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Cyclodextrins-Kaempferol Inclusion Complexes: Spectroscopic and Reactivity Studies

Carolina Jullian · Victor Brossard · Iván Gonzalez · Muriel Alfaro · Claudio Olea-Azar

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Abstract The slightly water-soluble flavonoid kaempferol (KAE) and its inclusion complexes with β-cyclodextrin (βCD), hydroxypropyl-β-cyclodextrin (HPβCD) or heptakis-2,6-*O*-dimethyl-β-cyclodextrin (DMβCD) were investigated. The stoichiometric ratios and association constants describing the extent of the formation of the complexes have been determined. Binding constants, estimated from fluorescence studies at different temperatures, were analyzed so as to gain information about the mechanisms involved in the association processes. The thermodynamic data for the inclusion of KAE in DMβCD and HPβCD indicated that it is mainly enthalpy-driven whereas for βCD it is an entropy-driven process. Complex formation was monitored by two-dimensional ROESY experiments through the detection of intramolecular dipolar interaction. ROESY experiments provided data indicating that the B-ring of kaempferol is immersed in the apolar cavity with the A- and C-ring protruding from the wider rim for the three cyclodextrins studied. The antioxidant studies of KAE and CDs complexes showed an increment in its antioxidant activity. The complexes behave as better antioxidants than kaempferol alone.

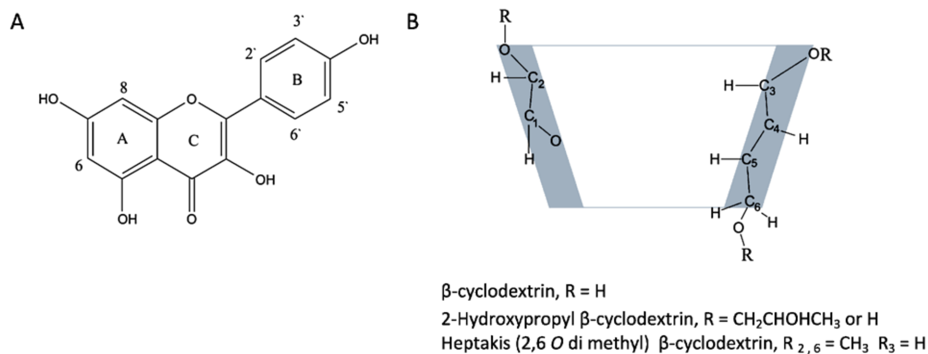
Keywords Kaempferol · Cyclodextrins · Inclusion complex · 2D-ROESY · Thermodynamic study · ORAC-FL

1 Introduction

Consumption of fruit and vegetables has been consistently associated with a reduced risk of human cancers [1]. Flavonoids are a group of potentially chemoprotective compounds

C. Jullian (✉) · V. Brossard · I. Gonzalez · M. Alfaro
Departamento Química Orgánica y Fisicoquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago de Chile, Chile
e-mail: cjullian@uchile.cl

C. Olea-Azar
Departamento Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Santiago de Chile, Chile



Scheme 1 (**A**) Molecular structure of kaempferol. (**B**) Schematic representation of β-cyclodextrin. (**B**) Schematic structure of β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, and heptakis-2,6-*O*-dimethyl-β-cyclodextrin

widely distributed in fruit, vegetables, and beverages of plant origin and have similar structures that consist of two phenolic benzene rings linked to a heterocyclic pyrone. More than 5000 individual flavonoids have been identified; these are classified into ten subgroups according to their chemical structure. Flavonoids of six principal subgroups—flavonols, flavones, anthocyanidins, catechins, flavanones, and isoflavones, are relatively common in human diets [2]. The flavonol moiety (2,3-double bond in conjugation with a 4-oxo group and a 3-hydroxyl group) and the 5,7-dihydroxylation at the A-ring (Scheme 1) are important structural features for significant antioxidant activity [3]. In addition to –OH moieties in the structural arrangements of flavonols, resonance of electrons between the A- and B-rings is very important for their antioxidant and biological activities [4]. The B-ring –OH moiety is the most significant factor in the scavenging of reactive oxygen species [5].

Flavonoids are particularly abundant in onions, apples, tea, and red wine. These natural products are of interest because of their proposed health-promoting effects as antioxidants [6] and as anticarcinogens [7]. An inverse association between the intake of flavonols and flavones and the risk of coronary heart disease and lung cancer has been shown in epidemiological studies [8, 9].

Kaempferol (KAE, 3,4′,5,7-tetrahydroxyflavone; Scheme 1) is a natural flavonoid that has been isolated from tea [10], broccoli, delphinium, witch-hazel, grapefruit, Brussels sprouts, apples and other plant sources. Owing to their phenolic nature, flavonoids are quite polar but poorly water-soluble, and their limited absorption is well known [11]; these aspects have limited their use in the pharmaceutical field [12].

