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Spironolactone and its Complexes with β-cyclodextrin: Modern NMR Characterization and Structural DFTB-SCC Calculations

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Abstract

In the present work, inclusion complexes of spironolactone (SP) with β-cyclodextrin (β-CD) in solid phase and aqueous solution were studied by solubility methods, NMR spectroscopy and thermal analysis. The results showed different kinds of complexations when freeze-drying and kneading methods were used. The freeze-drying product (1:1, SP:β-CD) showed lower degree of complexation and stability than the (1:2, SP:β-CD) compound obtained by kneading method. The spironolactone molecule was also studied by NMR spectroscopy at 400 MHz. The chemical shifts of all spironolactone atoms and their inclusion compounds were assigned. Extensive use of 1D and 2D NMR techniques, including ROESY experiment, allowed verifying the position of the spironolactone molecule inside the cyclodextrin cavity in both situations. In addition, DFTB-SCC quantum mechanical calculations of the inclusion compounds were performed. The predicted structural properties are in good agreement with ROESY NMR results.

Abbreviations: 2D: Two-dimensional; β-CD: β-cyclodextrin; COSY: Correlation Spectroscopy; DEPT: Distortionless Enhancement by Polarization Transfer; DFTB-SCC: Density Functional-Tight Binding- Self-Consistent Charge; HMBC: Heteronuclear Multiple Bond Correlation; HSQC: Heteronuclear Single Quantum Coherence; NMR: Nuclear Magnetic Resonance; ROESY: Rotating Frame Overhauser Enhancement Spectroscopy; S-Ac: Thio-acetyl group; SP: Spironolactone

Introduction

Spironolactone (SP) (**1**) (Figure 1) is a synthetic steroidal diuretic compound extensively used in medicine that has specific aldosterone antagonist effect and clinical value in the treatment of essential hypertension, congestive heart failure, and primary hyperaldosteronism [1]. Spironolactone shows limited oral activity because of its low solubility and dissolution rate. Various methods have been reported for the improvement of the dissolution and the bioavailability of spironolactone [2–4], among them, complexation with cyclodextrins. More recently, it was described the mechanism of cyclodextrin-catalyzed deacetylation of spironolactone at variable pH [5, 6]. The same work demonstrated that deacetylation is minimal at low pH. Although these insights on spironolactone deacetylation are very important, from a practical formulation point of view it is unpractical as it is quite impossible to apply formulations with pH around 2. In addition, some doubts about the structure and configuration of spironolactone and its inclusion compounds are still under discussion.

Thus, the purpose of this paper was to characterize the spironolactone molecule and its β-cyclodextrin inclusion compounds obtained by two different methods, namely, kneading and freeze drying, by employing different physical–chemical techniques and mainly the complete attribution of their NMR signals.

Modern 1D NMR and 2D shift-correlated NMR experiments, *i.e.*, HSQC [^1J(C, H)], HMBC [^nJ(C, H); $n = 2$, 3, and 4], ^{1}H-ROESY, and ^{1}H-COSY were used for the complete ^{1}H and ^{13}C – chemical-shift assignments and to investigate the formation of the inclusion complexes. The nuclear Overhauser effect was used to determine the position of the spironolactone molecule inside the cyclodextrin cavity, mainly by using two-dimensional ROESY experiment. The results obtained by the application of 1D and 2D NMR spectral methods were utilized to correct NMR data previously reported for the spironolactone molecule at physiological pH (pH 7.4) [4, 7]. Thermal analysis, solubility, and dissolution studies were also employed to analyze and characterize

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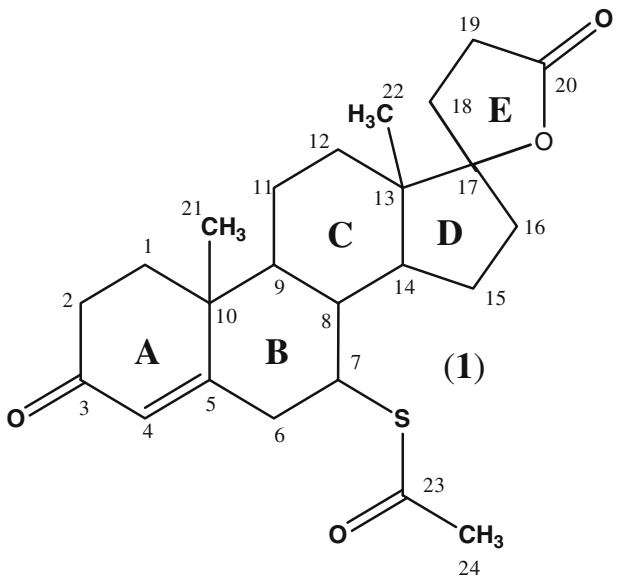

Figure 1. Chemical structure of spironolactone.

spironolactone inclusion complexes in solid phase. In addition, quantum DFTB-SCC chemical calculations provided information on the structural properties of the many possible arrangements of the inclusion compounds.

Experimental

Spironolactone was obtained from PURIFARMA Química e Farmacêutica, Brazil, β-cyclodextrin from CERESTAR®, USA. Deuterated water (D_2O), dimethyl – d_6 sulfoxide (DMSO-d_6) used in sample preparation was provided by CIL – Cambridge Isotope Laboratories, Inc.

The inclusion complexes of spironolactone with β-cyclodextrin were prepared through the following two methods: (1) *Kneading method* – Solid-state spironolactone complexes with β-cyclodextrin were prepared in a 1:1 molar ratio. The required amount of the physical mixtures of cyclodextrin with spironolactone was accurately weighed and transferred to a mortar. Water was added in small portions to the powder and the resulting slurry was kneaded in a mortar with a pestle for 60 min. The resulting paste was then dried at 30 °C for 24 h. (2) *Freeze-drying* – Solid-state spironolactone complexes with β-cyclodextrin were prepared in 1:1 molar ratio. The required amounts of spironolactone and β-cyclodextrin were accurately weighed and dispersed in Milli-Q water and the solution was magnetically stirred at room temperature for 48 h before freeze-drying.

