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ORIGINAL ARTICLE

NMR as a tool for simultaneous study of diasteroisomeric inclusion complexes, part 2: complexes formed by racemic mixture of 4′-hydroxyflavanone and two cyclodextrins

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Abstract Complexes formed by (±)-4′-hydroxyflavanone (OHFL) and the cyclodextrins β-cyclodextrin and (2-hydroxypropyl)-β-CD were obtained using the racemic mixture of the OHFL. These complexes were able to be studied due to their enantiodifferentiation by ^{1}H-NMR spectroscopy. Stoichiometry, association constants and thermodynamic parameters were obtained from these NMR data, and inclusion geometries were proposed from ROESY spectra. The results show a 1:1 stoichiometry, K_a values decrease with increasing temperature, a spontaneous and exothermic complexes formation, and that ROESY spectra cannot differentiate between enantiomers, and therefore the estimated inclusion geometries were proposed for the mixture of diasteroisomeric complexes.

Keywords Cyclodextrin · NMR · Enantiomeric differentiation · ROESY

Introduction

Currently, a number of chiral drugs are administrated in racemic form, and their demand and development is growing due to their particular biological effects [1, 2]. In an achiral environment, enantiomers have the same physicochemical properties (e.g., boiling point) [3], but in biological systems they can perform differently (e.g., distribution, metabolism and excretion) because of stereospecific interactions [4].

Due to the possibility of having different activities, differentiation and separation of enantiomers is a topic of interest, and techniques such as NMR [5–8] and HPLC [9, 10] have been used to discriminate and separate them. In these techniques, chiral selectors have to be used, and cyclodextrins (CDs) have become popular due to their relatively low cost and toxicity [11, 12].

Cyclodextrins are cyclic oligosaccharides composed by 6, 7 or 8 α-D-glucopyranose units, named α, β, or γ-cyclodextrin respectively. Their structure is a truncated cone, and as a consequence of the $^4C_1$ glucopyranose unit conformation, the primary hydroxyl groups are located in the narrow edge of the cone, and the secondary hydroxyl groups are situated in the wide edge. Therefore, the CDs' external surface is hydrophilic, while their inner surface is hydrophobic [13, 14] (Fig. 1). The latter gives the CDs the ability to include molecules, especially aromatic ones [15], to form inclusion complexes [11, 16–21]. Due to the α-D-glucopyranose chair conformation and their asymmetric centers, CDs are chirals entities. Consequently, they allow chiral recognition by forming a pair of diasteroisomeric complexes with the two enantiomers of a chiral substrate [22].

Due to the different activities that a couple of enantiomers could have, it is quite important to establish the characteristics of each diasteroisomeric complex independently, where this characterization involves obtaining stoichiometry, association constants, thermodynamic parameters and geometry estimation of the complexes. The study of diasteroisomeric complexes is usually performed using each enantiomer as a separate substrate, which forces their previous separation. Normally, this physical separation is expensive and could also take a long time. It has been described [23, 24], that the separation step can be avoided

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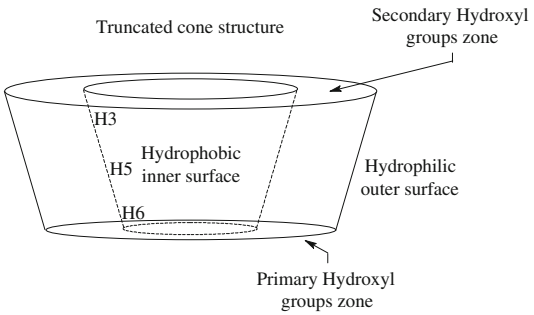


Fig. 1 Cyclodextrin truncated cone structure showing the hydrophobic and hydrophilic surfaces, and showing the primary and secondary hydroxyl groups zones

employing NMR, and then the enantiomers can be studied using their mixture.

To perform the simultaneous study of a mixture of diasteroisomeric complexes, it is necessary to differentiate them. In NMR, the discrimination relies on the differences in the chemical shifts of each complex as a consequence of the different magnitude of their association constants (K_a), diverse host-guest geometries and their different intrinsic chemical shifts (δ_c), where K_a is a measure of the equilibrium between the complex and its free components. The more different these parameters, more differentiation between enantiomers could be found [15, 22–24].

The NMR technique has the advantage that the geometry of the complexes can be estimated through ROESY experiments, which are able to detect the Nuclear overhauser effect (NOE). This effect is a dipolar spatial interaction between two nuclei that can be detected within 5 Å of distance [22, 25], and since the spatial positions of the internal protons of the CDs are well known (Fig. 1), their interaction with the substrate protons give an idea of the relative orientation between them, as it has been widely described before [7, 19, 21, 26–30].

In order to continue the investigation of the use of CDs to study diasteroisomeric complexes employing a racemic mixture of a substrate [24], the cyclodextrins β-cyclodextrin (βCD) and (2-hydroxypropyl)-β-CD (HPβCD) (Fig. 2) were chosen because of their low toxicity and relative low cost [12].

(±)-4′-hydroxyflavanone (OHFL) (Fig. 2) has been suggested as a potential target for the therapeutic treatment of diabetes type 2 [31], but since studies have been performed with the racemic mixture, this flavanone is an interesting chiral substrate to be included into the chosen CDs.