In pharmaceutical product development, β-cyclodextrins, a category of pharmaceutical excipients, have been widely used to improve solubility, chemical stability and bioavailability of a number of poorly soluble compounds. Cyclodextrins (CDs) (Scheme 1) are cyclic oligosaccharides composed of glucopyranose units and can be represented as a truncated cone structure with a hydrophobic cavity [13]. The cavities of CDs are relatively hydrophobic compared to water, while the external faces are hydrophilic [14]. The most extraordinary characteristic of a cyclodextrin is its ability to form inclusion complexes with a variety of compounds, i.e. caging foreign molecules (guest) in its cavity (host). Generally, hydrophobic molecules or some hydrophobic residues have the highest affinity with the CD's cavity in aqueous solution. It has been well established that the ability of β-cyclodextrin to enhance the stability and solubility of drugs is mediated through the formation of inclusion complexes [15]. The most widely used natural cyclodextrin, β-CD, is limited in its

pharmaceutical applications due to its limited aqueous solubility (18.5 $g\cdot L^{-1}$) [16]. Therefore, chemically modified β-CDs have been synthesized to overcome this problem. Examples include heptakis-(2,6-*O*-dimethyl)-β-cyclodextrin (DMβCD) and hydroxypropyl-β-cyclodextrin (HPβCD).

As a group, cyclodextrin complexes have been formed with several flavonoids (quercetin [17], morin [18], galangin [19, 20], and luteolin [21]) improving their solubility and their antioxidant properties. Also, we have determined the thermodynamic parameters which explain the difference in the association constants (K_a) obtained. Using NMR techniques and molecular modeling studies we have characterized the inclusion geometries for these complexes.

Here we report the preparation of the inclusion complexes of kaempferol with three different cyclodextrins (HPβCD, DMβCD and βCD) in solution. Association constants, estimated from fluorescence studies at different temperatures, were analyzed so as to gain information about the thermodynamic mechanisms involved in the associate processes. Nuclear magnetic resonance (NMR) spectroscopy was used to obtain detailed information about the structure of the inclusion complexes in aqueous solution. Also, the effect that the cyclodextrin has on the antioxidant capacity of kaempferol against reactive oxygen species (ROS) was studied by ORAC-fluorescein (ORAC-$_{FL}$) methodology [22].

2 Experimental

2.1 Materials

Kaempferol (3,4′,5,7-tetrahydroxyflavone) was purchased from Sigma (USA). βCD (β-cyclodextrin), DMβCD (heptakis-2,6-*O*-dimethyl-β-cyclodextrin), HPβCD (2-hydroxypropyl-β-cyclodextrin (MS = 1)), AAPH (2,2′-azobis(2-methylpropionamidine) dihydrochloride), FL (fluorescein disodium salt) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid were purchased from Sigma-Aldrich, Inc., St. Louis, MO. All solvents employed in the spectrophotometric analyses were of spectroscopic reagent grade, from Merck.

2.2 Method

2.2.1 Fluorescence Spectroscopy

Fluorescence spectra were recorded with a LS 55 Perkin–Elmer spectrofluorometer equipped with a xenon lamp source and thermostated bath. Fluorescence measurements were made with excitation and emission bandwidths of 15 and 20 nm, respectively.

The stoichiometry of the complexes was established by the methods of Benesi–Hildebrand and Job's plot. In the Benesi–Hildebrand's method, in the case of a 1:1 complex, the following equation is applicable:

$$\frac{1}{F - F_0} = \frac{1}{F_\infty - F_0} + \frac{1}{(F_\infty - F_0)K_a[\mathrm{CD}]_t} \quad (1)$$

where F_∞ is the fluorescence intensity when the total amount of kaempferol has been complexed in CDs, F_0 is the fluorescence of kaempferol in the absence of CDs and F is the observed fluorescence at each CD concentration tested. If the stoichiometry is 1:1, then the representation of $1/(F - F_0)$ versus 1/[CD] should give a linear plot. The K_a values were obtained by simply dividing the intercepts by the slopes.

The stoichiometry of the inclusion complexes was determined by the continuous variation method (Job's Plot) [23]. Equimolar solutions of KAE and CD were mixed to a standard volume while varying the molar ratio but keeping the total concentration of the species constant.

After stirring for 24 h, the absorbance at 386 nm was measured for all solutions. The collected data were analyzed by plotting ΔA [KAE], where $\Delta A = (A - A_0)$ is the difference in absorbance in the presence and in the absence of CDs, against r, where $r = [\text{KAE}]/\{[\text{KAE}] + [\text{CD}]\}$ is the molar fraction of the guest molecule.

For the determination of association constants (K_a), 300 μL of kaempferol (stock solution 5.0×10^{-4} mol·L^{-1} in methanol) was added to increasing buffered solutions of CDs. The final volume of the system was kept constant at 3 mL buffer, Britton–Robinson 0.1 mol·L^{-1} pH = 8. The resulting mixture was equilibrated in a Julabo thermostatic shaking water bath for 24 h at variable temperature (298–308 K) after which equilibrium was reached. Suitable aliquots of the CD solutions (10 mmol·L^{-1}) were added to the KAE solutions and the fluorescence spectra were recorded.

