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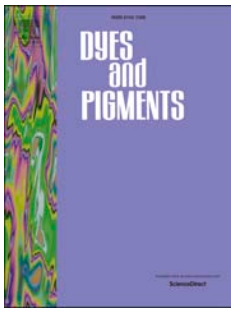

Photochromism of the Complex between4'-(2-Hydroxyethoxy)-7-Hydroxyflavylium and *β*-Cyclodextrin, studied by ^{1}H NMR, UV-Vis, Continuous Irradiation and Circular Dichroism

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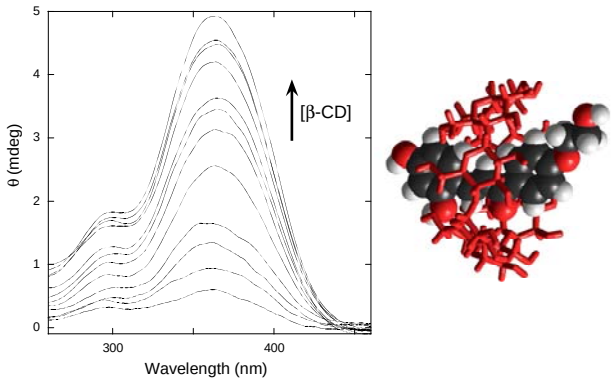
Graphical abstract



Incorporation of the chalcone into β-cyclodextrin leads to an induced circular dichroism signal providing direct evidence for the inclusion complex.

Photochromism of the Complex between4'-(2-Hydroxyethoxy)-7-Hydroxyflavylium and β-Cyclodextrin, studied by ^{1}H NMR, UV-Vis, Continuous Irradiation and Circular Dichroism.

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Abstract

The network of chemical reactions of the compound 4'-(2-hydroxyethoxy)-7-hydroxyflavylium and its photochromic properties were studied in the absence and presence of β-cyclodextrin. The system was characterized by ^{1}H NMR, UV-Vis, continuous irradiation and circular dichroism. In the presence of β-cyclodextrin 4.8 mM the pK'_a is reduced to 2.2. The association constants with β-cyclodextrin, follow the order ***trans*-chalcone>quinoidal base>flavylium cation**, respectively 1.7×10^3 M^{-1}; 5.6×10^2 M^{-1}; ≈0. The rate of inter-conversion of the network species increases in the presence of β-cyclodextrin circa 2.6 times. The appearance of an induced circular dichroism signal of

trans-chalcone inside the β-cyclodextrin is described for the first time. It gives information about the position of the guest inside the host and allows the calculation of the respective association constant.

Keywords: Flavylium, anthocyanins, photochromism, induced circular dichroism, cyclodextrin.

Graphical abstract

1. Introduction

2-phenyl-1-benzopyrilium (flavylium) derived chemical structures are a fascinating family of compounds, involved in a pH (and in many cases light) dependent reversible network of chemical reactions.While the qualitative behavior of the flavylium derivatives is the same, the relative pH dependent mole fraction distribution at the equilibrium and the interconversion kinetics are dramatically changed by the substitution pattern of the flavylium core. This is valid for anthocyanins, anthocyanidins and a series of other natural and synthetic flavylium compounds[1]. In Scheme 1 it is anticipated the network of chemical of the compound 4'-(2-hydroxyethoxy)-7-hydroxyflavylium, studied through this work.

(Scheme 1 here)

According to the general behaviour of the network, flavylium cation is the stable species only at very acidic medium. Raising pH, the quinoidal base, **A**, can be formed as well as hemiketal, **B**. These two species appear through two parallel reactions but due to the fact that proton transfer is the faster reaction of the network, **A** is formed immediately after the pH jump before formation of **B**. The species **A** is thus a kinetic product that can evolve to **B** and from this to the other species of the network thermodynamically more stable. An important detail of the kinetic mechanism was discovered by Brouillard and Dubois [2,3]:in acidic to neutral medium **A** does not react and the system evolves only through the mole fraction of $\mathbf{AH^+}$ available from the equilibrium $\mathbf{AH^+}$/ **A**. Consequently,the hydration reaction decreases by increasing pH. On the other hand, the central ring of the hemiketal, **B**, can open to give the *cis*-chalcone, **Cc**, and this last one isomerise to the *trans*-chalcone, **Ct**, Scheme 1.

β-cyclodextrin, ***β*–CD**, is a cyclic oligosaccharide containing α-(1–4)-linked glucopyranose units adopting a truncated cone structure with hydrophobic cavity which makes it ideal to form host-guest inclusion complexes.The host-guestcomplex has been shown to modify dramatically the equilibrium and rate constants of the flavylium network[4].In this work the network of the compound 4'-(2-Hydroxyethoxy)-7-hydroxyflavyliumis characterized in the absence and presence of ***β*–CD**. Profiting from the induced circular dichroism of **Ct** inside ***β*–CD**, a mathematical procedure to calculate the association constants of **A** with ***β*–CD** was developed.

