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Photochromism of the Natural Dye 7,4'-Dihydroxy-5-methoxyflavylium (dracoflavylium) in the presence of (2-Hydroxypropyl)-*β*-cyclodextrin.

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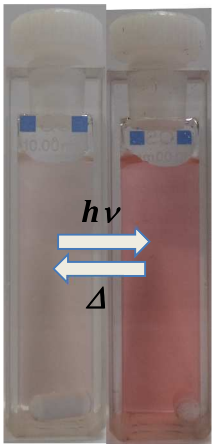
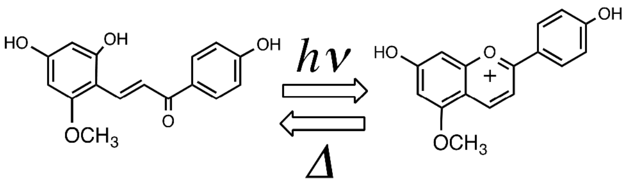


Improvement of the photochromism of the natural dye 7,4'-dihydroxy-5-methoxyflavylium (dracoflavylium) was achieved by incorporation in the cavity of hydroxypropyl-*β*-cyclodextrin.



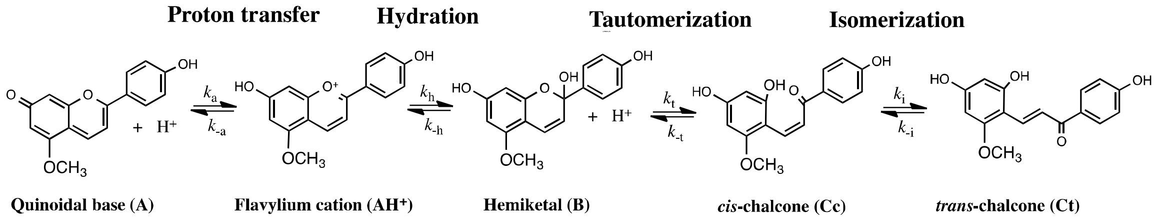
Abstract

A photochromic systems based on dracoflavylium a natural pigment extracted from Dragon's blood, a resin appearing in the injury parts of the tree *Dracaena draco*, is studied in water. The photochromism arises from the irradiation of the *trans*-chalcone, giving a mixture of flavylium cation/quinoidal base as photoproducts via *cis*-chalcone and hemiketal. The performance of the photochromic system can be improved in the presence of (2-Hydroxypropyl)-*β*-cyclodextrin. A mathematical model to account for the details of the kinetics and thermodynamics of the system was deduced. The model is general for all the host guest systems involving the flavylium network of chemical reactions with 1:1 stoichiometric association.

Key: words Photochromism; flavylium; cyclodextrins; anthocyanins; dragon's blood.

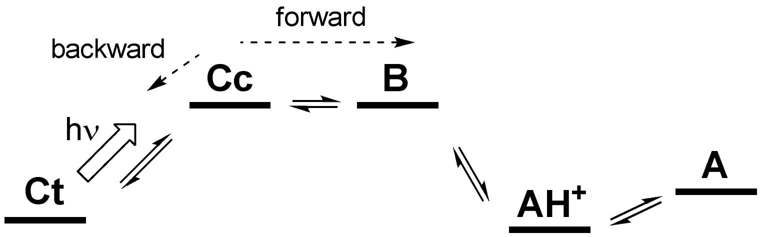
Introduction

Flavylium compounds have been identified as one of the recent families of photochromic compounds.[1] 2-phenyl-2-benzopyrylium (flavylium) is the core of many natural and synthetic compounds comprising anthocyanins, the dyes responsible for most of the red and blue color of flowers and fruits. In solution, practically all flavylium derivatives follow the same network of chemical reactions as shown in Scheme 1 for 7,4'-Dihydroxy-5-methoxyflavylium (Dracoflavylium). Actually, the flavylium cation is the dominant species only at very low pH values. As the pH increases other species are formed, the respective mole fraction distribution at the equilibrium depending on the pH as well as the substitution pattern of the flavylium, see, Fig. 1.



