer II in Scheme 1) remains the most probable ABZ structure at DS 1.3. On the other hand, no reported data on raw ABZ [2, 6] mimic NMR parameters of tautomer B.

Overall, spectral differences between raw ABZ and ABZ in spray-dried systems lead to the conclusion that strong structural changes occurred in the systems. However, tautomerism, possibility of several conformers, different hydrogen bonding rearrangements and molecular disorder, make ABZ:succinyl-β-CD systems difficult to be studied in the solid-state, even by advanced NMR techniques.

Secondly, the comparison of spectral data from ABZ:succinyl-β-CD systems with different DS reveals changes in the number and chemical shifts of C1 and C2 signals, respectively. At lower DS, just two signals were recorded which were designated as part of tautomer A (at 162 ppm and 153 ppm). By increasing DS, in addition to tautomer A resonances, signals from tautomer B (at 155 ppm and 149 ppm) are observed, as mentioned before (see Figs. 4 and 5). Such chemical shift changes may reflect the involvement of C1 in different hydrogen bonding schemes; also, because C2 is directly bound to three nitrogen atoms, those changes may be associated to different nitrogen protonation degrees. Therefore, these results imply different electronic environments being present in tautomers A and B. It must be pointed out here that an unique ABZ species was identified in ABZ:methyl-β-CD (DS 1.8) and ABZ:hydroxypropyl-β-CD (DS 0.9) spray-dried systems; 337 and 313 M^{-1} were the formation constants of the inclusion complexes, respectively [2]. Therefore, such CD-derivatives with higher hydrophobic cavities are able to stabilize amorphous ABZ. The spectral data reported on this ABZ species [2] are similar to the results shown here for tautomer B. In that study, the ABZ species was tentatively assigned to tautomer II (Scheme 1) with two C2 signals (at about 155 ppm and 149 ppm) and the carbamate ^{15}N tentatively assigned to a broad resonance (103 ppm). It is worth noting that we assign here to C1 the resonance at 155 ppm. The signal at 140 ppm, which until now has remained unidentified, is assigned to C5 (Table 3). In order to assist the identification of the corresponding ABZ tautomeric form, the predicted chemical shifts of the three tautomers shown in Scheme 1,

which are listed in Table 3, were examined. By comparing these values with C1, C2 and C5 experimental data obtained for tautomer B (155, 149 and 140 ppm), we can conclude now that ABZ must be in a tautomeric form similar to tautomer I (155, 148 and 137 ppm). Moreover, unlike raw ABZ and ABZ in the spray-dried system with DS 2.9, the high-magnetic field shifts obtained for C1 and C2 indicate that C1 and C2 are not involved in intra- or inter-molecular hydrogen bonds and, therefore, tautomer B is not a dimeric species [6]. Comparison of the present NMR data with earlier results demonstrates that an ABZ species similar to tautomer A was present in ABZ:β-CD and ABZ:citrate-β-CD and, in this system, was the single identified species; the corresponding spectral data were consistent with an ABZ inclusion complex formation (K_c ABZ:β-CD 68 M^{-1} - K_c ABZ:citrate-β-CD 1037 M^{-1}) [2, 11]. The formation of CD inclusion complexes is mainly controlled by van der Waals and hydrophobic interactions, as the major driving forces, although electrostatic interaction and hydrogen bonding may induce conformational changes of an inclusion complex [30].

Amorphous ABZ was stable in both systems over more than six months, based on the absence of any solid-state ^{13}C NMR spectral changes.

Table 3.

^{13}C chemical shifts obtained from ABZ in ABZ:succinyl-β-CD systems with two different DS (1.3 and 2.9). Data previously reported on raw ABZ are also shown for comparison. The predicted chemical shifts [28] for the tautomers shown in Scheme 1 are displayed as well.

