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Article

Study of the Mode of Inclusion for 7-Hydroxyflavone in β-Cyclodextrin Complexes

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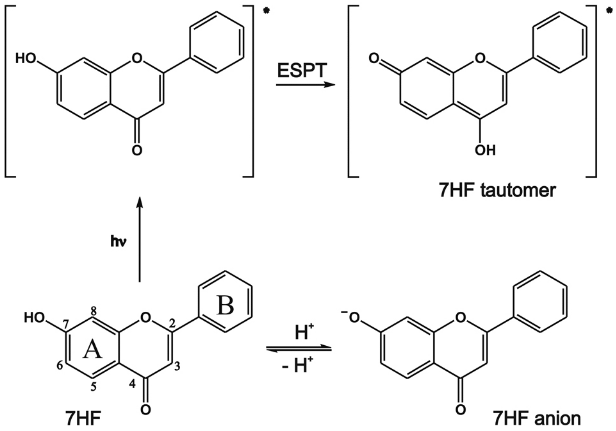
This paper aimed to study the mode of inclusion for 7-hydroxyflavone (7HF) in β-cyclodextrin (β-CD) complex in basic (pH 12) and acid (pH 4.5) media. The thermodynamic parameters of the complexation of 7HF with β-CD were evaluated by means of steady-state fluorescence and solubility measurements at several temperatures. The 7HF–β-CD inclusion complex was spontaneously formed at 1:1 stoichiometry in both basic and acid media. The high number of hydrogen bonds formed between the host and the guest molecules favored complexation in the basic medium, with a ground-state equilibrium constant (K) of 990 ± 130 mol^{-1} L, at 25 °C. The quantum-mechanics calculations showed that complexation of 7HF (neutral or anionic form) with β-CD occurred mainly via the phenyl group of the flavonoid. This result agreed with the induced circular dichroism signal of 7HF complexed with β-CD.

Keywords: 7-hydroxyflavone, β-cyclodextrin, fluorescence, solubility, quantum-mechanics calculations

Introduction

Flavonoids, such as 7-hydroxyflavone (7HF), are natural compounds that have excellent antioxidant properties and great potential to be applied as medicines.[1,2] Their molecular structures have an extensive electron conjugated π system that interact with various reactive oxygen intermediates, such as superoxide anion ($O_2^{\bullet -}$), hydroxyl ($HO^{\bullet}$) or peroxyl ($HO^{\bullet}_2$) radicals and efficiently quenches singlet oxygen.[3,4]

An important feature of 7HF described in the literature[5] is that it can undergo an excited state proton transfer (ESPT), although the proton donor and the acceptor site, the hydroxyl group at position 7 and the carbonyl oxygen, respectively, are not located in neighboring atoms (Scheme 1). Thus, the ESPT in 7HF is assisted by solvent and strongly depends on the nature of the environment.[5]



Scheme 1. 7-hydroxyflavone (7HF) and its anion. The light absorption leads to an intramolecular excited state proton transfer that forms a fluorescent tautomer. Adapted from Chaudhuri *et al.*[5]

In a recent published work,[6] the acid-base and spectral properties of 7HF in the ground and excited states have been investigated. It has been proposed that in ground state there is the equilibrium between 7HF and its anion (acid equilibrium constant, pK_a = 8.91 ± 0.02), depending on the acidity of the environment. In conditions of very high acidity (pKa = −1.01 ± 0.04), there is also the presence of 7HF cation, as described in Scheme 2.

Upon the excitation of 7HF cation and anion with appropriate energy (λ_{exc} = 367 and 361 nm, respectively), there is the fluorescence emission of 7HF cation (λ_{em} = 464 nm) and anion (λ_{em} = 545 nm), depending on the media acidity. The neutral 7HF emits phosphorescence rather than fluorescence in aprotic solvents of low basicity, where there is no possibility of formation of hydrogen bond between 7HF

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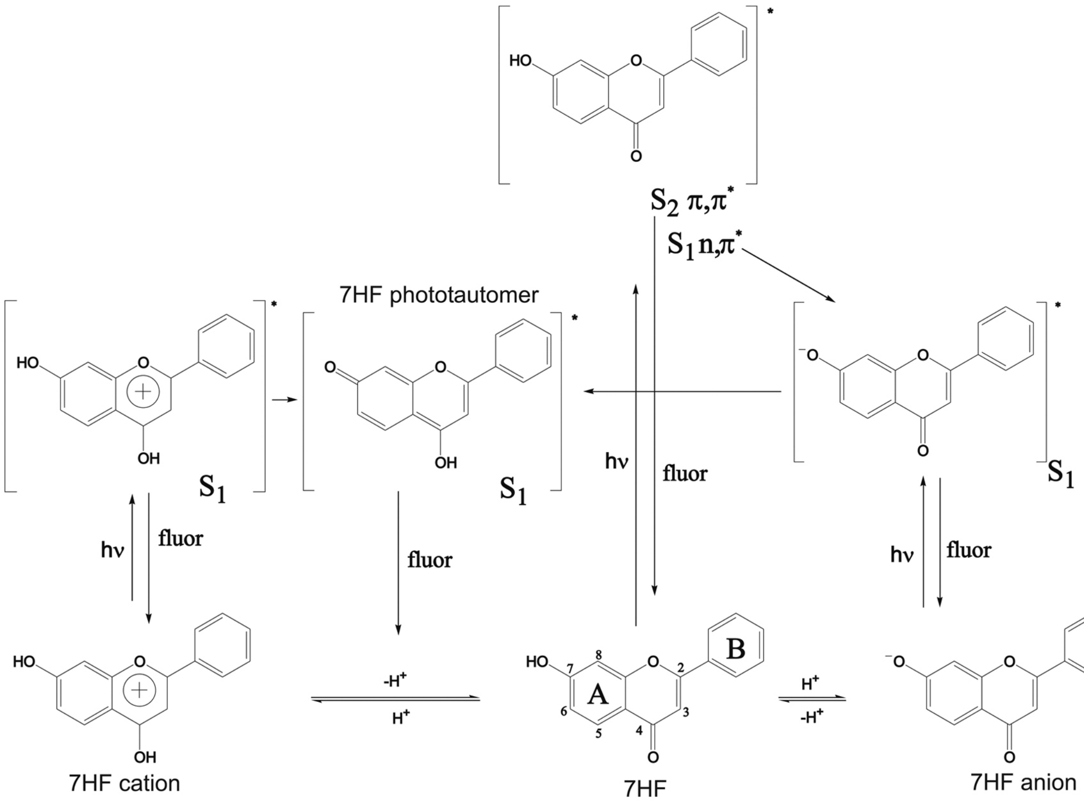

