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ORIGINAL ARTICLE

Thermodynamic and geometric study of diasteroisomeric complexes formed by racemic flavanones and three cyclodextrins through NMR

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Abstract The main purpose of this work was to study the chiral recognition thermodynamics of inclusion complexes formed by flavanones and β-cyclodextrins, and its relation with the inclusion geometries, through NMR experiments. By using the racemic mixtures of (±)-flavanone (FL) and (±)-2′-hydroxyflavanone (2′OHFL), diasteroisomeric complexes were formed employing β-cyclodextrin (βCD), (2-hydroxypropil)-β-cyclodextrin (HPβCD) and heptakis-(2, 6-*O*-dimethyl)-β-cyclodextrin (DMβCD). ^{1}H NMR experiments of the complexes showed enantiodifferentiation for FL/βCD, FL/HPβCD, FL/DMβCD, 2′OHFL/HPβCD and 2′OHFL/DMβCD complexes, so they were able to be studied by obtaining the stoichiometry (1:1 for each complex), association constants (Ka), Ka ratios, and thermodynamics (ΔH, ΔS and ΔG). The results show that Ka values decrease with increasing temperature and that Ka ratio values removed from 1 not always reflect better enantiodiscrimination by NMR. Thermodynamics (ΔH and ΔG) show an exothermic and spontaneous formation of the complexes. Since the results were established for each couple of diasteroisomeric complexes separately, comparison of thermodynamics between them was possible, concluding that one half of the couples of diasteroisomeric complexes present chiral recognition due to enthalpic phenomena and the other half due to entropic phenomena. Additionally, ROESY experiments were performed to estimate the inclusion geometry of the complexes, which are in good agreement with the thermodynamic and Ka results.

Keywords Cyclodextrins · Diasteroisomeric inclusion complex · ^{1}H NMR · Enantiomeric differentiation · Association constant · Inclusion thermodynamics · Chiral recognition thermodynamics · 2D-ROESY · Inclusion geometry

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Introduction

Cyclodextrins (CDs) are chiral oligosaccharides composed by 6, 7 or 8 α-D-glucopyranose units, named α, β and γ cyclodextrin, respectively. Since the α-D-glucopyranose units are connected by α-1,4-glycosidic bonds, these oligosaccharides have a truncated cone shape, where the glucopyranose units, having a $^4C_1$ conformation, place the 2-, 3- and 6-hydroxyl groups toward the external area of the cyclodextrins, originating an external hydrophilic surface. The inner face of cyclodextrins is covered by hydrogen atoms and the glycosidic oxygen, which generate a less polar microenvironment that an aqueous exterior, and with dipoles generated by the C–O internal bounds. Therefore, in recent times, the inner cavity of cyclodextrins has been considered moderately polar rather than nonpolar (Fig. 1). This allows the inclusion of small molecules, commonly orienting their polar portion toward the cavity exterior, forming inclusion complexes, where the interactions between the cyclodextrin and the guest are of noncovalent nature, and they could include hydrogen bonding, electrostatic, van der Waals and hydrophobic interactions, where the latter two have been described as the main ones [1–4].

Since molecules of different nature can be included, cyclodextrins have been widely studied not only as

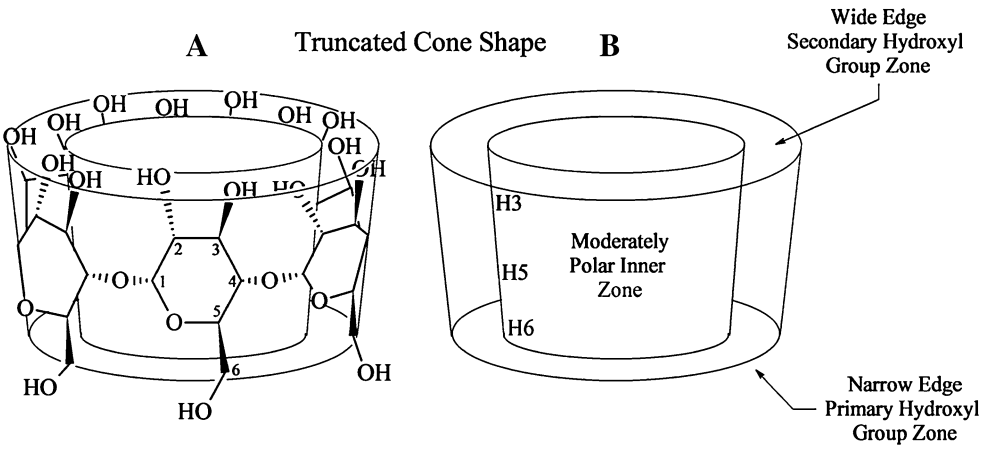

Fig. 1 Cyclodextrin scheme. **A** the glucopyranose units and the hydroxyl groups arrangement. Notice that the hydrogen atoms on the positions 1, 2 and 4 are oriented toward the exterior of the cyclodextrin, and that the hydrogen atoms at positions 3, 5 and 6 are oriented toward the inner cavity. **B** the internal hydrogen atoms, H3, H5 and H6, in addition to the primary and secondary hydroxyl group zone, located at the narrow and wide edges, respectively

solubility, bioavailability and stability enhancers of drugs [5–9], but also used in areas such as asymmetric synthesis and chiral recognition [10–13]. The latter uses are possible due to cyclodextrins being chiral molecules, so enantiomers could be recognized by forming diasteroisomeric complexes [11]. Some general and important observations concerning the chiral recognition with CDs have been pointed out [14], namely: (i) while the hydrophobicity around an asymmetric center is maintained, modifications in other portions of the guest do not disturb the enantioselectivity of the cyclodextrin; (ii) when hydrophilic substituents are present in a guest molecule, the farther this substituent, the more likely the chiral recognition; (iii) guests which are conformationally restricted show better chiral recognition. Despite these observations, a preferred affinity for a particular enantiomer cannot be predicted based on its stereochemistry; there are no general rules to correlate structural features and entropy- or enthalpy-driven chiral recognition, and there is no relationship or general tendency between the thermodynamic parameters and chiral recognition. Considering these observations, there still is lack of knowledge concerning the chiral recognition mechanism of CDs.

To study chiral recognition employing CDs as chiral selectors, several techniques have been used; among these HPLC [15–23], capillary electrophoresis (CE) [24–29] and NMR [11, 30–45]. These techniques are based on the formation of the diasteroisomeric complexes between each enantiomer and the CD. The former two techniques could give different retention and migration times for each enantiomer, respectively, thus characteristics as stoichiometry, association constants (Ka) and thermodynamics (ΔH, ΔS and ΔG) can be determined from these time data. In NMR, cyclodextrins could induce different chemical shifts (δ) for each enantiomer; this allows studying the formed complexes obtaining the same characteristics than in the previously named techniques (stoichiometry, Ka, ΔH, ΔS and ΔG). Moreover, the NMR technique has the additional advantage that it is able to estimate the relative orientation of the guest in respect to the CD cavity, or inclusion geometry. This structural feature can be estimated due to the existence of the nuclear overhauser effect (NOE), which is a through-space dipolar interaction that occurs between two nuclei, and that can be detected when the nuclei are at distances below 5 Å [11, 46]. Since the cyclodextrins' inner hydrogen atoms, being these H3, H5 and H6 hydrogen atoms (Fig. 1), are well characterized through NMR, by employing 2D-ROESY experiments, the NOE between these internal CD hydrogen atoms and the guest hydrogen can be detected and the inclusion geometry estimated, as it has been described for several CD inclusion complexes [33, 35, 37, 44, 47–52].

It has been described that the degree of chiral recognition can be evaluated by obtaining the Ka ratio between a diasteroisomeric complex and its counterpart [12, 53], and it has been proposed that the more different from 1 the value of the ratio, the better the chiral recognition could be. On the other hand, in NMR, the chiral recognition, based on the chemical shifts differences between diasteroisomeric complexes, depends on the magnitude of their association constants, the host–guest inclusion geometries and on their intrinsic chemical shifts (δ_c), where the latter corresponds to the chemical shift of the completely formed complex. The more different these parameters, the more differentiation between enantiomers could be found [11, 31].

When diasteroisomeric complexes are studied, these complexes are usually formed by using each enantiomer separately, separation which sometimes is a troublesome step in the study. In ^{1}H NMR, if the chemical shifts of an enantiomer and their counterpart by employing CDs as chiral selector are different enough, this separation inconvenient can be avoided and the complexes can be simultaneously studied by using the enantiomeric mixture, and stoichiometry, Ka and thermodynamics can be obtained for each complex [54–56].

The purpose of this study was to investigate the thermodynamics involved in the chiral recognition and in the formation of diasteroisomeric complexes constituted by three cyclodextrins, named β-cyclodextrin (βCD), (2-hydroxypropyl)-β-cyclodextrin (HPβCD) and heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin (DMβCD), and the racemic mixtures of two flavanones, (±)-flavanone (FL) and (±)-2′-hydroxyflavanone (2′OHFL) (Fig. 2), where the flavanones have been proposed as potential drugs for the treatment of diabetes type 2 [57]. By employing ^{1}H NMR experiments, stoichiometry, association constants, Ka ratios and thermodynamics were determined from the chemical shifts data, and from ROESY experiments, their inclusion geometry was estimated.

Materials and methods

NMR spectra were recorded on a Bruker Avance DRX 300 spectrometer (^{1}H at 300.13 MHz), in D_2O:CD_3OD 60:40, referencing the spectra to the MeOD residual signal at 3.2 ppm. ^{1}H NMR spectra were obtained by recording 128 scans, and coupling constants are given in Hz.

ROESY spectra were recorded in the same Bruker spectrometer in phase-sensitive mode by using the standard roesygpph19 pulse program. Spin-lock mixing time was set to 400 ms, relaxation delay to 2 s, and 32 scans were recorded, where each spectrum consisted of a matrix of 16 K (F2) by 8 K (F1) points, covering a spectral width of 3,000 Hz.

