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Study of ascorbic acid interaction with hydroxypropyl-β-cyclodextrin and triethanolamine, separately and in combination

Claudia Garnero, Marcela Longhi *

Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, 5000 Córdoba, Argentina

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Abstract

Complexation between ascorbic acid, hydroxypropyl-β-cyclodextrin (HP-β-CD) and triethanolamine (TEA), separately and in combination, was studied in solution and solid state. The freeze-drying method was used to prepare solid complexes, while physical mixtures being obtained by simple blending. These complexes were characterized in the solid state using differential scanning calorimetry (DSC) and infrared spectroscopy (IR). Nuclear magnetic resonance spectroscopy (^{1}H and ^{13}C NMR) was used in aqueous solutions to obtain information about the mode of interaction. The degradation rate of each complex in solution was determined, and the stability constant of the complexes and the degradation rate of the ascorbic acid within the complexes were obtained. NMR studies provided clear evidence of partial inclusion into the HP-β-CD cavity, but the stability constant value was very small indicating a weak host–guest interaction. The influence of complexation on the degradation rate of ascorbic acid was evaluated, and the data obtained showed a pronounced enhancement of aqueous stability with the TEA association complex, while this effect was lower with the HP-β-CD inclusion complex. NMR experiments showed evidence of the formation of aggregates.

Keywords: Ascorbic acid; Hydroxypropyl-β-cyclodextrin; Triethanolamine; Complexation; Characterization; NMR spectroscopy; Stability studies; Aggregation

1. Introduction

Cyclodextrins (CDs) are cyclic (α-1,4) linked oligosaccharides of α-D-glucopyranose containing a relatively hydrophobic central cavity and hydrophilic outer surface. They have been used extensively to form non-covalent inclusion complexes with many substances. The inclusion complex normally exhibits a higher aqueous solubility and a greater chemical stability than the pure drug [1]. However, it has been shown that CDs can accelerate the degradation of some drugs [2–4]. This ability to stabilize certain drugs while destabilizing others has been explained by the different structures of the formed inclusion complex [5]. Hydroxypropyl-β-cyclodextrin (HP-β-CD), a chemically modified β-cyclodextrin, has a greater aqueous solubility and safety compared to its parent compound. It is capable of forming soluble complexes with many drugs by taking up a whole drug molecule, or some part of it, into the cavity. Moreover, the addition of suitable auxiliary substances can significantly increase the CD complexing abilities by multicomponent complex formation. In our recent work, it has been shown that triethanolamine (TEA) can enhance the solubilizing power of HP-β-CD toward sulfisoxazole, as a result of a combined effect of salt formation and inclusion complexation [6].

Although ascorbic acid (vitamin C) has been extensively studied for several decades in different fields, interest in this vitamin has never waned and further aspects are currently being investigated. Moreover, the instability of ascorbic acid in aqueous solutions has caused much attention. It is highly sensitive to heat, alkali, oxygen and light, and also to contact with traces of copper and iron [7]. The rapid degradation of ascorbic acid clearly complicates the assay studies. It is therefore of interest to investigate the possibility of determining a suitable stabilizer and an optimum concentration in order to stabilize the ascorbic acid long enough for analysis to be carried out.

In previous works, the complexation of ascorbic acid with α- and β-CD was studied by ^{1}H NMR [8], calorimetry and densimetry [9], as well as by the interaction with HP-α-CD and HP-β-CD using solution calorimetry [10] and UV spectropho-

* Corresponding author. Fax: +54 351 4334127.
E-mail address: mrlcor@fcq.unc.edu.ar (M. Longhi).

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tometry. These studies have shown that ascorbic acid forms 1:1 molecular complexes with HP-β-CD and α-CD, but no complex with β-CD [11]. These last two results are inconsistent with those of Manzanares et al., who studied the stability of ascorbic acid in the presence of α- and β-CD by their electrochemical behaviour on platinum and gold electrodes [12,13].

The aim of this work focuses on two aspects. Our first goal was to obtain evidence of the interactions between ascorbic acid, HP-β-CD and TEA. To this purpose, selective physicochemical determinations based on differential scanning calorimetry (DSC), infrared spectroscopy (IR) and nuclear magnetic resonance spectrometry (^{1}H NMR and ^{13}C NMR) were used to analyze the binary and multicomponent complexes. Also, the formation of HP-β-CD aggregates was investigated. While the second aim was to improve the stability of ascorbic acid in solution by complexation, the values of the complex stability constants (K_c) and the degradation rates of the drug (k_c) for ascorbic acid were obtained from a series of degradation studies.

2. Experimental

2.1. Materials

Ascorbic acid was obtained from Anedra® (99%, Argentina); triethanolamine was purchased from Aldrich® (98%, USA); HP-β-CD (M_W = 1326–1400, degree of molar substitution 7.0) was kindly supplied by Roquette (France). All other materials and solvents were of analytical reagent grade. A Millipore Milli Q Water Purification System generated the water used in these studies.

2.2. Preparation of solid samples

The preparation of solid complexes ascorbic acid:HP-β-CD or ascorbic acid:TEA with 1:1 molar ratios, and ascorbic acid:TEA:HP-β-CD with 1:1:1:molar ratios, was performed by the freeze-drying method [14]. Appropriate amounts of each component were suspended in distilled water, sonicated in an ultrasonic bath at 25.0 ± 0.1 °C constant water temperature until the drug was dissolved completely, and finally the solutions were filtered through 0.45 μm membranes (Millipore, USA). Filtrates were frozen at −40 °C for 24 h to ensure complete solidification, before the freeze-drying was started (Freeze Dye 4.5 Labconco corp., Kansas City, MI).

Physical mixtures were prepared by the simple mixing of the corresponding components.

2.3. Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectra were recorded on potassium bromide disks on a Nicolet 5 SXC FT-IR Spectrophotometer (Madison, WI, USA).

2.4. Differential scanning calorimetry (DSC)

Thermal analyses were performed with a DSC TA 2920 (Newcastle, DE, USA), at a heating rate of 10 °C min^{-1}. The thermal behavior was studied by heating samples in aluminum hermetic pans under nitrogen gas flow, over a temperature range of 25–400 °C.