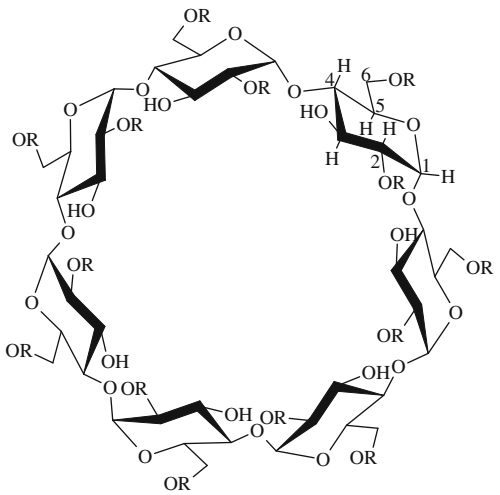
Experimental

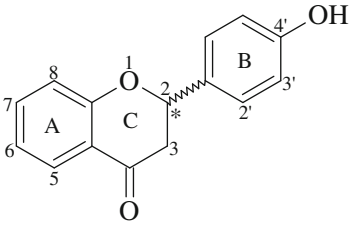
Apparatus

NMR spectra were recorded on a Bruker Avance DRX 300 spectrometer operating at 300.13 MHz for ^{1}H. Chemical shifts were measured relative to the residual MeOD signal at 3.2 ppm. 1D spectra were collected recording 128 scans.

Rotating-frame overhauser effect spectroscopy (ROESY) spectra were acquired in the phase sensitive mode with the same spectrometer and Bruker standard parameters (pulse program roesygpph19). Each spectrum consisted of a matrix of 16 K ($F2$) by 8 K ($F1$) points covering a spectral width of 3,000 Hz. Spectra were obtained with a spin-lock mixing time of 400 ms, relaxation delay 2 s, and 32 scans per increment.

Fig. 2 βCD, HPβCD, and OHFL structures. *Asterisk* indicates the OHFL asymmetric center, *Double asterisk* indicates random substitution





4'-hydroxyflavanone (OHFL)

R = H, β-cyclodextrin (βCD)
R = $CH_2CH(OH)CH_3$, (2-Hydroxypropyl)-β-cyclodextrin (HPβCD)**

Materials

(±)-2-(4-hydroxyphenyl)-2,3-dihydro-4H-chromen-4-one (OHFL), βCD and HPβCD, average $M_W = 1.540$, (HPβCD) were purchased from Aldrich (USA). D_2O and CD_3OD employed in the NMR analyses were of spectroscopic reagent grade, from Aldrich and Merck, respectively.

Methods

Complexes were formed by mixing the necessary quantity of OHFL and cyclodextrin in CD_3OD:D_2O and stirring for 24 h in a thermostatic water bath at constant temperature. Because of solubility reasons, methanol:water system was used in a ratio of 40:60, using methanol due to its low association constant with CDs [32].

Enantiomeric differentiation

1 mL solutions of complexes formed mixing 1 mg of OHFL and the necessary amount of CD to obtain 6 mM CD concentration at 25 °C, were stirred during 24 h and filtered to obtain a clear solution. ^{1}H-NMR spectra were acquired, and their examination allowed the observation of enantiomeric differentiation by establishing the protons which have different chemical shifts for each enantiomer. These protons are then employed to study the complexes by determining the stoichiometry, K_a and thermodynamics.

Stoichiometry determination

The equilibrium between the complex and its parts is given by the Eq. 1

$$S + a\mathrm{CD} \Leftrightarrow S - \mathrm{CD}_a \tag{1}$$

where S is the substrate, CD is the cyclodextrin, "a" its stoichiometric coefficient, and $S - \mathrm{CD}_a$ represents the formed complex.

It has been described that the inclusion complex stoichiometry can be obtained employing Eq. 2 [33, 34]

$$\frac{1}{\Delta\delta_{obs}} = \frac{1}{K_a \cdot \Delta\delta_c} \cdot \frac{1}{[\mathrm{CD}]_0^a} + \frac{1}{\Delta\delta_c} \tag{2}$$

where $\Delta\delta_{obs}$ is the observed chemical shift difference between the free and complexed substrate ($\delta_F - \delta_{obs}$), K_a is the association constant of the complex, $\Delta\delta_c$ is the chemical shift difference between the free and completely complexed substrate ($\delta_F - \delta_c$), $[\mathrm{CD}]_0$ is the initial concentration of CD, and "a" is the stoichiometric coefficient to be determined.

To obtain δ_{obs}, ^{1}H-NMR spectra were acquired at 25 °C to a set of complex solutions formed by OHFL (substrate) and CD in CD_3OD:D_2O 40:60 ratio, maintaining the OHFL concentration constant, [OHFL], and varying the CD initial concentration, $[\mathrm{CD}]_0$. For OHFL/βCD complexes, OHFL concentration was maintained constant at 0.3 mM, and the βCD concentration was varied from 0.6 to 6.0 mM, obtaining ten complex solutions. For OHFL/HPβCD complexes, OHFL concentration was maintained constant at 1.0 mM, and the HPβCD concentration was varied from 2.0 to 20.0 mM, obtaining 10 complex solutions. Additionally, a ^{1}H-NMR spectrum of pure OHFL was acquired to obtain δ_F.