The cyclodextrins, βCD (β-cyclodextrin), HPβCD ((2-hydroxypropyl)-β-cyclodextrin, average $M_W = 1.540$), DMβCD (heptakis-(2,6-di-*O*-methyl)-β-cyclodextrin), and the flavanone racemic mixtures, FL (2-phenyl-2,3-dihydro-4*H*-chromen-4-one; (±)-flavanone) and 2′OHFL (2-(2-hydroxyphenyl)-2,3-dihydro-4*H*-chromen-4-one; (±)-2′-hydroxyflavanone) were obtained from Aldrich (USA). D_2O and CD_3OD spectroscopic grade were obtained from Merck.

Diasteroisomeric complexes formation

Each diasteroisomeric complex was formed at 25, 30 and 35 °C using the required amount of cyclodextrin and flavanone in D_2O:CD_3OD 60:40 and stirring during 24 h in a thermostatically controlled water bath. CD_3OD was employed due to the flavanones having low water solubility, and since this solvent has a low affinity for cyclodextrins [23, 58, 59].

Chiral recognition

^{1}H NMR spectra were acquired to 1 mL solution of each diasteroisomeric complexes formed as follows: In D_2O:CD_3OD 60:40, 1 mg of flavanone (fl) and the required amount of CD to obtain a 6 mM solution concentration were mixed and stirred during 24 h at 25 °C, and finally filtered to obtain a clean solution of complex. ^{1}H NMR spectra of each complex solution were acquired and the flavanones' chemical shifts (δ_o) registered and compared with the corresponding pure flavanones' chemical shifts (δ_f), also obtained in D_2O:CD_3OD 60:40. Thus, signals which are split are evidence of chiral recognition, and they were used to obtain stoichiometry, Ka and thermodynamics.

As nomenclature for the split signals, the left signal, in respect to the spectrum, will be labeled "L" and it will be arbitrarily assigned to the "left" enantiomer ("L"-fl), and the right signal, in respect to the spectrum, will be labeled "R" and it will be arbitrarily assigned to the "right" enantiomer ("R"-fl). Employing this labeling, the complexes formed by the "left" enantiomer will be referred to as "L"-fl/CD and the complexes formed by the "right" enantiomer as "R"-fl/CD, where fl represents any flavanone (FL or 2′OHFL) and CD any cyclodextrin (βCD, HPβCD or DMβCD).

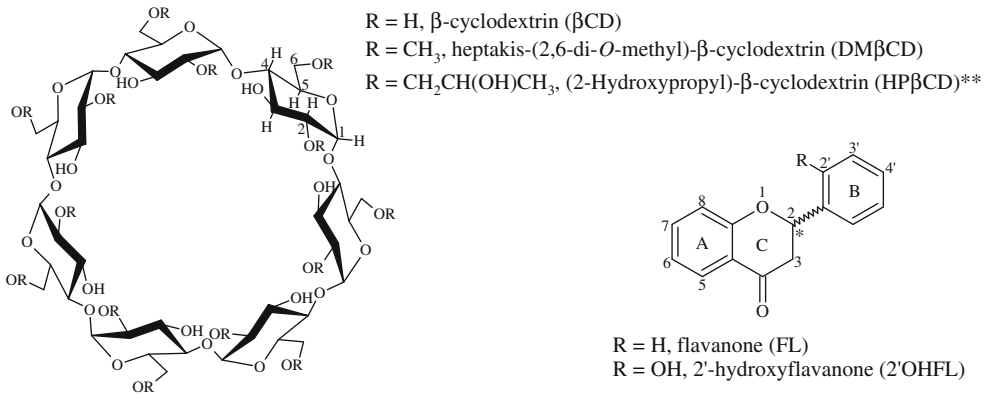

Fig. 2 βCD, DMβCD, HPβCD, FL and 2′OHFL structures. * Indicates the flavanones asymmetric center. ** Indicates random substitution

Stoichiometry determination

Stoichiometry of inclusion complexes was obtained graphically by employing Eq. 1, which has been adapted to NMR data [60, 61]

$$\frac{1}{\Delta\delta_o} = \frac{1}{Ka \cdot \Delta\delta_c} \cdot \frac{1}{[CD]_0^a} + \frac{1}{\Delta\delta_c} \quad (1)$$

where $\Delta\delta_o$ is the observed chemical shift difference defined by $\Delta\delta_o = \delta_f - \delta_o$, Ka is the complex association constant, $\Delta\delta_c$ is the intrinsic chemical shift difference defined by $\Delta\delta_c = \delta_f - \delta_c$, $[CD]_0$ is the initial cyclodextrin concentration, and "a" corresponds to the stoichiometric coefficient of the cyclodextrin, according to Eq. 2

$$fl + a \cdot CD \Leftrightarrow fl/CD_a \quad (2)$$

Eq. 2 corresponds to the equilibrium between the complex and its parts, where fl indicates one of the flavanone enantiomers as substrate, CD the cyclodextrin and fl/CD_a the formed diasteroisomeric complex.

According to Eq. 1, plots of $1/\Delta\delta_o$ vs. $1/[CD]_0^a$ will give the stoichiometry of the complex by testing different values of "a" until a straight line is obtained.

In order to obtain δ_o values, 10 solutions, in D_2O:CD_3OD 60:40, were obtained at 25 °C for each complex, varying the initial CD concentration and maintaining the flavanones' concentration constant, and then acquiring the complexes' ^1H NMR spectra. For βCD complexes, the solutions were formed maintaining the flavanones' concentration constant at 0.3 mM, and varying the CD initial concentration from 0.6 to 6 mM. For HPβCD complexes, the solutions were formed maintaining the flavanones' concentration constant at 1 mM, and varying the initial CD concentration from 2 to 20 mM. For DMβCD complexes, the solutions were formed maintaining the flavanones' concentration constant at 1 mM, and varying the CD initial concentration from 1 to 10 mM. Additionally, ^1H NMR spectra were acquired to pure flavanones in D_2O:CD_3OD 60:40 to obtain δ_f. Once obtained, these values (δ_f and δ_o) were used to determine $\Delta\delta_o$ ($\Delta\delta_o = \delta_f - \delta_o$), in Hz, from the split signals.

Association Constant and Ka Ratio Determination

The association constant is a measure of the equilibrium between the complex and their free components, (Eq. 2), which for a 1:1 stoichiometry is defined by Eq. 3

$$Ka = \frac{[fl/CD]}{[fl] \cdot [CD]} \quad (3)$$

where [fl/CD], [fl] and [CD] are the equilibrium concentrations of the diasteroisomeric complex, one enantiomer of the flavanone and the cyclodextrin, respectively.

By employing the non-linear method [62–64], association constants were determined at 25, 30 and 35 °C in agreement with Eq. 4

$$\Delta\delta_o = \frac{\Delta\delta_c}{2 \cdot [fl]_0}\left[\left([CD]_0 + [fl]_0 + \frac{1}{Ka}\right) - \sqrt{\left([CD]_0 + [fl]_0 + \frac{1}{Ka}\right)^2 - 4 \cdot [CD]_0 \cdot [fl]_0}\right] \quad (4)$$

where $[fl]_0$ and $[CD]_0$ are the initial concentrations of one enantiomer of the flavanone and the cyclodextrin, respectively.

The non-linear method consists in the iteration of Eq. 4, using approximated values of Ka and $\Delta\delta_c$ as initial parameters, which can be obtained from the Benesi-Hildebrand method, Eq. 5 [65].

$$\frac{1}{\Delta\delta_o} = \frac{1}{Ka \cdot [CD]_0 \cdot \Delta\delta_c} + \frac{1}{\Delta\delta_c} \quad (5)$$

If complex stoichiometry is 1:1, by plotting $1/\Delta\delta_o$ vs. $1/[CD]_0$ a straight line is obtained, and approximated Ka and $\Delta\delta_c$ values are determined from the slope and intercept, respectively.

It should be noted that Benesi-Hildebrand is a good method for Ka determination when $[CD]_0$ is at least 100 times larger than the substrate initial concentration ($[S]_0$) [66], therefore, for our experimental conditions the non-linear method is a better option.

In order to obtain δ_o values, 10 solutions of each complex were obtained at 25, 30 and 35 °C at the same concentration conditions as for stoichiometry determination, and ^1H NMR spectra were acquired at the same temperature of the complexes' formation. Additionally, and at the same temperature of the complexes' formation, ^1H NMR spectra were acquired to pure flavanones in D_2O:CD_3OD 60:40 to obtain δ_f. Once obtained, these values (δ_f and δ_o) were used to determine $\Delta\delta_o$ ($\Delta\delta_o = \delta_f - \delta_o$), in Hz, from the split signals.

The degree of chiral recognition was evaluated by obtaining the Ka ratio, at 25, 30 and 35 °C, between a diasteroisomeric complex and its counterpart, by dividing the Ka value of "R"-fl/CD by the Ka value of "L"-fl/CD, and designated $Ka^{"R"}/Ka^{"L"}$. Then, $Ka^{"R"}/Ka^{"L"}$ variations with temperature were studied, at 25, 30 and 35 °C.

Thermodynamic parameters determination

Thermodynamic parameters, ΔH, ΔS and ΔG, were calculated applying the van't Hoff equation [1, 67], Eq. 6

$$\ln(Ka) = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (6)$$

where Ka is the association constant, ΔH and ΔS are the enthalpy and entropy changes, respectively, R is the gas constant (8.314 J/K mol), and T represents the absolute temperature. By obtaining Ka at 25, 30 and 35 °C for each diasteroisomeric complex, ln(Ka) vs. 1/T were plotted and from the slopes and intercepts of the obtained straight lines, ΔH and ΔS were calculated, respectively.

Free energy change (ΔG) was obtained at 298 K in agreement with Eq. 7

$$\Delta G = \Delta H - T \cdot \Delta S \quad (7)$$

The equation $\Delta G = RT \ln(Ka)$ was employed to calculate the free energy changes as well, however the obtained results were the same as using Eq. 7.