Scheme 1. Network of chemical reactions involving 7,4'-Dihydroxy-5-methoxyflavylium (Dracoflavylium). Equilibrium constants defined as $K_n = \frac{k_n}{k_{-n}}$ $(n=a,h,t,i)$

Flavylium derivatives network of chemical reactions have another interesting property: the possibility of exhibit photochromism. A very useful way to visualize the photochromism is the use of an energy level diagram, Scheme 2.[2] The photochromism takes place upon irradiation of the *trans*-chalcone, leading to the *cis*-chalcone, which can give back the *trans*-chalcone or go forward to form flavylium cation/quinoidal base, depending on pH (and the pK_a of AH^+/A). The photoproducts, flavylium cation/quinoidal base are the network forms exhibiting the most intense colors.



Scheme 2. Photochromism of the flavylium network of chemical reactions.

The forward reaction depends on pH, is favoured at low pH values, and by consequence the efficiency of the photochromic system is modulated by the concentration of protons. However, there is an optimal pH value to carry out the irradiation; if the pH is very low, the flavylium cation is already present at the equilibrium and there is color prior to the irradiation. Fig.1 illustrates the situation.

The research regarding photochromic systems base on natural flavylium compounds is very appealing in the frame of the Green Chemistry approach. Anthocyanins, the most known flavylium derivatives, are not useful for this purpose. In anthocyanins the dominant species at the equilibrium at moderately acidic pH values is hemiketal (B), which does not present any steady state photochemistry. While the formalisms, procedures to solve all possible photochromic systems of the type AB have been nicely reviewed[3] the multistate systems pose other challenges. One of the aims of this work was the deduction of a simple mathematical model to account for the kinetic and thermodynamic behaviour of the system.

In particular, the photochromic system based on dracoflavylium displays color prior to irradiation due to the presence of quinoidal base at the equilibrium in a wide pH range around the neutral contitions, see Fig. 1B. In previous work it was observed that cyclodextrins complex preferentially *trans*-chalcone in comparison with quinoidal base.[4] This result prompted us to study the performance of the dracoflavylium system in the presence of (2-Hydroxypropyl)-β-cyclodextrin (HCD). The hydroxyl substituent confers a better solubility in comparison with the non-substituted cyclodextrin allowing the use of higher concentrations of the host and by consequence a higher extent of complexation. The system was used to test a new mathematical model to account for the host-guest behavior of flavylium compounds.

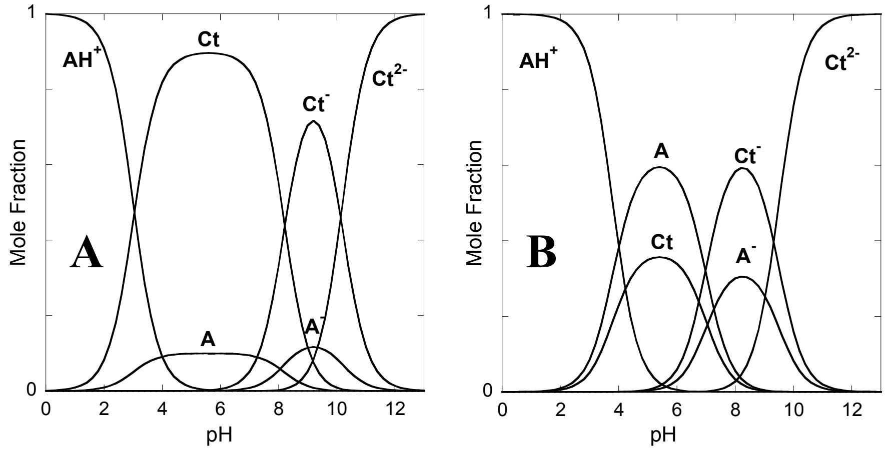


Figure 1. Mole fraction distribution of the flavylium network species at the equilibrium: **A**-7,4'-Dihydroxyflavylium[5]; **B**- 7,4'-Dihydroxy-5-methoxyflavylium (Dracoflavylium).[6]

Experimental Section

The compound 7,4'-Dihydroxy-5-methoxyflavylium (Dracoflavylium) was available from previous studies.[6] (2-Hydroxypropyl)-β-cyclodextrin (average molecular weight $\approx$ 1540 g.mol^{-1}) was purchased from a comercial supplier. All the reagents were used without any further purification. 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[7] 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 with 1 cm path length cuvettes at 21 ºC. All solutions were prepared in ultrapure water obtained from a Milli-Q water filtration system. Unless stated otherwise, the concentration of (2-Hydroxypropyl)-β-cyclodextrin and Dracoflavylium was 1.6x10^{-2} M and 2x10^{-5} M, respectively. Photoexcitation in continuous irradiation experiments were carried out using a xenon/medium pressure mercury arc lamp using a monochromator to select the excitation wavelength (366 nm). Flash photolysis experiments were performed according to a procedure previously described.[8] Data fitting were carried out in a Microsoft Excel spreadsheet using the SOLVER plug-in to minimize the sum of the squared residuals.[9]