Experimental chemical shift / ppm												
ABZ	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
DS=1.3 Tautomer A	162.11 (161.84±0.03, 159±5, 2.55±0.07)[a]	152.59 (152.46±0.07, 226±8, 2.30±0.07)[a]	131.5[d]	131.5[d]	131.5[d]	128[c]	118.2[d],115.6[d], 112.6[d], 111.5[d], 108[d]		52.78	39[f], 33.4	22[e]	16.46[c], 14.38
DS=2.9 Tautomers A and B	162.11, 155.36 (161.91±0.25, 155±35, 1.51±0.32)[a] (155.87±0.96)[b]	152.93, 149.13 (152.41±0.40, 148.98±0.76)[b]	131.3[d]	131.3[d]	131.3[d],140.48 (139.6±1.2)[b]	128[c]	119[e],116[d], 113[d],110[d],108[d]		52.78	39.11,32.89	22.86	14.38
Raw ABZ [2]	160.81	151.82	132.96	131.23	130.02	125.69	117.74, 111.86	114.97, 107.88	53.21, 52.87[c]	40.59, 39.90,32.11[g]	24.50,24.15, 22.08,20.87[c]	14.81,13.95, 11.70
Predicted chemical shift / ppm												
Tautomer I	155.3	148.4	137.0	137.0			120.0	114.1	52.2			
Tautomer II	152.8	154.7	131.0	131.0	137.2	128.2	110.1	117.3	55.5	34.5	21.7	13.2
Tautomer III	155.3	148.4	136.7	135.4	126.8	124.0	115.8	114.1	52.2			

Parameters of the Gaussian functions used in the sub-spectra deconvolution (see Fig. 5): [a] chemical shift (ppm), FWHM (Hz) and integral (arb. units) and [b] chemical shift (ppm), see other data in Fig. 5; [c] shoulder, tentatively assigned; [d] overlapping signals; [e] very broad; [f] low intense signal; [g] assigned to C11 in [2].

Additional information on these systems was obtained from ^{15}N NMR observations. It is worth mentioning that although being extremely sensitive to its local environment, this nuclide is difficult to observe at natural isotopic abundance. Key structural elucidations may however be obtained, as recently reported on several active pharmaceutical ingredients [31]. Intra- and inter-molecular interactions must be considered in the solid-state, particularly because of the influence of the orientation of the nitrogen lone pair electrons.

^{15}N CP/MAS spectra were recorded from ABZ and ABZ:succinyl-β-CD with DS 1.3 and 2.9 (Fig. 6).

The ^{15}N spectrum of pure ABZ reveals a broad signal (~150 ppm) and a narrow resonance (125.1 ppm) that were previously tentatively assigned to the imidazole ring and to the carbamate group, respectively [2]. It must be pointed here that, although the carbamate assignment agreed well with ^{15}N published data [32] it did not take into account intra- and inter-molecular interactions like hydrogen bonds present in ABZ dimeric species [6]. More recently was reported a different assignment on ^{15}N spectra of ABZ desmotropes: Form I signals at 126.7 ppm and 123.2 ppm were from the imidazole ring and 151.6 ppm from the carbamate moiety while Form II resonances at 140 ppm and 122.2 ppm were assigned to the imidazole ring and at 131.2 ppm to the carbamate group (referenced here in the liquid $^{15}NH_3$ scale) [6]. ^{15}N ABZ spectral analysis was based on considering that N3 protonation in both Forms I and II (Scheme 1) originates similar ^{15}N chemical shifts (123.2 ppm and 122.2 ppm in ABZ Form I and Form II, respectively) [6]. The following arguments do not support that full assignment: 1) protonated nitrogens were edited by comparing CP/MAS spectra acquired with different contact times (1 ms or 5 ms) and, due to less effective ^{1}H-^{15}N transfer of magnetization, was expected an intensity decrease of unprotonated nitrogens which, unpredictably, was

only clearly observed for the nitrogen in the carbamate moiety in ABZ Form II but not for N1 in ABZ Form I [6]; 2) that editing experiment is mainly controlled by ^{1}H molecular mobility in the kHz range and more reliable information could be obtained from dipolar dephasing experiments (by turning off the ^{1}H decoupler for a short time prior to ^{15}N signal acquisition); 3) N1 and N3 in the imidazole ring of tautomer II (Scheme 1) have similar electronic environments and therefore should resonate at comparable frequencies, unlike N1 and N3 in tautomer I (with different protonation states, as in tautomer III, Scheme 1). Accordingly, it would be reasonable to expect distinct ^{15}N chemical shifts for N1 and N3 in tautomer I; it has been reported on several imidazoles that, in solution, the resonances of the two nitrogens may differ by about 90 ppm when involved in a double versus a single bond [33]. However, in the solid-state, intra- and inter-molecular interactions must be considered, particularly because of the influence of the orientation of the nitrogen lone pair electrons. Based on the above considerations, we propose now the following ^{15}N assignments: N1, N2, N3 in Form I at ~150, 125.1 and 125.1 ppm (126.7 and 123.2 ppm in [6]) and at 131.2, 140 and 122.2 ppm in Form II (from ^{15}N data in [6]).