Inclusion geometry estimation

ROESY spectra were acquired to a 1 mL solution of each diasteroisomeric complexes formed in D_2O:CD_3OD 60:40, by adding 1 mg of flavanone and the required amount of CD to obtain a 6 mM solution concentration. The mixtures were stirred during 24 h at 25 °C, and finally filtered to obtain a clean solution of the diasteroisomeric complexes.

Results and discussion

In order to begin the study, ^{1}H NMR spectra were acquired to racemic mixtures of the flavanones, which were compared with the corresponding diasteroisomeric complexes' ^{1}H NMR spectra, as shown in Figs. 3 and 4, for FL/CDs and 2′OHFL/CDs complexes, respectively.

In Figs. 3A and 4A, ^{1}H NMR spectra of FL and 2′OHFL, respectively, are shown and compared with the corresponding complexes' ^{1}H NMR spectra, Fig. 3B–D for FL/CDs complexes, and Fig. 4B–D for 2′OHFL/CDs complexes. For all the complexes' ^{1}H NMR spectra, the cyclodextrins' signals appear between 3.2 and 4.0 ppm approximately (H2, H3, H4, H5 and H6 CDs protons), along with the signal about 5.0 ppm, corresponding to the anomeric proton, H1. Therefore, the flavanones' aromatic signals plus the H2 signal (about 5.5 ppm) could be used for the study since these do not overlap with the cyclodextrins signals.

To visualize the enantiomeric discrimination, aromatic zone ^{1}H NMR spectra of the flavanones and the corresponding complexes are shown in Figs. 5 and 6 for FL/CDs and 2′OHFL/CDs complexes, respectively.

Analyzing Figs. 5 and 6, it can be noticed that all the pure flavanones' aromatic and H2 signals change their

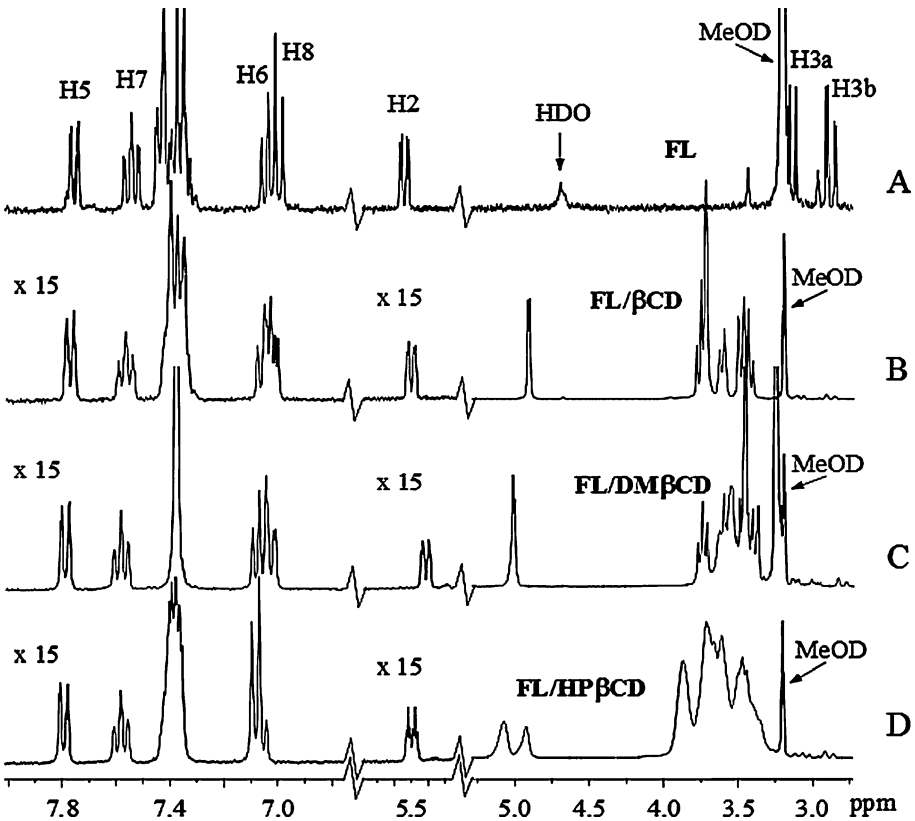

Fig. 3 ^{1}H NMR spectra of FL (**A**), FL/*β*CD (**B**), FL/DM*β*CD (**C**) and FL/HP*β*CD (**D**). Aromatic zone and H2 signal are amplified ×15 in respect to the aliphatic zone (3.0–5.0 ppm)

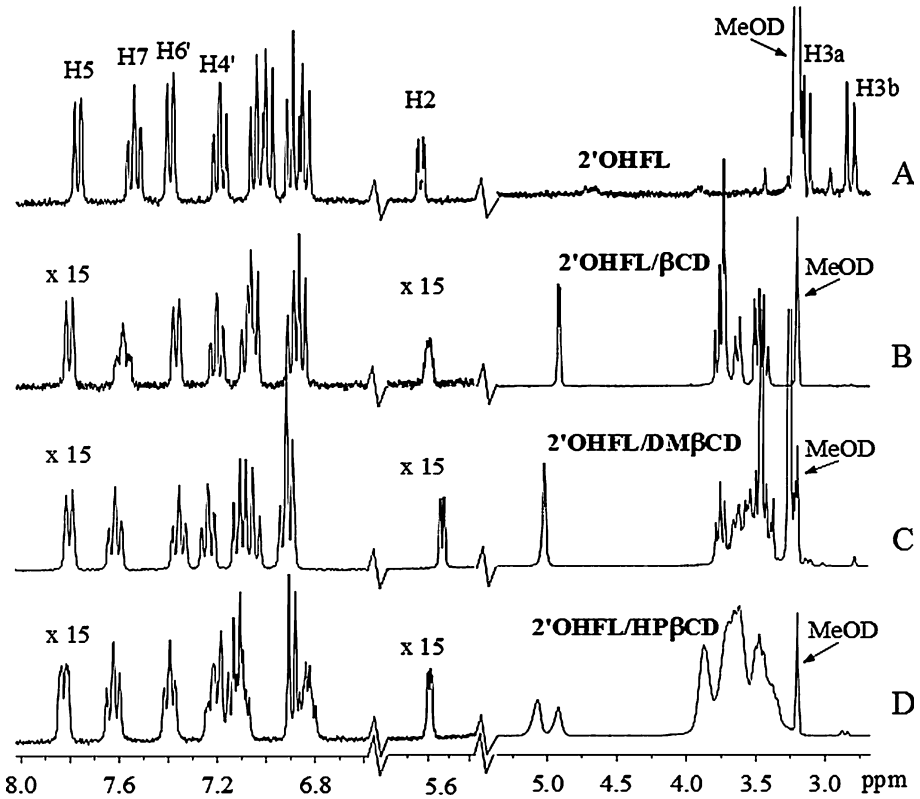

Fig. 4 ^{1}H NMR spectra of 2′OHFL (**A**), 2′OHFL/βCD (**B**), 2′OHFL/DMβCD (**C**) and 2′OHFL/HPβCD (**D**). Aromatic zone and H2 signal are amplified ×15 in respect to the aliphatic zone (3.0–5.0 ppm)

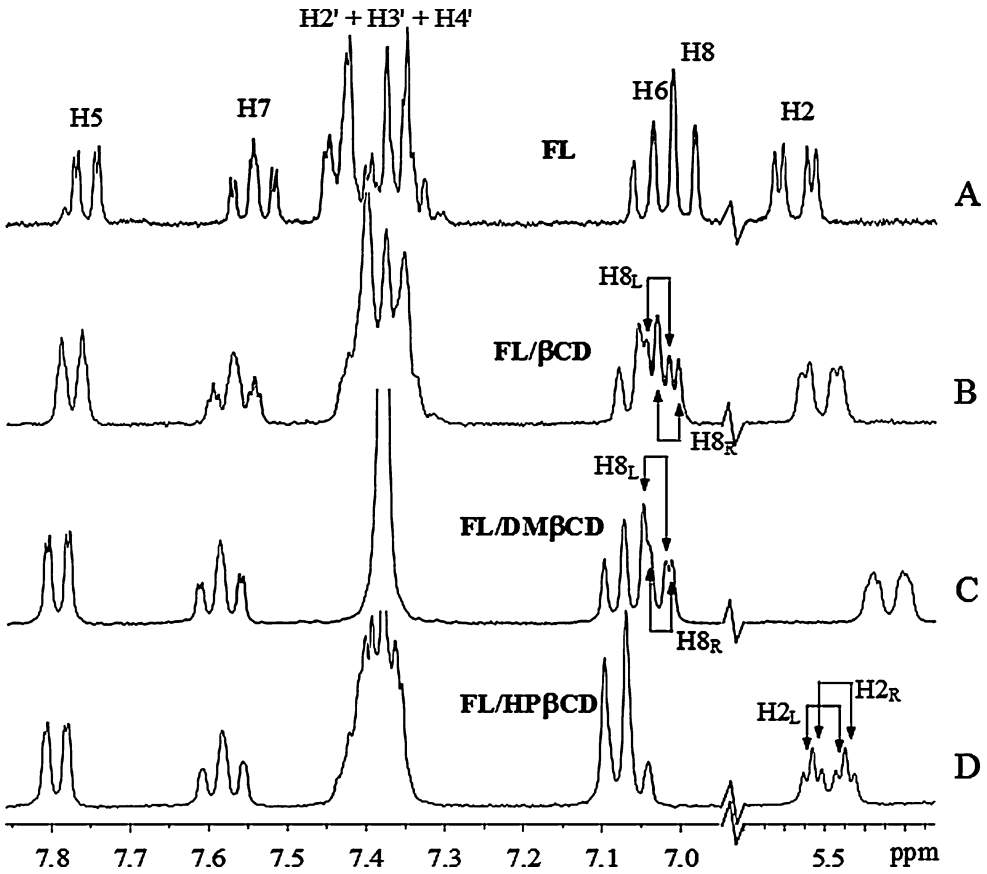

Fig. 5 ^{1}H NMR spectra of aromatic zone and H2 signal of FL (**A**), FL/βCD (**B**), FL/DMβCD (**C**) and FL/HPβCD (**D**)