2. Experimental

2.1. General for synthesis

The compound 4'-(2-hydroxyethoxy)-7-hydroxyflavylium was synthesized according to the method of Katritzky[5]. β-cyclodextrin (β-CD) was purchased from Sigma-Aldrich® (Madrid, Spain).All the reagents were used without any further purification. Elemental analyses were performed in a Thermofinnigan Flash EA 112 series. NMR spectra were run on a Bruker AMX 400 instrument operating at 400.13 MHz (^{1}H) and 100.61 MHz (^{13}C) and deuterated solvents were used as an internal reference.The purity of the compound 4'-(2-hydroxyethoxy)-7-hydroxyflavylium was confirmed by ESI-MS. This analysis was performed on a LTQ Orbitrap XL mass spectrometer (Thermo Fischer Scientific, Bremen, Germany) controlled by *LTQ Tune Plus 2.5.5* and *Xcalibur 2.1.0*. The capillary voltage of the electrospray ionization (ESI) was set to 3100 V. The capillary temperature was 275ºC and the sheath gas flow rate (nitrogen) was set to 5 (arbitrary unit). The capillary voltage was 36 V and the tube lens voltage 110 V.The mass spectrumwas obtained by ESI-MS in positive ion mode.

2.2. General for measurements

The solutions were prepared in Millipore water. The pH of the solutions was adjusted by addition of HCl, NaOH or universal buffer of Theorell and Stenhagen [6] and pH was measured in a Radiometer Copenhagen PHM240 pH/ion meter. UV-Vis absorption spectra were recorded in a Varian-Cary 100 Bio or 5000 spectrophotometer.

2.3. Synthesis

2.3.1. Synthesis of 1-(4-(2-hydroxyethoxy)phenyl)ethanone (This procedure was adapted from references [7,8])

(Scheme 2 here)

4'-hydroxyacetophenone (1.39 g, 10 mmol) and 2-bromoethanol (0.73 mL, 10 mmol) were dissolved in DMF (10 mL), followed by addition of 3 equivalents of potassium carbonate (34.14 g, 30 mmol). The mixture was heated at 100 ºC for 24h. The reaction was monitored by TLC (ethyl acetate:hexane, 1:1) and addition of 2-bromoethanol was needed. After completion of the reaction, the mixture was cooled to room temperature, filtered and the resulting solution combined with water and extracted with chloroform. The organic phase was dried over anhydrous sodium sulfate, filtered and the solvent removed on a rotary evaporator. The residue was purified by column chromatography on silica gel. (Yield: 1.35 g, 75%).

^{1}H-NMR ($CDCl_3$, 400.13 MHz, 298 K) δ (ppm): 7.95 (H_6 and H_2, d, ^{3}J= 8.8 Hz), 6.97 (H_5 and H_3, d, ^{3}J= 9.2 Hz), 4.17 (CH_2*(a)*, t, ^{3}J= 4.4 Hz), 4.02 (CH_2*(b)*, t, ^{3}J= 4.4 Hz), 2.58 (3H, CH_3, s).

2.3.2. Synthesis of 4'-(2-hydroxyethoxy)-7-hydroxyflavyliumhydrogensulfate

(Scheme 3 here)

A solution of 1-(4-(2-hydroxyethoxy)phenyl)ethanone (0.4 g, 2.2 mmol) with one equivalent of 2,4-dihydroxybenzaldehyde (0.31 g) in a mixture of glacial acetic

acid/concentrated sulfuric acid (4 mL:1mL) was stirred overnight at room temperature. Diethyl ether was added to the red solution and a precipitate was formed by freezing with liquid nitrogen. This dark orange solid was filtered, washed several times with diethyl ether and hexane and dried under vacuum. Yield: 0.60 g, 72%. ^{1}H-RMN (CD_3OD + DCl, 400.13 MHz, 298 K) δ (ppm): 9.14 (1H, H4, d, ^{3}J= 8.4 Hz), 8.49 (2H, H2', H6', d,^{3}J= 8.8 Hz), 8.38 (1H, H3, d, ^{3}J= 8.8 Hz), 8.19 (1H, H5, d, ^{3}J= 8.8 Hz), 7.54 (1H, H8, d, ^{4}J= 2.0 Hz),7.44 (1H, H6, dd, ^{3}J= 8.8 Hz, ^{4}J= 2.0 Hz),7.31 (2H, H5', H3', t, ^{3}J= 8.8 Hz),4.29 (CH_2*(a)*, t, ^{3}J= 4.4 Hz), 3.97 (CH_2*(b)*, t, ^{3}J= 4.4 Hz). ^{13}C NMR (CD_3OD + DCl, 100.61 MHz, 298 K) δ (ppm):173.5, 170.7, 168.0, 160.6, 155.0, 154.8, 134.2, 133.6, 122.6, 120.5, 117.4, 113.6, 103.7, 71.7 (O*C*H_2CH$_2$OH), 61.4 (OCH$_2$*C*H$_2$OH). ESI-MS: m/z (%): calcd for $C_{17}H_{15}O_4^+$ (AH^+ species), 283.10; found, 283.09 [M+] (100%). Elemental analysis (%) calcd for $C_{19}H_{19}O_3{\cdot}HSO_4.H_2O$ (M_r= 398.38): C 51.25, H 4.55; Found: C 51.44, H 4.30.

2.4. Circular dichroism measurements

Circular dichroism spectra were recorded in the UV/visible region (260-600 nm) with a Jasco J810 spectropolarimeter (Jasco, Japan) equipped with a temperature control unit Julabo F25. The 4'-(2-hydroxyethoxy)-7-hydroxyflavyliumhydrogensulfate concentration was kept constant ($7.7x10^{-5}$M) and the concentration of *β*-cyclodextrin increased from 0 to $4.67x10^{-3}$M. The CD spectra were recorded with 1 cm (linear) path length quartz cuvette at 20 ^{o}C. For each spectrum, three scans were averaged and the baseline was subtracted from all measurements.