Scheme 2. 7-Hydroxyflavone (7HF) and its cation and anion in ground and excited state. The light absorption of the neutral 7HF can lead to S_1 n,π^* and S_2 π,π^* states. The decay mechanism of S_1 n,π^* state of the neutral 7HF is radiationless. S_1 n,π^* state can interconvert into the fluorescent anion or T_1 state. S_2 π,π^* is poorly fluorescent and depending on the conditions of the environment. 7HF fluorescent phototautomer can be produced from the 7HF S_1 states of the cation or anion. Adapted from Serdiuk *et al.*[6]

and solvent molecules. This is an indication that neutral 7HF S_1 is n,π^*, and the authors refer to this neutral 7HF as N.[6,7] On the other hand, in aprotic basic environments and in alcohols, where the neutral 7HF can form hydrogen bond between solvent molecules, which they call N_H, there is a weak fluorescence emission (λ_{em} ca. 407 nm). This fluorescence emission is attributed to the close proximity of the energy between π,π^* and n,π^* excited state, allowing the radiative deactivation of N_H S_2 π,π^* excited state.[6]

In this work,[6] the authors also detect the formation of the 7HF fluorescent tautomer (λ_{em} at ca. 570 nm, estimated lifetime of ca. 140 ps) under conditions of very high acidity from H_0 1.3 to −4.5, due to dissociation of the 7-hydroxy group of the 7HF cation S_1. The rate of this cationic pathway to lead to the phototautomer is four times higher than the second and possible anionic way. The latter involves a double ESPT from 7HF N_H^* to 7HF anion S_1 excited state. Then, protonation of the 7HF anion S_1 excited state to produce the same phototautomer.[6] Surely, the pathway of phototautomer formation depends on the acidity of the envinroment under investigation.

However, the solubility of flavonoids such as 7HF in aqueous solutions is usually very low. Complexation with cyclodextrins (CDs) extends their industrial applications by improving not only their aqueous solubility but also their stability and bioavailability.[8] In addition, photodegradation of drugs may occur during storage and/or manipulation and either decrease their therapeutic efficiency or cause adverse effects due to their administration. Hence, the knowledge of the photostability of these molecules in the presence or absence of CDs is highly desirable.

CDs are cyclic oligosaccharides composed of α-D-glucopyranose (D-glucose) units connected by α-1,4 linkages. The most common CDs, produced from starch and cellulose, are the α-, β-, and γ-CD consisting of 6, 7 and 8 glucopyranose units, respectively.[8] CDs have an external hydrophilic surface and a hydrophobic internal cavity, whose size varies according to the number of D-glucose units. The high electron density of the D-glucose oxygen atoms promotes the CD cavity hydrophobicity.[8] CDs have the ability to form reversible complexes with nonpolar molecule. Therefore, several researchers have been studying the complexation with CDs either to explain how a confined environment can affect the guest molecule[9-11] or to improve their solubility in aqueous solution.[12,13] The latter approach is a very interesting alternative to solve solubility problems related to the administration of hydrophobic drugs in aqueous solutions.

Our research group has previously studied the complexation of fisetin (Fis; 3,3',4',7-tetrahydroxyflavone; Figure 1) with β-CD in acid and basic aqueous solutions by means of steady-state fluorescence, ultraviolet-visible (UV-Vis) absorption, induced circular dichroism (ICD) signal, proton nuclear magnetic resonance (^{1}H NMR) measurements, and quantum-mechanics calculations.[9] At neutral or acid pH, Fis preferentially inserts itself into β-CD via its phenyl group, due to formation of a higher number of intermolecular bonds between Fis 3' and 4' hydroxyl groups and β-CD.