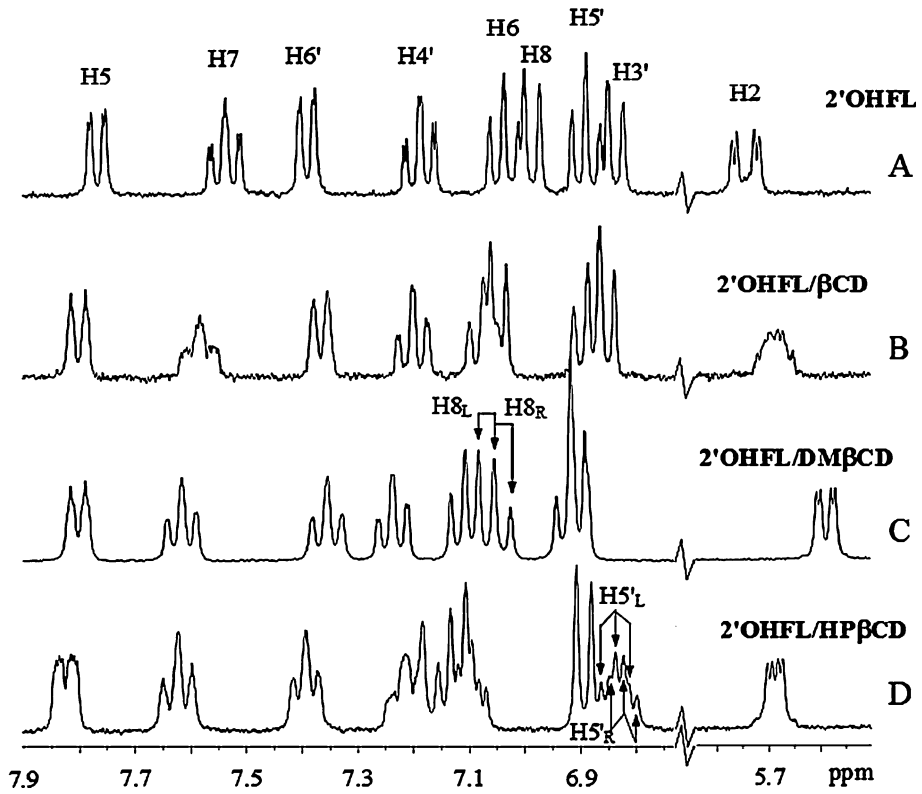

Fig. 6 ^{1}H NMR spectra of aromatic zone and H2 signal of 2′OHFL (**A**), 2′OHFL/βCD (**B**), 2′OHFL/DMβCD (**C**) and 2′OHFL/HPβCD (**D**)

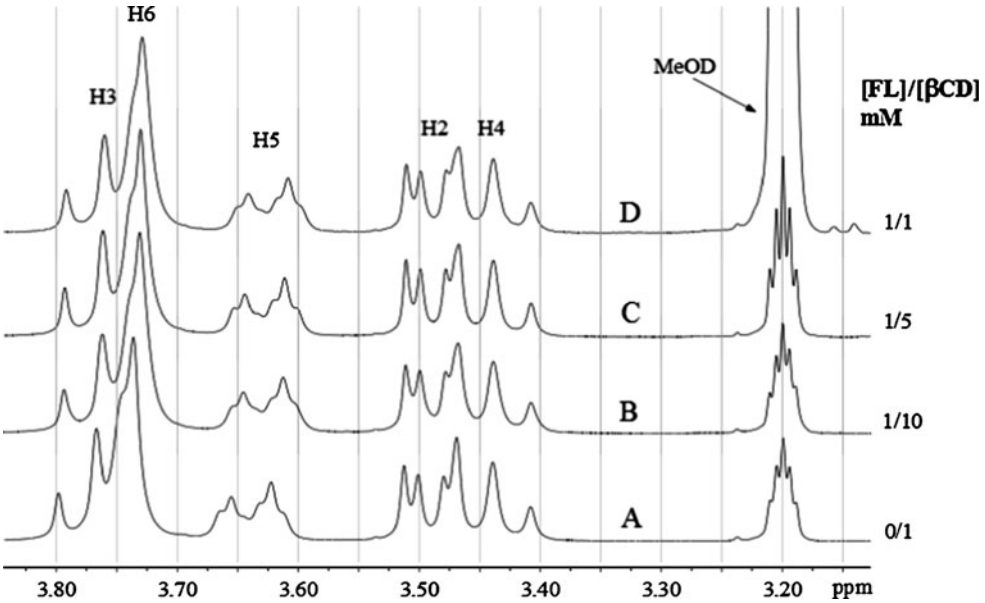

Fig. 7 ^{1}H NMR spectra of the aliphatic zone of FL/βCD complexes at different FL and βCD concentrations (mM). The spectra show the chemical shift changes of the βCD protons from its pure form (**A**) to their 1/1:[FL]/[βCD] concentration ratio (**D**)

chemical shifts due to the cyclodextrin' presence, e.g. in Fig. 6, the H7 hydrogen signal of the pure 2′OHFL (Fig. 6A) changes its chemical shift to downfield when any cyclodextrin is present, as it is seen by comparison of Fig. 6A with B, C and D. Similar results are observed for each aromatic and H2 signals for each flavanone, which confirms interactions between the cyclodextrins and flavanones. Additionally, H3, H5 and H6 inner hydrogen atoms of the pure cyclodextrins change their chemical shifts when flavanones are present (e.g. Fig. 7 for FL/βCD), which implies that the interactions occur between the flavanones and the inner zone of the cyclodextrins, confirming the formation of the diasteroisomeric complexes.

For both FL/βCD and FL/DMβCD complexes, H8 FL hydrogen signal splits due to the presence of these cyclodextrins (Fig. 5B, C), which is indicated in the spectra by $H8_L$ and $H8_R$, for the left and right signals, respectively. Therefore, each enantiomer presents different chemical

shifts, implying enantiomeric differentiation. A similar result can be seen for H2 FL hydrogen signal in FL/HPβCD complexes (Fig. 5D), where each enantiomer displays different chemical shifts ($H2_L$ and $H2_R$, left and right signals, respectively), so enantiomeric differentiation occurred.

According to Fig. 6B, 2′OHFL/βCD complexes do not present enantiomeric differentiation for any signal; therefore, subsequent results will be the ones determined by employing the data obtained for the mixture of diastereoisomeric complexes.

Observing spectra C (2′OHFL/DMβCD) and D (2′OHFL/HPβCD) of Fig. 6, H8 and H5′ 2′OHFL hydrogen signals split, respectively, which in the spectra is indicated by $H8_L$ and $H8_R$ for the left and right signals, respectively, and $H5'_L$ and $H5'_R$ for the left and right signals, respectively.

For all the split signals, L and R subscripts (e.g. $H8_L$ and $H8_R$) will indicate the left and right signals in respect to the spectrum, respectively, and they will be arbitrarily designed to the "left" and "right" enantiomers, respectively.

According to the results for enantiomeric differentiation, H8 hydrogen split signals of FL/βCD, FL/DMβCD and 2′OHFL/DMβCD complexes; and H2 and H5′ hydrogen split signals of FL/HPβCD and 2′OHFL/HPβCD complexes, respectively, will be used to obtain stoichiometry, association constants, Ka ratio and thermodynamics, by measuring the chemical shifts differences, in Hz, between the signals of the pure flavanone and the same in presence of the cyclodextrins at different concentrations of the latter.

Inclusion stoichiometry of diasteroisomeric complexes

In order to determine the complexes' stoichiometry, the linearity of $\Delta\delta_o^{-1}$ vs. $[CD]_0^{-a}$ plots was examined in agreement with Eq. 1, as shown in Fig. 8 for the "L"-2′OHFL/DMβCD complex ($H8_L$ hydrogen).

Figure 8 displays $\Delta\delta_o^{-1}$ vs. $[CD]_0^{-a}$ plots using a = 1, 2, 3 and 4 for "L"-2′OHFL/DMβCD complex ($H8_L$). The results show that when "a" values of 2, 3 and 4 are employed, non-linear curves are obtained, while when a = 1 is used, a linear tendency is observed. Similar results were obtained for all the studied diasteroisomeric complexes, which imply, for all the cases, a 1:1 stoichiometry.

Association constants of diasteroisomeric complexes

According to Eq. 5, by obtaining the slope and intercept of $\Delta\delta_o^{-1}$ vs. $[CD]_0^{-1}$ plots, approximated values of Ka and $\Delta\delta_c$, respectively, were obtained for each diasteroisomeric complex, which corresponded to about 500 M^{-1} for Ka and 20 Hz for $\Delta\delta_c$. Then, $\Delta\delta_o$ vs. $[CD]_0$ plots were obtained and iterated according to Eq. 4, initializing the process by using the approximated values of Ka and $\Delta\delta_c$ indicated above. This is represented in Fig. 9 for "L"-2′OHFL/DMβCD and "R"-2′OHFL/DMβCD complexes.