2.5. NMR studies

NMR experiments were recorded on a BrukerAvance II spectrometer, operating at 400.13 MHz (^{1}H), equipped with 5 mm PABBO BB Probe and pulse gradient units, capable of producing magnetic field pulsed gradients in the z-direction of 53 G/cm. The NMR measurements have been done with standard BRUKER pulse sequences, in deuterium oxide (D_2O), at 300K and at pD3.9.^{1}H NMR experiments were performed with water suppression using excitation sculpting with gradients [9], acquisition time 2.56 s, relaxation delay 1 s, 128 or 256 transients of a spectral width of 5342 Hz were collected into 32 K time domain points. Typical measuring conditions for the ^{1}H/^{1}H and 2D spectra (COSY and ROESY) recorded in phase sensitive mode and with water suppression were: relaxation delay 2 s, 64-128 scans, a total 2K data points in *F*2 and 256 data points in *F*1 over a spectral width of 5342 Hz. ROESY experiments were carried out using an intermediate mixing time of 500 ms in the phase-sensitive mode. 2D ^{1}H/^{13}C HSQC and HMBC experiments were carried out with a spectral width of *ca*6410 Hz for 1H and 20125 Hz for 13C, relaxation delay 1.5 s, Fourier Transform (FT) size 2 K × 1K.

For the study of the complex formation between the co-existing equilibrium species of 4'-(2-hydroxyethoxy)-7-hydroxyflavylium and **β–CD**,2.0 mM of the former was dissolved in 99.9% D_2O at room temperature. **β-CD**was added at a molar ratio of 1:1. pD was adjusted to 3.9[10]by the addition of NaOD and samples were allowed to equilibrate for at least 12 hours before performing the NMR experiments.

3. Results and Discussion

The network of chemical reactions shown in Scheme 1 can be simplified by a global acid-base equilibrium (acidity constant K'_a) defined by eq.(1) and eq.(2) where CB refers to all species formed from $\mathbf{A}H^+$, eq.(3).

(**Scheme 4 here)**

$$K'_a = \frac{[CB][H_3O^+]}{[AH^+]} \qquad (1)$$

$$K'_a = K_a + K_h + K_h K_t + K_h K_t K_i \qquad (2)$$

$$[CB]=[Ct]+[Cc]+[B]+[A] \qquad (3)$$

The absorption spectra of equilibrated solutions of the compound 4'-(2-hydroxyethoxy)-7-hydroxyflavylium in the presence of **β-CD** 4.5 mM is shown in Fig. 1A. The shape and position of the absorption bands are compatible with an equilibrium between the acidic species $\mathbf{AH}^+$ and the basic species **Ct** (major) and **A** (minor), permitting to calculate pK'_a=2.2. As in other 7-hydroxyflavylium flavylium compounds the mole fraction of **B** and **Cc** is negligible at the equilibrium [1].

(Figure 1 here)

Kinetic information regarding the interconversion between the network species was achieved by carrying out direct pH jumps defined as a fast addition of base containing buffer in order to change the pH of equilibrated solutions of the flavylium cation, $\mathbf{AH}^+$ at pH=1, to higher pH values. Fig. 1 B reports the absorption spectra taken immediately

after the pH jump. As mentioned above the first product to be formed is the quinoidal base, **A**, with a pK_a=3.7. From the absorption spectra of Fig. 1B to those equilibrated, Fig. 1A, a kinetic process takes place as represented in Fig. 2A. The absorption due to**A/AH**$^+$ disappears to give the absorption spectra of the species at the equilibrium, **Ct** (major) and **A** (minor).

An example of the obtained traces of the absorption of **AH**$^+$/**A** (their relative fraction is dependent on the final pH)is shown Fig 2A. The absorbance decreasing follows a first order kinetic rate. Representing these rate constants as a function of pH leads to a bell shaped curve, Fig. 2B black circles. As mentioned above, in the present compound the mole fraction of the species **B** and **Cc** are negligible at the equilibrium, have not be detected, and by consequence the following approximation is valid

(Scheme 5 here)

$$K_a = \frac{k_a}{k_{-a}}; \qquad K_{Ct} = \frac{k_h k_t k_i}{k_{-h} k_{-t} k_{-i}} \tag{4}$$

$$K_a^{'} = K_a + K_h K_t K_i \tag{5}$$

$$X_{AH+} = \frac{[H^+]}{[H^+] + K_a^{'}} \qquad X_A = \frac{K_a}{[H^+] + K_a^{'}} \qquad X_{Ct} = \frac{K_h K_t K_i}{[H^+] + K_a^{'}} \tag{6}$$

where X_n is the mole fraction of the species n.

In spite of the impossibility of detecting the species **B** and **Cc** this does not mean that they do not have a role in the kinetics of the network. In other words they behave as

transient species, see below Scheme 6. The bell shaped curve is accounted for by eq.(7). Fitting was achieved by means of the parameters presented in Table 1 [11].

$$k_{obs} = \frac{\frac{[H^+]}{[H^+]+K_a} K_h K_t K_i + k_{-i}[H^+]}{[H^+] + \frac{k_i K_t}{k_{-h}}} \quad (7)$$

(Figure 2 here)

(Table 1 here)

3.1. NMR spectral characterization of the inclusion complex between 4'-(2-hydroxyethoxy)-7-hidroxyflavylium and β-cyclodextrin.