The fluorescence intensity at any wavelength (F) can be related to the CDs concentration by a non-linear regression described by the following equation [24]:

$$F = F_0 + \frac{(F_\infty - F_0)K_a[\text{CD}]_t}{1 + K_a[\text{CD}]_t} \tag{2}$$

Experimental data of F as a function of [CD] can be fitted to Eq. 2, using as initial parameters K_a and F_0 values obtained from the analysis of the experimental data using the Benesi–Hildebrand equation for 1:1 complexes.

2.2.2 ORAC$_{FL}$ Assay

The ORAC analyses were carried out on a Synergy HT multidetection microplate reader, from Bio-Tek Instruments, Inc. (Winooski, VT), using 96-flat polystyrene microplates with clear bottoms, purchased from Nalge Nunc International. Fluorescence was read through the clear bottom, with an excitation wavelength of 485/20 nm and an emission filter of 520/20 nm. The plate reader was controlled by Gen 5 software. The oxygen radical absorbance capacity was determined as described by Ou et al. [22], with slight modifications. The reaction was carried out in 575 mmol·L^{-1} sodium phosphate buffer (pH = 7.4), and the final reaction mixture was 200 μL. FL (150 μL; 52 nmol·L^{-1} final concentration) and kaempferol in the absence or presence of CDs (70 μL) solutions were placed in the wells of the microplate. The mixture was preincubated for 30 min at 37 °C, before rapidly adding the AAPH solution (30 μL; 19 mmol·L^{-1}, final concentration) using a multichannel pipet. The microplate was immediately placed in the reader and the fluorescence recorded every 1 min for 80 min. The microplate was automatically shaken prior to each reading. A blank with FL and AAPH using sodium phosphate buffer instead of the antioxidant solution and eight calibration solutions using Trolox C (1, 2, 3, 4, 5 and 6 μmol·L^{-1}) as antioxidant were also used in each assay. All reaction mixtures were prepared in triplicate, and at least three independent assays were performed for each sample. In order to avoid a temperature effect, only the inner 60 wells were used for experimental purposes, while the outer wells were filled with 200 μL of distilled water. The results were expressed as relative fluorescence with respect to the initial reading. The area under the fluorescence decay curve (AUC) was calculated by the equation:

$$\text{AUC} = 1 + \sum_{i=1}^{i=80} \frac{f_i}{f_0} \tag{3}$$

where f_0 is the initial fluorescence reading at 0 min and f_i is the fluorescence reading at time i. The net AUC corresponding to the sample was calculated by subtracting the AUC corresponding to the blank.

2.2.3 NMR Spectroscopy

One-dimensional ^{1}H NMR spectra were recorded at 300 K on a Bruker Avance DRX operating at a proton NMR frequency of 300.13 MHz in unbuffered D_2O solutions. Acquisition parameters consisted of a spectral width of 3000 Hz, an acquisition time of 2.67 s and a relaxation delay of 1 s. 128 scans were recorded. FIDs were Fourier transformed with LB = 0.3 Hz and GB = 0. The resonance at 4.7 ppm due to partially deuterated water (HDO) was used as the internal reference.

Rotating-frame Overhauser Effect SpectroscopY (ROESY) spectra were acquired in the phase sensitive mode using 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 3000 Hz. Spectra were obtained from the samples solutions prepared for the ^{1}H NMR studies, using a spin-lock mixing time of 400 ms, relaxation delay 2 s, and 32 scans were recorded.

3 Results and Discussion

In order to verify the stoichiometry of the complex formation, we used the method of continuous variations, often known as the Job's Plot [23], commonly used for determining the stoichiometry of two interacting components. With this method, the total molar concentration of the two components is held constant while their molar ratios are continuously varied. A measurable parameter that is linearly proportional to the complex formed is plotted against the mole fractions to generate a curve. The binding stoichiometry is then determined from the ratio of the mole fractions of the two components found at the maximum of the curve. The stoichiometry of the three complexes was found to be the same for all three CDs utilized, as show in Fig. 1, where there is a turning point at $R = 0.5$, which leads to a consistent conclusion that the molar ratio between kaempferol and the CDs utilized is 1:1.

To quantify the interaction between kaempferol with natural and derivatized CDs, the K_a values were determined using, as an analysis technique, steady-state fluorescence, which takes into account changes in the physicochemical state of this compound with the concentration of CD. The addition of increasing concentrations of CDs resulted in a corresponding decrease in the fluorescence signal [25], in contrast to the behavior observed by other flavonoids [19, 21, 26].