Results and discussion

The data regarding the equilibrium constants of dracoflavylium in the absence of the host was reported previously and correspond to equilibrium between **AH^+**, **A** and **Ct**, Table 1 and Fig. 1B.[6]

The absorption spectra taken immediately after a pH jump from equilibrated solutions of the flavylium cation (λ_{max} = 479 nm) in the presence of HCD 16 mM to higher pH values (direct pH jump) is shown in Fig. 2A. The spectral variations are also compatible with formation of the quinoidal base, **A** (λ_{max} = 505 nm). Scheme 1, with an apparent acidity constant equal to $pK_a*=3.4$. The absorption spectra of the same solutions upon thermal equilibration, Fig. 2B, indicate an equilibrium between flavylium cation **AH^+**, *trans*-chalcone, **Ct** (λ_{max} = 374 nm), and quinoidal base **A**. As previously demonstrated, the system can be treated as a single acid-base equilibrium between the flavylium cation and a conjugate base, CB, defined as the sum [CB] = [A]+[B]+[Cc]+[Ct].[1] This equilibrium is defined by an acidity constant K'_a. In the specific case of dracoflavylium the following approximation can be made: [CB] ≈ [A]+[Ct]. Table 1 summarize the data used to fit the eequilibrium and kinetic data obtained for dracoflavylium in the presence and absence of the HCD, see below the respective calculation details.

Table 1. Kinetic and thermodynamic parameters of dracoflavylium (DF) and dracoflavylium:(2-hydroxypropyl)-*β*-cyclodextrin inclusion complex (DF:HCD) obtained from pH jump experiments. See scheme 1 for definition of the equilibrium and kinetic constants.

	pK'_a	pK_a	$K_hK_tk_i$ (M s^{-1})	k_iK_t/k_{-h} (M)	k_{-i} (s^{-1})	k_h (s^{-1})
DF	3.8[a]	4[a]	(2.2±1.0)x10^{-8}	(3.0±1.5)x10^{-6}	(5.5±1.0)x10^{-4}	(7±5)x10^{-3}
DF:HCD	2.4±0.3[b]	3.3±0.2[b]	(1.3±0.3)x10^{-7}	(1.5±0.5)x10^{-6}	(4±2)x10^{-5}	(9±5) x10^{-2}

[a] Values taken from ref. 6. [b] Calculated from equations 3 and 4.

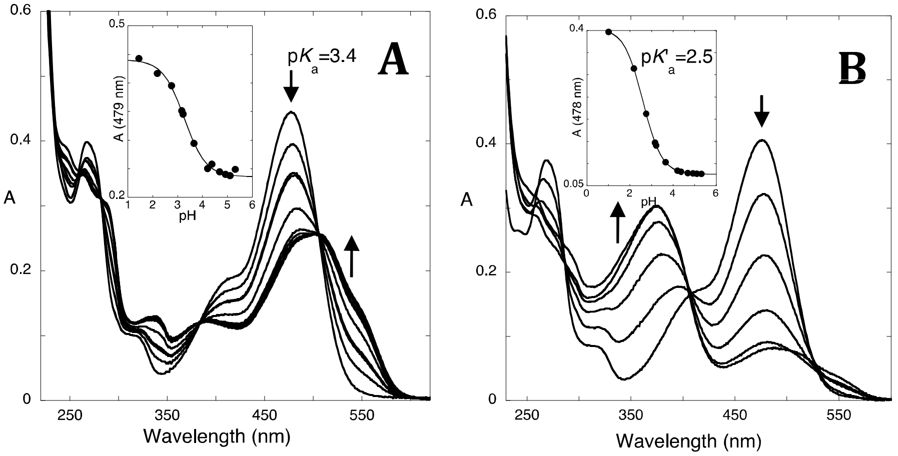


Figure 2.-A. Spectral variations of dracoflavylium (2×10^{-5} M) in the presence of HCD (1.6×10^{-2}M) upon a direct pH jump from pH=1 to higher pH values taken immediately after the mixing (pK_a* = 3.4±0.2, R^2 = 0.99); **B**- the same upon thermal equilibration in the dark (pK_a* = 2.5±0.1, R^2 = 0.99). All spectra were acquired in 1 cm path length disposable plastic cuvettes at 21 ºC. The arrows indicate the appearance or disappearance of the absorption bands upon pH increase.