Due to their enantiomeric differentiation, chemical shifts changes of H3′ and H2′ OHFL protons were monitored for plotting $\Delta\delta_{obs}^{-1}$ versus $[\mathrm{CD}]_0^{-a}$, for OHFL/βCD and OHFL/HPβCD complexes, respectively. Plots using $a = 1$, $a = 2$, and $a = 3$ were examined to determine the stoichiometry of the complexes, which is given by the linearity of the plots.

Association constant

The association constant, K_a, in analogy with an equilibrium constant, is defined in agreement with Eq. 3

$$K_a = \frac{[S - \mathrm{CD}]}{[S] \cdot [\mathrm{CD}]} \tag{3}$$

where $[S - \mathrm{CD}]$, $[S]$ and $[\mathrm{CD}]$, are the complex, substrate and CD equilibrium concentrations, respectively.

For each diasteroisomeric complex, association constants at 25, 30, and 35 °C were determined employing the non-linear method which consists in the iteration of Eq. 4 [35–37]

$$\Delta\delta_{obs} = \frac{\Delta\delta_c}{2 \cdot [S]_0}\left[\left([S]_0 + [\mathrm{CD}]_0 + \left(\frac{1}{K_a}\right)\right) - \left[\left([S]_0 + [\mathrm{CD}]_0 + \left(\frac{1}{K_a}\right)\right)^2 - 4[S]_0[\mathrm{CD}]_0\right]^{1/2}\right] \tag{4}$$

where $\Delta\delta_{obs}$ is the observed chemical shift difference between the free and complexed substrate ($\delta_F - \delta_{obs}$), $\Delta\delta_c$ is the chemical shift difference between the free and completely complexed substrate ($\delta_F - \delta_c$), $[S]_0$ is the initial concentration of the substrate, $[\mathrm{CD}]_0$ is the initial concentration of CD, and K_a is the association constant of the complex.

Since this is an iterative procedure, approximated K_a and $\Delta\delta_c$ values are required; these values were obtained from the Benesi-Hildebrand method, Eq. 5

$$\frac{1}{\Delta\delta_{obs}} = \frac{1}{K_a \cdot [\mathrm{CD}]_0 \cdot \Delta\delta_c} + \frac{1}{\Delta\delta_c} \tag{5}$$

To determine $\Delta\delta_{obs}$, ^{1}H-NMR spectra were acquired to the same kind of complexes' solutions than for stoichiometry, by

maintaining the OHFL concentration constant (0.3 and 1 mM to form complexes with βCD and HPβCD, respectively) and varying the CD concentration (from 0.6 to 6.0 mM for βCD complexes, and from 2.0 to 20 mM for HPβCD). Additionally, a ^{1}H-NMR spectrum of pure OHFL was acquired to obtain δ_F. Formation of complexes was sustained for 24 h at 25, 30, and 35 °C, and the ^{1}H-NMR spectra were recorded at the same formation temperature.

Using the chemical shift changes of H3′ and H2′ protons of the OHFL/βCD and OHFL/HPβCD complexes, respectively, the slopes and intercepts of the $\Delta\delta_{obs}^{-1}$ versus $[CD]_0^{-1}$ plots were determined, obtaining approximated values of $\Delta\delta_c$ and K_a, which were used to initiate the iteration of the Eq. 4, and determining the final values of K_a.

Inclusion thermodynamics

For each diasteroisomeric complex, enthalpy (ΔH) and entropy (ΔS) changes were calculated using the van't Hoff Equation (Eq. 6) [38–40]

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

By determining K_a at different temperatures, $\ln(K_a)$ versus T^{-1} plots were obtained for each diasteroisomeric complex, and from the slopes and intercepts and using $R = 8.314$ J/K mol, values of ΔH and ΔS were calculated.

Additionally, free energy changes (ΔG) at 298 K for each diasteroisomeric complex were obtained employing Eq. 7

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

ROESY experiments

ROESY spectra were acquired at 25 °C to solutions of complexes formed by the necessary amount of OHFL and CD to obtain a 3 mM concentration of each component. Complexes were formed in CD_3OD:D_2O, 40:60 and stirring during 24 h at 25 °C.

Results and discussion

In order to study the diasteroisomeric complexes formed by βCD, HPβCD and the racemic mixture of OHFL, ^{1}H-NMR spectra of the pure substrate and the corresponding CD complexes were acquired. OHFL ^{1}H-NMR spectrum shows signals in the aromatic zone, between 6.8 and 7.85 ppm, H2 proton in 5.44 ppm, and H3a and H3b protons in 3.15 and 2.80 ppm, respectively. ^{1}H-NMR spectra of complexes show that the chemical shifts range of the CDs is narrow, between 3.2 and 5.1 ppm (data not shown), so the OHFL aromatic protons signals are not overlapped by the CDs' signals, and the OHFL aromatic signals are able to be used for the study.

^{1}H-NMR spectra of the aromatic zone of OHFL, OHFL/βCD complexes and OHFL/HPβCD complexes are shown in Fig. 3.