2.5. NMR spectroscopy

^{1}H NMR and ^{13}C NMR were performed using a Bruker AC 200 spectrometer at 200.13 MHz. NMR spectra of pure components and their equimolar combinations were taken in D_2O. The concentrations of the components were 11 mg/ml for ascorbic acid, 76.4 mg/ml for HP-β-CD and 25 mg/ml for TEA.

For ^{13}C NMR measurements, a small amount of methanol (∼1 mM) was added to all samples as a reference, because it has been shown that methanol is a good internal reference due to its low association constant with CDs [15].

All spectra were recorded with 5 mm tubes immediately after preparation in order to avoid degradation of ascorbic acid. The chemical shifts (δ) were reported as ppm, and were referenced to the residual water signal (4.80 ppm) or methanol signal (49.1 ppm) for ^{1}H and ^{13}C NMR experiments, respectively.

The critical CD concentration (cc) for the aggregate formation and the aggregation number were estimated using ^{1}H NMR measurements by the method of Ruso et al. [16].

2.6. Stability studies

The kinetics of degradation of ascorbic acid was studied both in the presence and absence of ligands in aqueous solutions at different pH values ranging from 1.34 to 11.04. In addition, the influence of both pH and HP-β-CD concentrations on the degradation was studied at pH 2.50, 4.20 and 8.50; and the effect of TEA concentration was studied at pH 8.50.

The studies were performed using $NaHCO_3$/NaOH and McIlvaine buffers [17]. The pH values were adjusted by addition of phosphoric acid or sodium hydroxide. Measurements of pH were performed by using an ORION SA520 pH-meter.

Stock solutions of ascorbic acid (0.5 mg/ml) were prepared in water. Test solutions were prepared by diluting the stock solutions to a final concentration of 1.8 10^{-2} mg/ml in water or buffer solutions. Then, HP-β-CD or TEA or their combination was added in increasing concentrations (0.01–10% and 0.9749–3.5450 mM, respectively). These solutions were stored at 25.0 ± 0.1 °C under continuous shaking (Haake DC10 thermostat) and protected from light during the kinetic runs in order to diminish photolytic effects. At suitable time intervals, samples were withdrawn and were immediately analyzed for remaining ascorbic acid by monitoring spectrophotometrically (Shimadzu UV-160A spectrometer) the decrease in absorbance at a suitable wavelength (266 nm in aqueous solutions and buffer pH 8.50; 259 nm in buffer pH 4.20 and 246 nm in buffer pH 2.50), since interference from degradation products was negligible at these wavelengths. The experiments were performed in triplicate. The observed pseudo first-order rate constants (k_{obs}) for the degradation were obtained from a linear regression analysis of the natural logarithm of the remaining ascorbic acid plotted against time.

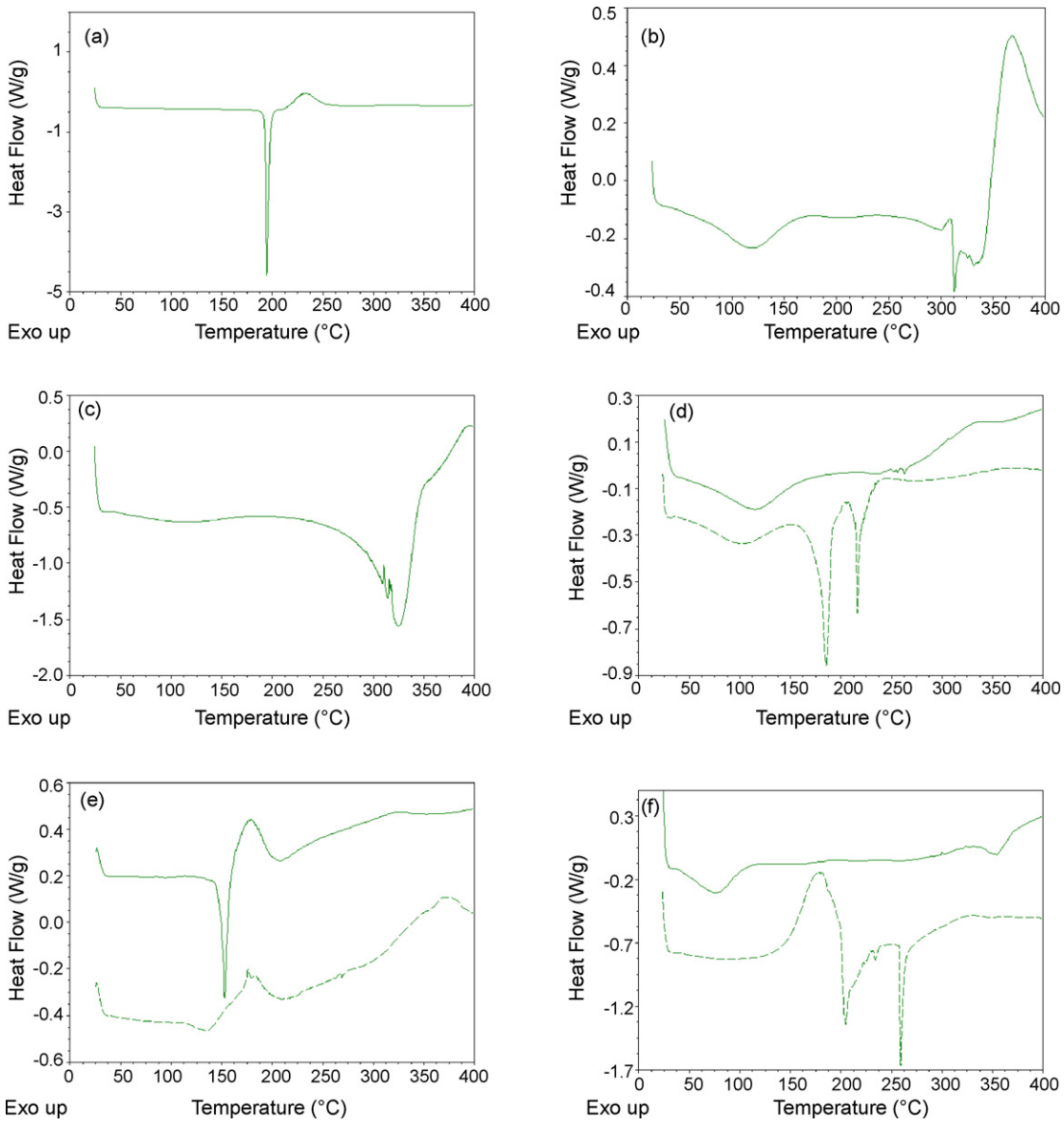

Fig. 1. DSC curves of ascorbic acid (a); HP-β-CD (b); TEA (c); ascorbic acid:HP-β-CD freeze-dried complex (solid line) and physical mixture (short dashed line) (d); ascorbic acid:TEA freeze-dried complex (solid line) and physical mixture (short dashed line) (e); ascorbic acid:HP-β-CD:TEA freeze-dried complex (solid line) and physical mixture (short dashed line) (f).