To obtain the association constant, K_a, we used non-linear least-squares regression analysis [24] as an alternative and more appropriate approach than the graphical methods, Fig. 2. The initial parameter estimates needed for this method were obtained from the linear plots. The double-reciprocal plots tend to place more emphasis on lower concentrations in comparison to higher ones. As a result, the value of the slope is dependent upon the ordinate value corresponding to the point having the smallest cyclodextrin concentration. The association constants K_a of the complexes with this methodology at 298 K are 580, 6175 and 5432 L·mol^{-1} for βCD, HPβCD and DMβCD, respectively. This association constant was determined in basic medium (pH = 8) containing 10% methanol, which was needed to improve the 'aqueous' solubility of kaempferol in a CD-free medium. The K_a values calculated by this spectroscopic method were found to be less than those obtained by solubility method [25], due to the fact that for this spectroscopic experiment we need to dissolve KAE

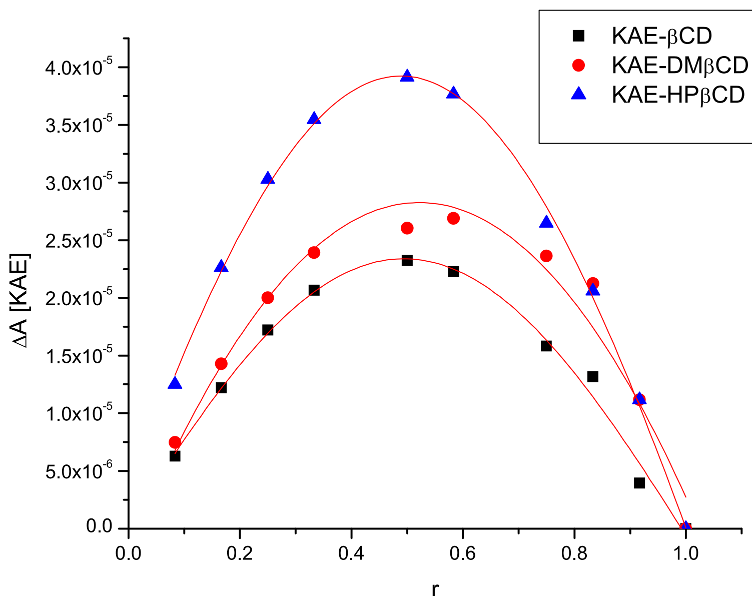


Fig. 1 Continuous variation plot for the KAE-βCD, KAE-HPβCD and KAE-DMβCD systems from absorbance measurements

in organo-aqueous medium to solubilize the flavonoid completely. Methanol was the solvent of choice due to its low affinity for binding with CD.

The association constants, K_a, of the complexes at different temperatures (298, 303 and 308 K) were determinated and the results are summarized in Table 1. As shown in Table 1, the association constants for the KAE-HPβCD and KAE-DMβCD complexes decrease with increasing temperature as expected for an exothermic process, which might be interpreted as a lower degree of interaction at higher temperatures, possibly due to the fact that hydrogen bonds are usually weakened by heating. However, for KAE-βCD, the association constants increase as the temperature rises, as expected for an endothermic process. These types of temperature effects on the association constant were also found for the galangin [19] and luteolin [21] cyclodextrin complexes.

Thermodynamic parameters were calculated based on the temperature dependence of the association constant for KAE-CDs binding. The thermodynamic parameters standard enthalpy changes (ΔH°) and entropy changes (ΔS°) of binding reaction are important to confirm the driving force of interactions of kaempferol with cyclodextrins. It has been generally accepted that the main driving forces for complex formation are hydrogen binding between the hydroxyl groups of the CD and the guest, van der Waals force interactions between host and guest molecules, hydrophobic interaction, and the release of "high energy water" molecules from the cavities of CD to bulk water. Hydrophobic interaction essentially involves favorable positive entropy change together with a slightly positive enthalpy change, while the other forces involve negative ΔH° and ΔS°. The thermodynamic parameters (ΔG°, ΔH° and ΔS°) for the formation of inclusion complexes were determined from the temperature dependence of the association constants, by using classical van't Hoff equation and plotting ln K_a versus $1/T$. The thermodynamic parameters of the host–guest inclusion compounds are listed in Table 1. The negative value for standard state Gibbs energy change (ΔG°) of the three complexes means that the bind-

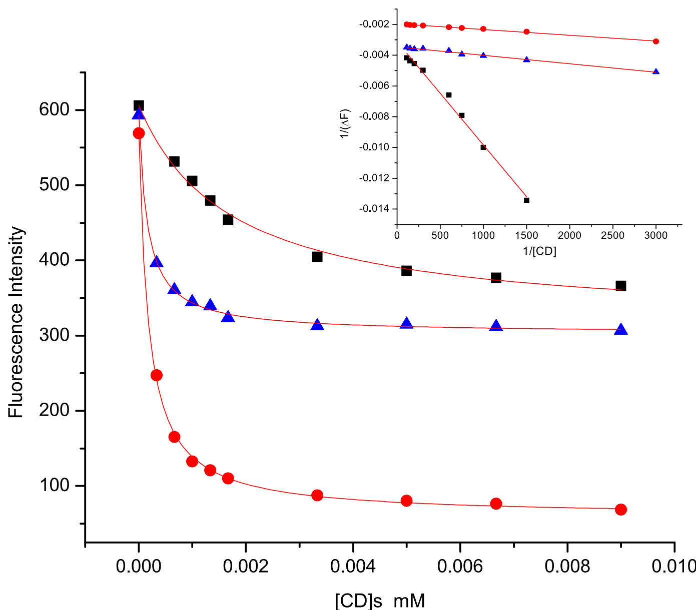


Fig. 2 Fluorescence intensities of kaempferol-CD complexes versus different concentration of CDs. The *insert* shows the double reciprocal plots for kaempferol complexed with (■) βCD, (▲) HPβCD and (●) DMβCD for 1:1 binding stoichiometries at 298 K