Figure 3 shows the aromatic zone of the OHFL ^{1}H-NMR spectrum and its chemical shift variations when forming complexes with βCD (B) and HPβCD (C). When comparing spectra (B) and (C) with spectrum (A), it is possible to observe a subtle signal broadening and chemical shifts variations of H5, H7, H6 and H8 OHFL protons to downfield and the H2′ and H3′ OHFL protons to upfield due to the presence of the CDs. Also the displacement of the inner protons of CDs due to the anisotropic effect of the aromatic rings (data not shown) confirm the OHFL inclusion into the CDs cavity, where the broadening may be due to the restricted motion of the 4′OHFL molecule [41, 42], the increasing of the solution viscosity when the CD is present and by the complexation association-dissociation process. On the other hand, the H3′ OHFL proton signal splits when the flavanone is included into the βCD cavity, and the same effect is observed for the H2′ OHFL proton when the

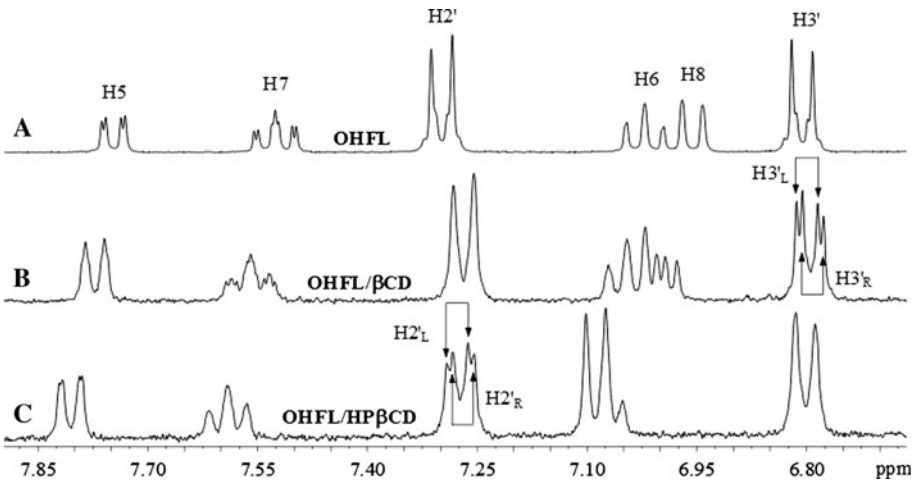


Fig. 3 Aromatic zone ^{1}H-NMR spectra of **A** OHFL, **B** OHFL/βCD, and **C** OHFL/HPβCD. *L* and *R* subscripts represent *left* and *right*, respectively

flavanone is included into the HPβCD cavity, indicating enantiomeric differentiation. Therefore, the splitting of H2′ and H3′ signals allow the determination of the stoichiometry, K_a, and complexes' thermodynamics, and the study of the diasteroisomeric complexes by using the OHFL racemic mixture.

For the OHFL/βCD complexes, and in respect to the spectrum, the left and right signals will be labeled $H3'_L$ and $H3'_R$, and they will correspond to the "left" and "right" enantiomers, respectively. Similarly, for the OHFL/HPβCD complexes, and in respect to the spectrum, the left and right signals will be labeled $H2'_L$ and $H2'_R$, and they will correspond to the "left" and "right" enantiomers, respectively.

Inclusion stoichiometry

Stoichiometry of diasteroisomeric complexes was determined by obtaining the chemical shift differences between the pure and complexed OHFL, as shown in Fig. 4 for OHFL/HPβCD complexes.

By determining $\Delta\delta_{obs}$ for all the diasteroisomeric complexes (as shown in Fig. 4), $\Delta\delta_{obs}^{-1}$ versus $[CD]_0^{-a}$ plots were obtained using $a = 1$, $a = 2$, and $a = 3$. Since the only linear plots were obtained using a = 1, the stoichiometry of all the complexes was 1:1. OHFL/HPβCD complexes plots are shown in Fig. 5.

Association constants

Association constants were determined employing the non-linear procedure, which implies Eq. 4 iteration. For this purpose, approximated K_a and $\Delta\delta_c$ values were obtained from the Benesi-Hildebrand method. This method consists in applying Eq. 5, by plotting $\Delta\delta_{obs}^{-1}$ versus $[CD]_0^{-1}$, and from the slope and intercept obtaining approximated K_a and $\Delta\delta_c$ values, respectively.

To obtain $\Delta\delta_{obs}$ values, an analogous procedure to stoichiometry determination was employed (Fig. 4) for each diasteroisomeric complex, and $\Delta\delta_{obs}$ values were obtained using different CD concentrations.

To employ the non-linear procedure, $\Delta\delta_{obs}$ versus $[CD]_0$ plots are obtained and iterated in agreement with Eq. 4 to obtain the K_a values (Fig. 6).

K_a determination for all the complexes was similar to OHFL/HPβCD complexes at 25 °C (Fig. 6), and they were obtained at 25, 30 and 35 °C. The results are displayed in Table 1.