Before the study of the formation of the complex between this synthetic flavylium and *β*-cyclodextrin, ^{1}H NMR spectral characterization of this compound in aqueous solution was performed (pD 3.9). Similarly to other 7-hydroxy substituted flavylium compounds the flavylium cation and the *trans*-chalcone were the two forms detected. The assignment of the resonances due to these forms were established on the bases of the analysis of the mono and bi dimensional NMR spectra (COSY, HSQC, HMBC) and are in agreement with data already published for similar compounds [12].

Briefly, the isomer *trans* was recognized based on the large coupling constant of 16 Hz between the vicinal protons, H-4 and H-3. Additionally, long range correlations were observed in the ^{1}H/^{13}C HMBC spectra between protonsH-2',6', H-3 and H-4 with the carbon C2 at 193 ppm (carbonyl group). Assignment of resonances due to protons H-

2',6'and protons H-3',5' in the two different forms follows from the relative intensity of two protons expected from these equivalent nuclei. The proton chemical shifts, coupling constants and carbon chemical shifts for the two equilibrium forms of 4'-(2-hydroxyethoxy)-7-hydroxyflavylium are presented in Table 2.

(Table 2 here)

In order to study the intermolecular interactions between 4'-(2-hydroxyethoxy)-7-hydroxyflavylium co-existing equilibrium forms (AH^+ and C*t*) with β-CD and to define a more detailed structure of the 1:1 complex, a proton-proton NOE experiment was performed. Rotating frame nuclear Overhauser spectroscopy (ROESY) was applied in a D_2O solution containing 4'-(2-hydroxyethoxy)-7-hydroxyflavylium and: ***β*–CD** at equimolar amounts and at pD 3.9. An intermediate mixing time (500 ms) was selected and the evaluation of the intermolecular proximity of protons in the guest specieto the protons located inside of the cyclodextrin cavity ($H\text{-}3_{CD}$, $H\text{-}5_{CD}$ and $H\text{-}6_{CD}$) was performed. An expanded region of 2D ROESY (400 MHz) spectra of 4'-(2-hydroxyethoxy)-7-hydroxyflavylium: ***β*–CD** complex (1:1) showing the selected intermolecular ROE's between ***β*–CD** protons and the aromatic protons of the C*t* equilibrium form of 4'-(2-hydroxyethoxy)-7-hydroxyflavylium is shown in Fig.3.

(Figure 3 here)

In the 2D ROESY spectra of the complex 4'-(2-hydroxyethoxy)-7-hydroxyflavylium: ***β*–CD** strong intermolecular interactions between ***β*–CD** internal protons and the *trans*-chalcone were clearly observed. Also, no interactions between the outer protons (H-$2_{CD}$and H-4_{CD}) and the *trans*-chalcone protons were detected, a clear evidence of inclusion complex formation. For the flavylium cation no intermolecular interactions were detected, a clear evidence of the lack of contact between these two molecules.

Regarding the *trans*-chalcone A ring, correlations between H-6 and H-8 and H-6_{CD} (primary interaction) and H-5_{CD} (weaker interaction) of ***β*–CD** were clearly distinguished. For the H-5 proton a strong interaction with H-5_{CD} and a weaker interaction with H-6_{CD} was detected, which suggests that this part of the molecule is deeply included in ***β*–CD**'s cavity. Regarding protons H-4 and H-3, strong correlations were detected with H-5_{CD}. The former is also slightly correlated with $H_{6\text{-}CD}$ from *β-cyclodextrin* suggesting again a higher penetration in the macrocycle. For the protons located in the *trans*-chalcone B ring, strong intermolecular interactions were detected with ***β*–CD**'s H-3_{CD} and H-5_{CD} protons. Collectively, the ROEs correlations observed propose that the A ring and proton H-4 are deeply included in the cavity being closer to the narrow rim while protons belonging to the B ring are located closer to ***β*–CD**'s wide rim. Concerning the 4' substituent (4.14-3.85 ppm) no interaction was detected with ***β*–CD**'s protons and it could be assumed that this part of the molecule is outside ***β*–CD**'s cavity.

Compared to similar compounds (4',7-dihydroxyflavylium and 3',4',7-trihydroxyflavylium) the results obtained in the 2D ROESY experiments suggest a higher penetration of this compound and a more efficient interaction between the guest and the

host molecule [12]. This proposal is also in agreement with the data obtained in the UV-Vis experiments, which reflect a higher increase in the hydration reaction for 4´-(2-hydroxyethoxy)-7-hydroxyflavylium when compared to 3',4',7-trihydroxyflavylium.

Based on the intermolecular interaction observed in the 2D ROESY a possible structure of the inclusion complex 4'-(2-hydroxyethoxy)-7-hydroxyflavylium: ***β*–CD** is shown on Fig.4.

(Figure 4 here)

3.2. Circular dichroism

Figure 5 shows the circular dichroism spectra obtained upon addition of ***β*–CD** to the *trans*-chalcone form of 4'-(2-hydroxyethoxy)-7-hydroxyflavylium at pH = 4.8. This signal can be interpreted as a manifestation of induced circular dichroism (ICD). This phenomenon takes place when a chiral transparent compound causes a structural perturbation or a coupling of the transition moments with achiral UV-vis absorbing molecule [13]. The technique is particularly useful to investigate inclusion complexes formed between chiral hosts and multistate guest systems since the complexed species show ICD signals while the uncomplexed ones are silent [14].