3. Results and discussion

3.1. Solid-state studies

Some information on solid-state interactions between ascorbic acid, HP-β-CD and TEA was obtained by DSC and FT-IR.

Fig. 1 shows the calorimetric curves of individual components and their complexes or physical mixtures. The DSC thermogram of ascorbic acid showed a melting endothermic peak at 194.6 °C. HP-β-CD exhibited a typical broad endothermic peak between 50 and 175 °C assigned to a dehydration process, with TEA showing an endothermic event between 250 and 350 °C, which was attributed to its decomposition.

Comparison of the DSC curves of the systems prepared by freeze-drying with those obtained by physical mixture confirms an interaction between the components. In fact, the disappearance of the melting peak of ascorbic acid in the thermograms of the freeze-dried ascorbic acid:HP-β-CD and ascorbic acid:HP-β-CD:TEA systems indicates the formation of complexes. In the thermogram of ascorbic acid:HP-β-CD freeze-dried, the endothermic peak present at around 250 °C is probably due to the fusion of a new solid phase. In the physical mixtures, however both characteristic endothermic processes were evident for the drug and for the CD. Also, the appearance of a new endothermic peak may be explained by a solid-state interaction between the two components in the physical mixture at high temperature. In the freeze-dried ascorbic acid:TEA system, the endothermic peak of the acid was observed at 152.8 °C, with this lowering effect of TEA being due to lower crystalline lattice energy [18]. Nevertheless, an examination of their physical mixture thermograms revealed an evident interaction, probably due to the liquid state of the samples.

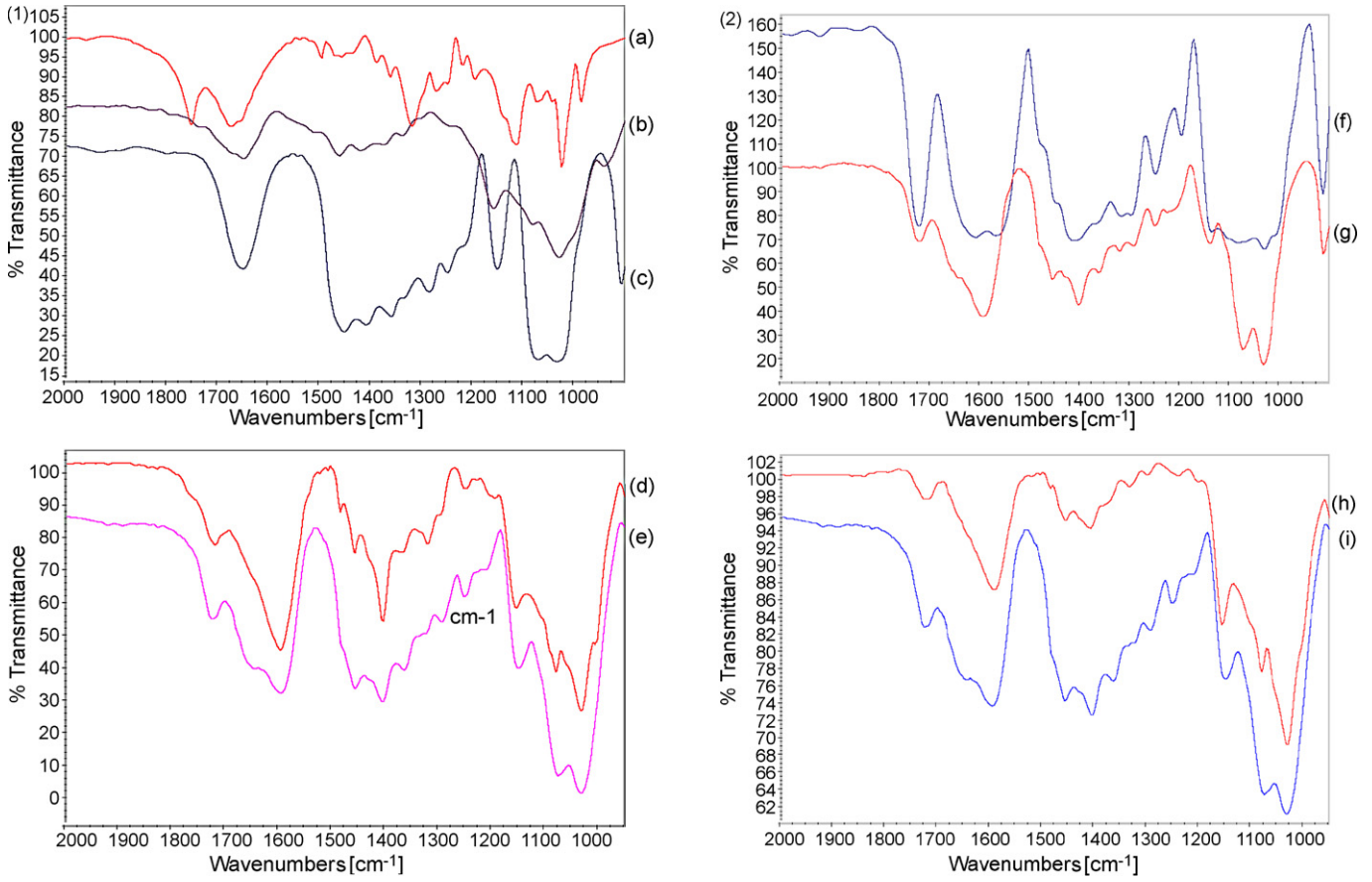

Fig. 2. (1) FT-IR spectra of ascorbic acid (a); HP-β-CD (b); TEA (c); ascorbic acid:HP-β-CD freeze-dried complex (d) and physical mixture (e). (2) Ascorbic acid:TEA freeze-dried complex (f) and physical mixture (g); ascorbic acid:HP-β-CD:TEA freeze-dried complex (h) and physical mixture (i).