Table 1 Apparent stability constant (K_a), thermodynamic parameters and antioxidant capacity (T_{eq}) of kaempferol forming complexes with cyclodextrin

	K_a (L·mol^{-1}) 298 K	K_a (L·mol^{-1}) 303 K	K_a (L·mol^{-1}) 308 K	ΔH° (kJ·mol^{-1})	ΔS° (kJ·mol^{-1}·K^{-1})	ΔG° (kJ·mol^{-1})	T_{eq}
KAE							9.91 ± 0.39
KAE-βCD	580	688	994	41.02	0.19	−15.68	11.95 ± 0.38
KAE-DMβCD	5432	4847	4482	−14.68	0.02	−21.29	11.53 ± 0.36
KAE-HPβCD	6175	4048	3559	−42.16	−0.07	−21.51	12.85 ± 0.32

ing process is a spontaneous process and thermodynamically favored. By inspection of Table 1, ΔH° and ΔS° for KAE-HPβCD are negative in the experimental temperature range whereas for KAE-DMβCD ΔH° is negative and ΔS° is positive, which indicates that for both complexes the inclusion process is exothermic and enthalpically controlled. Different behavior is exhibited by the KAE-βCD complex, where the complexation gave a positive enthalpic change and positive entropic term, indicating that this inclusion is mainly entropically driven. This same type of effect was observed for the complexation of morin [16], galangin [24] and luteolin [21] with βCD. Although the behavior of these flavonoids complexed with DMβCD are enthalpically favored, the entropy changes may be positive or negative. However, for these same flavonoids with HPβCD, the behavior is not similar, in some cases the complexes formation is enthalpy driven (luteolin) and in other cases entropy driven (galangin, morin). No reason for these differences was found.

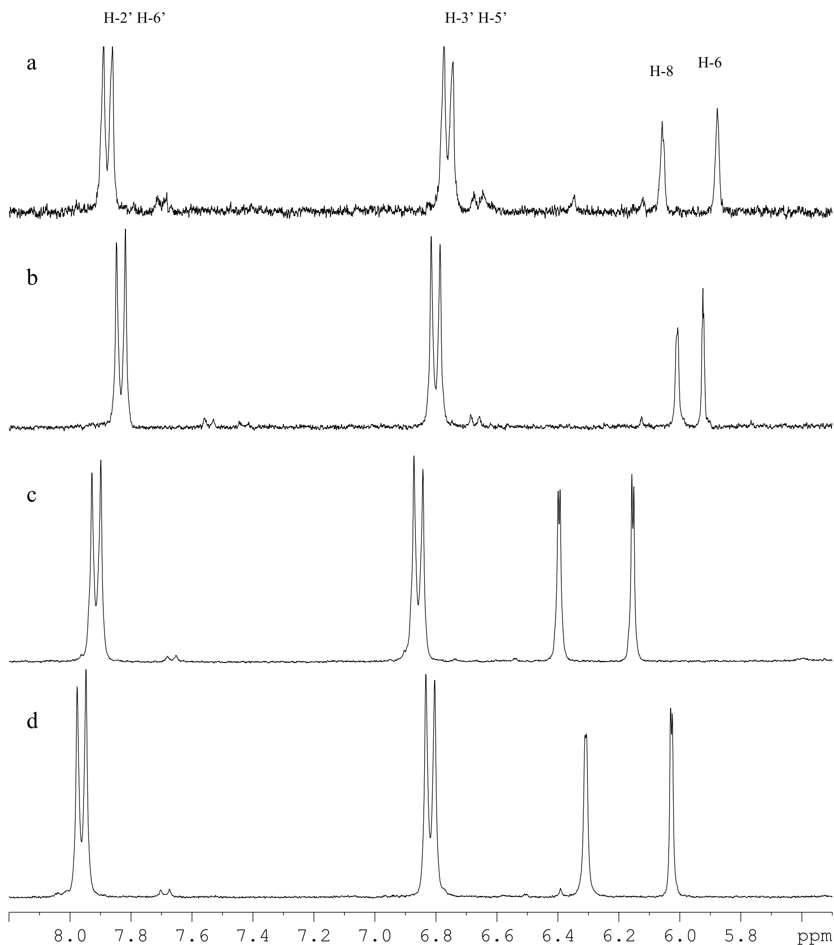


Fig. 3 Chemical shifts of aromatic protons of KAE in the absence and presence of CDs in 10% v/v MeOD in D_2O: (**a**) KAE, (**b**) KAE-βCD, (**c**) KAE-DMβCD and (**d**) KAE-HPβCD

Further support for the inclusion of kaempferol inside the CDs cavity can be obtained using proton nuclear magnetic resonance spectroscopy (^{1}H-NMR), which has proved to be a useful tool in the study of cyclodextrin inclusion complexes. The information gained from NMR spectroscopy relies on the observation of selective line broadening and/or chemical shift displacement of ^{1}H-NMR spectral signals of the guest and host protons.