An SDT 29600 SILMUTANEOUS DTA/TGA (TA Instruments) instrument was used to record DTA/TGA curves of raw spironolactone as well as inclusion complexes. Samples were heated in alumina crimp cells at 10 °C min^{-1} under nitrogen purge at 100 m min^{-1} over 25–800 °C.

NMR spectra were recorded on Bruker DRX400-*AVANCE* spectrometer operating at 400 and 100 MHz at 27 °C equipped with a direct detection 5 mm $^1H/^{13}C$ dual probe and a 5 mm inverse probe with z-gradient coil and with DMSO-d_6 as a solvent. The chemical shifts are reported in ppm using TMS (0 ppm) as internal standard. One-dimensional ^1H and ^{13}C NMR spectra were acquired under standard conditions. Two-dimensional inverse hydrogen-detected heteronuclear shift correlation spectra were obtained by HSQC pulse sequence [^1J(C, H)] and HMBC pulse sequence [^nJ (C, H), $n = 2$, 3, and 4], ^1H homonuclear correlation spectroscopy (COSY) and homonuclear 2D-ROESY experiment were used to confirm the assignments of all carbons and hydrogens of the spironolactone molecule and of the inclusion compounds (ROESY spinlock pulse = 600 ms) [10, 11].

Solubility measurements were carried out according to Higuchi and Connors' method. Briefly, excess amounts of spironolactone were added to screw-cap vials with aqueous solutions containing different concentrations of β-cyclodextrin, the suspensions were shaken for 48 h at room temperature. The vial contents were centrifuged and aliquots of clear supernatant were diluted with Milli-Q water. The drug concentration in solution was measured by ultraviolet spectrophotometry by using a calibration curve with spironolactone concentration between 0.4 and 2.0×10^{-5} mol/l and $R = 0.999$ [10].

The dissolution rates of spironolactone inclusion compounds were measured according to the dispersed amount method [11]. An amount of 10 mg of spironolactone-β-cyclodextrin complex was added to the buffer solution (pH 7.4) and stirred at 90 rpm at 37 °C. At appropriate time intervals (0.25, 0.5, 1, 2, 4, 8, 24 and so on up to 260 h), 1.5-mL aliquots were taken out and spironolactone concentration was measured spectrophotometrically at 240 nm with the calibration curve described above.

Inclusion complexes 1:1 and 1:2 spironolactone-β-cyclodextrin were pre-optimized by UFF (Universal Force Field) [14]. Density Functional – Tight Binding – Self-Consistent-Charge (DFTB-SCC) [15–17] was used to perform all calculations. Recently we suggested an efficient "a posteriori" treatment for van der Waals interactions, which was included in the DFTB-SCC method [18]. This method can be considered as an approximation to Density Functional Theory (DFT) [19]. It treats short-range atomic potentials and neglects three-center terms in the Hamiltonian. The generalized gradient approximation for the exchange-correlation functional due to Perdew, Burke, and Erzenholf [20] (PBE) was used. The DFTB-SCC method is known as a fast quantum-chemical computational tool. It has been successfully applied to treat many carbon-based systems [18, 21, 22], including those with non-negligible van der Waals interactions. The DFTB-SCC implemented in the present experimental version of the deMon computer code [23] was used in calculations.

Results and discussion

Solubility studies

The phase solubility data of spironolactone with β-cyclodextrin shows that spironolactone solubility increased linearly as a function of β-cyclodextrin concentration. The beginning of the curve is followed by a plateau region where the total spironolactone concentration decreases with the complex precipitation (Data not shown); the solubility curve can be classified as B_s type [12]. This behavior is probably due to a product mixture in aqueous solution as our phase-solubility experiment was performed without pH control and spironolactone might have been partially or fully degraded during equilibration period, which is consistent with earlier reports in which the phase-solubility diagram was jointly produced by spironolactone and its main degradation products [3, 6, 13].

Dissolution studies

The dissolution curve of the spironolactone-β-cyclodextrin complex is depicted in Figure 2. It is evident that the dissolution rate of spironolactone improved significantly through complex formation. The results clearly show the existence of a spironolactone-β-cyclodextrin complex in aqueous solution and the higher dissolution rate for the complex prepared by kneading method when compared to that of the freeze-dried one.

Thermal analysis

TGA and DTA data clearly indicates that the complex exists in solid state as shown by the DTA curves in Figure 3 (TGA not shown). In this case, the endothermic peak, the melting point of spironolactone (213.3 °C), was not observed in the inclusion complex prepared by either kneading or freeze-drying methods [Figure 3:(3) and 3:(4), respectively]. The DTA curve of the spironolactone-β-cyclodextrin complex prepared by freeze-drying method shows three endothermic events around 334 °C, in contrast with the complex prepared by kneading method, which displays only one event. These results reinforced the hypothesis that two kinds of complexes were formed by the preparation methods used. Finally, the results above clearly indicate that the spironolactone-β-cyclodextrin complexes exist in solid state and show some differences due to preparation methods.

NMR spectroscopy

Modern NMR techniques based on gradient-pulsed field was used in this study to determine and assign the structures of spironolactone and its inclusion compounds [10, 11]. ^{1}H and ^{13}C NMR resonance assignments of the spironolactone molecule were carried out by 2D shift-correlated NMR techniques. The results of this study were also used to confirm and refute some wrong spironolactone assignments reported in literature [7–9]. Hydrogen and carbon atom chemical shifts, including