The spectra depicted in Figure 5 are readily attributed to the complex ***β*–CD-Ct**. The absence of ICD signal for the quinoidal base (minor species at pH = 4.8) confirms that ***β*–CD**displays higher affinity for the *trans*-chalcone and drives the equilibrium toward this state. This feature can also be confirmed by observing the equilibrated spectra of 4'-

(2-hydroxyethoxy)-7-Hydroxyflavylium at pH = 4.8 that upon addition of increasing concentration of **β–CD** show gradual fading of the band assigned to the quinoidal base.

Due to the small changes observed in the UV-Vis absorption spectra of the *trans*-chalcone upon addition of the host, the induced circular dichroism (ICD) spectroscopic technique proven to be the best choice to directly measure the apparent binding constant of the *trans*-chalcone and**β–CD** complex. Fitting the maxima of the ICD band as a function of the host concentration to a 1:1 binding model a binding constant of K_{ass} = $1.7x10^3$ M^{-1} can be obtained. This value is in relatively good agreement with that obtained by the indirect method based on the observed pK_a shifts (K_{ass} = $1.7x10^3$ M^{-1})

(Figure 5 here)

Besides of its usefulness for the determination of the binding constants, the ICD has been recognized as a suitable sensitive tool for structural assignment of the relative alignment of chromophoric molecule inside the **β–CD** chiral cavity [13]. The magnitude and sign of the ICD spectrum of a molecule interacting with a chiral cavity such as the one existing in **β–C**D can unveil its conformation with respect to that microenvironment. The ICD spectrum sign varies depending on the type and depth of the molecule inclusion in the β-cyclodextrin cavity and the orientation of its electronic transition moment relative to the β-cyclodextrin n-fold axis. If it is in a parallel orientation gives a positive ICD band. If it is in a perpendicular orientation gives rise to a negative ICD band. Both types of ICD bands become opposite sign when the chromophore is located partially outside. The observation of a positive signal shows that the transition moment of the *trans*-chalcone is

parallel to the n-fold axis in agreement with the structure suggested from the NMR study described above. Careful comparison of the ICD band with the UV spectrum of the *trans*-chalcone under same conditions (pH = 4.8, [***β*–CD**] = 4.7 mM) shows that the ICD band is distorted and maximum slightly blue-shifted relatively to the UV-Vis absorption spectra. In addition the relative intensity of the shoulder observed at ca. 311 nm is considerably lower in the case of the ICD band. This spectral ICD distortion suggests that the guest experiences considerable conformational flexibility and can also result from the fact that it cannot be completely immersed inside the cavity of **CD** due to his large size.

3.3. Network of chemical reactions of 4'-(2-hydroxyethoxy)-7-hydroxyflavylium inside and outside the host

The equilibrium constants of the network reported in Table 1 allow the construction of a energy level diagram where the respective species are positioned from the relationship $\Delta G^0 = -RT \ln K$ where ΔG^o is the standard Gibbs energy, R the gas constant, T the absolute temperature and K the equilibrium constant for each process of the network, Scheme 6 black color [11].

Identical experiments were carried out in the presence of ***β*–CD**, Scheme 6 red color. The two schemes can be superimposed by means of the association constant between the species of the network and the ***β*–CD**. As in the case of other flavylium compounds, the interaction between the flavylium cation of 4'-(2-hydroxyethoxy)-7-hydroxyflavylium, and ***β*–CD** seems to be very weak so that in the millimolar concentration range the formation of complex can be neglected. This allows to superimpose the two energy level diagrams from a common energy level of the flavylium cation, Scheme 6.

(Scheme 6 here)

Considering the association constant between *trans*-chalcone with β-**CD** defined by scheme 7 and eq.(8)

(**Scheme 7 here)**

$$K_{assCt} = \frac{[Ct-\beta-CD]}{[Ct][\beta-CD]} \quad (8)$$

The following relation is obtained

$$\begin{aligned} -RT\ln K_{assCt} &= -RT\ln[Ct-\beta-CD] + -RT\ln[Ct] + RT\ln[\beta-CD] \\ \Delta G^0_{assCt} &= \Delta G^0_{Ct-CD} - \Delta G^0_{Ct} + RT\ln[\beta-CD] \end{aligned} \quad (9)$$

or

$$\Delta G^0_{Ct-CD} - \Delta G^0_{Ct} = \Delta G^0_{assCt} - RT\ln[\beta-CD] \quad (10)$$

Eq.(10) shows that the difference between the energy levels of the host-guest complex and the free guest in water are dependent on the concentration of the host. They have the same energy level when [***β*–CD**]=K_{assCt} and for each order of magnitude higher or lower (for example [***β*–CD**]=0.1K_{assCt}) are separated by 5.7 kJ mol^{-1}.

According to eq.(10) the differences between the standard Gibbs energy of the species **Ct** inside and outside the host can be calculated from the association constant obtained by circular dichroism, for a determinate host concentration, in the present case 4.5 mMleading to -5 kJ mol^{-1}. This value compares with -4.5 kJ mol^{-1} from the data of Scheme 6, which is a good agreement taking into account the error associated to the

determination energy levels reported in Scheme 6. On the other hand, the use of eq.(10) considering the energy level differences of the quinoidal base inside and out side the host in Scheme 6allows to calculate an association constant for the interaction quinoidal base and **β–CD** K_{assA}=562 M^{-1}.