In the case of aromatic compounds, some of the most important spectral changes that occur upon complexation come from the diamagnetic shielding of the aromatic host on the nearby spins of the guest molecule. In the structure β-cyclodextrin, only hydrogens H-3 and H-5 are located inside the cavity (Scheme 1). H-3 are located near the wider rim of the cyclodextrin cavity while the H-5 hydrogens form a ring near the narrower rim of the methylene (H-6) bearing the primary hydroxyl groups. All other hydrogens (H-1, H-2 and H-4) are located on the exterior of the cavity. In general ^{1}H-NMR spectroscopy provides evidence for inclusion of the drug inside the cyclodextrin cavity, as well as providing information about the geometry and orientation of the incorporated drug molecule. Figure 3 illustrates that most of the aromatic protons of KAE are influenced by the presence of CDs.

Table 2 Change of the ^{1}H-chemical shift ($\Delta\delta$) of KAE-CDs complexes at 300 K. Complexation shifts $\Delta\delta = (\delta_{complex} - \delta_{free})$

Kaempferol Proton	$\Delta\delta$ (ppm) KAE-βCD	$\Delta\delta$ (ppm) KAE-DMβCD	$\Delta\delta$ (ppm) KAE-HPβCD
H-8	0.05	0.28	0.16
H-6	−0.06	0.33	0.25
H-2′	−0.05	0.04	0.08
H-3′	0.04	0.09	0.06
CD proton			
H-3	−0.12	−0.12	−0.02
H-5	−0.14	−0.15	−0.05
H-6	−0.10	−0.09	−0.03

Table 2 lists the detailed variation of the aromatic chemical shifts of KAE before and after forming inclusion complexes with CDs. The induced shift, $\Delta\delta$, is defined as the difference in chemical shift in the absence and presence of the other reactants. In the present case, the induced shifts were calculated by the following equation: $\Delta\delta = (\delta_{(complex)} - \delta_{(free)})$. In this convention, negative and positive signs show high and low frequency shifts, respectively. The formation of inclusion complexes can be proved from the changes of chemical shifts of KAE or CDs in the ^{1}H-NMR spectra. Practically, for the three complexes studied, the B-ring proton doesn't show a variation upon complexation. Moreover, major deshielding is observed for the A-ring protons of KAE-DMβCD. In contrast the KAE-HPβCD complex shows minor changes in chemical shifts and for the KAE-βCD complex the difference in chemical shifts decreases further.

Additional information about the inclusion mode of KAE in the cyclodextrin cavity can be derived from the evidence of spatial proximities between protons of CD and KAE. Two-dimensional NMR is a powerful tool for investigating inter- and intra-molecular interactions. The presence of NOE cross-peaks between protons from two species indicates spatial contacts within 5 Å. To gain more conformational information, we used 2D Rotating-frame Overhauser Effect SpectroscopY (2D-ROESY) to study the inclusion complexes, and the effects were only qualitatively used.

Figure 4 shows a partial contour plot of 2D ROESY spectra of the inclusion complex of KAE-βCD. This spectrum exhibited intermolecular cross peaks between the phenyl ring protons (H-2′,6′ and H-3′,5′) and βCD cavity protons (H-3 and H-5). Other KAE ring protons did not show any cross peaks with βCD cavity protons. These data suggest that the interactions of the B-ring take place in the cyclodextrin cavity, with the rest of the flavonol protruding from the secondary rim, which is in agreement with the results presented by Bergonzi et al. [12].

An expansion of the ROESY spectrum of KAE-DMβCD is reported in Fig. 5. The bidimensional spectrum shows several intermolecular cross-peaks between H-3 and H-5 of DMβCD and protons of the A- and B-ring of kaempferol, demonstrating the inclusion of these groups in the hydrophobic cavity. Moreover, the correlation observed for the B-ring is more intense than that with H-8 of the A-ring, confirming that the guest molecule is introduced within the CD cavity through the wider end, with the rest of the molecule protruding from the secondary face. This is in agreement with the major induced deshielding observed for H-6 and H-8 of KAE-DMβCD where the flavonoid is deeply inserted in the cavity.