From Table 1, it can be noted that the association constant values range from 0.30×10^2 M^{-1} (OHFL/βCD "left" enantiomer complex at 35 °C) to 3.17×10^2 M^{-1} (OHFL/

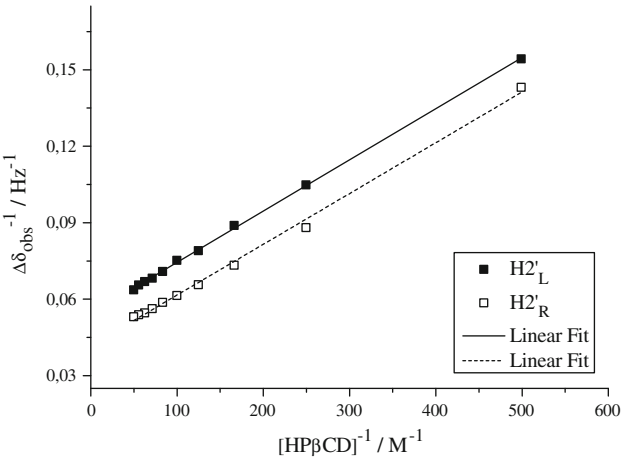


Fig. 5 $\Delta\delta_{obs}^{-1}$ versus $[HP\beta CD]_0^{-1}$ plots for OHFL/HPβCD complexes

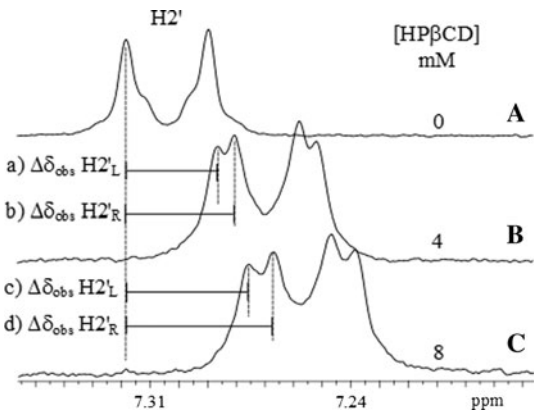


Fig. 4 ^{1}H-NMR spectra of H2' OHFL proton of **A** pure OHFL, **B** OHFL/HPβCD complexes using 4 mM of HPβCD, and **C** OHFL/HPβCD complexes using 8 mM of HPβCD. [OHFL] = 1 mM. In spectra **B** and **C**, observed chemical shift differences ($\Delta\delta_{obs}$) are indicated for $H2'_L$ (*a* and *c*) and $H2'_R$ (*b* and *d*)

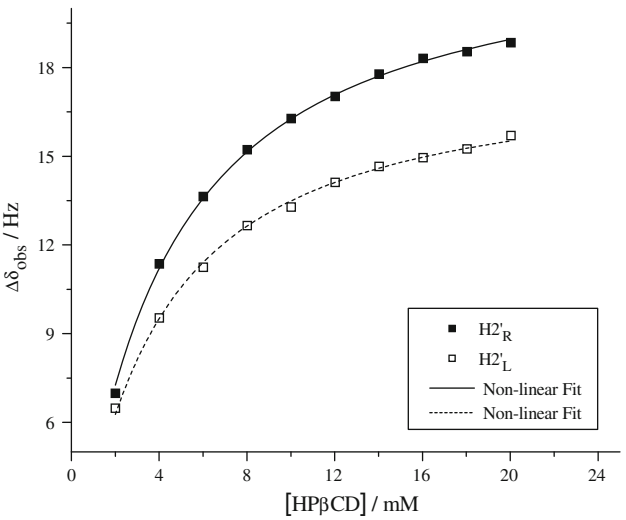


Fig. 6 $\Delta\delta_{obs}$ versus $[HP\beta CD]_0$ plots for OHFL/HPβCD complexes at 25 °C

Table 1 Association constants for OHFL/βCD and OHFL/HPβCD complexes at 25, 30, and 35 °C

Temperature (°C)	OHFL/βCD		OHFL/HPβCD	
	H3′$_L$ $K_a \times 10^2$ (M^{-1})	H3′$_R$ $K_a \times 10^2$ (M^{-1})	H2′$_L$ $K_a \times 10^2$ (M^{-1})	H2′$_R$ $K_a \times 10^2$ (M^{-1})
25	2.54	1.45	3.17	2.57
30	1.12	0.79	2.57	1.94
35	0.32	0.30	2.23	1.67

HPβCD "right" enantiomer complex at 25 °C) and that association constants decrease while temperature increases, which means that at higher temperatures, complexes' formation is less favored. On the other hand, and comparing at the same temperature, complexes formed by "left" enantiomers have larger K_a values than complexes formed by "right" enantiomers. This result indicates that complexes formed by "left" enantiomers are more stable than complexes formed by "right" enantiomers. Additionally, and at the same temperature, OHFL/HPβCD complexes have larger K_a values than OHFL/βCD complexes, indicating that HPβCD is able to promote stronger interactions with OHFL than βCD. Considering CDs' structure, HPβCD substituents could be responsible for these interactions, because βCD lacks any substituent. This conclusion is also supported by the fact that in a previous work [24], association constants ranging from 5.42×10^2 to 8.53×10^2 M^{-1} were obtained for diastereoisomeric complexes formed with another βCD derivative (heptakis-(2,6-*O*-dimethyl)-β-cyclodextrin (DMβCD)) and the same flavanone of this work (OHFL). These values, larger than the obtained for OHFL/βCD complexes, indicate that substituents could be responsible for larger K_a*s*.