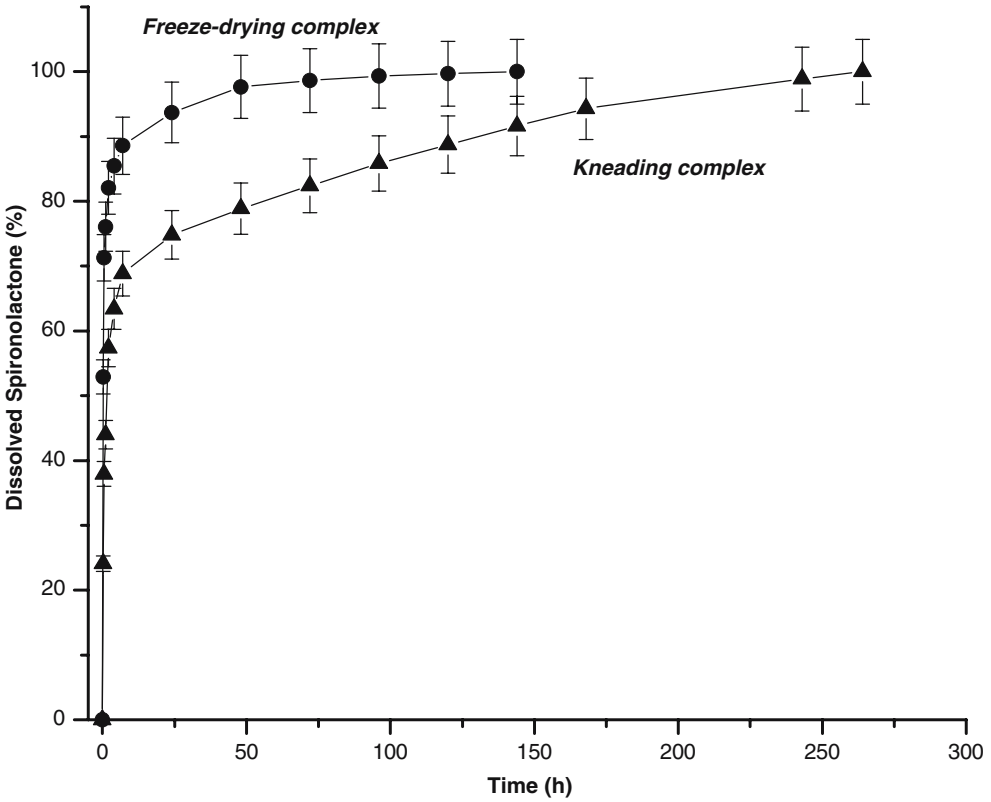

Figure 2. Dissolution curves of spironolactone-β-cyclodextrin complex in water at 37°C. (▲ kneading complex, ● freeze-drying complex).

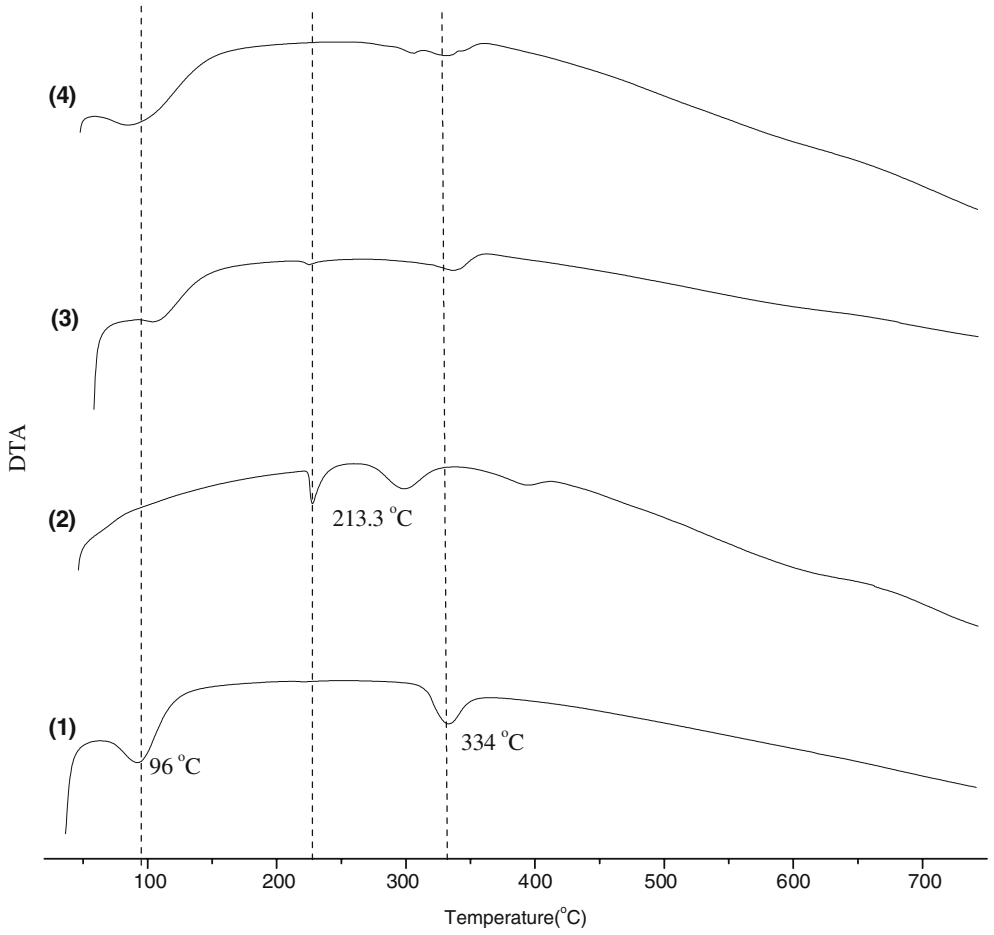

Figure 3. DTA of (1) *β*-cyclodextrin; (2) spironolactone; (3) spironolactone-*β*-cyclodextrin complex by Kneading method, and (4) spironolactone-*β*-cyclodextrin by Freeze-drying method.

2D heteronuclear correlations, are summarized in Tables 1 and 2.

The chemical shifts of the non-hydrogenated and several hydrogenated carbons were made based mainly on HMBC spectra.