The freeze-dried complexes, and the physical mixtures in the same molar ratios, were also examined by FT-IR spectroscopy. They were then compared to the pure components to try to obtain supporting evidence of complexation.

As shown in Fig. 2(1) (a), characteristics bands of ascorbic acid were found at 1755 cm^{-1} (C=O), 1500 cm^{-1} (C=C) and 1117 cm^{-1} (C–O–C), which were not superimposed with HP-β-CD (Fig. 2(1) (b)) or TEA (Fig. 2.1 (c)) bands. Differences between the spectrum of ascorbic acid and those of its systems were found in these regions.

As shown in Fig. 2(1) (d), in the spectrum of ascorbic acid:HP-β-CD freeze-dried system the band of the carbonyl group of the guest at 1755 cm^{-1} changed to 1770 cm^{-1}, with a decrease in its intensity being observed, whereas the bands corresponding to C=C and C–O–C (at 1500 cm^{-1} and 1117 cm^{-1}, respectively) were shifted to 1490 and 1159 cm^{-1}, respectively. These events could be attributed to the formation of hydrogen bonds between the encapsulated drug and the CD, suggesting that the ring moiety of the ascorbic acid molecule interacts with the CD. On the other hand, the spectrum of the physical mixture (Fig. 2(1) (e)) corresponded simply to the superposition of the FT-IR spectra of the two components.

For the ascorbic acid:TEA freeze-dried system (Fig. 2(2) (f)) as well as for its physical mixture (Fig. 2(2) (g)), the appearance of the carbonyl band differed from that of the pure ascorbic acid. It shifted to 1729 cm^{-1} in both systems, but it increased in intensity in the freeze-dried.

In the case of the ascorbic acid:HP-β-CD:TEA systems, the intensity of the carbonyl band of ascorbic acid was significantly diminished in the freeze-dried system (Fig. 2(2) (h)) relative to the physical mixture (Fig. 2(2) (i)), and at the same time, it was shifted towards lower frequencies in both systems (1719 and 1726 cm^{-1}, respectively).

The observed shifts for the carbonyl band in the systems containing TEA are indicative of strong intermolecular interactions between ascorbic acid and the TEA.

Taking into account these spectral changes, there was undoubtedly clear evidence of interactions between ascorbic acid and the other two compounds, suggesting the formation of a complex.

3.2. NMR studies

Solid-state characterization gives information about the interaction between the drug and the CD that can only involve the external surface of the CD. Hence, the guest molecule could be accommodated externally between two or more CD molecules. To clarify the existence of the complexes, other techniques can be used such as NMR spectroscopy. NMR spectra were used to attempt to provide direct proof for the complex conformation

Table 1
^{1}H NMR chemical shifts (δ) of the individual components and changes ($\Delta\delta$) in the presence of the binary and ternary systems (see Fig. 3)

Assignment	δ free drug (ppm)	$\Delta\delta$ (δ complexed − δ free)		
		AA:HP-β-CD	AA:TEA	AA:HP-β-CD:TEA
Ascorbic acid				
H5	5.030	−0.032	−0.473	−0.477
H6	4.142	−0.017	−0.074	−0.048
H7	3.823	−0.006	−0.030	−0.044
TEA				
H1	2.800		0.393	0.718
H2	3.747		0.142	0.259
HP-β-CD				
H1	5.214	−0.006		−0.006
H2	3.681	−0.005		−0.005
H3	4.058	−0.042		−0.004
H4	3.541	−0.008		−0.095
H5	3.735	−0.010		−0.005
H6	3.936	−0.005		−0.006
CH_3	1.229	−0.001		−0.005

between ascorbic acid and the ligands. Evidence of the inclusion of ascorbic acid in the cavity of the HP-β-CD, as well as its interaction with TEA in aqueous solution, was based on the modification of the NMR spectra of the mixtures with respect to the spectra for the individual components. For ascorbic acid and HP-β-CD, assignment of peaks to protons was performed following Yang et al. [19] and Loukas et al. [20], respectively.

The chemical shifts (δ) for the protons of the individual components, and the changes induced on them as a result of the interactions in the binary and ternary systems, are summarized in Table 1 and Fig. 3. In the ^{1}H NMR spectra of the systems studied, appreciable shifts were observed in the ascorbic acid signals, probably due to conformational changes as a result of complexation.

^{1}H NMR results for the ascorbic acid:HP-β-CD system (Table 1) revealed that the signals due to HP-β-CD protons shifted upfield, induced by interaction with ascorbic acid. This suggests that a hydrophobic interaction prevails between the ascorbic acid and the CD. The internal H3 and H5 protons and the external H4 proton underwent greater displacements than the external H1, H2, and H6 protons. These changes are critical functions of the ascorbic acid position in the molecule. The magnitude of the H3 and H5 shifts and their relationship ($\Delta\delta$H5/$\Delta\delta$H3) can be used as quantitative measurements of the

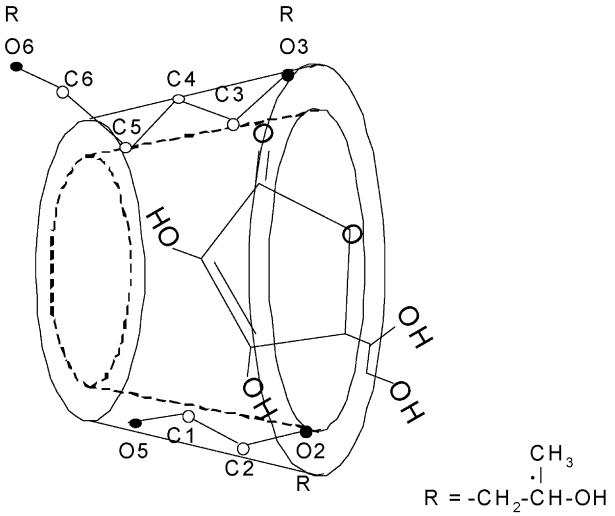

Fig. 4. Graphic illustration of the proposed structure of the ascorbic acid:HP-β-CD inclusion complex.