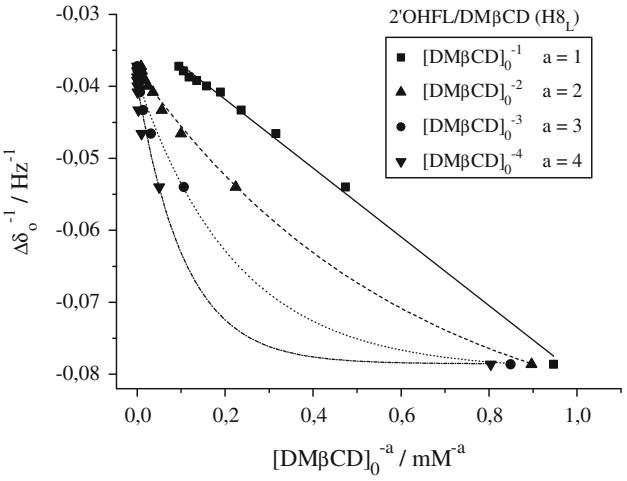

Fig. 8 $\Delta\delta_o^{-1}$ vs. $[DM\beta CD]_0^{-a}$ plots for "left" enantiomer of 2′OHFL/DMβCD complexes

Similar plots to those displayed in Fig. 9 were obtained for each diasteroisomeric complex, and their Ka values at 25, 30 and 35 °C are indicated in Table 1.

In agreement with Table 1, for each of the studied complexes, the higher the temperature, the lower the Ka values. This tendency indicates that the formation of the complexes is disfavored with increasing temperature, which would be consistent with an exothermic behavior.

When comparing all the Ka values at the same temperature, it can be seen that the lower values are for the complexes formed by βCD, and DMβCD complexes have the larger values, while the complexes formed by HPβCD are in the middle of the tendency. For example, at 25 °C and for the complexes formed by the "left" enantiomer, Ka values of 1.84×10^2, 2.51×10^2 and 4.96×10^2 M^{-1}

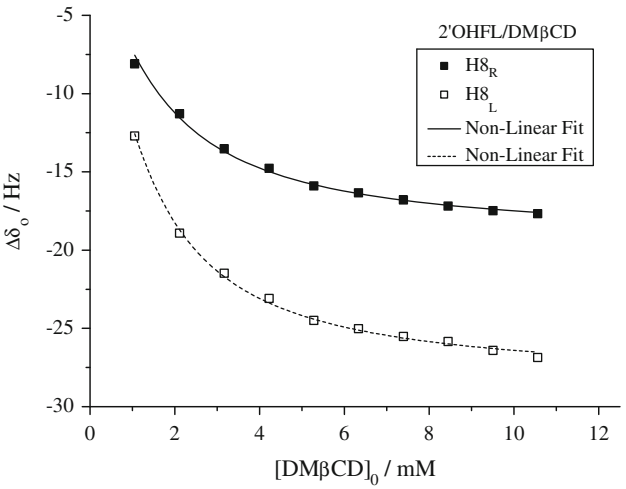

Fig. 9 $\Delta\delta_o$ vs. $[DM\beta CD]_0$ plots for 2′OHFL/DMβCD complexes

Table 1 Diasteroisomeric complexes Ka values (M^{-1}) at 25, 30 and 35 °C, and Ka^R/Ka^L ratio

Complex	FL/βCD		
Hydrogen T/°C	$H8_L$ Ka × 10^2 M^{-1}	$H8_R$ Ka × 10^2 M^{-1}	Ka^{H8R}/Ka^{H8L}
25	1.84	2.22	1.20
30	0.96	1.44	1.50
35	0.49	0.99	2.02
Complex	FL/HPβCD		
Hydrogen T/°C	$H2_L$ Ka × 10^2 M^{-1}	$H2_R$ Ka × 10^2 M^{-1}	Ka^{H2R}/Ka^{H2L}
25	2.51	2.40	0.96
30	1.72	1.94	1.13
35	1.31	1.63	1.24
Complex	FL/DMβCD		
Hydrogen T/°C	$H8_L$ Ka × 10^2 M^{-1}	$H8_R$ Ka × 10^2 M^{-1}	Ka^{H8R}/Ka^{H8L}
25	4.96	5.64	1.14
30	3.70	4.50	1.22
35	2.65	3.72	1.40
Complex	2′OHFL/HPβCD		
Hydrogen T/°C	$H5'_L$ Ka × 10^2 M^{-1}	$H5'_R$ Ka × 10^2 M^{-1}	$Ka^{H5'R}/Ka^{H5'L}$
25	4.99	5.08	1.02
30	3.29	3.39	1.03
35	2.30	2.43	1.06
Complex	2′OHFL/DMβCD		
Hydrogen T/°C	$H8_L$ Ka × 10^2 M^{-1}	$H8_R$ Ka × 10^2 M^{-1}	Ka^{H8R}/Ka^{H8L}
25	13.0	10.9	0.84
30	7.55	8.02	1.06
35	4.53	5.84	1.29

were obtained for "L"-FL/βCD, "L"-FL/HPβCD and "L"-FL/DMβCD complexes, respectively. Since the complexes formed with the CD derivatives are the ones which have the larger Ka values, it would then be the presence of their substituents groups the responsible for this behavior.

On the other hand, when comparing between flavanones at the same temperature and with the same cyclodextrin, complexes formed with 2′OHFL have higher Ka values than FL, which could be a consequence of the presence of the OH group in the 2′OHFL molecule. For example, at 35 °C and for the complexes formed by the "right" enantiomer, Ka values of 5.84 × 10^2 and 3.72 × 10^2 M^{-1} were obtained for "R"-2′OHFL/DMβCD and "R"-FL/DMβCD, respectively.

$Ka^{"R"}/Ka^{"L"}$ ratios have been used as a measure of the chiral recognition degree [12, 53], and it has been proposed that the more different from 1 the ratio value, the more chiral recognition should be displayed. $Ka^{"R"}/Ka^{"L"}$ ratio results indicate that the higher the temperature, the higher the ratio, and the opposite (the lower the temperature, the lower the ratio). This tendency would imply that at extreme temperatures, larger differences between Ka values should be found, passing through the value of 1, where $Ka^{"R"}$ and $Ka^{"L"}$ should be equal. However, diasteroisomeric complexes' ^{1}H NMR spectra show less enantiomeric differentiation at higher temperatures than at lower ones (data not shown), since for the split signals, the chemical shifts of one enantiomer and its counterpart are more similar between them at higher temperatures than at lower temperatures, having then in the latter case larger chemical shifts differences between enantiomers, and consequently better chiral recognition. The reason for this behavior would be that complexes' observed chemical shifts (δ_o), and hence enantiomeric differentiation, depend on the Ka values as follows[11, 65]

$$\delta_o = \chi_c \delta_c + \chi_f \delta_f \tag{8}$$

where χ_c and χ_f are the mole fraction of the complexed flavanone and the mole fraction of the pure flavanone, respectively, and δ_c and δ_f are the chemical shifts of the completely complexed flavanone and the pure flavanone, respectively. Since χ_c is directly dependent on the association constant value, the smaller the Ka value, the smaller χ_c and larger χ_f values are, and then, according to Eq. 8, the δ_o value would be closer to the δ_f value, which is the same for both enantiomers. Therefore a poorest enantiomeric differentiation is displayed at higher temperatures, due to the lower Ka values. Consequently, for the complexes of this work, at lower temperatures a better enantiomeric differentiation is displayed in the ^{1}H NMR spectra. These results also suggest that large Ka ratios not always imply better chiral recognition.

Thermodynamics of diasteroisomeric complexes

The thermodynamic parameters of the complexes, ΔH, ΔS and ΔG, were obtained according to Eqs. 6 and 7. Enthalpy and entropy changes were calculated from the slope and intercept of ln(Ka) vs. T^{-1} plots, respectively. For example, ln(Ka) vs. T^{-1} plots for both enantiomers of FL/DMβCD complexes are displayed in Fig. 10.

Similar plots to Fig. 10 were obtained for all the complexes. Subsequently, free energy change values (ΔG) were obtained from ΔH and ΔS values and T = 298.15 K by employing Eq. 7. Results are pointed out in Table 2.

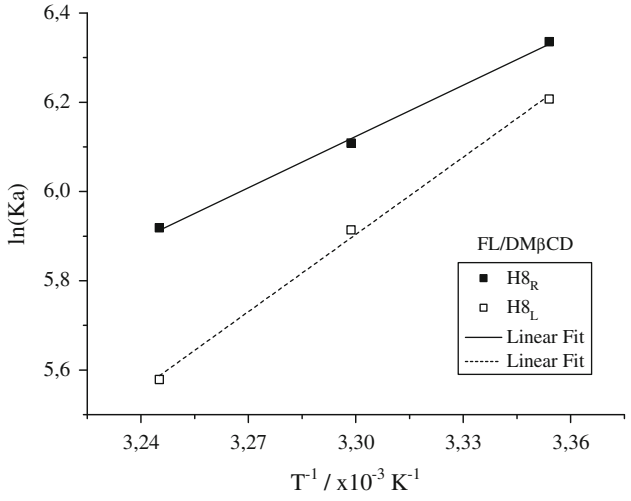

Fig. 10 Ln(Ka) vs. T^{-1} plots for FL/DMβCD complexes

In agreement with Table 2, ΔG values for all the studied complexes are negative, which implies the spontaneous formation of all of them. Similarly, all ΔH and T·ΔS values are negative, indicating not only an exothermic behavior, but also that all the interactions promoting the complexes' formation could be analogous in nature.

In order to understand the origin of the thermodynamic parameter values, different kinds of interactions and phenomena have been proposed according to the chemical nature of the complexes of this work. Two of the more recurrently described interactions in literature are the van der Waals and hydrophobic ones. The former interaction is characterized by negative values of enthalpy changes [4], which is in complete agreement with the thermodynamic values obtained in this work. This result would be also supported by the fact that flavanones have the ability to delocalize electrons, and cyclodextrins to polarize bonds [56], phenomena which promote dipolar interactions that are part of the van der Waals interactions. Therefore, van der Waals interactions would make an important contribution to the formation of the complexes of this work.

In the case of hydrophobic interactions, this is characterized by positive values of entropy and enthalpy changes, which has been considered evidence of this kind of interaction [2, 4, 68]. Given that the entropy and enthalpy changes obtained for the complexes of this work, are all negatives, if hydrophobic interactions do occur, they would not make an important contribution to the complexes' formation process.