3.4. Photochemistry

Irradiation of the compound 4'-(2-hydroxyethoxy)-7-Hydroxyflavyliumin the presence and absence of **β–CD** is shown in Fig. 6. The absorption spectrum of *trans*-chalcone disappears with concomitant formation of an absorption in the visible due to the flavylium cation and some quinoidal base.

(Figure 6 here)

The efficiency of photochromism is improved in the presence of the host due to the increasing of the quantum yield.

4. Conclusions

The photochromism of flavylium derivatives can be modulated by complexation with β-cyclodextrin. The quantum yield for formation of the photoproduct increases in the presence of this host and the thermal back reaction is accelerated circa 2.6 times. The pH domain of the photochromism is shifted by almost one pH unit to the acidic region. One interesting aspect described for the first time in this work is the existence of a circular dichroism signal of the *trans*-chalcone induced by the β-cyclodextrin, permitting to

calculate the respective association constant as well as to get information about the structure of the host-guest complex.

Acknowledgments

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Scheme 1. Network of chemical reactions involving the compound 4'-(2-hydroxyethoxy)-7-hydroxyflavylium $K_n=k_n/k_{-n}$ (n=a,h,t,i).

Scheme 2. Numbering scheme for the assignment of the ^{1}H NMR spectrum of 1-(4-(2-hydroxyethoxy)phenyl)ethanone.

Scheme 3. Numbering scheme for the assignment of the ^{1}H NMR spectrum of 4'-(2-hydroxyethoxy)-7-hydroxyflavyliumhydrogensulfate.

Fig. 1.(A) Spectral variations of the compound 4'-(2-hydroxyethoxy)-7-hydroxyflavylium $1.5x10^{-5}$ M in the presence of *β*-cyclodextrin 4.5 mM. (B) the same immediately after the pH jump.

Fig. 2. (A) Spectral variations after a direct pH jump from pH=1 to 4.2 of the compound 4'-(2-hydroxyethoxy)-7-hydroxyflavylium, $1.2x10^{-5}$ M; (B**)** Bell shape curve in the absence of *β*-cyclodextrin (black circles) and in the presence of of*β*-cyclodextrin 4.5 $x10^{-3}$ M (open circles). Fitting was achieved with eq.(4), see Table 1.

Fig. 3. Expanded region of 2D ROESY (400 MHz) spectra of 4´-(2-hydroxyethoxy)-7-hydroxyflavylium:*β*-CD complex (1:1) showing the selected intermolecular ROE's between *β*-CD protons and the aromatic protons of 4'-(2-hydroxyethoxy)-7-hydroxyflavylium (Ct equilibrium form).

Fig. 4. Possible structure of the inclusion complex of the *trans*-chalcone form of 4'-(2-hydroxyethoxy)-7-hydroxyflavylium and *β-cyclodextrin* in D_2O.

Fig.5. Circular dicroism spectra of the compound 4'-(2-hydroxyethoxy)-7-Hydroxyflavylium in the presence of increasing concentrations of *β*-cyclodextrinat pH = 4.8.

Scheme 6. Representation of the network of chemical reactions of the compound 4'-(2-hydroxyethoxy)-7-hydroxyflavylium in water (black); in the presence of :□□*β*□-CD 4.5 mM (red). The interaction of the host with flavyliumcation is neglected.

Fig. 6.(A)Spectral variations of the compound 4´-(2-hydroxyethoxy)-7-hydroxyflavylium, $1.5x10^{-5}$ M in water at pH=3.1 upon irradiation at 375 nm, Φ=0.05. (B) Identical variations take place in the presence of *β*-CD 4.5 mM, Φ = 0.17, pH=3.1.

Table 1. Fitting parameters of 4'-(2-hydroxyethoxy)-7-hydroxyflavylium in the absence and presence of **β-CD** 4.5 x10^{-3} M.

β-CD /mM	**pK'_a**	**pK_a**	**$K_hK_tk_i$/M s^{-1}(1)**	**k_{-i}/s^{-1}***	**K_tk_i/k_{-h}/M(1)**	**k_h/s^{-1}(1)**
-	3.0±0.05	4.1±0.05	3.1x10^{-7}	3.0x10^{-4}	4.0x10^{-6}	0.08
4.5	2.2±0.05	3.7±0.05	1.6x10^{-6}	3.0x10^{-4}	1.5x10^{-6}	0. 11

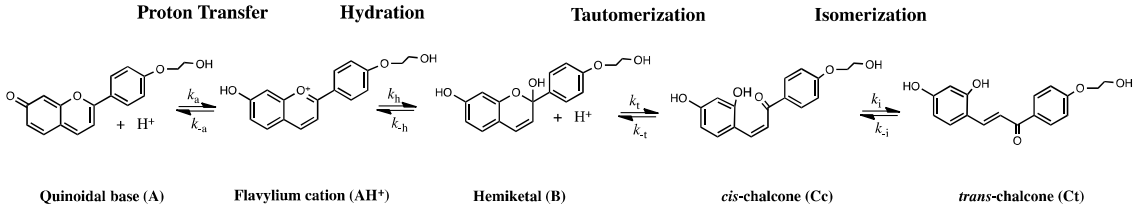
(1) Estimated error 20 %

Table 2

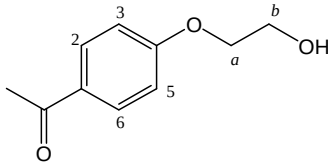
Proton chemical shifts, coupling constants and carbon chemical shifts for the two equilibrium forms of 4'-(2-hydroxyethoxy)-7-hydroxyflavylium in D_2O at pD 3.9.