complex stability and of the depth of inclusion of the ascorbic acid inside the cavity [21], respectively. The shields of the HP-β-CD internal protons are probably due to diamagnetic anisotropy effects inside the cavity produced by groups very rich in π electrons of the guest [22], which suggests that the ascorbic acid ring penetrates in the cavity, since this possesses the only groups with π electrons in the molecule. The small value obtained for the $\Delta\delta$H5/$\Delta\delta$H3 relationship (0.238) suggests that ascorbic acid penetrates partially into the HP-β-CD cavity from the wider side (Fig. 4). Also, the H6 proton, located in the narrow border, was not significantly affected by the presence of ascorbic acid. Nevertheless, because the external H4 proton was also influenced in the presence of the guest, we cannot exclude a probable interaction between ascorbic acid and the external surface of the CD [23]. On the other hand, in the presence of HP-β-CD, the H5 and H6 protons of ascorbic acid showed a pronounced upfield shift, suggesting their association with the oxygen atoms of the CD, which are rich in π electrons [22]. These findings allow us to speculate that only the lactone cyclic portion was inserted into the cavity of the CD.

The inclusion was also confirmed by ^{13}C NMR as it provides considerable information on the environment of individual carbons and intermolecular interactions, and is therefore very useful for analyzing inclusion phenomena [24]. Remarkable changes were observed in the ^{13}C signals of ascorbic acid in the presence of HP-β-CD (Table 2). The C6 of the alkyl side chain shifted

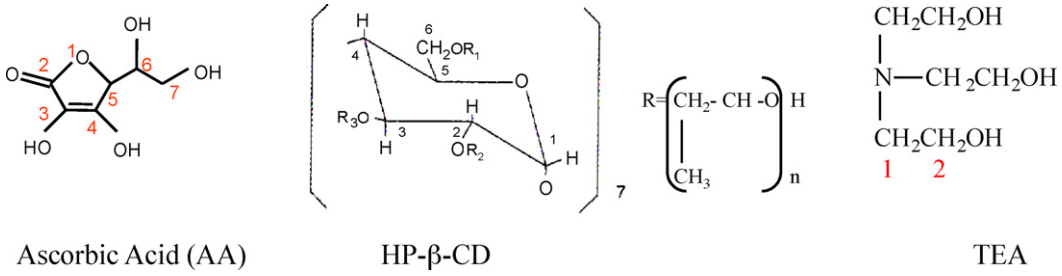

Fig. 3. Chemical structures and labeling of the compounds used in this study.

Table 2
^{13}C NMR chemical shifts (δ) of ascorbic acid and ascorbic acid:HP-β-CD complex

Carbon	Ascorbic acid δ_0 (ppm)	Ascorbic acid: HP-β-CD δ_c (ppm)	$\Delta\delta$ ($\delta_c - \delta_0$)
C2	173.6740	173.2157	−0.4583
C3	118.2059	118.3149	−0.1090
C4	155.9662	155.5080	−0.4582
C5	76.4028	76.2950	−0.1078
C6	69.1526	69.1795	0.0269
C7	62.3067	62.3067	0

to lower fields whereas the others shifted to higher fields. This behaviour was well correlated with ^{1}H NMR results, since both demonstrated the introduction of the ascorbic acid ring into the CD cavity.

On the other hand, in the ^{1}H NMR spectrum of ascorbic acid:TEA system marked changes were observed in the signals of both molecules (Table 1). This demonstrated a strong interaction between TEA and ascorbic acid, suggesting the creation of an association complex by the formation of hydrogen bonds between the nitrogen atom of the aminoalcohol and the enolic hydrogen of the ascorbic acid (Fig. 5). The greater upfield shift observed for H5 of ascorbic acid could be due to a pH effect, since it was reported in Ref. [19] that this proton signal shifted upfield for an increase in the pH value. Furthermore, the ^{1}H NMR spectrum of the ascorbic acid:HP-β-CD:TEA system presented a considerable complexity (Table 1). The H3 and H5 protons located within the CD cavity were unaffected, suggesting no involvement in the complexation. The upfield shifts of the ascorbic acid protons and the external H4 proton of the HP-β-CD, and the downfield shifts of the TEA signals, permit us to postulate that ascorbic acid forms an association complex with TEA, which then interacts with the hydroxyl groups on the external surface of the CD to form a ternary complex.

The largest downfield shifts were observed for the TEA protons in the presence of ascorbic acid, with these shifts being more pronounced when HP-β-CD was added to the binary system.

3.3. Formation of aggregates

The CDs and their complexes show a tendency to self-associate by forming aggregates in aqueous solution. Studies have indicated that the aggregates are formed with two or more CD molecules or complexes [25]. The possible formation of aggregates or non-inclusion complexes was investigated using ^{1}H NMR spectroscopy. Critical concentrations (cc) and aggregation numbers were determined from chemical shift data [16,26].

The chemical shift of the CH_3 group of the HP-β-CD molecule was measured from the ^{1}H NMR spectra of solutions for a range of HP-β-CD concentrations (1.33–211.50 mg/ml), combined with a constant concentration of other components.

The plots of the chemical shifts for the pure HP-β-CD solution, the HP-β-CD solution containing ascorbic acid, and the HP-β-CD solution containing ascorbic acid and TEA, as a function of the inverse of the total HP-β-CD concentration (Fig. 6),

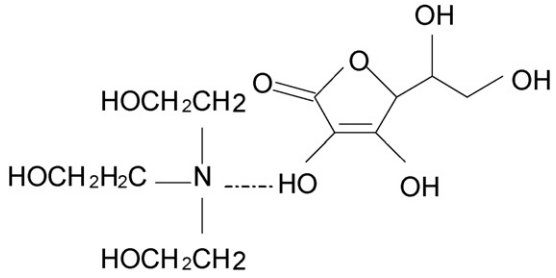

Fig. 5. Proposed structure of the ascorbic acid:TEA association complex.