The behavior of the dracoflavylium network in presence of HCD is similar to that observed in bulk water, but the mole fraction of **A** at the equilibrium (K_a/K'_a) changes from 0.63 to 0.13 in the absence and presence of HCD respectively. The lower percentage of **A** in the presence of the HCD is a good result to the performance of the photochromic system, because reduces the color prior to the irradiation, leading to a high contrast between the dark form and the photoproduct, see below.

The concentrations of the species hemiketal and *cis*-chalcone at the equilibrium are very small. This can be confirmed from the ratio $K_h(1+K_t)/K'_a$ which, gives the fraction of these two species for $[H^+]>>K'_a$ equal 0.2 % and an estimation of 0.3% respectively in the absence and presence of the HCD 16 mM, Table 1.

Full thermodynamic and kinetic characterization of the system is required to rationalize the experimental observations and optimize the photochromic properties of the flavylium-cyclodextrin host-guest complex. The association constant for the flavylium cation- HCD inclusion complex can be obtained from UV-Vis absorption spectroscopy. The spectral variations taking place when solutions of dracoflavylium at pH=1.0 are titrated with HCD are shown in Fig. 3. The interaction of the flavylium cation with the HCD gives rise to a small red shift of the flavylium absorption band, according to a 1:1 adduct with association constant 180±40 M^{-1}.

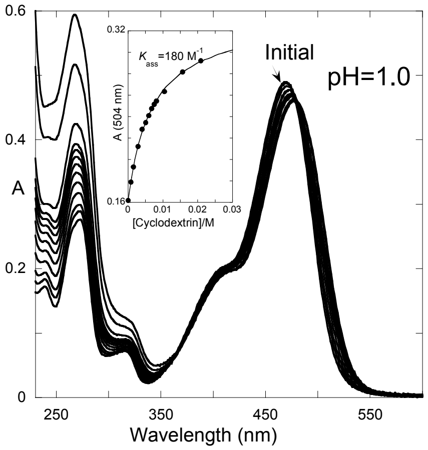


Figure 3. Spectral modifications of dracoflavylium 2x10^{-5} M upon addition of increasing quantities of (2-Hydroxypropyl)-β-cyclodextrin (HCD) at pH=1.0 (K_{AH+CD} = 180±40 M^{-1}, R^2 = 0.99). All spectra were acquired in 1 cm path length disposable plastic cuvettes at 21 ºC.

Knowledge of the association constant for the complexation of the flavylium cation together with the apparent pK_a* and pK'_a* values obtained in the presence and absence of cyclodextrin allows the determination of the association constants for the complexation of the quinoidal base [(7±4)x10^2 M^{-1}] and the *trans*-chalcone [(1.1±0.6)x10^4 M^{-1}] (eq. 1 and 2).[10] It is worth noting that the association constants for the complexation of dracoflavylium species with HCD are approximately one order of magnitude higher than those reported for 7,4'-dihydroxyflavylium with β-CD but follow the same trend: $K_{CtCD}>K_{ACD}>K_{AH+CD}$.[11] The higher affinity of the cyclodextrin for the uncoloured *trans*-chalcone leads to the so-called anti-copigmention effect and profit can be taken, as we show below, to increase the contrast of the photochromic system. With the values of these association constants in hand, together with $K_hK_tK_i = K'_a - K_a\ K_hK_tK_i$, the p$K_a$ and pK'_a ($K'^{CD}_a = K^{CD}_a + K^{CD}_h K^{CD}_t K^{CD}_i$) of the inclusion complex can be directly determined (eq. 3 and 4.). The values are presented in Table 1. When compared with free guest, the inclusion complex displays lower values of pK_a and pK'_a, reflecting the higher stabilization of the neutral species inside the ciclodextrin hydrophobic cavity.