The spectra of ABZ in the presence of the carrier show an intense peak assigned to NH in the imidazole ring (124.7 and 125.5 ppm for DS 1.3 and 2.9, respectively). Other weaker signals are observed only from the ABZ:succinyl-β-CD system with DS 1.3 (132.7 ppm, 142 ppm and 146 ppm). These peaks, at lower magnetic field, could originate from unprotonated nitrogens in the imidazole ring and in the carbamate moiety because, conversely, the contribution of the sp^2-hybridized nitrogen lone pair would induce upfield ^{15}N protonation shifts, as demonstrated recently [34]. For DS 2.9, the presence of several ABZ species, as revealed by ^{13}C NMR, generates a broad signal at

about 128 ppm that overlaps the NH well resolved peak but no other resonance was observed.

Some ^{15}N chemical shifts of ABZ:succinyl-β-CD with DS 1.3, apart from 146 ppm, are comparable to those reported on ABZ Form II desmotrope [6] but differently assigned here, as already pointed out: N1, N2, N3 at 131.2, 140 and 122.2 ppm (referenced in the liquid $^{15}NH_3$ scale). However, those ABZ:succinyl-β-CD resonances are all observed at about 2 ppm towards lower magnetic field referenced to the ABZ desmotrope Form II corresponding data [6]; this result along with the signal at 146 ppm is consistent with involvement of the nitrogens in hydrogen bonds. Consequently, these observations lead to the conclusion that tautomer II is the ABZ tautomeric form designated as tautomer A, with the involvement of the nitrogens in the imidazole ring and carbamate moiety in hydrogen bonds most possibly with succinyl-β-CD hydroxyl groups.

Finally, as already mentioned, were proposed dimeric structures for raw ABZ Forms I and II, in agreement with NMR evidences for the presence of inter-molecular hydrogen bonds connecting N1 and N2 (Scheme 1) [6]. In addition, such rearrangement, favours intra-molecular hydrogen bond involving N3-H3 and O1 due to spatial proximity between the ABZ aromatic rings and the methoxy group [6]. Unlike for tautomer B present in ABZ:succinyl-β-CD with DS 2.9, formation of dimers cannot be discarded in the system with DS 1.3. As for the former, ^{13}C evidences of hydrogen bonds involving C1 and C2 were not obtained, which were considered a signature of the presence of ABZ dimeric structures [6]. Molecular dynamics simulations performed to elucidate the hydrogen bond orientations of β-CD and head-to-head dimerization of β-CD monomers with or without a guest molecule, in solvents with different polarity, show that the less stable dimer is expected in polar hydrogen bond accepting solvents, capable of interrupting inter-molecular hydrogen bonds between β-CD components; particularly in

those solvents, included guest molecules reinforce the binding affinity among the two monomers [35].