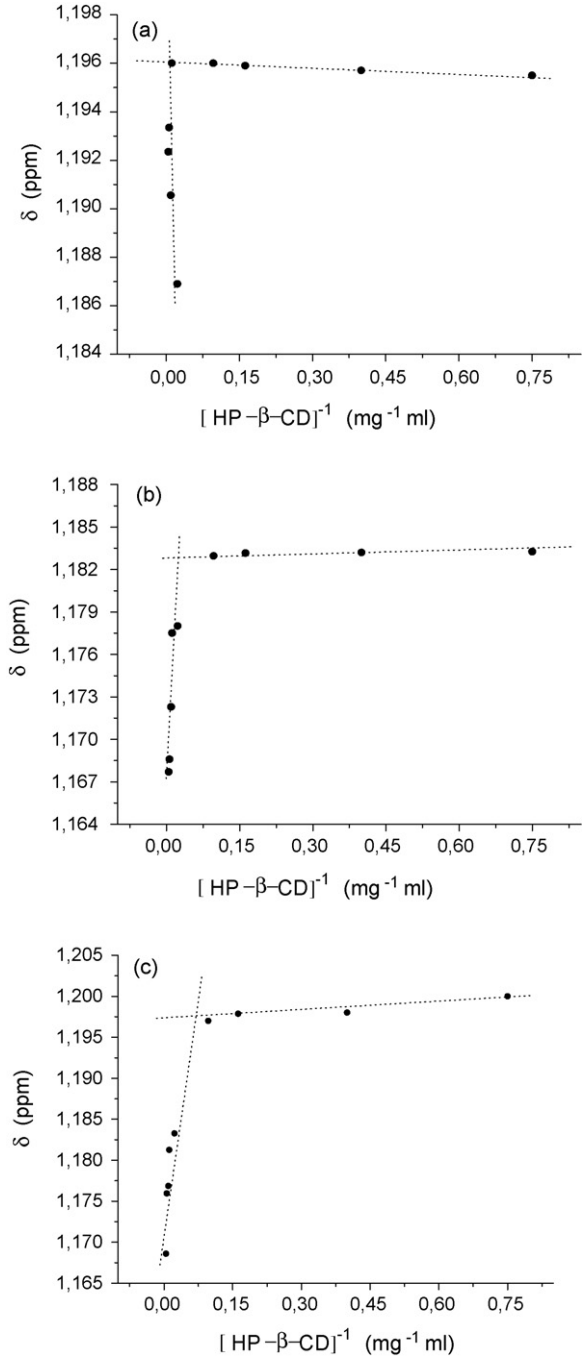

Fig. 6. The proton chemical shift of CH_3 of the HP-β-CD molecule for the pure HP-β-CD solution (a); HP-β-CD solution containing ascorbic acid and TEA (b); HP-β-CD solution containing ascorbic acid (c).

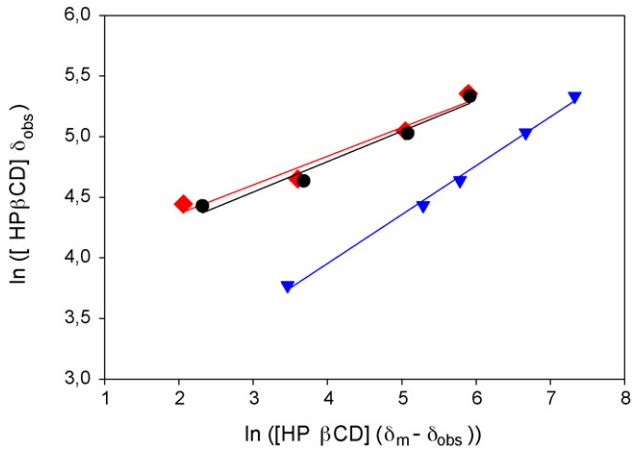

Fig. 7. NMR chemical shift data for determination of the aggregation number for HP-β-CD (♦), AA:HP-β-CD:TEA (●) and AA:HP-β-CD (▼).

show pronounced upfield shift upon aggregation. These results indicated that below critical concentration (cc) the chemical shift was concentration independent, suggesting an absence of any appreciable pre-aggregation, but when the concentration was higher than cc, the chemical shift was inversely proportional to the total HP-β-CD concentration. The cc values were determined from the intersection of the linear portions of the plots at concentrations, both above and below the inflection region. The cc of HP-β-CD is 77.7 mg/ml, with the effect of ascorbic acid and TEA on the cc value being insignificant (75.67 mg/ml). However, the presence of only ascorbic acid yielded a marked decrease in the cc value (25.28 mg/ml). This suggests that ascorbic acid induced the formation of aggregates of HP-β-CD. We assume that the chemical shifts observed (δ_{obs}) are the weighted averages between the chemical shift of free HP-β-CD in the monomeric state (δ_s) and the chemical shift of HP-β-CD in the aggregate (δ_m). The aggregation number "n" can be deduced from the variation of δ_{obs} as a function of total HP-β-CD concentration (C_t) [26]. Plots of $\ln(C_t\delta_{obs})$ against $\ln[C_t(\delta_m - \delta_{obs})]$ (Fig. 7) show that the aggregation numbers are between 3 and 4 for pure HP-β-CD, and the ternary system, and between 2 and 3 for the binary system.

Due to the small diameter of the HP-β-CD molecule, it is difficult to characterize the aggregates, which consist of a small number of units. However, changes in the physicochemical properties due to the formation of such aggregates can easily be observed. Any kind of self-association between the complexes, as well as between free HP-β-CD molecules and the complexes, might explain the shape of the profile obtained for ascorbic acid degradation.