$$K_a{}^* = K_a \frac{1+K_{ACD}[HCD]}{1+K_{AH+CD}[HCD]} \qquad (1)$$

$$K'_a{}^* = \frac{K'_a + \left(K_a K_{ACD} + K_i K_t K_h K_{CtCD}\right)\left[HCD\right]}{1 + K_{AH+CD}\left[HCD\right]} \tag{2}$$

$$K_a^{CD} = K_a \frac{K_{ACD}}{K_{AH+CD}} \tag{3}$$

$$K_h^{CD} K_t^{CD} K_i^{CD} = K_h K_t K_i \frac{K_{CtCD}}{K_{AH+CD}} \tag{4}$$

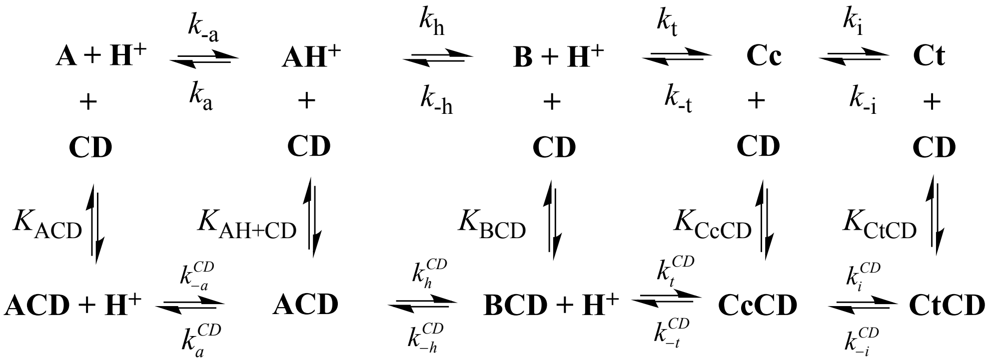
Kinetics of the network

As shown above, the mole fraction distribution of the network species are pH dependent, Fig. 1. The interconversion kinetics can be studied by changing the pH and following the absorbance changes with time (pH jumps). The kinetics is dependent on the existence or not of a thermal *cis-trans* isomerization barrier.[1]

Dracoflavylium is an example of a compound possessing a low *cis-trans* isomerization kinetic barrier. The kinetics of interconversion between **AH^+/A** and **Ct**, which occurs when solutions of flavylium cation at pH=1 are made more basic (direct pH jump) or solutions of **Ct** at moderately acidic solutions are made very acidic (reverse pH jumps, is pH dependent and follows eq.(5).[12] The observed rate constants obtained from several pH jumps are represented in Fig. 4. The experimental data can be satisfactorily fitted with eq.5 showing that the kinetics of the interconversion behaves according to the proposed mechanism (i.e. low *cis-trans* isomerization kinetic barrier). In order to increase the accuracy of the parameters obtain from the fitting procedure K_a was held constant (10^{-4} M) and the following restriction was imposed: $K_a+K_hK_tK_i = K'_a$ ($10^{-3.8}$ M). The obtained parameters are shown in Table 1. The k_h value can be directly obtained from the division of $K_hK_tk_i$ by K_tk_i/k_{-h}.

$$k_{obs} = \frac{\dfrac{[H^+]}{[H^+]+K_a} K_h K_t k_i + k_{-i}}{[H^+] + \dfrac{K_t k_i}{k_{-h}}} \tag{5}$$

Scheme 3 shows all interactions of the flavylium network with HCD. The rates of formation and dissociation of β-cyclodextrin inclusion complexes with common organic molecules are usually above 10^8 $M^{-1}s^{-1}$ and 10^4 M^{-1}, respectively.[13] Therefore, the complexation reactions of HCD with the different network species are considered to be in fast equilibrium in our kinetic analysis.



Scheme 3. The network of chemical reactions of a flavylium system in the presence of a host.

According to Scheme 3 there are two possibilities of reaction, outside and inside the host. These two processes should be taken into account because the association constants of the flavylium species and HCD are relatively low. In appendix 1 the deduction of the kinetic equations to account for the global process is presented, eq.(6) regarding the kinetics in the bulk water and eq.(7) inside the host. The experimentally observed rate constants are given by the sum of these two equations.