Inclusion thermodynamics

Thermodynamics of diasteroisomeric complexes was determined by plotting $\ln(K_a)$ versus T^{-1} and obtaining the linear fit. According to van't Hoff's equation (Eq. 6), enthalpy (ΔH) and entropy (ΔS) variations were obtained from the slope and intercept, respectively, as shown in Fig. 7 for OHFL/HPβCD complexes.

Using ΔH and ΔS obtained values, $T = 298$ K and $R = 8.314$ J/K mol, free energy variation (ΔG) was determined according to Eq. 7. Thermodynamic values are displayed in Table 2.

In agreement with Table 2, for all the diasteroisomeric complexes all the thermodynamic parameters are negative, being these ΔH, ΔS and ΔG. Since ΔG values are negative, formation of complexes is spontaneous. On the other hand, negative ΔH values indicate the exothermic formation of the complexes.

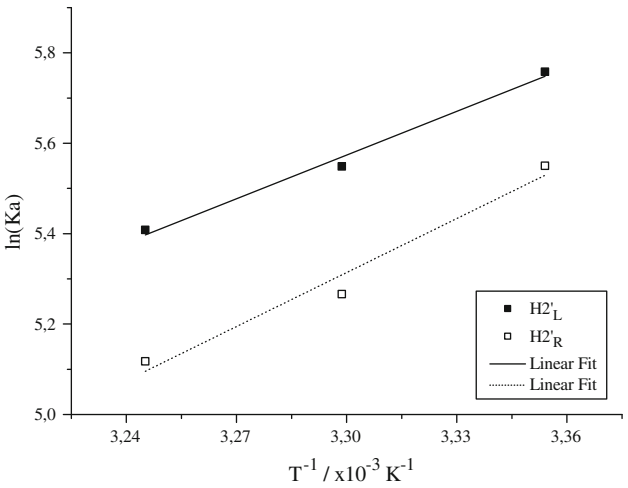


Fig. 7 $\ln(K_a)$ versus T^{-1} plots of OHFL/HPβCD complexes

Table 2 Thermodynamic parameters for OHFL/βCD and OHFL/HPβCD diasteroisomeric complexes

Thermodynamic parameter	OHFL/βCD	
	"Left" enantiomer H3′$_L$	"Right" enantiomer H3′$_R$
ΔH (kJ mol^{-1})	−158.6	−120.4
ΔS/J $(mol\ K)^{-1}$	−0.485	−0.362
$T{\cdot}\Delta S$ (kJ mol^{-1})	−144.7	−107.9
ΔG (kJ mol^{-1})	−13.9	−12.5
Thermodynamic parameter	OHFL/HPβCD	
	"Left" enantiomer H2′$_L$	"Right" enantiomer H2′$_R$
ΔH (kJ mol^{-1})	−26.8	−33.1
ΔS $(J(mol\ K)^{-1})$	−0.042	−0.065
$T{\cdot}\Delta S$ (kJ mol^{-1})	−12.5	−19.4
ΔG (kJ mol^{-1})	−14.2	−13.7

The used temperature for obtaining $T{\cdot}\Delta S$ and ΔG is 298 K

It has been reported [13], that van der Waals and hydrophobic interactions are the main driving forces that promote the formation of complexes. These interactions are characterized by negative ΔH and ΔS values for van der Waals interactions, and positive ΔH and ΔS for hydrophobic interactions. For the complexes of this work, both ΔH and ΔS values are negative, so van der Waals interactions should be the forces that mostly contribute to the formation of these complexes. This conclusion is also supported by the fact that OHFL is able to present electron delocalization, and inductive effects for both molecules, OHFL and CDs. These effects induce dipolar interactions, which are part of van der Waals forces.

Another interaction which is able to contribute to the formation of complexes is the hydrogen bond. It has been

described [13, 14] that this interaction is able to occur between (i) the substrate and the solvent molecules around the complex, (ii) the substrate and the CD OH groups, and (iii) the substrate and the CD substituents groups. Since hydrogen bonds contribute with both ΔH and ΔS negative values to the complexes' formation, only negative thermodynamic values were found in this work, and that OHFL molecule has an OH group which could be available to form hydrogen bonds, it is possible that this interaction contributes to the stabilization of the complexes in this work.

Another interesting result involves the obtained ΔG values, which are very similar for all the complexes, even with the large differences of ΔH and $T{\cdot}\Delta S$ values between complexes formed with different CDs. For example, ΔH and $T{\cdot}\Delta S$ for OHFL/βCD complex formed with the "left" enantiomer have values of −158.6 and −144.7 kJ/mol, respectively. These values are smaller than the results for OHFL/HPβCD complex formed with "left" enantiomer, having ΔH and $T{\cdot}\Delta S$ values of −26.8 and −12.5 kJ/mol, respectively. In spite of the differences between ΔH values and $T{\cdot}\Delta S$ values, ΔG values are very similar between them, having −13.9 and −14.2 kJ/mol for OHFL/βCD and OHFL/HPβCD complexes formed both with "left" enantiomers, respectively. These results reveal enthalpy-entropy compensation, because as ΔH decreases, ΔS also decrease, so ΔG is maintained. This is reflected in the K_a values as well, since in spite of the fact that OHFL/βCD complexes have the larger ΔH values (which should promote larger $K_a s$ values), they also have the smaller K_a values, which is because of the lower $T{\cdot}\Delta S$ values.