3.4. Stability studies

3.4.1. Chemical stability in aqueous solution

As discussed previously, ascorbic acid is rapidly decomposed in aqueous solutions with half-lives ranging from a few hours to a few minutes. Therefore, one goal of this research was to ascertain whether the stability of ascorbic acid could be enhanced by

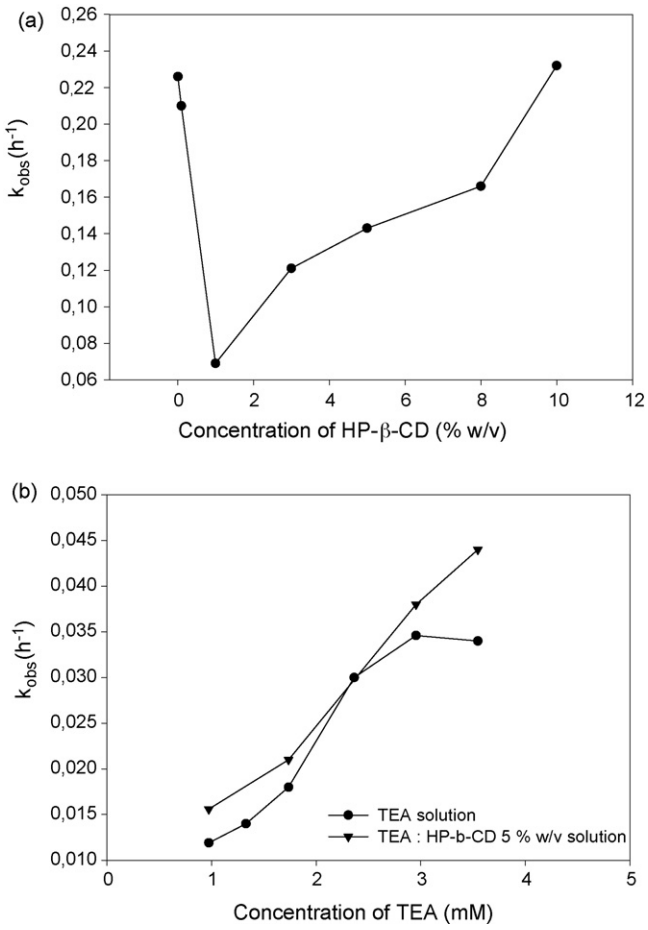

Fig. 8. Effect of (a) HP-β-CD, (b) TEA, separately or in combination, on the rate constant of the ascorbic acid degradation.

complexation with HP-β-CD and TEA, either separately or in combination.

The effect of the ligands on the stability of the ascorbic acid in aqueous solution is depicted in the plots of the observed rate constants (k_{obs}) as a function of the ligand concentration (Fig. 8a and b). Among the ligands examined, TEA had a pronounced positive effect on the degradation of ascorbic acid, whereas HP-β-CD had a minor effect. These figures clearly show the significant improvement in the stability of ascorbic acid upon the addition of TEA. Moreover, the multicomponent complex gives a significantly better stabilization than that of HP-β-CD alone. The linear relationship obtained between the natural logarithmic percentage of the remaining concentration of ascorbic acid and time unambiguously indicates that the degradation of ascorbic acid, irrespective of the concentration of ligand being employed, follows a pseudo-first-order kinetic.

The k_{obs} and the corresponding half-life values are presented in Table 3. There was approximately a 17-fold increase in the stability of ascorbic acid in the presence of 0.9749 mM of TEA. This corresponds to an increase in the half-life of ascorbic acid from 3.4 h in aqueous solution to 58.2 h in the presence of TEA. Also, in the presence of 1% HP-β-CD the half-life was 10.0 h.

The quantitative relationship between k_{obs} and the total concentration of ligand was obtained by the Lineweaver–Burke plot

Table 3
Degradation rate constants and corresponding half-life values for ascorbic acid complexed with different ligands in aqueous solution at 25 °C

Ligand (concentration)	k_{obs} (h^{-1})	t_{50} (h)
Aqueous solution	0.206 ± 0.005	3.4
HP-β-CD (1%, w/v)	0.069 ± 0.003	10.0
TEA (0.9749 mM)	0.0119 ± 0.0009	58.2
HP-β-CD (5%, p/v):TEA (0.9749 mM)	0.0156 ± 0.0006	44.4

[27], constructed using the relationship:

$$\frac{1}{(k_0 - k_{obs})} = \frac{1}{K_c}(k_0 - k_c)[L] + \frac{1}{(k_0 - k_c)}$$

where k_0 and k_c are the rate constants for the degradation of the free and the complex drug, respectively, with K_c being the stability constant for the complex, and [L] the concentration of the ligand. The values of k_c and K_c, calculated from the intercept and slope of the linear line constructed by plotting $1/(k_0 - k_{obs})$ versus 1/[L] are shown in Table 4. This method is used for estimating K_c values when the guest molecule is so chemically labile that the K_c values cannot be determined by any other method [2,28]. The results obtained clearly show that the degradation of ascorbic acid in the complex forms at lower concentrations of ligands is much slower than that of free ascorbic acid. Using K_c for the complexes a different interaction can be deduced for each ligand, with the interaction for TEA being the largest. The K_c value for ascorbic acid:HP-β-CD is relatively low, indicating that this host–guest complex has relatively small driving forces for inclusion. This implies that at the CD concentrations used, a large fraction of the acid remains uncomplexed.

Concerning ascorbic acid in a TEA complex (Fig. 8), its profile shows a stabilizing effect, but with k_{obs} increasing for a corresponding rise in the TEA concentration, whereas, the degradation profile for ascorbic acid in the complex form with HP-β-CD (Fig. 8) shows two different behaviours. At the beginning, for lower CD concentrations, the degradation rate decreased with increasing CD concentration, but then k_{obs} accelerated upon further increases in CD concentration. The interpretation of these kinetic results could be made by taking into account the cc value, since it was shown that the ascorbic acid:HP-β-CD complex could form higher-order systems with additional cyclodextrin molecules. From this, we can postulate that HP-β-CD has a stabilizing effect until its concentration reaches the cc, but subsequently its effect diminishes proportionally to an increase in the concentration. These differences in the stability behaviour can be explained by the formation of aggregates. At HP-β-CD concentrations higher than the cc value for the complex, we postulate that the catalysis of the degradation is favoured by the approach of aggregated cyclodextrin molecules to the reactive site. These results are also coherent with the geometry for the complex postulated from the NMR experiments. Indeed, the partial inclusion of ascorbic acid, with the lactone ring being located in the cavity rim at the wider end of the cyclodextrin molecule, allows a favourable approach of the ester cyclic group to the hydroxyl groups of the cyclodextrins forming the aggregates. In such cases, the observed values of K_c and k_c are the weighted averages of the different forms of all the complexes formed.