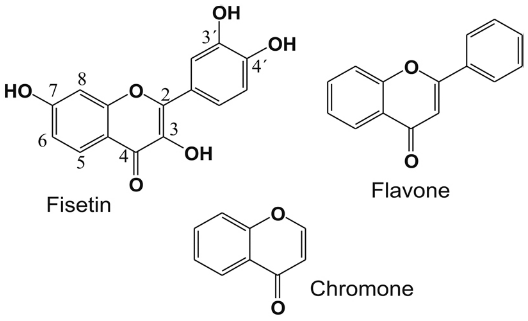

Figure 1. Chemical structures of fisetin (3,3',4',7-tetrahydroxyflavone), flavone and chromone.

However, theoretical data analysis did not totally rule out the possibility of Fis inclusion in β-CD via the chromone group. These findings raised the following question: does only the number of intermolecular bonds between the phenolic protons of Fis and the β-CD structure determine its preferential mode of inclusion? To clarify this issue, the present study examined the complexation of 7HF with β-CD in acid (pH 4.5) and basic (pH 12) aqueous solutions by analyzing the 7HF-β-CD complex through UV-Vis absorption, steady-state fluorescence, induced circular dichroism measurements, and theoretical data obtained from quantum-mechanics calculations. 7HF has only one hydroxyl group at the chromone part (position 7). The hypothesis here is that the single hydroxyl group in the chromone moiety but not the phenyl group can guide the complexation of 7HF with β-CD.

Experimental

Materials

7-Hydroxyflavone (≥ 98%) was obtained from Sigma-Aldrich and recrystallized from methanol. β-Cyclodextrin (99%, Lot A006223601) and sodium hydroxide (p.a.) were purchased from Acros Organic and Synth, respectively. Anhydrous Na_2HPO_4, $NaH_2PO_4.H_2O$, ethanol and methanol (all p.a.) were acquired from J. T. Baker. All reagents, except 7HF, were used as received.

Equipments

Steady-state emission and excitation fluorescence spectra were measured with a Hitachi F7000 fluorimeter (Tokyo, Japan) (λ_{exc} = 340 or 360 nm, emission and excitation slits = 5 or 10 nm). ICD and UV-Vis absorption measurements (from 15 to 40 °C) were recorded using a Jasco J-810 spectropolarimeter (Easton, USA) equipped with a Jasco PTC-423S coupled device for temperature control, with baseline correction. The baseline was recorded using an acid or basic solution (phosphate buffer) of 7HF containing methanol at a maximum concentration of 0.5% (v/v). An average of two spectra was recorded for each sample. The spectrum of an aqueous solution of β-CD in the absence of 7HF was subtracted from those acquired for β-CD in the presence of the guest.

Methods

Preparation of 7HF and β-CD complexes in buffer solutions

First, phosphate buffer solutions were prepared at pH 4.5 and 12. The acid buffer solution was prepared by adding a ratio of 400 mol of NaH_2PO_4 to 1 mol of Na_2HPO_4, while the basic buffer solution was prepared by adding equal volumes of aqueous solutions of 0.2 mol L^{-1} Na_2HPO_4 and 0.1 mol L^{-1} NaOH. Second, a 7HF stock solution (2 mmol L^{-1}) was prepared in methanol and further diluted in both buffer solutions (pH 4.5 and 12) to the concentrations of 5 or 10 µmol L^{-1}, with methanol content less than 0.5% (v/v). Third, β-CD (12 mmol L^{-1}) was mixed with 7HF in methanol (5 or 10 µmol L^{-1}) at pH 4.5 and 12, and stirred for 4 h in the dark. The mixture of the buffer solutions with 7HF in methanol (5 or 10 µmol L^{-1}) in presence and absence of β-CD were used to prepare by dilution several β-CD concentrations (from 0 to 12 mmol L^{-1}). All solutions were stirred for at least 24 h, protected from light, before any experimental measurement.

Determination of equilibrium constants and stoichiometry of the complex 7HF-β-CD

At a fixed emission wavelength in the fluorescence spectra, the 7HF fluorescence emission intensity in the presence of β-CD/ buffer solution was subtracted from the solution containing 7HF/ buffer solution, in absence of β-CD (ΔI). Data from the curves of the plot of ΔI as function of β-CD concentration were analyzed according to the Benesi-Hildebrand equation[14] (equation 1), which gives the ground-state equilibrium constant (K):

$$= \frac{[7HF] \quad \varphi K[CD]}{1 + K[CD]} \qquad (1)$$

where, Δϕ represents the difference between the emission quantum yields of the free and complexed 7HF.

The linear relationship (equation 2) was obtained from the double-reciprocal plot of equation 1:

$$\frac{1}{\ } = \frac{1}{[7HF]_T} + \frac{1}{[7HF]_T \qquad [CD]} \qquad (2)$$

Experimental data collected at different temperatures were analyzed to obtain the thermodynamic parameters of the complexation of 7HF with β-CD in acid and basic media.

Solubility analysis of 7HF in β-CD in acid media

The 7HF solubility in β-CD in acid media was studied following the method described by Higuchi and Connors.[15] Excess of 7HF (ca. 1 mg) was added to β-CD (0 to 10 mmol L^{-1}) in phosphate buffer pH 4.5, stirred for 24 h at room temperature, and further incubated in a thermostatized bath for ca. 2 h at 30 °C. To determine 7HF concentration, absorbance of the filtered solutions was recorded at 308 and 314 nm. The molar absorptivities at these maximum absorbance wavelengths have been previously determined experimentally. The same procedure was repeated at 15, 20 and 25 °C. The experiments were carried out twice.