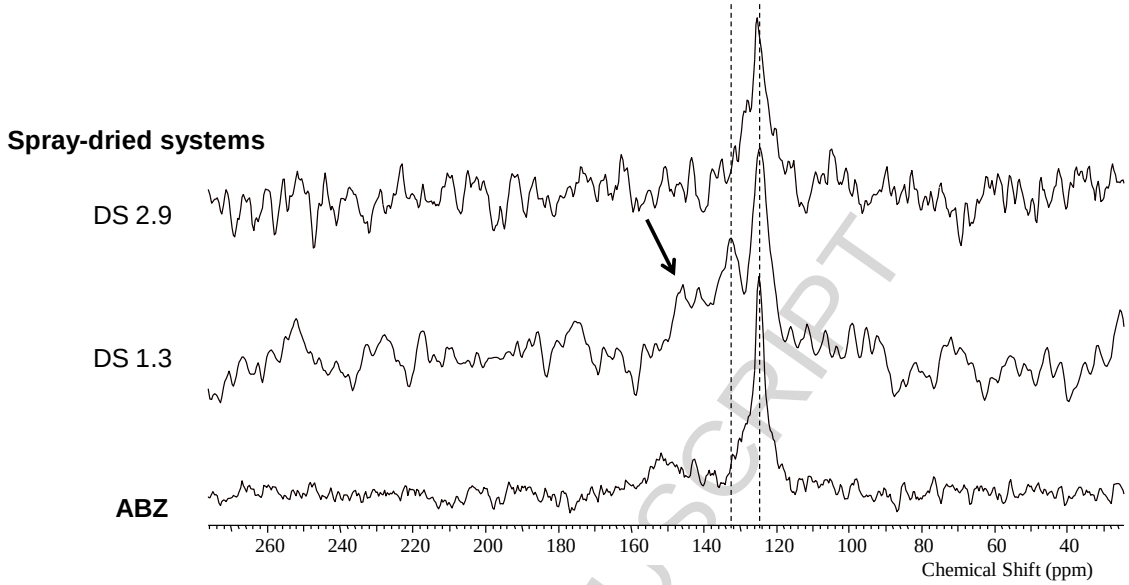


Fig. 6. ^{15}N CP/MAS spectra obtained from ABZ and ABZ:succinyl-β-CD spray dried systems with DS of 1.3 and 2.9 (from bottom to top). The arrow indicates the signal recorded at 146 ppm from ABZ:succinyl-β-CD with DS 1.3.

4. Conclusions

This study provided evidences for the presence of ABZ:succinyl-β-CD inclusion complexes in spray-dried samples. The aqueous solubility of the drug was particularly enhanced using succinyl-β-CD with DS 2.9. It is noteworthy that this effect of solubilization was additive to the spray-drying technique, which increased free ABZ solubility three times and produced ABZ in amorphous state. Two amorphous ABZ species, structurally different, were identified in solid-state NMR spectra from ABZ:succinyl-β-CD with DS 2.9; just one of them was observed at lower DS (1.3), designated here as tautomer A and most probably corresponding to tautomer II in Scheme 1. Because DS is an average value, we hypothesize here that for ABZ:succinyl-β-CD systems with DS 2.9, succinyl-β-CD molecules with DS<2.9 favour the formation

of an inclusion complex and the guest is tautomer A; as for DS>2.9, it is reasonable to expect the presence of tautomer B.

Overall, depending on the DS of β-CD, different ABZ amorphous species can be stabilized. Therefore, the affinity and solid-state structure of ABZ:succinyl-β-CD complexes are directly affected by DS.

Work is now in progress in order to study the influence of temperature on the molecular dynamics and stability of ABZ in the inclusion complexes.

Acknowledgements

This work has been carried out with financial aid from "Fundação para Ciência e Tecnologia" (FCT, Portugal) through the projects RECI/QEQ-QIN/0189/2012. MJGF thanks FCT for the fellowship RECI/QEQ-QIN/0189/2012. JP and AG are grateful to Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET, Argentina) for a Doctoral and Posdoctoral Fellowship. The authors also gratefully acknowledge the Universidad Nacional de Rosario and CONICET (Project N° PIP 112-201001-00194) and Agencia Nacional de Promoción Científica y Tecnológica (Project N° PICT 2006-1126) for the financial support. We would like to thank the staff from the English Department (Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario) for the language correction of this manuscript.

APPENDIX - SUPPLEMENTARY MATERIAL

^{1}H NMR spectra of succinyl-β-CD with DS 1.3 and 2.9 in D_2O are included in the Appendix.

^{13}C SOLID-STATE NMR SPECTRA OF ABZ AND ABZ:SUCCINYL-β-CD PHYSICAL MIXTURES CAN BE FOUND IN THE APPENDIX.