Inclusion geometry

Inclusion geometry of the CD complexes was studied by acquiring ROESY spectra and analyzed qualitatively. ROESY spectrum is an experiment that is able to detect the NOE, which is a dipole–dipole spatial interaction between two nuclei. In NMR, this interaction is manifested as an intensity signal change as a result of the perturbation of another signal. In ROESY experiments, NOE is able to be detected at distances smaller than 5 Å, so protons at this distance or less could correlate and the ROESY experiment will present cross peaks [22, 25]. Inner CDs protons are H3, H5, and H6 (Fig. 1), and their chemical shifts are well known. Therefore, if these CD protons are close enough to the substrate protons, ROESY experiment will present cross peaks, and the orientation of the substrate in respect to the CD can be determined. Partial contour plot of OHFL/βCD complexes' ROESY spectrum is shown in Fig. 8.

In the vertical scale of OHFL/βCD complexes' ROESY spectrum, βCD ^{1}H-NMR spectrum appears, showing H3, H5, and H6 protons, being H3 protons a triplet which is overlapped with H6 protons. In the horizontal scale, OHFL ^{1}H-NMR spectrum appears, showing the aromatic zone. Due to interactions between βCD protons and OHFL protons, cross peaks appear as is shown in the spectrum, but no differences are able to be seen for each enantiomer, so the inclusion geometry is determined for the complexes as a mixture of enantiomers and not separately. According to the ROESY spectrum of OHFL/βCD complexes, H5, H6 and H8 OHFL protons from "A" ring, and H2' OHFL proton from "B" ring, correlate with both H3 and H6 βCD

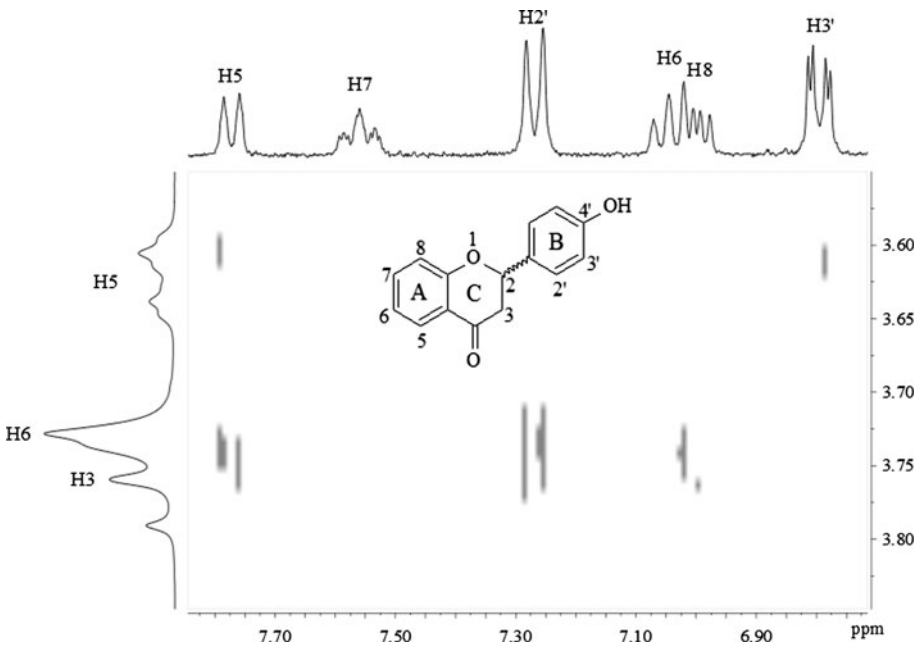


Fig. 8 ROESY spectrum of OHFL/βCD complexes

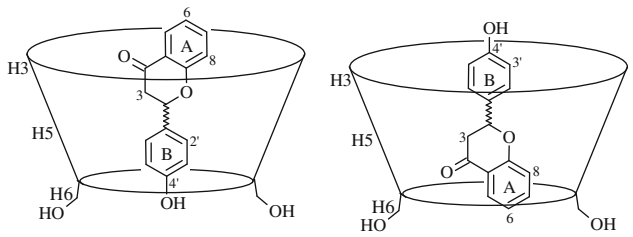


Fig. 9 Scheme of proposed OHFL/βCD complexes′ inclusion geometries

protons. Since the "A" and "B" rings are too far away from each other, and they both have interactions with both opposed H3 and H6 βCD protons, it is very unlikely that only one geometric orientation can explain the resulting cross peaks. This result is in agreement with the cross peaks of H5 and H3′ OHFL protons, because they are from different rings, and both correlate with H5 βCD protons. Again, H5 and H3′ OHFL protons are too far away from each other to explain the results. Therefore, two inclusion geometries are proposed for OHFL/βCD complexes, one opposed to the other as shown in Fig. 9

In both proposed OHFL/βCD complexes' inclusion geometries, the entire OHFL molecule is inserted into the βCD cavity, orienting the hydroxyl group toward the exterior of the complex, being able to interact with the exterior solvent molecules, possibly through hydrogen bond, as it was suggested in the thermodynamic discussion.