The starting point for spironolactone assignments was the hydrogen resonance located at δ_H 3.89, which showed correlation in ^1H–^{13}C HSQC experiment with the ^{13}C signal at δ_C 44.99. This resonance was correlated to atom 9 signals, which had been wrongly attributed in earliest studies. The assignments of H-9 were confirmed based on the connectivity observed in 2D homo- and hetero-nuclear NMR experiments. Hydrogen H-9, which split into a broad multiplet in the 1D ^1H NMR spectrum (Figure 4), showed homonuclear correlations with H-1_{eq} (δ_H 3.00, dd), H-1_{ax} (δ_H 2.29), and H-2_{eq} (δ_H 2.40–2.52, m) in the COSY experiment, and with carbons C-1 (δ_C 39.77), C-5 (δ_C 166.46), and C-7 (δ_C 48.97) in HMBC, respectively.

In the same way, C-1 showed a correlation with H-4 (δ_H 5.59; δ_C 125.74), and C-4 showed correlations with H-1, H-2, and a long-range correlation via ^4J with H-21 (δ_H 1.19; δ_C 17.15), which confirmed the assignments of A-ring of the spironolactone molecule.

Based on HMBC data, the methyl group C-21 showed long-range correlation with hydrogens H-6 (δ_H 1.63 and δ_H 1.39–1.52) via ^3J, H-4 (δ_H 5.59; δ_C 125.74) via ^4J and H-7 (δ_H 0.93–0.99) located in the B-ring via ^5J in the HMBC experiment modified with a different long range evolution delay ($d = 100$ ms $\equiv$ $^1J_{C-H} = 10$ Hz).

The attribution of hydrogen H-7 was also modified in comparison with a previously published data. It was confirmed through out the connectivity observed between this signal and the rest of the molecule in the COSY and HMBC experiments. Hydrogen H-7 (δ_H 0.93–0.99) showed homonuclear correlations with hydrogen H-6 and H-8 (δ_H 1.32–1.41; δ_C 45.38) in COSY experiment, and heteronuclear long-range correlations with C-9 via ^3J (δ_C 44.99), C-6 (δ_C 20.04) via ^2J, C-8 (δ_C 45.38) via ^2J and C-14 (δ_C 37.76) via ^3J in HMBC.

The resonance signal attributed to C-8 (δ_C 45.38) showed heteronuclear correlations with H-11 (δ_H 1.37–1.51; δ_C 21.55) via ^3J, H-14 (δ_H 2.11–2.17; δ_C 37.76) via ^2J, and H-15 (δ_H 2.05–2.14 and 1.82–1.92; δ_C 34.40) via ^3J of rings C and D in HMBC experiment.

The assignment of the spiro carbon, C-17 (δ_C 94.88) was made based on the assignment of C-15. It showed heteronuclear long-range correlations with H-16, H-18, H-19, and H-22 in HMBC spectrum. Thus, complete ^1H and ^{13}C chemical shift assignments of spironolactone were made.

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of spironolactone (**1**), including results obtained by heteronuclear 2–D shift-correlated HMBC (DMSO-d_6)

Atom	δ_H (ppm)	δ_C (ppm)	HMBC		
			$^{2}J_{C\text{-}H}{}^{a}$	$^{3}J_{C\text{-}H}{}^{a,b}$	$^{n}J_{C\text{-}H}{}^{b}$; n = 4; 5
1	2.29 (td, H_{ax}) 3.00 (dd, H_{eq})	39.77	–	H-9	H-4
2	2.18–2.26 (m, H_{ax}) 2.40–2.52 (m, H_{eq})	33.51	H-1	H-4	H-6; H-8
3	–	197.50	H-4	H-1	H-6
4	5.59 (s br)	125.74	–	H-2_{ax}	H-1
5	–	166.46	H-6	H-9; H-1; Me-21	H-2
6	1.39–1.52 (m, H_{ax}) 1.63 (H_{eq})	20.04	H-7	H-8	–
7	0.93–0.99 (m)	48.97	H-6; H-8	H-9; H-14	–
8	1.32–1.41 (m)	45.38	H-14	H-15; H-11	–
9	3.89 (q, br)	44.99	–	H-1	H-4
10	–	38.01	H-1	H-4; H-2	–
11	1.37–1.51 (m, H_{ax} and H_{eq})	21.55	H-12	–	H-14
12	1.33–1.51 (m, H_{ax} and H_{eq})	30.46	–	Me-22	H-18
13	–	44.85	Me-22; H-12	H-11; H-16	–
14	2.11–2.17 (m)	37.76	–	H-12; H-16	–
15	1.82–1.92(m, H_{ax}) 2.05–2.14(m, H_{eq})	34.40	H-14	–	H-18
16	1.58–1.68 (m H_{ax}) 2.01–2.09 (m, H_{eq})	34.96	–	H-18; H-14	–
17	–	94.98	H-18; H-16	H-19; H-12; Me-22	–
18	1.87–1.97(m, H_{ax}) 2.130–2.42(m, H_{eq})	30.18	H-19	–	H-15
19	2.39–2.49(m, H_{ax}) 2.53–2.64(m, H_{eq})	28.62	H-18	–	–
20	–	176.08	H-19	H-18	–
21	1.20 (s)	17.15	–	–	H-7; H-4; H-6
22	0.90(s)	14.38	–	H-12	–
23	–	194.06	Me-24	–	–
24	2.35 (s)	31.15	–	–	–

(s) simplet; (d) duplet; (t) triplet; (m) multiplet; (br) broad; (a) long-range delay = 65 ms; (b) long-range delay = 100 ms

To know the host: guest interaction, the ^{1}H NMR spectrum of the spironolactone-β-cyclodextrin complex in aqueous solution was obtained, Figure 5. The comparison of these spectra with those of spironolactone reveals some differences in the NMR signals (Figure 4).

These differences were due to the complexation effects and the molecule degradation of spironolactone. The most important differences observed are the disappearance of the singlet due to the S-Ac group (Me-24 in structure 1) and the shift of the multiplets attributed to H-7 (δ_H 1.39–1.47) and H-9 (δ_H 3.34), assigned by 2D correlations experiments (COSY and HSQC). The ^{13}C NMR experiment confirmed the loss of the S-Ac group. The resonances assigned to C-24 and C-23 were absent in the spectra of the inclusion complex and the signal at δ_C 21.48 was attributed to the S–CH_3 group. The resonances of C-7 (δ_C 48.97) and C-9 (δ_C 44.99) were shifted to δ_C 45.39 and δ_C 39.89 ppms as a consequence of the loss of the thioester carbonyl group upon inclusion as reported in literature [3].