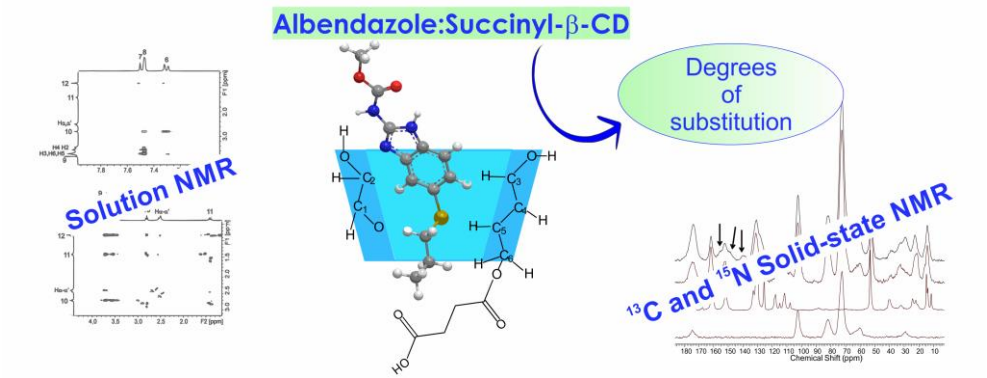
References

[1] WHO, Preventive chemotherapy in human helminthiasis: coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers, WHO Press, Geneva, Switzerland, 2006.
[2] M.J.G. Ferreira, A. García, D. Leonardi, C.J. Salomon, M.C. Lamas, T.G. Nunes, 13C and 15N solid-state NMR studies on albendazole and cyclodextrin albendazole complexes, Carbohydr. Polym., 123 (2015) 130-135.
[3] Y. Kasetti, P.V. Bharatam, Tautomerism in drugs with benzimidazole carbamate moiety: an electronic structure analysis, Theor. Chem. Acc., 131 (2012) 1160.
[4] M.B. Pranzo, D. Cruickshank, M. Coruzzi, M.R. Caira, R. Bettini, Enantiotropically related albendazole polymorphs, J. Pharm. Sci., 99 (2010) 3731-3742.
[5] B. Stanovnik, New Developments in Heterocyclic Tautomerism: Desmotropes, Carbenes and Betaines, Chapter 8 in: Advances in Heterocyclic Chemistry, 119 (2016) 209-239.
[6] A.K. Chattah, R. Zhang, K.H. Mroue, L.Y. Pfund, M.R. Longhi, A. Ramamoorthy, C. Garnero, Investigating albendazole desmotropes by solid-state NMR spectroscopy, Mol. Pharm., 12 (2015) 731-741.
[7] N.L. Calvo, J.M. Arias, A.B. Altabef, R.M. Maggio, T.S. Kaufman, Determination of the main solid-state form of albendazole in bulk drug, employing Raman spectroscopy coupled to multivariate analysis, J. Pharm. Biomed. Anal., 129 (2016) 190-197.
[8] A. Garcia, M.G. Barrera, G. Piccirilli, M.D. Vasconi, R.J. Di Masso, D. Leonardi, L.I. Hinrichsen, M.C. Lamas, Novel albendazole formulations given during the intestinal phase of Trichinella spiralis infection reduce effectively parasitic muscle burden in mice, Parasitol. Int., 62 (2013) 568-570.
[9] A. Thakur, R. Thipparaboina, D. Kumar, K. Sai Gouthami, N.R. Shastri, Crystal engineered albendazole with improved dissolution and material attributes, CrystEngComm, 18 (2016) 1489-1494.
[10] M. Descamps, Amorphous Pharmaceutical Solids, Adv. Drug Deliv. Rev, 100 (2016) 1-212.
[11] A. Garcia, D. Leonardi, M.O. Salazar, M.C. Lamas, Modified beta-cyclodextrin inclusion complex to improve the physicochemical properties of albendazole. complete in vitro evaluation and characterization, PLoS One, 9 (2014) e88234.
[12] A. Singh, G. Van den Mooter, Spray drying formulation of amorphous solid dispersions, Adv. Drug Deliv. Rev, 100 (2016) 27-50.
[13] J. Szejtli, J.L. Atwood, J.M. Lehn, Comprehensive supramolecular chemistry, Pergamon Elsevier Oxford, 1996.
[14] M.L. Bender, M. Komiyama, Cyclodextrin Chemistry, Springer-Verlag, Berlín, 1978.
[15] E. Dienst, B.H. Snellink, I.M. Piekartz-Eissink, M.H. Grote Gansey, F. Venema, M.C. Feiters, R.J. Nolte, J.F. Engbersen, D.N. Reinhoudt, Selective functionalized and