The linear part of the plot of 7HF concentration as function of β-CD concentration gave the equilibrium constant for the complex formation by using the Higuchi and Connors equation[15] (equation 3):

$$K = \frac{\text{slope}}{\text{intercept}\ (1 \quad \text{slope})} \qquad (3)$$

The thermodynamic parameters of the formation of 7HF–β-CD complex were obtained from the integrated Van't Hoff equation (equation 4). The slope and the intercept of the plot of ln K as function of the inverse of temperature gave the changes in enthalpy (ΔH) and entropy (ΔS) in acid media, respectively.

$$\ln K = -\frac{H}{RT} + \frac{S}{R} \qquad (4)$$

The free energy variation (ΔG) was calculated from the Gibbs equation (equation 5):

$$\Delta G = \Delta H - T.\Delta S \qquad (5)$$

Results and Discussion

Determination of the complexation constant at different temperatures

The pK_a of 7HF is ca. 7.1,[16] which means that this compound is in equilibrium between its anion and neutral forms in neutral pH. For this reason, we have studied the complexation behavior at two pH, an acid (4.5) and a basic one (12). To ensure that equilibrium of the complexation of 7HF with β-CD was reached, the solutions were stirred for 24 h prior to the experimental measurements. The results reported herein are the average of duplicate experiments.

Figure 2a depicts the 7HF emission fluorescence spectra as function of β-CD concentration at pH 12, at 35 °C. Figure 2b shows the corresponding data fitted using the Benesi-Hildebrand equation.[14]

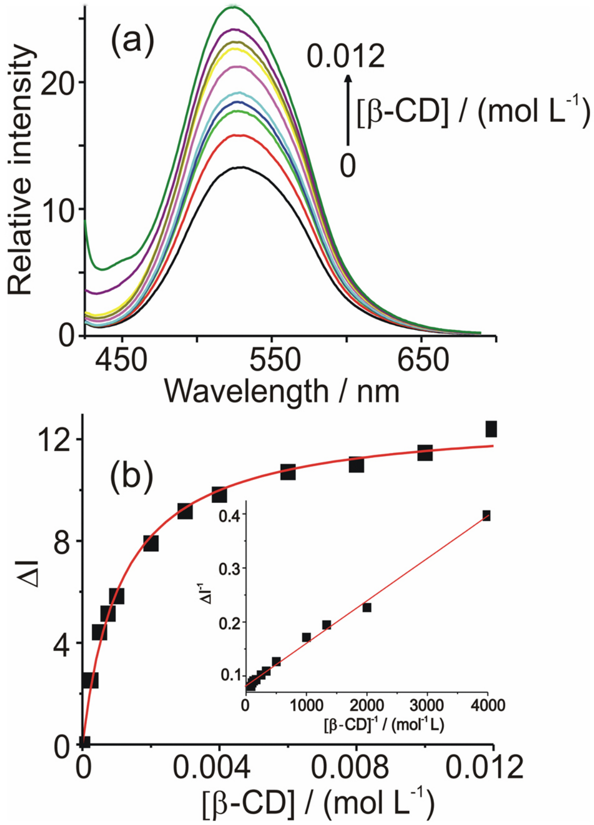

Figure 2. Fluorescence emission spectra of 7-hydroxyflavone (7HF; 5 µmol L^{-1}) at 35 °C and pH 12 in the presence of different β-cyclodextrin (β-CD) concentrations (λ_{exc} = 360 nm) (a); and plot of fluorescence emission intensity (ΔI) as function of β-CD concentration (mol L^{-1}) at 530 nm. Experimental data were fitted applying the Benesi-Hildebrand equation (solid line, red). Inset: the double reciprocal plot and the linear fit (solid line, red) (b).

The Benesi-Hildebrand equation (equation 1) was used to determine the ground-state equilibrium constant (K) by evaluating the steady-state fluorescence measurements at pH 12. The application of this method was feasible, since β-CD concentration was in excess compared to the photophysical probe, 7HF, concentration. The fluorescence emission intensity (ΔI) increased with increasing β-CD concentrations. Table 1 shows the obtained complexation constants, K, in basic and acid pH media at several temperatures.

Table 1. Equilibrium constant (K) of the complexation of 7-hydroxyflavone (7HF) with β-cyclodextrin (β-CD), performed at different temperatures and pH

Temperature / °C	K / (mol^{-1} L)	
	pH 12[a]	pH 4.5[b]
15	1070 ± 150	670 ± 10
25	990 ± 130	640 ± 10
35	880 ± 60	620 ± 20
45	710 ± 50	570 ± 30

Data obtained through [a]steady-state fluorescence measurement or [b]solubility analysis.

Comparison of the obtained K values for the formation of the 7HF–β-CD complex and literature values (Figure 1 and Table 2) revealed that the K for 7HF is 990 ± 130 mol^{-1} L, in basic medium at 25 °C, which is similar to values of flavone (1090 ± 80 mol^{-1} L),[17] and fisetin (860 ± 70 mol^{-1} L)[9] at 23 °C, in acid or neutral medium. Note that the literature K value for flavone has been determined in aqueous solutions, without pH or temperature controls. The values showed in Table 1 are four times higher than those reported for chromone (240 ± 40 mol^{-1} L),[17] which does not bear the phenyl group. In basic medium, Fis has low equilibrium constant (240 ± 90 mol^{-1} L),[9] because the presence of four deprotonated hydroxyl groups favors its solubilization in aqueous solution.