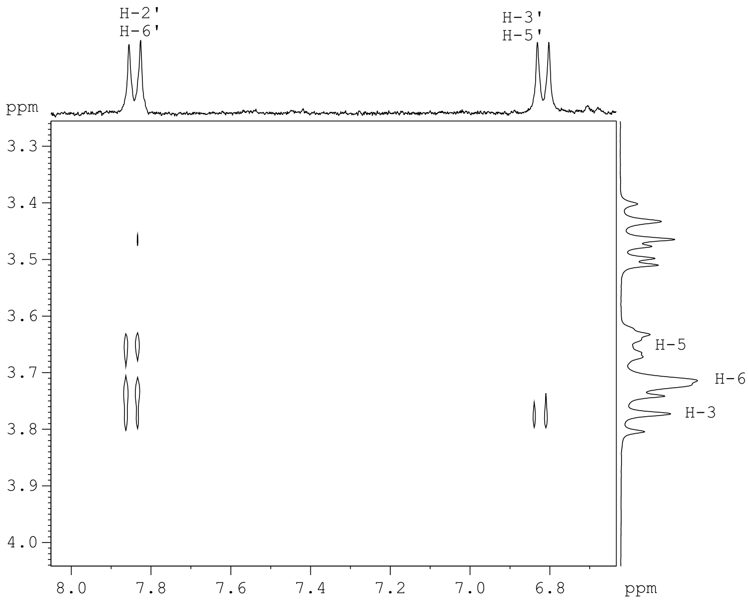


Fig. 4 Partial contour plot of the two-dimensional ROESY spectrum of kaempferol in the presence of βCD in D_2O

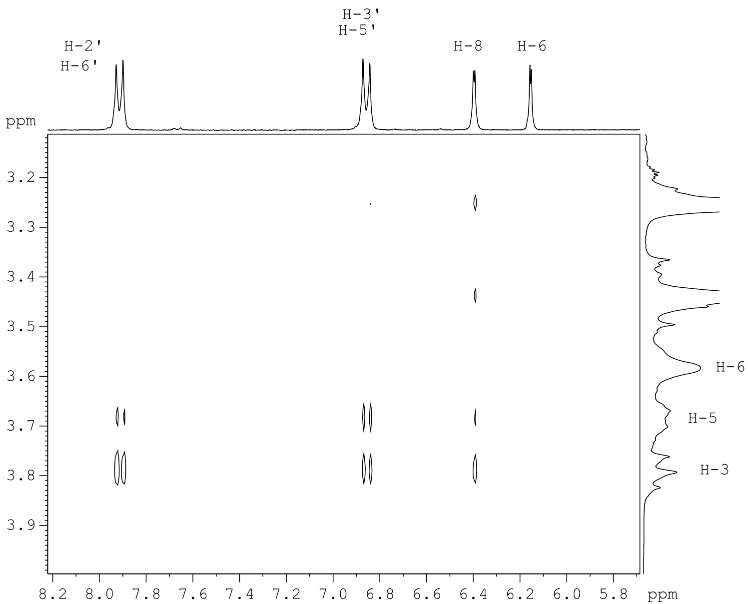


Fig. 5 Partial contour plot of the two-dimensional ROESY spectrum of kaempferol in the presence of DMβCD in D_2O

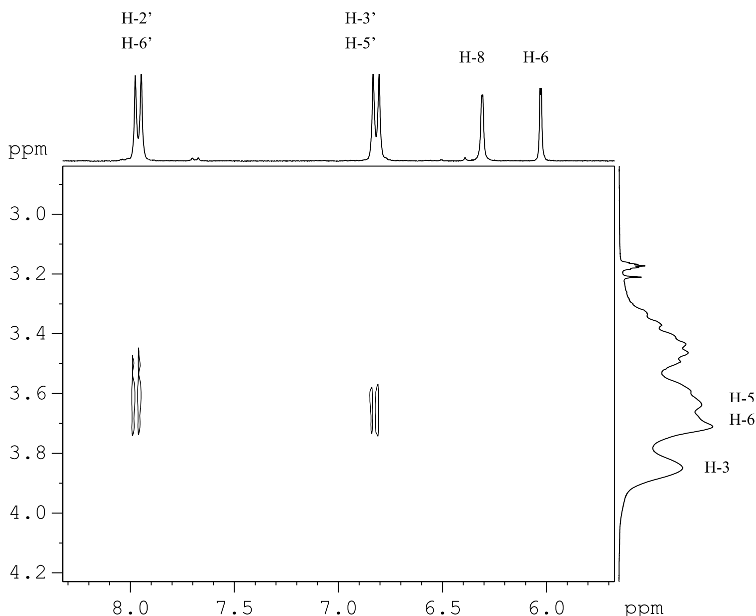


Fig. 6 Partial contour plot of the two-dimensional ROESY spectrum of kaempferol in the presence of HPβCD in D_2O

The HPβCD derivative consists of a mixture of number of closely related species with different degrees of substitution and isomeric forms, which produce very broad NMR peaks. To obtain definite information about the inclusion of KAE inside the HPβCD cavity, a Heteronuclear Single Quantum Coherence (HSQC) spectrum of the KAE-HPβCD system was measured in the same conditions as those used for the ROESY spectrum to attribute unambiguously the inner protons H-3, H-5 and H-6 of HPβCD (data not shown). The ROESY spectrum of the KAE-HPβCD complex, Fig. 6, shows correlation between the B-ring protons with H-5 and/or H-6 of the cyclodextrin, which indicates that the B-ring is inserted in the cavity with the A and C-ring protruding from the secondary rim of the cyclodextrin.