4'-(2-hydroxyethoxy)-7-hydroxyflavylium				
	Flavylium cation (AH^+)		*Trans*-chalcone (C*t*)	
	^{1}H δ (ppm) / *J* (Hz)	^{13}C δ (ppm)	^{1}H δ (ppm) / *J* (Hz)	^{13}C δ (ppm)
H-3	8.04 *d (9.8)*	112.0	7.42 *d (16.2)*	118.6
H-4	8.84 *d (9.8)*	153.0	7.76 *d (16.2)*	141.4
H-5	7.94 *d (9.2)*	132.5	7.43 *d (9.0)*	131.1
H-6	7.26 *dd (9.1; 2.2)*	121.2	6.36 *dd (8.8; 2.3)*	108.3
H-8	7.29 *d (2.2)*	102.3	6.26 *d (2.3)*	102.3
H-2',6'	8.22 *d (9.3)*	132.1	7.85 *d (9.1)*	130.8
H-3',5'	7.10 *d (9.3)*	116.1	6.98 *d (9.3)*	114.3
a	4.14 *t (4.4)*	69.6	4.10 *t* (4.4)	69.3
b	3.85 *t (4.6)*	59.5	3.85 *t* (4.6)	59.6

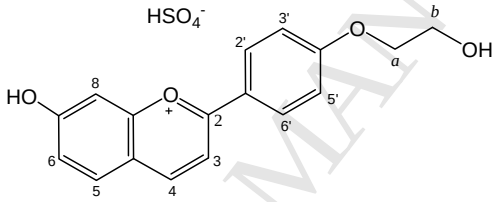
d, doublet; *dd*, double doublet; *t*, triplet



Scheme 1.



Scheme 2.



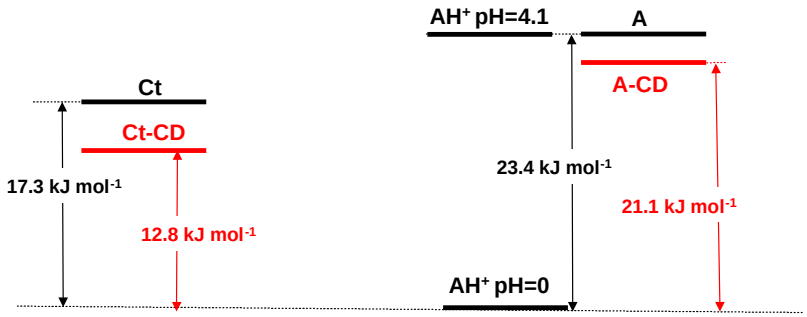
Scheme 3.

$$\mathbf{A}H^+ + H_2O \rightleftharpoons \mathbf{CB} + H_3O^+$$

Scheme 4.

$$\mathbf{A} + \mathbf{H}^+ \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} \mathbf{AH}^+ \underset{k_{-h}k_{-t}k_{-i}}{\overset{k_hk_tk_i}{\rightleftharpoons}} \mathbf{Ct} + \mathbf{H}^+$$

Scheme 5.



Scheme 6.

$$\mathrm{Ct} + \beta\text{-CD} \overset{K_{\mathrm{assCt}}}{\rightleftharpoons} \mathrm{Ct}\text{-}\beta\text{-CD}$$

Scheme 7.

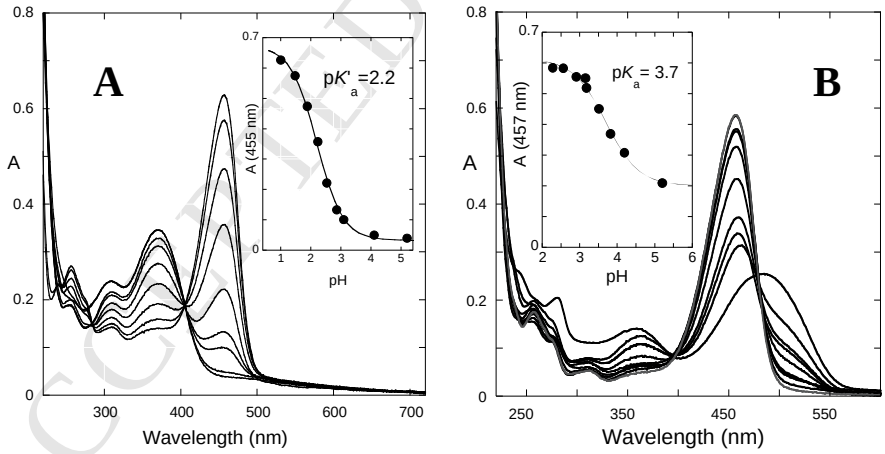


Fig. 1.