The linear relationship of the Benesi-Hildebrand equation gave us straight lines (inset plot of Figure 2b). This means that the complex formed by 7HF and β-CD has a stoichiometry of 1:1 (7HF:β-CD) like other flavonoids such as Fis,[9] and flavone.[17]

Table 2. Complexation constants (K) for flavonoids described in the literature

Flavonoid	K / (mol^{-1} L)
Chromone[a]	240 ± 40
Fisetin/acid or neutral medium[b]	860 ± 70
Fisetin/basic medium[b]	240 ± 90
Flavone[a]	1090 ± 80

Obtained from the publication of [a]Christoff *et al.*[17] and [b]Guzzo *et al.,*[9] at 23 °C.

The stability of the 7HF-β-CD complex formed decreased with the temperature increase in basic medium (Table 1). The K value of the complexation reaction in acid medium at 25 °C was 715 ± 74 mol^{-1} L; however, it was not possible to obtain reliable K values by changing the complexation temperature. It is worthy of note that 7HF is prone to undertake an ESPT,[5,6] despite the high distance between the hydroxyl proton at position 7 and the carbonyl group. The ESPT is a very fast process that researchers have studied for other molecules complexed with CD using ultrafast spectroscopic techniques, such as time-resolved fluorescence in picoseconds (ps) or femtosecond (fs).[11,18]

In order to assure which fluorescence species we were observing in acid solutions, we examined the 7HF steady-state fluorescence excitation and emission spectra in the presence and absence of β-CD, by varying the emission or excitation wavelength, respectively, for both pH (4.5 and 12; Figures S1-S4). We noted that in basic or acid solutions, the excitation and emission spectra were all the same, despite the variation of the emission and excitation wavelength, respectively. There was no shift in the excitation and emission of fluorescence spectra in presence of β-CD either in basic or acid buffer aqueous solution. The sharp peaks observed in these spectra (Figures S1-S4) are related to artifacts from the harmonic of the emission or excitation wavelength used for measurements acquisitions and they do not arise from the 7HF fluorescence. Thus, we assumed that the 7HF fluorescence emission spectra is due to one exclusive specie, the 7HF anion (λ_{em} = 530 nm). This finding agrees with data reported by Roshal and co-workers,[6] who have conducted a very comprehensive study of 7HF spectral properties in 20 solvents and frozen solvents, as well as spectrophotometric and spectrofluorimetric titrations in methanol-water (4:1, v/v) from pH 11.0 to H_0 −4.5. They found that in alcoholic environments, like those provided by the inner β-CD cavity,[19] there is the presence of 7HF N_H (i.e., the protonated form of 7HF due to the hydrogen-bonded complex with the solvent molecules).[6] The N_H fluorescence emission intensity is very weak (λ_{em} = 420 nm) at pH < 8, and it is comparable to the Raman scattering band at 380 nm. However, at the pH range to 2.0-6.0 in alcohol-water solutions, the predominant fluorescent specie is the 7HF anion (λ_{em} = 545 nm), which is derivated from the N_H.[6] This fact was supported by the excitation spectra recorded at both fluorescence maxima (420 and 545 nm, related to, respectively, 7HF N_H and anion), that were similar to the absorption spectra of the N_H. The main difference between 7HF anion fluorescence emission wavelength of our work (530 nm) and the Roshal and co-workers[6] value (545 nm) is that they used a mixture of methanol-water (4:1, v/v) to evaluate it. Besides of this, in the same reference,[6] 7HF anion fluorescence wavelength is reported to be 531 and 530 nm in iso-propanol and iso-butanol, respectively. In our experimental condition (pH 4.5), we did not notice the formation of phototautomer in the steady-state fluorescence spectra. Nevertheless, due to the complexity of the system, we decided to obtain K values for the equilibrium of the complexation of 7HF with β-CD in acid media through measurements of solubility at different temperatures.

Guzzo *et al.*[9] did not show the excitation fluorescence spectra for the complexation of Fis with β-CD, which would probably have provided a more detailed picture of the differences between 7HF and Fis mode of inclusion.

Measurement of solubility at acid pH

Figure 3a depicts a representative solubility diagram, where solubility of 7HF increased as function of the β-CD concentration. A similar behavior was observed at all temperatures assessed in this work.