The $ORAC_{FL}$ assay expresses antioxidant activity relative to a standard (Trolox) while measuring the oxidation of the fluorescent substrate by peroxy radicals generated during the reaction. This method follows a hydrogen atom transfer pathway, where the antioxidant and a peroxy radical form a stable antioxidant radical that breaks the radical chain oxidation. In order to discard any antioxidant effect of cyclodextrins per se, the disappearance of fluorescence signal of FL by the attack of the AAPH radical were measured in the presence of increasing concentrations of CDs (in the absence of kaempferol or Trolox). In this case, no effect was observed as the CDs concentrations increased, indicating that CDs, at the concentrations studied, do not act as antioxidants. Figure 7 shows the FL fluorescence decay curves of kaempferol in the presence of AAPH. The linear relationship between net area and antioxidant concentration was calculated at different concentrations. The regression analysis points to the linear response between the kaempferol concentration and the net AUC ($R = 0.999$). Table 1 reports the Trolox equivalent, T_{eq}, for kaempferol free and forming inclusion complexes with the different cyclodextrins referenced to Trolox. We can see that, for the three KAE complexes, the Trolox equivalents are higher than for free kaempferol.

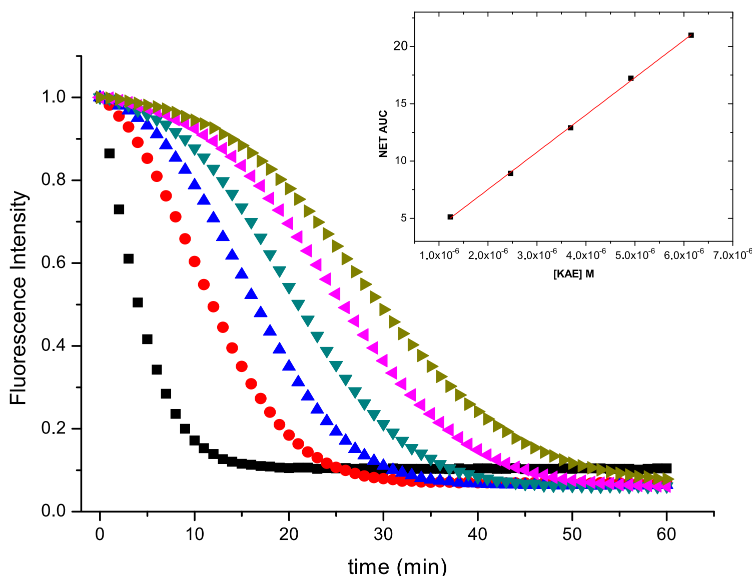


Fig. 7 FL fluorescence decay curves induced by AAPH in the presence of kaempferol at different concentrations: (■) blank, (●) 0.13 μmol·L^{-1}, (▲) 0.27 μmol·L^{-1}, (▼) 0.40 μmol·L^{-1}, (◀) 0.54 μmol·L^{-1}, (▶) 0.67 μmol·L^{-1}. *Insets*: net AUC of kaempferol on different concentrations of kaempferol. The net AUC = $AUC_{sample} - AUC_{blank}$; the AUC were calculated by Eq. 3

The complexes behave as better antioxidants than kaempferol alone. This increment in the antioxidant activity is practically equal for the three complexes. This enhancement of the antioxidant activity could be due to a modification in the redox behavior of the phenol group and/or due the stabilization of the radical in the cyclodextrin cavity [21]. Besides increasing the solubility of flavonoids, cyclodextrins may also have an effect on their antioxidant capacity. Our earlier results indicated that for morin complexes [18] the antioxidant activity increased due to stabilization of the radical inside the apolar cavity. However, for luteolin complexes [21] the antioxidant activity is maintained, and for galangin [19] the antioxidant activity is maintained with βCD but decreases with DMβCD and HPβCD.

4 Conclusion

The association constant and thermodynamic parameters for the inclusion complexes were evaluated by fluorescence spectroscopy. Thermodynamic studies of cyclodextrin complexes indicated that for DMβCD and HPβCD, inclusion is mainly an enthalpy driven process while for βCD it is an entropy driven process. Complex formation was monitored by two-dimensional ROESY experiments through the detection of intramolecular dipolar interactions. ROESY experiments provided data indicating that the B-ring of kaempferol is immersed in the apolar cavity with the A and C-rings protruding from the secondary rim for the three cyclodextrins studied. The antioxidant studies of KAE and CDs complexes showed an increase in their antioxidant activity. The complexes behave as better antioxidants than kaempferol alone; this enhancement of the antioxidant activity could be due to a stabilization of the radical in the cyclodextrin cavity.

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