Table 4
The stabilization of ascorbic acid by complexation

Ligand	pH	k_0 [a] (h^{-1})	k_c [b] (h^{-1})	K_c (M^{-1})
HP-β-CD	4.20	0.206 ± 0.005	0.187	82.87 ± 0.06
TEA	7.51	0.549 ± 0.002	0.080	7342.01 ± 0.03
HP-β-CD:TEA	7.59	0.549 ± 0.002	0.093	6676.81 ± 0.04

[a] k_0 the observed first-order rate constant for the degradation of the free ascorbic acid.
[b] k_c the observed first-order rate constant for the degradation of the ascorbic acid within the complex.

3.4.2. *Influence of pH and stabilization with ligands*

The degradation rate profile for ascorbic acid at 25 °C, determined in the pH range from 1.34 to 11.04 is shown in Fig. 9. In aqueous solutions, ascorbic acid showed better stability between pH 1 and 6. In addition, the combined effect of pH and CD concentration on the stability of ascorbic acid was studied at pH values where the drug molecules were either fully ionized (pH 8.50) or fully unionized (pH 2.50), and also at a pH near p*K*a (pH 4.20). This was due to the fact that charged forms of the drug molecules are more hydrophilic and carry a larger number of tightly bounded water molecules than the unionized forms, thus making it difficult for them to enter the narrow and relatively lipophilic CD cavity. In Table 5 are shown the results obtained at different CD concentrations for the three selected pH values. These indicate that the stabilizing effect of HP-β-CD is pH-dependent, since it stabilized ascorbic acid in the acidic pH region but destabilized it in the alkaline pH region.

The stabilizing effect of CD was more significant at pH 2.50, because the unionized form of ascorbic acid interacted more strongly with the cavity than its ionized form. However, in alkaline solution the degradation was accelerated in the presence of CD, indicating that the catalytic combined effect was synergistic (i.e. a greater acceleration was achieved when alkaline media and CD were used together), suggesting that this process was

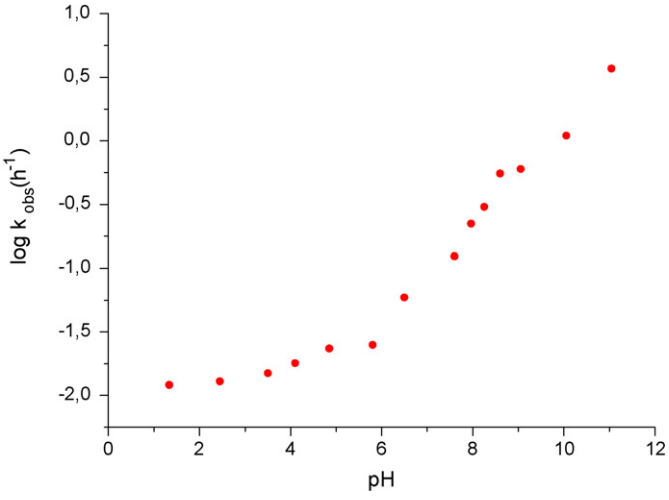

Fig. 9. Effect of pH on the rates of ascorbic acid degradation at 25 °C in the absence of HP-β-CD.

Table 5
The stabilization of ascorbic acid at different pHs by complexation

Ligand	pH 2.50		pH 4.20		pH 8.50	
	k_{obs} (h^{-1})	t_{50} (h)	k_{obs} (h^{-1})	t_{50} (h)	k_{obs} (h^{-1})	t_{50} (h)
	0.029 ± 0.004	23.70	0.032 ± 0.002	21.60	0.231 ± 0.002	3.00
HP-β-CD (%w/v)						
0.50%	0.0176 ± 0.0002	39.32	0.0325 ± 0.0001	21.35	0.4217 ± 0.0001	1.64
1.00%	0.0167 ± 0.0003	41.55	0.0316 ± 0.0005	21.91	0.3888 ± 0.0009	1.78
3.00%	0.0162 ± 0.0003	42.71	0.0298 ± 0.0005	23.26	0.3202 ± 0.0006	2.16
8.00%	0.0195 ± 0.0006	35.53	0.0315 ± 0.0001	22.00	0.3748 ± 0.0001	1.85
TEA						
0.9749 mM					0.1129 ± 0.0001	6.14
1.7365 mM					0.1142 ± 0.0009	6.07
2.3633 mM					0.1173 ± 0.0009	5.91
2.9542 mM					0.1251 ± 0.0009	5.54

favoured by the interaction of the ester part of the ascorbic acid with the hydroxyl groups of the CD, which existed as alkoxide ions in this pH region. This observed behaviour could be associated with the presence of aggregates, which might have catalyzed the hydrolysis in this media through non-inclusion complexation. Also, our data suggest that the aggregate formation is unaffected by acid (pH 2.50) and basic (pH 8.50) conditions.

Furthermore, the rate of degradation of ascorbic acid in aqueous solution was higher than in the pure buffer solution of identical pH. This effect on the rate of degradation reflects a significant ionic interaction, possibly with the buffer ions acting as stabilizing agents of ascorbic acid. In addition, by using the buffers, the stabilizing effect of the CD on ascorbic acid degradation at 4.20 pH value is negligible This could be due to the displacement of the drug molecules away from the CD cavity, by the buffers ions.

Moreover, the combined effect of complexation with TEA and the pH control was evaluated. As shown in Table 5, the rate of degradation is reduced in relationship to the control solution, with the stabilizing effect being independent of the TEA concentration. However, the TEA effect was greater in the aqueous solution, suggesting a possible interaction between the buffer ions and TEA, which interfered in the process with ascorbic acid.

4. Conclusion

In conclusion, the results obtained in this study demonstrate that the ligands HP-β-CD and TEA are capable of interacting with ascorbic acid, thereby producing binary and ternary complexes. Furthermore, these complexes can markedly increase the chemical stability of the ascorbic acid. However, HP-β-CD stabilized ascorbic acid for acid pH but destabilized it at alkaline pH, with the pH control having a negative influence on the TEA stabilizing effect.

This study clearly showed that the preparation of complexes in the solid state is feasible. The inclusion was tested in the solid state by DSC and FT-IR, and in solution by NMR spectroscopy. The results obtained from these NMR studies provide significant information about the species involved in the interactions, and indicated the partial complexation of ascorbic acid into the HP-β-CD cavity.

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