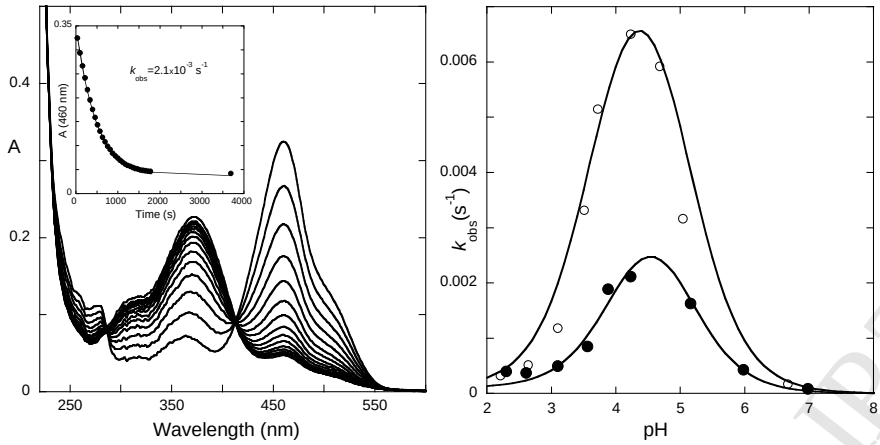

A
Wavelength (nm)
A (460 nm)
Time (s)
k_{obs}=2.1x10^{-3} s^{-1}
k_{obs}(s^{-1})
pH

Fig. 2.

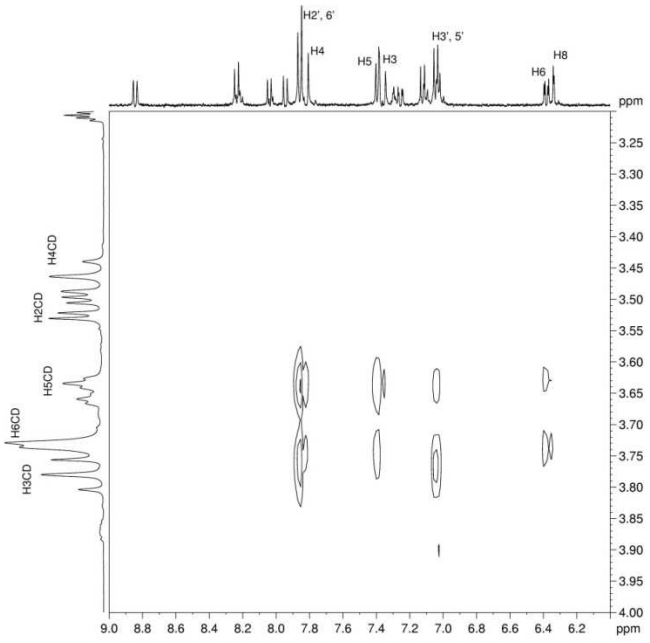

H2', 6'
H4
H5
H3
H3', 5'
H6
H8
H4CD
H2CD
H5CD
H6CD
H3CD
ppm

Fig. 3.

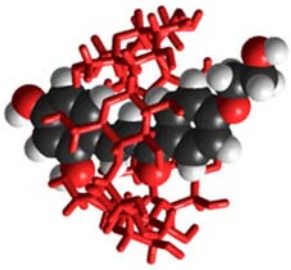


Fig. 4.

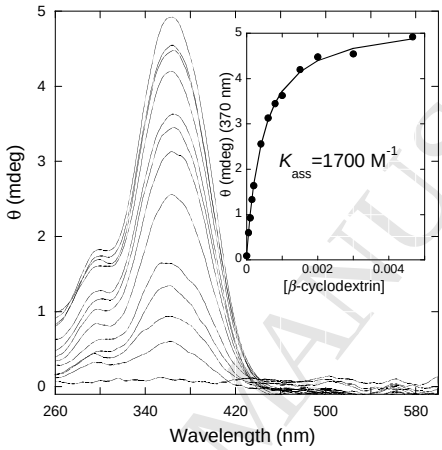


Figure 5

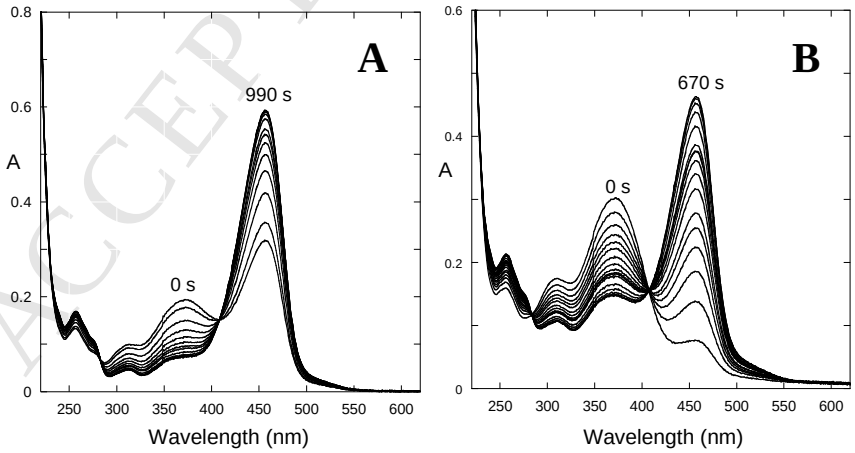


Fig. 6.

The chalcone derived from a flavylium compound forms an inclusion complex with β-cyclodextrin.

The host-guest complex was characterized by rotating frame nuclear Overhauser spectroscopy (ROESY) and circular dichroism.

A mathematical model was deduced to account for the thermodynamic and kinetics of the photochromic host-guest system.