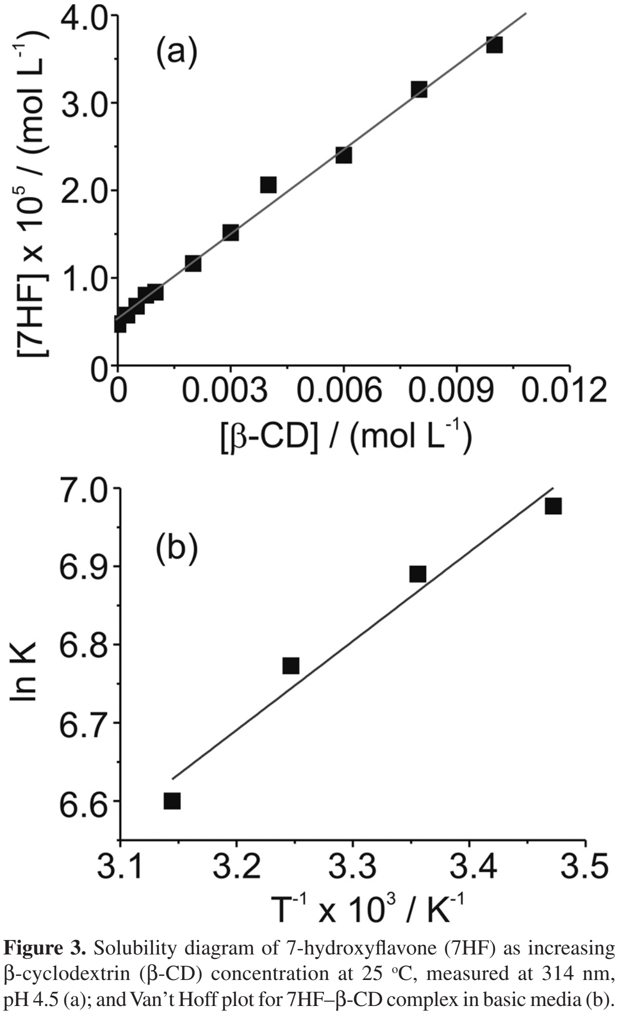

Figure 3. Solubility diagram of 7-hydroxyflavone (7HF) as increasing β-cyclodextrin (β-CD) concentration at 25 °C, measured at 314 nm, pH 4.5 (a); and Van't Hoff plot for 7HF–β-CD complex in basic media (b).

Values of K at different temperatures in acid media (Table 1) was obtained applying equation 3 to graphics similar to Figure 3a. The analysis was made at 308 and 314 nm and showed similar results. Table 1 data represent the average of duplicate experiments.

It is worth to mention that the K values of Fis in acid or neutral (860 ± 70 mol^{-1} L) and basic (240 ± 90 mol^{-1} L) media were determined by two techniques: fluorescence and measurements of solubility.[9] Table 2 presents the average of the results obtained using both methods. In addition, the fluorescence properties of flavone and chromone are similar to those displayed by the neutral 7HF. The deactivation of S_1 excited state is radiationless, but there is phosphorescence emission, since the lowest excited state of these compounds is of n,π^* type.[6,7] For this reason, the K values for chromone and flavone (Table 2) were determined on the basis of UV-visible absorption spectra changes. These evidences allowed comparison between data from steady-state fluorescence and solubility measurements.

The stability of the 7HF-β-CD complex in acid medium decreased with the temperature increase (Table 1). This finding was similar to that reported for the complex prepared in basic medium. Therefore, the temperature acts positively on the final solubility degree and negatively on the stability of the 7HF-β-CD complex. In addition, in the acid medium, the obtained K of 640 ± 10 mol^{-1} L was lower than that described for Fis (Figure 1) in acid pH (860 ± 70 mol^{-1} L).[9] Compared with 7HF, Fis bears additional hydroxyl groups at positions 3, 3' and 4', which they could contribute to this difference.

Determination of the thermodynamic parameters

We used K values of the 7HF-β-CD complex in basic and acid media (Table 1) to plot ln K as function of inverse of temperature (Figure 3b). Further, these experimental data were analyzed using the integrated form of Van't Hoff equation (equation 4). The linear coefficient and the slope provided the thermodynamic parameters, described in Table 3.

Table 3. Thermodynamic parameters of the 7-hydroxyflavone (7HF)-β-cyclodextrin (β-CD) complex (7HF–β-CD) in acid and basic media

Thermodynamic parameter	pH 4.5	pH 12
ΔH / (kJ mol^{-1})	–7.62	–9.46
ΔS / (J mol^{-1} K^{-1})	27.7	25.4
ΔG / (kJ mol^{-1})	–15.9	–17.0

Analysis of the thermodynamic parameters (Table 3) revealed that formation of the inclusion complex is favored in both acid and basic media (ΔG = –15.9 and –17.0 kJ mol^{-1}, respectively, calculated at standard temperature of 298 K). The inclusion of a molecule in the β-CD inner cavity must take into account the dependence of various factors of the guest and the CD,[8] such as: (*i*) the replacement of an energetically unfavourable polar-apolar interaction between the included water and the CD by a more favourable non-polar-non-polar interaction between the guest and the CD cavity, and a polar-polar interaction between the bulk water and the water molecules released from the cavity; (*ii*) the CD ring forces relax on the complexation; (*iii*) Van der Waals interactions, and; (*iv*) in the case of some guest molecules, hydrogen bonds between the guest and the host.

Comparison between the change of enthalpy (ΔH) for the 7HF-β-CD complex (–7.62 and –9.46 kJ mol^{-1} for pH 4.5 and 12, respectively) and the Fis-β-CD complex (–27 ± 4 kJ mol^{-1})[9] shows that the values of the former are ca. three times lower and the complexation is exothermic. The complex dissociates as the temperature rises. In this work, the change of entropy (ΔS; 27.7 and 25.4 J mol^{-1} K^{-1}, for acid and basic solutions, respectively) exhibited an increase of disorder, perhaps due to the release of the highly ordered water molecules of the CD inner cavity or surrounding 7HF. This result differs from that of the Fis-β-CD complex, that has a negative ΔS (–40 ± 10 J mol^{-1} K^{-1}).[9] The complexation of 7HF with β-CD enhances the disorder degree of the system.