Another interaction that might make take place is the hydrogen bond, which is a directional interaction that develops negative values of both entropy and enthalpy changes and that has been described to occur between (i) the substrate and the solvent surrounding the complex, (ii) the substrate and the CD hydroxyl groups, and (iii) the substrate

Table 2 ΔH, T·ΔS and ΔG values for diasteroisomeric complexes formation (T·ΔS and ΔG at T = 298.15 K)

Complex	FL/βCD	
Hydrogen	$H8_L$	$H8_R$
ΔH (kJ/mol)	−101.31	−61.75
T·ΔS (kJ/mol)	−88.36	−48.37
ΔG (kJ/mol)	−12.95	−13.38
Complex	FL/HPβCD	
Hydrogen	$H2_L$	$H2_R$
ΔH (kJ/mol)	−49.77	−29.72
T·ΔS (kJ/mol)	−36.11	−16.14
ΔG (kJ/mol)	−13.66	−13.58
Complex	FL/DMβCD	
Hydrogen	$H8_L$	$H8_R$
ΔH (kJ/mol)	−47.99	−31.85
T·ΔS (kJ/mol)	−32.59	−16.15
ΔG (kJ/mol)	−15.40	−15.70
Complex	2′OHFL/HPβCD	
Hydrogen	$H5'_L$	$H5'_R$
ΔH (kJ/mol)	−58.96	−56.28
T·ΔS (kJ/mol)	−43.58	−40.86
ΔG (kJ/mol)	−15.38	−15.42
Complex	2′OHFL/DMβCD	
Hydrogen	$H8_L$	$H8_R$
ΔH (kJ/mol)	−80.74	−47.50
T·ΔS (kJ/mol)	−62.97	−30.16
ΔG (kJ/mol)	−17.77	−17.34

and substituent groups of CD derivatives [2, 4]. Since all ΔH and ΔS values of the complexes of this work are negative, hydrogen bonds are a possibility in their formation. In order to evaluate this possibility, and since hydrogen bonds usually provoke conformational changes, NMR coupling constants (J) have been analyzed, because this parameter depends on the dihedral angle (θ), according to Karplus equation, which has the general form as follows [69, 70]

$$^3J = A \cdot \cos^2\theta + B \cdot \cos\theta + C \quad (9)$$

where ^3J is the coupling constant at three bounds, A, B and C are tabulated coefficients for different kinds of chemical structures and θ is the dihedral angle, in this case formed between H2 hydrogen and H3a or H3b hydrogens. ^{1}H NMR spectra of 2′OHFL and their complexes are shown in Fig. 11.

According to the aliphatic zone ^{1}H NMR spectra of 2′OHFL and their complexes (Fig. 11), H2 and H3

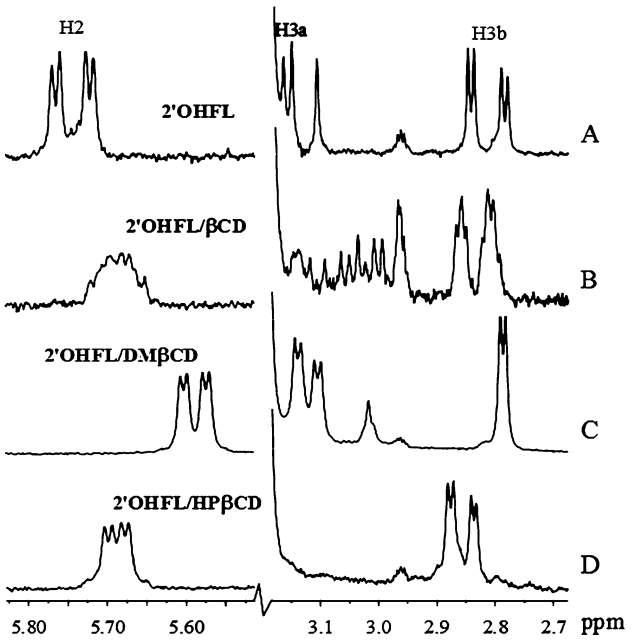

Fig. 11 ^{1}H NMR spectra of aliphatic zone of 2′OHFL (**A**), 2′OHFL/βCD (**B**), 2′OHFL/DMβCD (**C**) and 2′OHFL/HPβCD (**D**)

hydrogens' coupling constants (^{3}J) of 2′OHFL change from the pure form to the complexed one, except for 2′OHFL/βCD and FL/CDs complexes (spectra not shown). ^{3}J values for each hydrogen are summarized in Table 3.

Analysis of Table 3 shows that all the aliphatic hydrogens (H2, H3a and H3b) of the pure 2′OHFL have the largest ^{3}J values, and the lower ones when 2′OHFL is included into the CD derivatives. These variations in ^{3}J indicate that the value of the dihedral angle between H2 and H3 hydrogens changes, which is evidence that 2′OHFL molecules change their conformation from the pure form to the included one, which could be consequence of the formation of hydrogen bonds. This presumption is also supported by their estimated inclusion geometry (Fig. 16A, B), which indicates that the hydroxyl group of 2′OHFL is oriented toward the CD substituents, so a hydrogen bond could be formed between this hydroxyl group and the CD substituents.

Table 2 shows that all ΔS values are negative, which would imply a decrease in the number of the freedom degrees of the formed complexes, in respect to their free components. Additionally, for the complexes formed by CD derivatives, 2′OHFL have lower ΔS values than FL, being these results also consistent to the hydrogen bond proposal, since FL has no hydroxyl groups (e.g. $\Delta S = -62.97$ kJ/mol for "L"-2′OHFL/DMβCD and $\Delta S = -32.59$ kJ/mol for "L"-FL/DMβCD).

Table 3 H2, H3a and H3b hydrogen coupling constant (J) of 2′OHFL free and complexed

Hydrogen	^{3}J H2	^{3}J H3a	^{3}J H3b
2′OHFL	2.7; 12.8	12.9; 17.3	3.0; 17.2
2′OHFL/HPβCD	2.9; 6.3	–	2.8; 11.9
2′OHFL/DMβCD	2.5; 8.5	3.2; 10.2	2.6

Since signals correspond to doublet-doublet, there are informed two J for each hydrogen

Another interesting result is the one related to FL/βCD complexes, which present the lower values of both ΔH and ΔS compared to all the other complexes. This behavior could be explained with the high energy water molecules expulsed from the CD cavity, which is associated with negative values of ΔH and ΔS [2, 4]. Since βCD has no substituent groups and has a smaller inner volume than CD derivatives [71, 72], formation of βCD complexes would be susceptible to remove more solvent molecules from their inner zone than the formation of HPβCD and DMβCD complexes. Therefore, formation of βCD complexes would have lower ΔH and ΔS values.

Referring to ΔG values, it is remarkable that in spite of the fact that ΔH and ΔS values differ from one complex to another, ΔG values are very similar between all of them. This would come as consequence of the so called enthalpy-entropy compensation, which is an empirical phenomenon characterized by the partial cancellation of the enthalpy gain with the entropic loss, that has been repeatedly reported, but cannot be deducted from the fundamental equations of thermodynamics [1].

Inoue et al. [14, 32, 73–76]. have carried out a systematic study of the enthalpy-entropy compensation, and they have reported a linear relationship between ΔH and $T\cdot\Delta S$, which means that the resulting change in $T\cdot\Delta S$ is proportional to the accompanying change in ΔH. With this reasoning, they have suggested that from the slope and intercept of $T\cdot\Delta S$ vs. ΔH plots, quantitative information about the phenomenon can be obtained. The relationship between $T\cdot\Delta S$ and ΔH comes from Eq. 10

$$T\cdot\Delta S = \alpha\cdot\Delta H + T\cdot\Delta S_0 \quad (10)$$

where α indicates the magnitude of the enthalpy gain (induced by structural changes in the substrate, CD and/or solvent molecules) which is cancelled by the entropic loss, and ΔS_0 is the inherent complex stability. Therefore, the closer to 1 the value of α, the lower the contribution of the structural changes to the enthalpic gain, and therefore to the decrease of the ΔG values (which implies larger Ka values).

In order to achieve $T\cdot\Delta S$ vs. ΔH plots, the thermodynamic values of this work have been employed along with previous reported values by our research group for complexes formed between the same CDs of this study and (±)-4′-hydroxyflavanone (4′OHFL), which corresponds to FL molecule substituted in position 4′ by a hydroxyl group [55, 56]. $T\cdot\Delta S$ vs. ΔH plots are displayed in Fig. 12

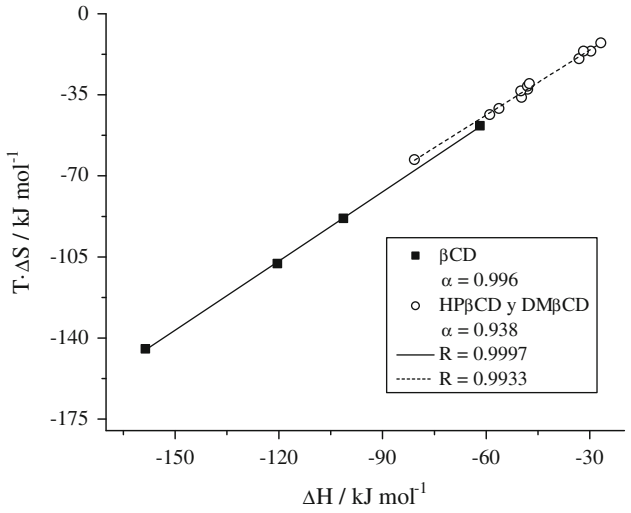

Fig. 12 T·ΔS vs. ΔH plots for βCD complexes in *solid line* (*filled square*), and HPβCD and DMβCD complexes in *dashed line* (*open circle*)

According to T·ΔS vs. ΔH plots (Fig. 12), $\alpha = 0.996$ is obtained for βCD complexes, and for HPβCD and DMβCD complexes $\alpha = 0.938$. The former α value would indicate that a 0.4 % of the enthalpic gain would contribute to obtain lower ΔG values for the complexes formed by βCD due to flavanones' structural variations. On the other hand, and for HPβCD and DMβCD complexes, a 6.2 % of the enthalpic gain would contribute to obtain lower ΔG values due to CDs and flavanones' structural variations. If the relation $\Delta G = RT \cdot \ln(Ka)$ is employed, these results would imply that at a given temperature, βCD complexes would have smaller Ka variations compared to the complexes formed with CD derivatives, which would have larger Ka variations. For instance, at 25 °C "L"-4′OHFL/βCD and "L"-FL/βCD complexes have Ka values of 2.54×10^2 [56], and 1.84×10^2 M^{-1}, respectively, with a difference of 0.70×10^2 M^{-1}, while at 25 °C "L"-2′OHFL/DMβCD and "L"-2′OHFL/HPβCD complexes have Ka values of 13.0×10^2 and 4.99×10^2 M^{-1}, respectively, with a difference of 8.01×10^2 M^{-1}.