The degradation of spironolactone in the presence of β-cyclodextrin was previously reported in nuclear magnetic resonance studies. Spironolactone reacts with ionized β-cyclodextrin hydroxyl groups, leading to deacetylated products [4–6]. Besides this process, it was also verified the formation of inclusion compounds with the same spironolactone steroidal skeleton in the presence of cyclodextrin.

^{1}H and ^{13}C chemical shift assignments of the spironolactone molecule in its inclusion complexes are given in Table 2.

Nuclear Overhauser effect measurement is one of the most important tools to prove the formation of the host: guest complex. Additionally, it is very useful to get insights on the supramolecular geometry of the complex. The NOEs observed between the spironolactone and β-cyclodextrin hydrogens, as detected in the 2D-ROESY experiments, could only arise if a spironolactone-β-cyclodextrin complex had been formed.

NOEs between hydrogens H-1 ($\delta_{H(ax)}$ 2.29–2.36; $\delta_{H(eq)}$ 2.99) and H-2 ($\delta_{H(ax)}$ 2.17; $\delta_{H(eq)}$ 2.36–2.42) of

Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of spironolactone in spironolactone-β-cyclodextrin complex, (D_2O).

Atom	δ_H (ppm)	δ_C (ppm)
1	2.29–2.36 (m, H_{ax})	41.09
	2.99 (dd, H_{eq})	
2	2.17 (dt, H_{ax})	33.63
	2.36–2.42 (m, H_{eq})	
3	–	197.0
4	5.65	126.15
5	–	166.65
6	1.58–1.63 (m, H_{ax} and H_{eq})	19.75
7	1.58–1.64 (m, H_{ax})	45.38
8	1.31–1.39 (m)	44.76
9	3.34	39.89
10	–	38.53
11	1.31–1.38 (m, H_{ax})	21.48*
	1.60–1.67 (m, H_{eq})	
12	1.30–1.37 (m, H_{ax})	30.67
	1.39–1.46 (m, H_{eq})	
13	–	44.80
14	1.89–1.98(m)	38.58
15	1.83–1.92 (m, H_{aq})	34.58
	2.03–2.10 (m, H_{eq})	
16	1.83–1.91 (m, H_{ax})	34.90
	2.03–2.10 (m, H_{eq})	
17	–	95.09
18	1.86–1.95 (m, H_{ax})	30.31
	2.26–2.35 (m, H_{eq})	
19	2.35–2.46 (m, H_{ax})	28.72
	2.51–2.59 (m, H_{eq})	
20	–	176.28
21	2.35–2.45 (m, H_{ax})	17.28
	2.50–2.59 (m, H_{eq})	
22	0.89(s)	14.63
23	1.99 (s, S-CH_3)	21.48*
24	–	–

(s) simplet; (d) duplet; (t) triplet; (m) multiplet; (br) broad;
* overlapping signals identified by DEPT experiment.

spironolactone and hydrogens H-3 (δ_H 3.89) and H-5 (δ_H 3.69–3.80) of β-cyclodextrin were observed for the freeze-dried complex (Figure 6). These results suggest the formation of a spironolactone: β-cyclodextrin complex with a 1:1 molar ratio mainly via its A ring.

Different results were observed for the complex prepared by kneading method. In this case, a higher NOE interaction between host and guest molecules was verified when compared to that of the supramolecular complex prepared by freeze-dried method.

As shown in Figure 7, NOE between protons H-1, H-2, H-6, H-8, H-11, H-12, H-15, H-18, H-19, H-21, and H-22 of spironolactone and protons H-3 and H-5 of β-cyclodextrin was observed. These data suggest that the spironolactone A-ring and part of the D- and E-rings are situated deep in the torus cavity of cyclodextrin. These results strongly suggest the formation of the spironolactone: β-cyclodextrin complex with 1:2 molar ratio.

Molecular modeling

DFTB-SCC quantum mechanical methods were carried out to verify the ROESY NMR experimental data and to get in-depth information on the supramolecular geometry of the spironolactone: cyclodextrin complexes obtained by the two different methods.

Four possible inclusion compounds may be formed from the interaction of one β-cyclodextrin and one spironolactone. Either rings A or DE of spironolactone can be included in head or tail arrangements, namely A-tail, A-head, DE-tail, and DE-head. If two β-cyclodextrin, are involved in the inclusion complexes, four other arrangements are possible, namely, head–head, tail–head, head–tail, and tail–tail. These structures have been fully optimized and the complexation energies have been estimated.

All structures were stable and the inclusion reaction energies were about −43 and −87 kcal mol^{-1} for 1:1 and 1:2 (spironolactone: β-cyclodextrin) complexes, respectively. The exceptions are the DE-tail and tail–head, which lie about 40 and 100 kcal mol^{-1} higher in energy, respectively. Solvent effects have an important role in the inclusion phenomena and the relative order of the reaction energies can be changed. Nevertheless, structural properties are only slightly affected by solvent effects.

The most important structural properties of the inclusion compounds are shown in Tables 3 and 4. The distances of the selected spironolactone hydrogens and the hydrogens of H3 and H5 β-cyclodextrin are in the range of 2.0–3.0 Å for the A-tail and A-head structures of 1:1 complexes.

Figure 8a shows the optimized structures of the A-head complex. The DE-head and DE-tail have much larger distances between the spironolactone and β-cyclodextrin hydrogens, which is in agreement with the ROESY NMR observations. For the 1:2 spironolactone: β-cyclodextrin complex, the head–head arrangement presents the shortest distance between the protons observed in the NOE experiments, which is an evidence that the head-head (1:2) structure is preferable, Figure 8b.