Induced circular dichroism signal

The ICD signal (Figure 4) appears because 7HF is achiral and β-CD is chiral, and indicates formation of the 7HF-β-CD complex. The ICD intensity and the signal of the Cotton effect provide information about the orientation and depth of 7HF when inserted into the β-CD hydrophobic cavity.[20,21] It is possible that the axial or equatorial mode of inclusion occurs[22] (Figure S5) due to orientation of the electronic transition moment of the molecule relative to the axis of symmetry of β-CD. A positive ICD spectrum sign indicates that electric dipole moment of the molecule coincides with the β-CD n-fold axis, which means an axial inclusion. The equatorial inclusion shows a perpendicular orientation between the guest molecule and the β-CD axis.[21]

In Figure 4, the positive sign at 200 to 250 nm can be associated to the π-π* transition absorbance of the benzoyl system of the 7HF, which comprises the A ring and the carbonyl group at C_4 (Scheme 1). The positive band at 314 nm is related to the cynnamoyl system composed by the carbonyl group at C_4 conjugated through C_3 and C_2 to phenyl ring B (Scheme 1).[23,24] Both signs suggest an axial inclusion of 7HF into β-CD, and raise the possibility of 7HF insertion via its phenyl or chromone group, in a manner similar to the Fis inclusion into β-CD.[9]

Theoretical calculations

The theoretical calculations were performed to clarify (*i*), which portion of the 7HF molecule, the phenyl group or the chromone, preferentially inserts into the β-CD cavity to form the inclusion complex; and (*ii*) which medium, basic or acid, is more effective to form the 7HF-β-CD inclusion complex. In other words, we applied these calculations to establish the geometry of the minimum energy configuration of the inclusion complexes formed, but not to obtain quantitative parameters related to the formation of hydrogen bonds between 7HF and β-CD in the inclusion complex. We found that the anionic and neutral forms of 7HF predominate in the basic and acid media, respectively.

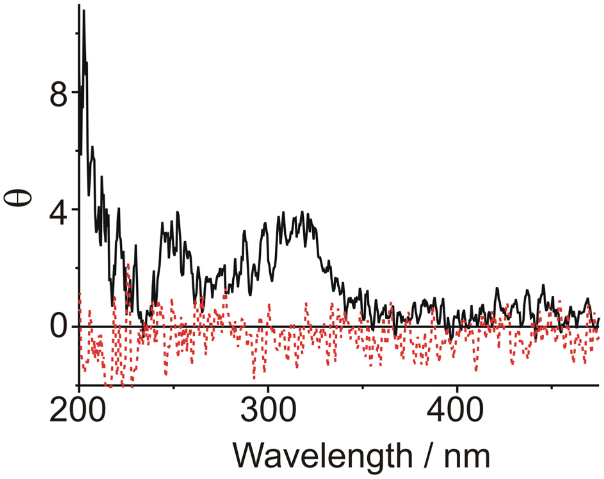

Figure 4. Induced circular dichroism (θ) spectra with 7-hydroxyflavone (7HF) excess, alone (short dash, red) or in combination with 8 mmol L^{-1} of β-cyclodextrin (β-CD; full line, black) in buffer solution pH 4.5.

The optimizations and frequency calculations (to estimate the thermodynamic parameters) were made using the B3LYP hybrid functional from density functional theory (DFT) in combination with the atomic basis 6-31G(d) to describe each atom and the polarized continuum model (PCM) to simulate the aqueous environment. All these functions were present in the software Gaussian 09.[25] Before the optimizations, the structures were adjusted using the semi-empirical PM3 method in combination with the COSMO algorithm for simulation of aqueous medium. Semi-empirical calculations were done using the software AMPAC™ version 9.1.2.[26]

We considered the model below to calculate the thermodynamic parameters (equation 6):

$$7HF_{(aq)} + \beta\text{-}CD_{(aq)} \rightarrow complex_{(aq)} \quad (6)$$

To examine the entry of 7HF into β-CD cavity, we took into account that it might occur via either the chromone or the phenyl group of the flavonoid. We performed the same analysis for the 7HF tautomer-β-CD complex. Table 4 shows the theoretical thermodynamic parameters for all the 7HF structures investigated.

Figure 5 shows the optimized structures of 7HF complexed with β-CD, assuming that its chromone or

Table 4. Theoretical thermodynamic parameters estimated from density functional theory (DFT) calculations at 298.15 K

	Insertion mode via	ΔH[b] / (kJ mol^{-1})	ΔS[c] / (J mol^{-1} K^{-1})	ΔG[d] / (kJ mol^{-1})
7HF[a] neutral	chromone	+4158	–394.8	+143.8
	phenyl	+4143	–308.1	+130.8
7HF[a] anion	chromone	+6513	–405.0	+618.4
	phenyl	+3878	+963.9	+113.4
7HF[a] tautomer	chromone	+2.07	+201.6	+62.18
	phenyl	+54.67	–74.26	+109.1

[a]7-Hydroxyflavone; [b]enthalpy; [c]entropy; [d]Gibbs free energy variation.