In order to understand the thermodynamic contribution to chiral recognition, ΔG changes (ΔΔG) between diasteroisomeric complexes were obtained, and are indicated in Table 4.

Table 4 shows |ΔΔG| values for each couple of diasteroisomeric complexes, varying their magnitudes between 0.04 and 1.43. These |ΔΔG| values correspond to the additional free energy which one diasteroisomeric complex has in relation to its counterpart. To understand the thermodynamic parameter which induces this free energy gain (|ΔΔG|), and consequently a larger Ka value at a determined temperature, enthalpy and entropy changes were related between diasteroisomeric complexes to their free

Table 4 |ΔΔG| values for each couple of diasteroisomeric complexes in kJ/mol

	βCD	HPβCD	DMβCD
FL	0.43	0.08	0.30
4′OHFL	1.4[a]	0.6[a]	0.2[b]
2′OHFL	–	0.04	0.43

[a] ΔΔG values calculated from Table 2 of ref. [56]

[b] ΔΔG value calculated from Table 2 of ref. [55]

energy changes (ΔG) at 298.15 K considering Eq. 7. For instance, "R"-FL/βCD complex formation has a larger ΔH value (−61.75 kJ/mol) than the formation of "L"-FL/βCD complex (−101.31 kJ/mol), which favors the formation of the latter by enthalpy phenomena. Additionally, the formation of the "R"-FL/βCD complex is favored by entropy phenomena because its ΔS value (−48.37 kJ/mol) is larger than the ΔS value of "L"-FL/βCD complex formation (−88.36 kJ/mol). Since "R"-FL/βCD complex formation is the favored one because it has the smaller ΔG value (−13.38 kJ/mol) in respect to its counterpart (−12.95 kJ/mol), it would then be the entropy contribution promoting the formation of the "R"-FL/βCD complex to be favored over the formation of the "L"-FL/βCD complex, so entropy phenomena would contribute more to their chiral discrimination. The opposite occurs for FL/HPβCD complexes, where the entropy contributes more to the formation of the "R"-FL/HPβCD complex, while the enthalpy contributes more to the formation of the "L"-FL/HPβCD complex. Since ΔG is smaller for the formation of the latter complex, it would then be the enthalpy contribution promoting the formation of this complex to be favored over its counterpart, and then enthalpy phenomena would be contributing more to their chiral discrimination. In order to visualize the contribution of ΔH and ΔS values to the chiral discrimination of each couple of the studied diasteroisomeric complexes, ΔH, T·ΔS and ΔG change modules (|ΔΔH|, T·|ΔΔS| and |ΔΔG|) were obtained, due to the free energy gain (|ΔΔG|) corresponding to the difference between |ΔΔH| and T·|ΔΔS|. Thus, the parameter (|ΔΔH| or T·|ΔΔS|) having the largest magnitude will be causing the free energy gain (|ΔΔG|). Values of |ΔΔH|, T·|ΔΔS| and |ΔΔG| are summarized in Table 5.

According to Table 5, four couples of diasteroisomeric complexes show a larger contribution from entropy phenomena to their diasteroisomeric differentiation: FL/βCD, FL/DMβCD, 4′OHFL/HPβCD and 2′OHFL/HPβCD; and four couples of diasteroisomeric complexes show a larger contribution from enthalpy phenomena to their diasteroisomeric differentiation: FL/HPβCD, 4′OHFL/βCD, 4′OHFL/DMβCD and 2′OHFL/DMβCD.

Table 5 |ΔΔH|, T·|ΔΔS| and |ΔΔG| values for all couples of diasteroisomeric complexes

	|ΔΔH| kJ/mol^{-1}	T·|ΔΔS| kJ/mol^{-1}	|ΔΔG| kJ/mol^{-1}	Favored by
FL/βCD	39.56	**39.99**	0.43	Entropy
FL/HPβCD	**20.05**	19.97	0.08	Enthalpy
FL/DMβCD	16.14	**16.44**	0.30	Entropy
4′OHFL/βCD[a]	**38.2**	36.8	1.4	Enthalpy
4′OHFL/HPβCD[a]	6.3	**6.9**	0.6	Entropy
4′OHFL/DMβCD[b]	**0.7**	0.5	0.2	Enthalpy
2′OHFL/HPβCD	2.68	**2.72**	0.04	Entropy
2′OHFL/DMβCD	**33.24**	32.81	0.43	Enthalpy

Values in bold correspond to the larger value between |ΔΔH| and T·|ΔΔS|

[a] ΔΔG values calculated from Table 2 of ref. [56]

[b] ΔΔG value calculated from Table 2 of ref. [55]

Inclusion geometry estimation of diasteroisomeric complexes

The NOE is a dipole–dipole spatial interaction that occurs between two nuclei, and in ROESY experiments it can be detected when the distance between these nuclei is smaller than 5Å, thus, these hydrogen nuclei will correlate presenting cross peaks in the 2D-ROESY spectrum [11, 46]. Since the chemical shifts of H3, H5 and H6 inner hydrogen of CDs are well known; the inclusion geometry of the diasteroisomeric complexes was estimated by acquiring the 2D-ROESY spectra and then qualitatively analyzed by correlating the ROESY's cross peaks. With this analysis the relative orientation of the flavanone molecule in respect to the CD structure was proposed. As example, the partial contour plot of 2D-ROESY spectrum of FL/βCD complexes is shown in Fig. 13.

Partial contour plot of 2D-ROESY spectrum of FL/βCD complexes (Fig. 13) shows the βCD ^{1}H NMR spectrum in vertical scale, where H3, H5 and H6 hydrogens appear, being the H3 hydrogen signal a triplet overlapped with the H6 hydrogen signal. The aromatic zone of FL ^{1}H NMR spectrum appears in horizontal scale. Due to the NOE between βCD and FL hydrogen, cross peaks can be seen, and unfortunately no differences are able to be seen for each enantiomer, so the inclusion geometry was estimated for the complexes as a mixture instead of separately. This occurred for all the studied complexes, so inclusion geometries were estimated to the mixture of them. In agreement with the 2D-ROESY spectrum of FL/βCD complexes, H5, H6 and H8 FL hydrogens from "A" ring and H2′, H3′ and H4′ FL hydrogens from "B" ring correlate with both H3 and H6 βCD hydrogens. Since "A" and "B" rings are too far away from each other to induce cross peaks with the opposed H3 and H6 βCD hydrogens (Fig. 1) with only one inclusion geometry, two inclusion geometries have been proposed (Fig. 14). Along with these correlations, H5, H6 and H8 FL hydrogens from "A" ring and H2′, H3′ and H4′ FL hydrogens from "B" ring present

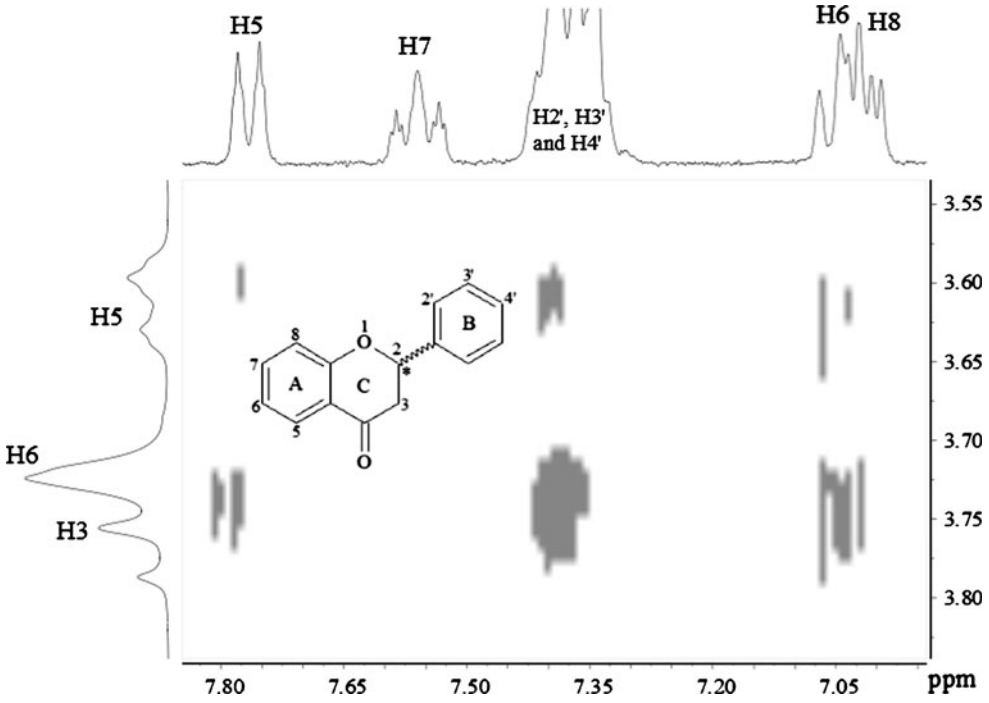

Fig. 13 Partial contour plot of 2D-ROESY spectrum of FL/βCD complexes

cross peaks with the H5 βCD hydrogen, which would indicate that all these hydrogens are close to the middle of the βCD, meaning that the FL is completely inserted into the βCD cavity. Therefore, according to the evidence given by the ROESY spectrum, the estimated geometry for FL/βCD complexes is shown in Fig. 14.