Conclusion

Complexation of spironolactone with β-cyclodextrin was accomplished by both freeze-drying and kneading methods. The results showed that freeze-drying and kneading methods yielded two kinds of host: guest complexes. The freeze-drying product (1:1) showed a lower degree of complexation and stability than the kneading method compound (1:2). The dissolution curves of spironolactone and its β-cyclodextrin complexes clearly showed a higher dissolution rate for the complex obtained by kneading method when compared to that of the spironolactone free one. TG/DTA results suggested two kinds of inclusion compounds in solid state when freeze-drying and knead-

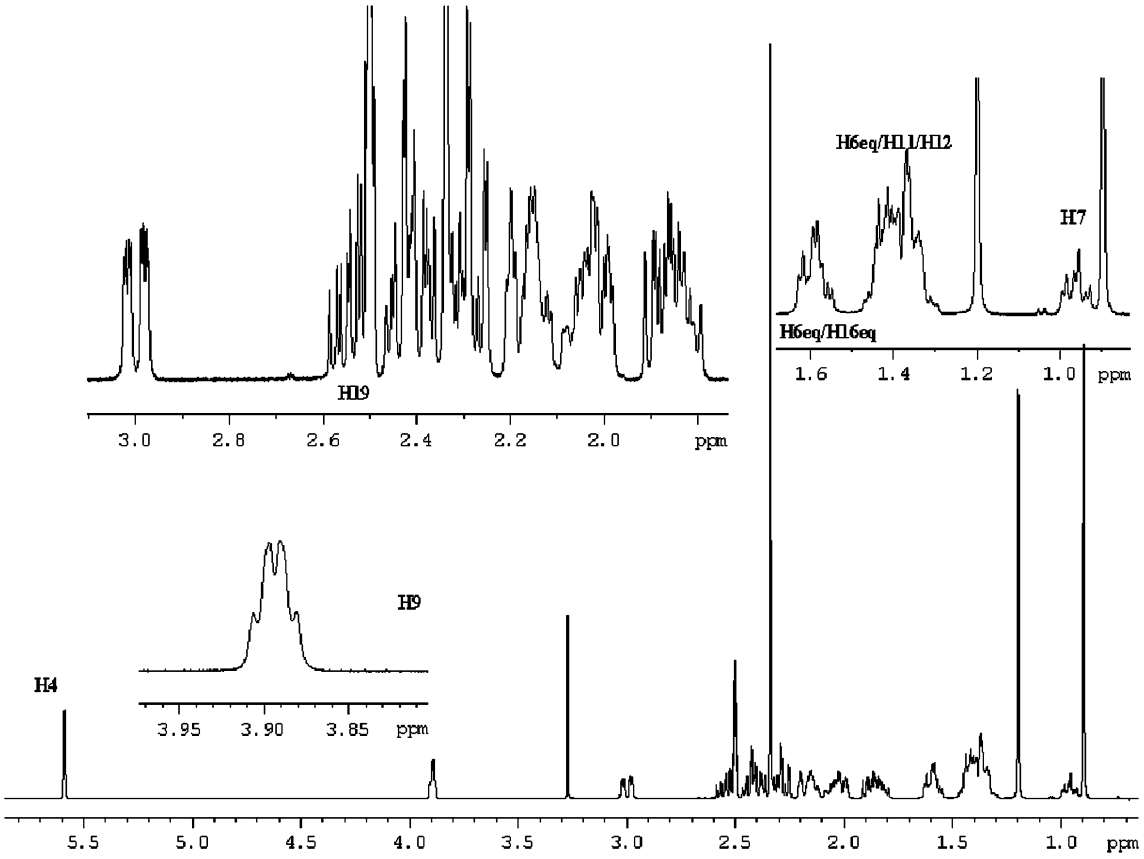

Figure 4. ^{1}H NMR of spironolactone (DMSO-d_6, 400 MHz).

ing methods were used. NMR analyses allowed correcting some misassignments of spironolactone reported in literature. The NOEs results obtained in two-dimensional ROESY experiment gave more insights on the complex supramolecular geometry of the inclusion compounds prepared by different methods. These NMR findings were

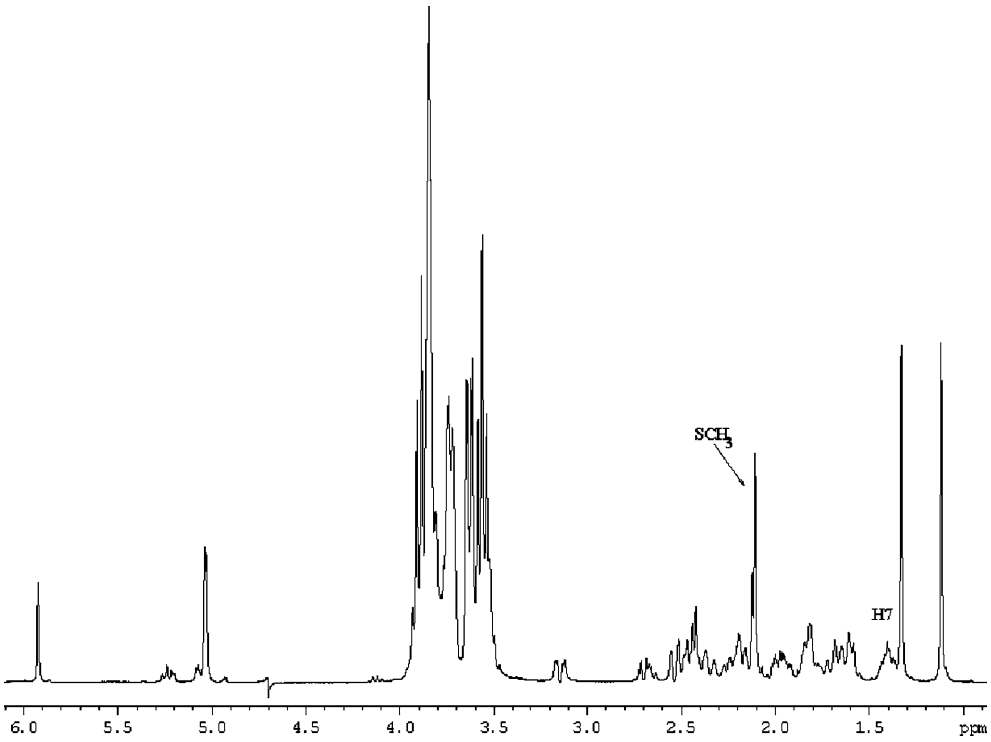

Figure 5. ^{1}H NMR of spironolactone-β-cyclodextrin complex, kneading method (D_2O, 400 MHz).