flexible coupling of cyclo-dextrins at the secondary hydroxyl face, J. Org. Chem., 60 (1995) 6537-6545.
[16] K. Uekama, Design and evaluation of cyclodextrin-based drug formulation, Chem. Pharm. Bull. (Tokyo), 52 (2004) 900-915.
[17] R. Challa, A. Ahuja, J. Ali, R.K. Khar, Cyclodextrins in drug delivery: An updated review, AAPS PharmSciTech, 6 (2005) E329-E357.
[18] H.E. Dodziuk, Cyclodextrins and their complexes: chemistry, analytical methods, applications, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, FRG, 2006.
[19] L. Zerkoune, A. Angelova, S. Lesieur, Nano-Assemblies of Modified Cyclodextrins and Their Complexes with Guest Molecules: Incorporation in Nanostructured Membranes and Amphiphile Nanoarchitectonics Design, Nanomaterials, 4 (2014) 741.
[20] A. Garcia, D. Leonardi, M.C. Lamas, Promising applications in drug delivery systems of a novel beta-cyclodextrin derivative obtained by green synthesis, Bioorg. Med. Chem. Lett., 26 (2016) 602-608.
[21] B.W. Müller, U. Brauns, Solubilization of drugs by modified β-cyclodextrins, Int. J. Pharm., 26 (1985) 77-88.
[22] J. Pitha, J. Milecki, H. Fales, L. Pannell, K. Uekama, Hydroxypropyl-β-cyclodextrin: preparation and characterization; effects on solubility of drugs, Int. J. Pharm., 29 (1986) 73-82.
[23] T. Loftsson, B.J. Ólafsdóttir, H. Friðriksdóttir, S. Jónsdóttir, Cyclodextrin complexation of NSAIDSs: physicochemical characteristics, Eur. J. Pharm. Sci., 1 (1993) 95-101.
[24] T. Higuchi, A. Connors, Phase-solubility techniques, Adv. Anal. Chem. Instr., 4 (1965) 117-212.
[25] W.T.S. Dixon, J.; Sefcik, M. D.; Stejskal, E. O.; McKay, R. A. , Total suppression of sidebands in CPMAS C-13 NMR, J. Magn. Reson., 49 (1982) 341-345.
[26] H.J. Schneider, F. Hacket, V. Rudiger, H. Ikeda, NMR Studies of Cyclodextrins and Cyclodextrin Complexes, Chem. Rev., 98 (1998) 1755-1786.
[27] S. Rowland, V. Cirino, A. Bullock, Structural components in methyl vinyl sulfone modified cotton cellulose, Can. J. Chem., 44 (1966) 1051-1058.
[28] A.M. Castillo, L. Patiny, J. Wist, Fast and accurate algorithm for the simulation of NMR spectra of large spin systems, J. Magn. Reson., 209 (2011) 123-130.
[29] G. Merino, T. Heine, G. Seifert, The induced magnetic field in cyclic molecules, Chem. Eur. J., 10 (2004) 4367-4371.
[30] L. Liu, Q.-X. Guo, The Driving Forces in the Inclusion Complexation of Cyclodextrins, J. Incl. Phenom. Macrocycl. Chem., 42 (2002) 1-14.
[31] S.L. Veinberg, K.E. Johnston, M.J. Jaroszewicz, B.M. Kispal, C.R. Mireault, T. Kobayashi, M. Pruski, R.W. Schurko, Natural abundance (14)N and (15)N solid-state NMR of pharmaceuticals and their polymorphs, Phys. Chem. Chem. Phys., 18 (2016) 17713-17730.
[32] S. Maeda, Oumae, S., Kaneko, S., Kunimoto, K-K., Formation of carbamates and cross-linking of microbial poly(ε-l-lysine) studied by 13C and 15N solid-state NMR, Polym. Bull., 68 (2012) 745–754.
[33] S. Cheatham, M. Kline, E. Kupce, Exploiting natural abundance 13C-15N coupling as a method for identification of nitrogen heterocycles: practical use of the HCNMBC sequence, Magn. Reson. Chem., 53 (2015) 363-368.
[34] V.A. Semenov, D.O. Samultsev, L.B. Krivdin, Theoretical and experimental study of 15N NMR protonation shifts, Magn. Reson. Chem., 53 (2015) 433-441.

[35] H. Zhang, T. Tan, W. Feng, D. Spoel, Molecular recognition in different environments: β-cyclodextrin dimer formation in organic solvents, J. Phys. Chem. B, 116 (2012) 12684-12693.

Graphical abstract



Highlights

- Succinyl-β-cyclodextrins obtained by green synthesis with two substitution degrees.
- Spray-dried solid dispersions of albendazole and succinyl-β-cyclodextrins.
- Stable complexes with similar NMR data in solution regardless the substitution degree.
- Structural elucidation provided using ^{13}C and ^{15}N solid state NMR
- Substitution degree influenced the number and type of complexes in the solid state.