$$k_{obs}=\frac{\dfrac{\dfrac{1}{(1+K_{AH+CD}[CD])}[H^+]}{[H^+]+\dfrac{K_a(1+K_{ACD}[CD])}{(1+K_{AH+CD}[CD])}}K_hk_iK_t+\dfrac{k_{-i}}{1+K_{CDCt}}[H^+]}{[H^+]+k_i\dfrac{K_t}{k_{-h}}} \qquad (6)$$

$$k_{obsCD}=\frac{\dfrac{\dfrac{K_{AH+CD}[CD]}{(1+K_{AH+CD}[CD])}[H^+]}{[H^+]+\dfrac{K_a(1+K_{ACD}[CD])}{(1+K_{AH+CD}[CD])}}K_h^{CD}k_i^{CD}K_t^{CD}+k_{-i}^{CD}\dfrac{K_{CtCD}[CD]}{(1+K_{CtCD}[CD])}[H^+]}{[H^+]+\dfrac{k_i^{CD}K_t^{CD}}{k_{-h}^{CD}}} \qquad (7)$$

In eq.(7) the superscript CD corresponds to the processes taking place inside the host. Eq.(6) and (7) are very similar to eq.(5) and still behave as a bell shaped curve. In order to increase the accuracy of the fitting, previously determined values are held constant in equations 6 and 7. This reduces the number of fitting parameters to three: $K_h^{CD}K_t^{CD}k_i^{CD}$, k_{-i}^{CD} and $\dfrac{K_t^{CD}k_i^{CD}}{k_{-h}^{CD}}$. In addition, and considering that the mole fraction distribution of the B and Cc are negligible, the following constraint can be introduced in order to restrict the number of possible solutions to a minimum: $K'^{CD}_a = K_a^{CD} + K_h^{CD}K_t^{CD}K_i^{CD}$.

The association constants for the formation of inclusion complexes between HCD and AH^+, A and Ct were previously determined (see above).

A global fitting taking into account eq.(6) and (7) as well as the restrictions described above was achieved for the parameters reported in Table 1 and is represented in Fig. 4.

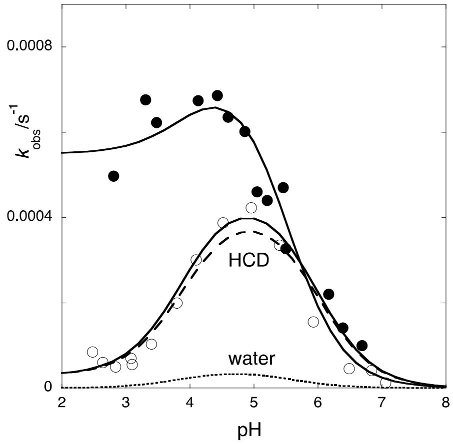


Figure 4. Observed rate constants of the interconversion between AH^+/A and Ct of dracoflavylium in the absence (●) and presence (○) of HCD 1.6x10^{-2} M. Fitting (eq.5) was achieved for $K_hK_tk_i$ = (2.2±1.0)x10^{-8} M s^{-1}; k_{-i} = (5.5±1.0)x10^{-4} s^{-1} and k_iK_t/k_{-h} = (3.0±1.5)x10^{-6} M (R^2 = 0.77) . In the presence of HCD a global fitting was achieved with eq.(6) in bulk water and (7) inside HCD for $K_h^{CD}K_t^{CD}k_i^{CD}$.= (1.3±0.3)x10^{-7} M s^{-1}, k_{-i}^{CD}= (4±2)x10^{-5} s^{-1} and $k_i^{CD}K_t^{CD}/k_{-h}^{CD}$ = (1.5±0.5)x10^{-6} M (R^2 = 0.86). Dashed line contribution from the kinetics inside the host; pointed line contribution from the compound in the bulk water.

Inspection of Table 1 and Fig. 4 shows a small contribution of the reactions taking place in bulk water. The most interesting feature is the increase in the hydration rate constant inside the HCD when compared with pure water. This behavior is consistent with the lower micropolarity of the cyclodextrin nanocavity and previous studies on the effect of solvent polarity in the flavylium network of reactions.[14] On the other hand the decreasing of *trans-cis* isomerization rate constant (k_{-i}) can be ascribed to the constraint effect of the cavity. This does not exclude a possible contribution of the cavity micropolarity. For example, in the case of the *cis-trans* isomerization, a less efficient stabilization of the twisted dipolar transition state could occur when the polarity of the medium decreases. The isomerization of substituted azobenzenes is inhibited by the presence of *β*-cyclodextrin.[15] These results were also interpreted on the basis of polarity arguments but the authors recognized that the structure of the inclusion complexes plays an important role in the isomerization rate constant.