An analogous analysis as for the estimation of inclusion geometry of the FL/βCD complexes was employed for all the studied complexes and the obtained estimated inclusion geometries are shown in Figs. 15 and 16.

Figure 14 shows that FL/βCD complexes present two inclusion geometries, while FL/HPβCD, FL/DMβCD, 2′OHFL/HPβCD and 2′OHFL/DMβCD complexes (Figs. 15A, B, 16A, B) manifest only one inclusion geometry. The fact that complexes formed by CD derivatives are the ones which exhibit only one inclusion geometry could be explained by the presence of the substituent groups of the CD derivatives, which could promote stronger interactions with the flavanones, thereby favoring only one inclusion geometry instead of two. This is consistent with the Ka

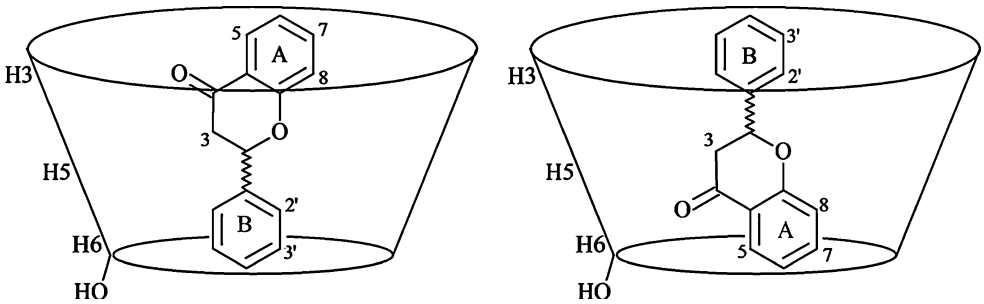

Fig. 14 Estimated inclusion geometry of FL/βCD complexes

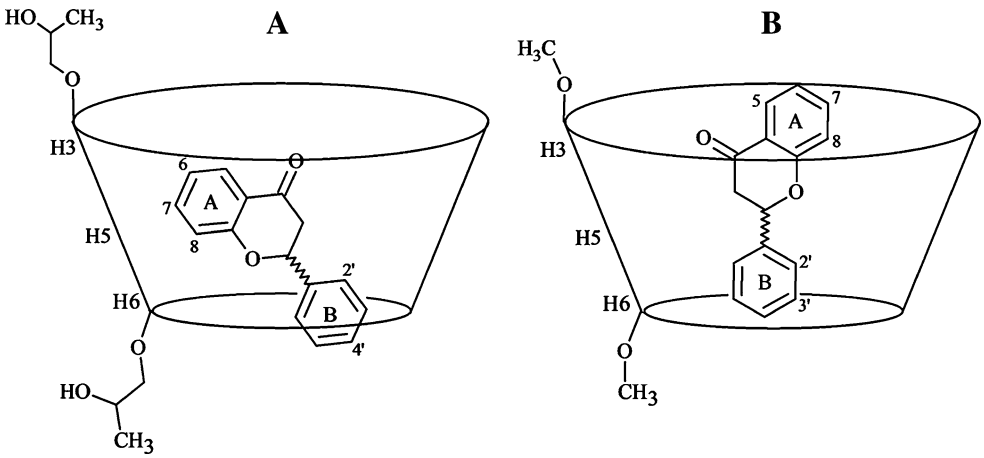

Fig. 15 Estimated inclusion geometry of **A** FL/HPβCD and **B** FL/DMβCD complexes

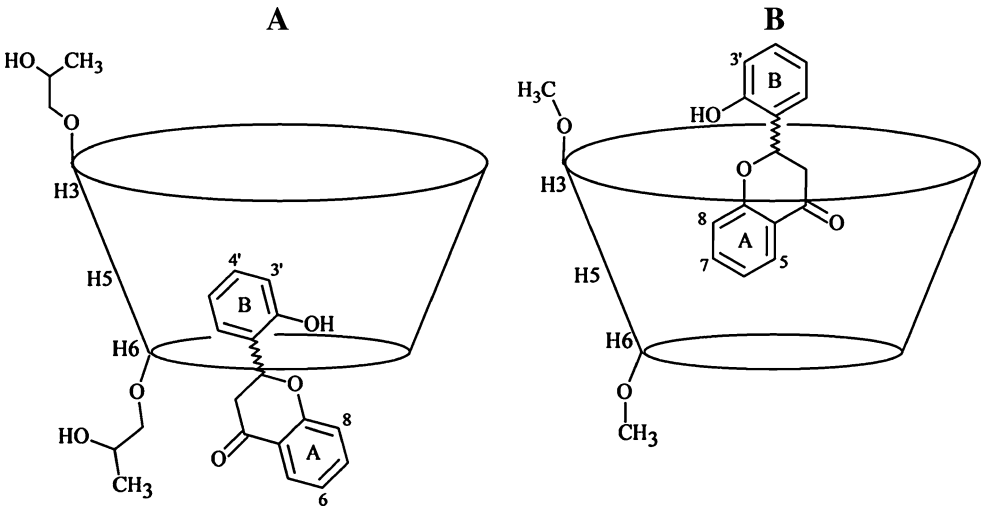

Fig. 16 Estimated inclusion geometry of **A** 2′OHFL/HPβCD and **B** 2′OHFL/DMβCD complexes

values, which are larger for the complexes formed by the CDs derivatives than for the βCD complexes. To provide further experimental support to this explanation, 2′OHFL/βCD inclusion geometry was estimated (data not shown), and as a results two inclusion geometries were found. Additionally, the inclusion geometry of 4′OHFL/βCD complexes has been reported, resulting again in two inclusion geometries, while 4′OHFL/HPβCD and 4′OHFL/DMβCD complexes have one [55, 56].

According to Fig. 14 and 15, the FL molecule is deeply inserted into the CD' truncated cone cavity regardless of which CD it is, while complexes formed by 2′OHFL have a portion of this flavanone oriented toward the CD substituents (Fig. 16). Since the only structural difference between the FL and 2′OHFL molecules is the OH group in the latter, it would then be the presence of this group conditioning the insertion depth of the flavanones into the CD cavity.

From the estimated inclusion geometries of 2′OHFL/HPβCD and 2′OHFL/DMβCD complexes (Fig. 16), it can be noticed that 2′OHFL hydroxyl group is oriented toward the edge of the truncated cone, where the CDs substituents are located. This orientation would indicate the existence of strong interactions between the hydroxyl group and the CDs substituents, which would be consistent with the hydrogen bond suggestion presented in the thermodynamic section and with the Ka values, which are the larger for these complexes.

In spite of the fact that the non-polar portion of a substrate is usually inserted into the CD cavity, thereby orienting their polar portion toward the solvent polar molecules [2, 40, 42, 67], this behavior is developed for all the complexes of this work except for one of them, 2′OHFL/HPβCD, where the 2′OHFL "B" ring, having the polar hydroxyl group, is inserted into the HPβCD cavity (Fig. 16A). This uncommon orientation would indicate interactions between the 2′OHFL polar portion and the inner zone of the HPβCD, which could be possible due to the presence of inner dipolar interactions that would be consistent with the inner semi-polar microenvironment that has been already suggested in previous reports [2, 4, 56].

Conclusion

It has been demonstrated in this work that the racemic mixtures of flavanone (FL) and 2′hydroxyflavanone (2′OHFL) are included into the cavity of the cyclodextrins βCD, HPβCD and DMβCD forming diasteroisomeric complexes. With the exception of 2′OHFL/βCD, all the complexes of this work show chiral recognition in ^{1}H NMR spectra by obtaining split signals, allowing the study of each diasteroisomeric complex by obtaining their stoichiometry—1:1 for all the complexes—association constants and thermodynamics.

Ka values show a decrease as temperature increases, which indicates that formation of the complexes is not favored by high temperatures. Additionally, and due to the obtained Ka values, the complexes formed by CD derivatives have more stability than βCD complexes, which would be consequence of the presence of the substituent groups of the CD derivatives.

Besides finding a direct correlation between $Ka^{"R"}$/$Ka^{"L"}$ values and temperature, it was determined that $Ka^{"R"}/Ka^{"L"}$ values removed from 1 not always reflect better enantiodiscrimination by NMR, and that enantiomeric differences also depend on the magnitude of the Ka value in this technique.

Thermodynamics show a spontaneous and exothermic complex formation, where van der Waals interactions are proposed to have the larger contribution. Additionally, hydrogen bonds would be a possibility between 2′OHFL hydroxyl group and CD substituents in the complexes formed by 2′OHFL and HPβCD and DMβCD, which would be supported by the changes in the aliphatic hydrogen coupling constants of this flavanone, and their inclusion geometries.

Remarkable enthalpy-entropy compensation was found for all the complexes, with free energy change values notably similar for all the complexes.

Chiral recognition thermodynamics shows that one half of the couples of diasteroisomeric complexes present a larger contribution from enthalpic phenomena to their recognition and the other half presents a larger contribution from entropic phenomena.

2D-ROESY experiments were unable to differentiate between enantiomers, so inclusion geometries were obtained for the mixture of diasteroisomeric complexes. The estimated inclusion geometries show two modes of inclusion for βCD complexes, while the CD derivative complexes show only one. This amount of inclusion modes (1 or 2) have been proposed to be consequence of the presence -or lack- of substituent groups in the CD structures.

It was found that 2′OHFL "B" ring, the polar portion of this flavanone, is inserted into the HPβCD cavity, which would be evidence of the semi-polar nature of the inner zone of the cyclodextrins.

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