Previous to obtaining ROESY spectra of OHFL/HPβCD complexes, HSQC spectrum was acquired in order to unambiguously determine HPβCD chemical shifts while forming the complexes (data not shown), so the ROESY spectra could be correctly analyzed.

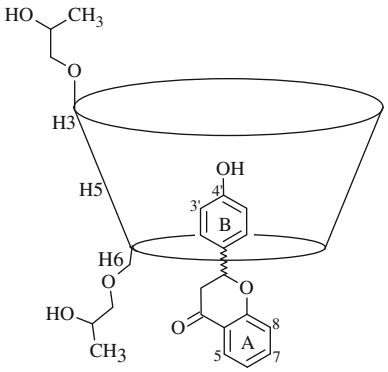
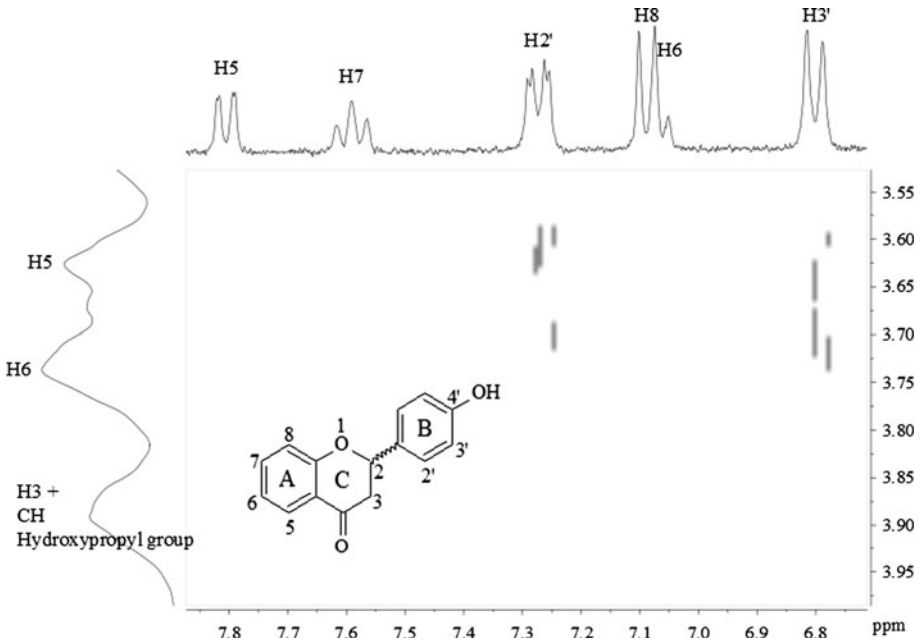


Fig. 11 Scheme of proposed OHFL/HPβCD complexes inclusion geometry

According to the OHFL/HPβCD complexes′ ROESY spectrum (Fig. 10), H2′ and H3′ OHFL protons from "B" ring correlate with both H5 and H6 HPβCD protons, indicating that the OHFL "B" ring is inserted into the HPβCD cavity by their narrow side. According to the analysis above, only one inclusion geometry is proposed for OHFL/HPβCD complexes, and this is shown in Fig. 11.

According to the estimated inclusion geometry for OHFL/HPβCD complexes, the entire OHFL "B" ring is inserted into the truncated cone of the HPβCD, orienting "A" and "C" rings to the HPβCD 2-hydroxypropyl substituent groups located in the narrow edge. As can be seen, the polar portion of OHFL is inserted into the HPβCD truncated cone cavity, which is uncommon in CD chemistry, where the substrate's nonpolar portion is usually inserted into the CD cavity [14, 19, 21, 43]. This result suggests that the inner HPβCD cavity is able to interact with polar substrates, it actually being a semi-polar

Fig. 10 ROESY spectrum of OHFL/HPβCD complexes



microenvironment interior. This could be possible due to the presence of inner dispersion interactions, as it has been reported before [13, 14].

Conclusions

It has been demonstrated in this work that diasteroisomeric complexes formed by OHFL and the two CDs βCD and HPβCD, are able to be studied by using the racemic mixture of OHFL. It was possible the determination of stoichiometry, being of 1:1, K_a values and thermodynamic parameters. The values of association constants prove that the higher the complex formation temperature, the lower the complex stability.

Thermodynamic values are in agreement with spontaneous and exothermic formation of complexes, and also suggest that van der Waals interactions are the primary contributor. Additionally, formation of hydrogen bonds could be possible in the case of OHFL/βCD complexes, which is in agreement with their inclusion geometry.

Inclusion geometries indicate that for OHFL/βCD complexes two orientations are possible, being one opposed to the other. For OHFL/HPβCD complexes only one geometry was found, and it suggests that the inner HPβCD truncated cone cavity is able to present a semi-polar microenvironment, since the OHFL "B" ring is inserted into it, developing dispersion interactions.

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