Photochromic system

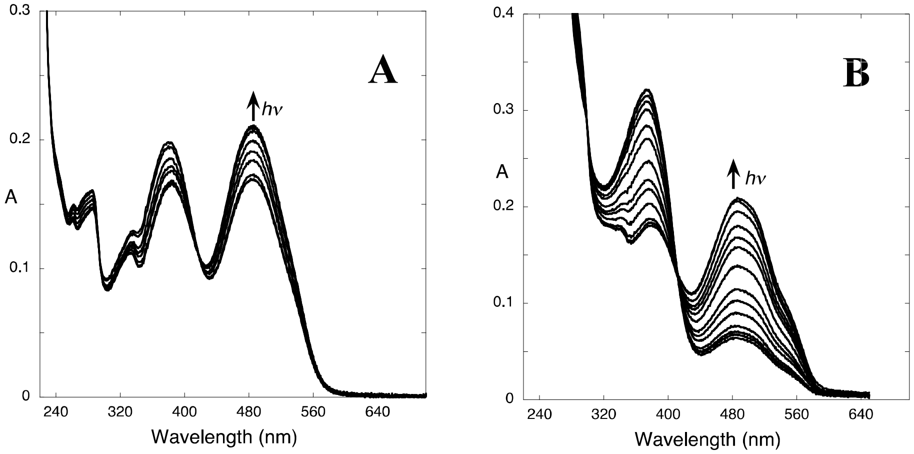


Figure 5. A- Spectral variations upon irradiation at 366 nm of dracoflavylium (2x10^{-5} M) at pH=4.1; **B**- the same at pH=3.1 in the presence of the HCD (1.6x10^{-2} M). Quantum yield is 0.15 at pH= 3.5 in the absence and 0.4 at pH=4 in the presence of 16 mM HCD .[16] Both irradiation and spectral measurements were performed in 1 cm path length quartz cuvettes at ambient temperature.

The preferential stabilization of the *trans*-chalcone in the presence of the *β*-cyclodextrin, increases the performance of the photochromic system, because reduces the percentage of the quinoidal base *i.e.* color prior to the irradiation, increasing in this way the color contrast. As can be observed in Fig. 5, light irradiation at 366 nm leads to an increase of the absorbance in the visible region of the spectrum accompanied by a concomitant decrease of the absorbance in the UV region. This behavior is compatible with the photochemical transformation of Ct into AH$^+$/A. The effect of HCD in the photochromic system can be readily observed since a much higher ΔA is obtained in the presence of the host. It can be also observed that in the presence of HCD the fraction of quinoidal base (maximum absorbance at ≈ 490 nm) is much lower than in the absence of host.

The quantum yield for the photoconversion of the *trans*-chalcone to the quinoidal base/flavylium cation is pH dependent. The values 0.15 (pH=3.5) and 0.4 (pH=4) correspond to the maximum in the absence and presence of [HCD]=16 mM, respectively.

The flash photolysis is another very useful tool to study the flavylium derived photochromic systems, Fig. 6. After the flash the absorption at 385 nm shows a bleaching due to the formation of the *cis*-chalcone, which absorbs less than the *trans*. As shown in Scheme 2 the *cis*-chalcone is not thermodynamically stable and two parallel reactions take place: i) one to recover flavylium cation (trace at 470 nm) and the other, ii) to give back the *trans*-chalcone (trace at 385 nm). As expected from two parallel reactions the observed rate constant is the sum of both processes.