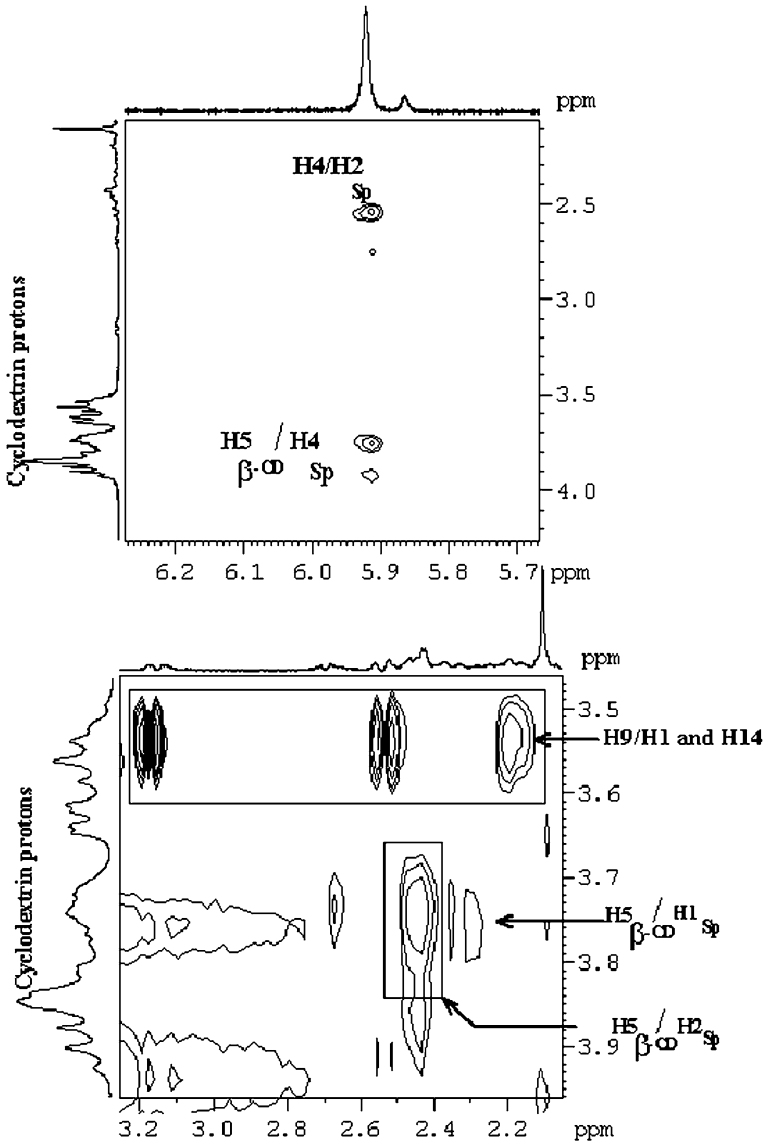

Figure 6. Two-dimensional ROESY contour map of spironolactone-β-cyclodextrin complex, freeze-drying method (D_2O, 400 MHz). (SP = spironolactone).

confirmed by structural analysis through DFTB-SCC quantum chemical calculations for the spironolactone: β-cyclodextrin complexes, which showed the A-head of 1:1 spironolactone: β-(cyclodextrin complex and the head–head of 1:2 spironolactone: β-cyclodextrin complex as preferred conformations.

Table 3. Structural properties of the the spironolactone-β-cyclodextrin complex (1:1).

Spironolactone atom	β-cyclodextrin atom	Distance (Å)	
		A-Tail	A-Head
H1	H3	2.048/2.737	2.909/2.936
	H5	4.013/4.446	2.603/3.017
H2	H3	2.576/4.296	2.253/3.458
	H5	2.564/3.989	4.206/5.110
H6	H3	3.075/3.210	2.029/2.083
	H5	2.283/2.437	3.509/3.543
		D/E-Tail	DE-Head
H15	H3	7.083/7.189	4.328/5.849
	H5	3.984/5.477	6.097/7.164
H18	H3	4.577/4.651	2.421/3.099
	H5	2.760/4.577	2.426/2.766

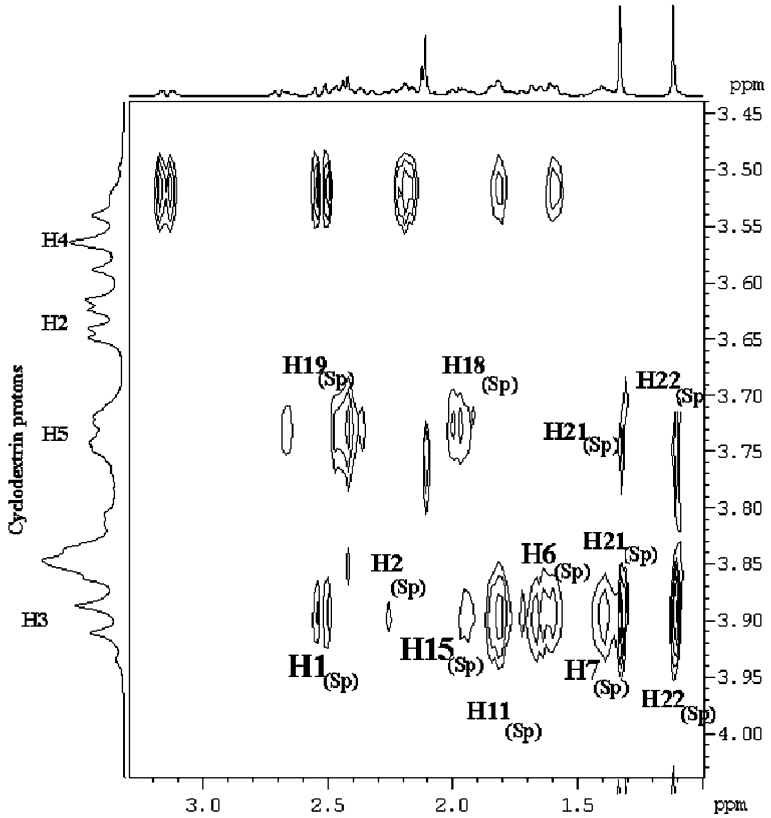

Figure 7. Two-dimensional ROESY spectrum of spironolactone-β-cyclodextrin complex, kneading method (D_2O, 400 MHz). (SP = spironolactone).