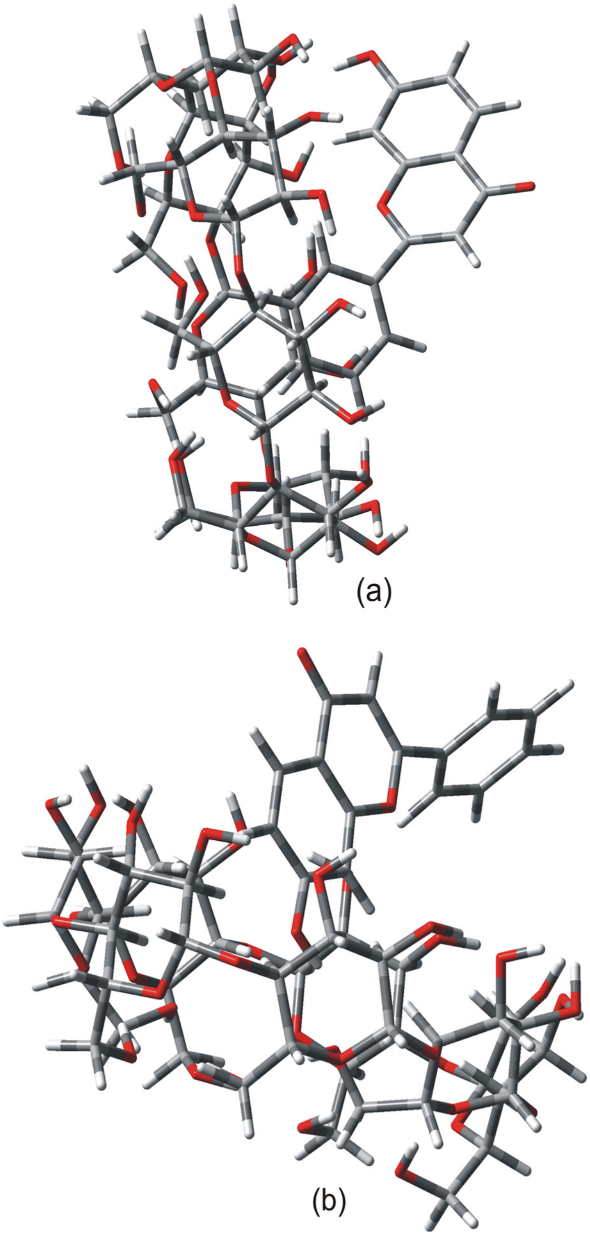

Figure 5. Optimized structures of 7-hydroxyflavone (7HF)-β-cyclodextrin (β-CD) complex (7HF-β-CD) assuming that inclusion occurred via phenyl (a) and chromone (b) part of the flavonoid molecule.

phenyl group is inserted into the CD cavity. The insertion mode of 7HF into the β-CD cavity via the phenyl group was much more effective.

Analysis of the theoretical thermodynamic parameters (Table 4) indicates that the complexation of 7HF with β-CD in basic and acid media occurs preferentially via insertion of the phenyl group, which features lower variation of the Gibbs free energy and enthalpy, as compared to the insertion mode via the chromone group. The low complexation constant value (240 ± 40 mol^{-1} L) of chromone in neutral medium[17] is another evidence that 7HF preferentially interacts with the β-CD cavity via its phenyl group. All the experimental values obtained in this work were about four times higher than the chromone K value (Table 3). For the neutral form of 7HF, the difference between the Gibbs free energy for the insertion via phenyl group (130.8 kJ mol^{-1}) and chromone (143.8 kJ mol^{-1}) was lower when compared with the same parameter calculated for the 7HF tautomer or its anionic form.

Compared with complexation of the neutral form of 7HF, complexation of the anionic form of 7HF with β-CD exhibited lower variation in the Gibbs free energy (113.4 kJ mol^{-1}). It can be attributed to the formation of a higher number of hydrogen bonds between the anionic form of 7HF and β-CD.

In contrast to the results obtained for the neutral (acidic medium) and deprotonated (basic medium) forms of 7HF, theoretical calculations indicated that the 7HF tautomer was more effectively included in β-CD via its chromone group. Such group of the 7HF tautomer allows the formation of closer hydrogen bonds between the guest and host molecules, which favors this mode of inclusion.

Conclusions

This paper stressed the importance of the study of the complexation of flavonoids with β-CD. The complexation of the neutral and anionic forms of 7HF with β-CD occurs preferentially via the flavonoid phenyl group and is more efficient in basic media. This result means that the hydroxyl group at the position seven of the flavonoids does not guide their complexation with β-CD. It suggests that flavone, a non-hydroxylated flavonoid, also complexes with β-CD via its phenyl group. However, the theoretical thermodynamic parameters revealed only a small difference between the Gibbs free energy of the complexation of the neutral form of 7HF via the phenyl and the chromone moiety of its structure.

The excitation spectra evidenced that the 7HF anion is the predominant fluorescent species (λ_{em} = 530 nm) at acidic pH, due to the excitation of the protonated form of 7HF as consequence of the formation of hydrogen bond with the β-CD or the water molecules.

Despite our experimental data did not detect the presence of phototautomer, the theoretical calculations showed that, when present, the 7HF tautomer preferentially complexes with β-CD via the chromone part in contrast to the neutral and anion forms of 7HF. The 7HF tautomer-β-CD complex is the most effectively formed due to the indicative of shorter distance between the host and the guest hydrogen bonds.

Supplementary Information

7HF steady-state fluorescence excitation and emission spectra in the presence and absence of β-CD, changing the emission or excitation wavelength, respectively, for both pH (4.5 and 12); and a scheme of the axial or equatorial mode of inclusion in β-CD are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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