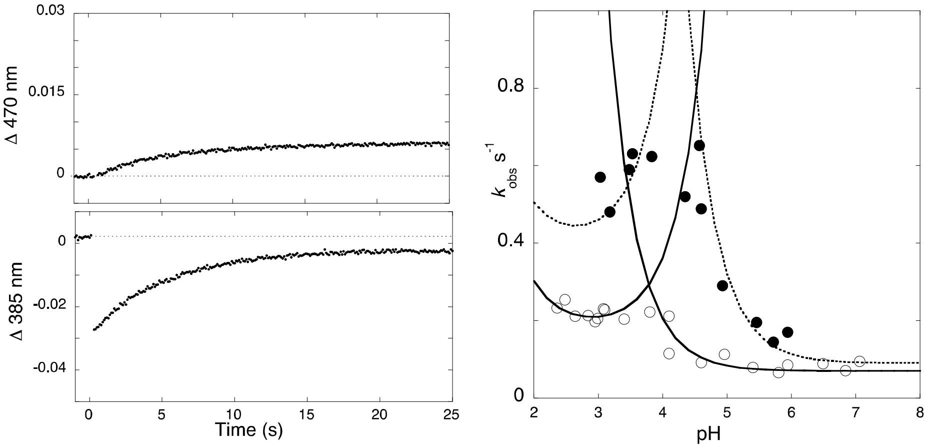


Figure 6. **A**- Flash photolysis traces of dracoflavylium (2x10^{-5} M) in the presence of the HCD (1.6x10^{-2} M) at pH=2.95.**B**- Observed rate constants of the flash photolysis in the absence (●) and presence (○) of (2-Hydroxypropyl)-*β*-cyclodextrin 16 mM. Fitting was achieved with eq.(8-10) for the parameters reported in Table 2 (●R^2 = 0.92 and ○ R^2 = 0.89) All experiments were conducted at 21 ºC.

Representation of the observed flash photolysis rate constants is shown in Fig. 6B. Again, according to the rate-determining step, the kinetic equations are different, see appendix 1 for the respective deduction.

In the case of control by tautomerization occurring at very acidic pH values eq.(8) is followed:[17,18]

$$k_{obs} = \frac{\left(k_i + k_{-t} + k^{H}[H^+] + k^{OH}[OH^-]\right) + \left(k_i^{CD} + k_{-t}^{CD} + k^{CDH}[H^+] + k^{CDOH}[OH^-]\right)K_{CcCD}[CD]}{1 + K_{CcCD}[CD]} \tag{8}$$

In the absence of cyclodextrin eq. (8) reduces to eq. (9)

$$k_{obs} = k_i + k_{-t} + k^{H}[H^+] + k^{OH}[OH^-] \tag{9}$$

When the hydration is the rate determining step eq.(10) is followed.

$$k_{obs} = m[H^+] + b \tag{10}$$

with

$m = \frac{k_{-h}}{1 + K_t}$; $b = \frac{K_t k_i}{1 + K_t}$ in the absence and $m = \frac{k_{-h} + K_{BCD}k_{-h}^{CD}[CD]}{1 + K_t + \left(K_{BCD} + K_{CcCD}K_t\right)[CD]}$;

$b = \frac{K_t k_i + K_{CcCD}K_t k_i^{CD}[CD]}{1 + K_t + \left(K_{BCD} + K_{CcCD}K_t\right)[CD]}$ in the presence of HCD.

Table 2. Kinetic and thermodynamic parameters of dracoflavylium (DF) in the abscence and in presence of (2-hydroxypropyl)-β-cyclodextrin 0.016 M, obtained from flash photolysis experiments.

	m **(M^{-1} s^{-1})**	***b*** **(s^{-1})**	**k_{-t}+k_i (s^{-1})**	**k^H(M^{-1} s^{-1})**	**k^{OH} (M^{-1} s^{-1})**
-	(2.4±0.5)x10^4	(10±5)x10^{-2}	0.55±0.10	≈20	≈1x10^9
HCD	(4±1)x10^3	(7±3)x10^{-2}	< 0.2[a]	≈10	≈0.2x10^9

[a] This parameter is strongly correlated with K_{CcCD}. It can be < 0.2 for K_{CcCD} < 1000 M^{-1}.

The fitting of both equations was achieved with the parameters reported in Table 2. Despite the fact that the obtained parameters are function of rate and equilibrium constants and those cannot be individually evaluated, the results indicate that the overall rate of Cc consumption to give Ct and AH^+/A is lower in the presence of the HCD. In principle more information can be obtained if the flash photolysis parameters are obtained for different concentrations of HCD but this is behind the scope of the present work.

Conclusions

Host-guest interaction of flavylium derived compounds is a useful way to tune the photochromic properties of these compounds. The relative stabilization of the *trans*-chalcone makes the system more acid shifting the pK'_a and pK'_a to lower pH values. The kinetics inside the host compared with water evidences the greater difficulty of the isomerization and tautomerization constituting an interesting example of the effect of the constraint medium. Conversely the hydration of the flavylium cation is faster inside the host probably due to the higher activity of water in the less polar cavity of the cyclodextrin.

Acknowledgements

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