Table 4. Structural properties of the spironolactone-β-cyclodextrin complex (1:2).

Spironolactone atom	β-cyclodextrin atom	Distance (Å)			
		Tail-Tail	Tail-Head	Head-Tail	Head-Head
H1	H3	2.162/3.238	6.356/6.608	3.321/3.479	2.909/3.241
	H5	4.216/4.980	4.090/4.120	2.394/2.642	2.675/3.083
H2	H3	2.843/3.253	1.951/3.333	4.645/5.654	2.387/3.544
	H5	2.450/3.045	4.207/4.803	2.270/3.511	4.116/5.224
H6	H3	4.724/4.853	6.360/7.452	2.342/2.484	2.103/2.312
	H5	2.686/2.790	3.604/4.871	2.380/2.551	3.341/3.769
H15	H3	6.304/6.896	2.742/2.752	4.859/5.783	2.814/2.920
	H5	3.899/4.829	3.949/4.424	3.077/3.430	2.003/3.263
H18	H3	6.757/8.501	3.746/4.122	4.415/4.616	2.455/3.895
	H5	4.171/5.923	3.870/4.010	2.730/3.445	2.513/2.656

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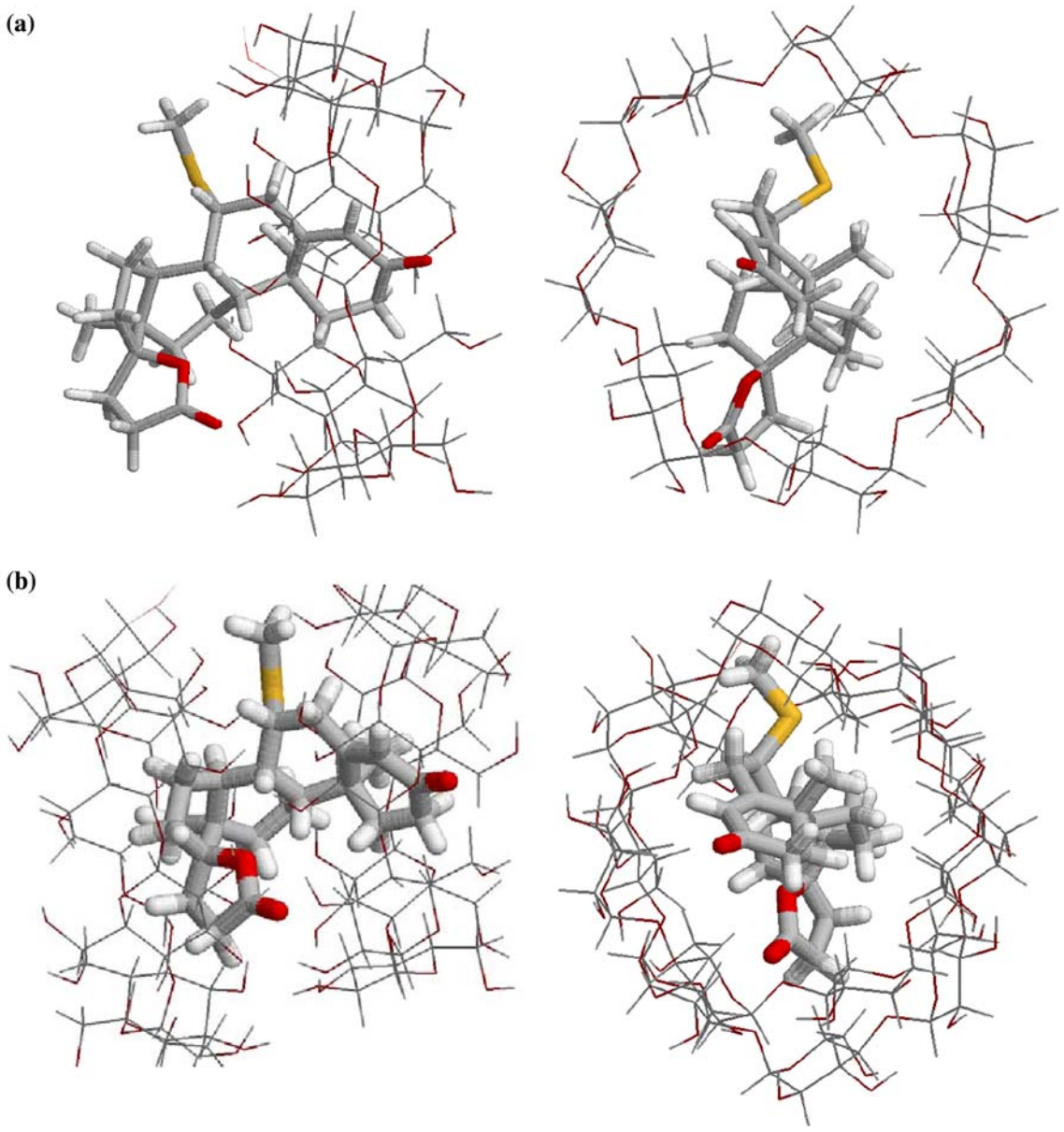

Figure 8. DFTB-SCC-optimized structures of spironolactone-β-cyclodextrin complex. (a) 1:1 complex. (b) 1:2 complex.

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