

Photochemistry of 5-Hydroxy-4'-Dimethylaminoflavylum in the Presence of SDS Micelles. The Role of Metastable States of Flavylum Cation-Quinoidal Base and *Trans*-Chalcones

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Abstract

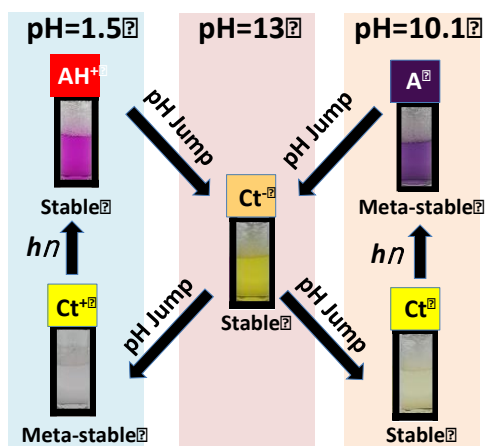
The multistate of species originated by the compound 5-hydroxy-4'-dimethylaminoflavylum was studied in the presence of sodium dodecyl sulfate micelles 0.1 M. A series of pH jumps obtained by addition of base to the equilibrated solutions of the flavylum cation results in a metastable equilibrium between the flavylum cation and the quinoidal base with $pK_a=7.6$. In neutral to moderately basic medium, only at 60° C and after 5 days a new distribution involving the flavylum cation and *trans*-chalcones is achieved. On the other hand, the anionic *trans*-chalcone can be formed in few minutes in very basic medium at room temperature and from this, another meta-stable state constituted by protonated, neutral and anionic *trans*-chalcones, $pK_{Ct^+/Ct}=2.6$ and $pK_{Ct/Ct^-}=10.7$ is reached. The equilibrium is apparently

attained between flavylum cation, neutral and anionic *trans*-chalcones respectively for $pK_{AH^+/Ct}=4.1$ and $pK_{Cv/Ct}=10.7$.

Irradiation of the neutral (stable or metastable) and protonated (meta-stable) *trans*-chalcones yields to the appearance of the quinoidal base or the flavylum cation in an extended pH region $1 < \text{pH} < 10$. The peculiar properties of this flavylum based photochromic system are explained by means of an energy level diagram where *cis*-chalcone and hemiketal lay above the quinoidal base.

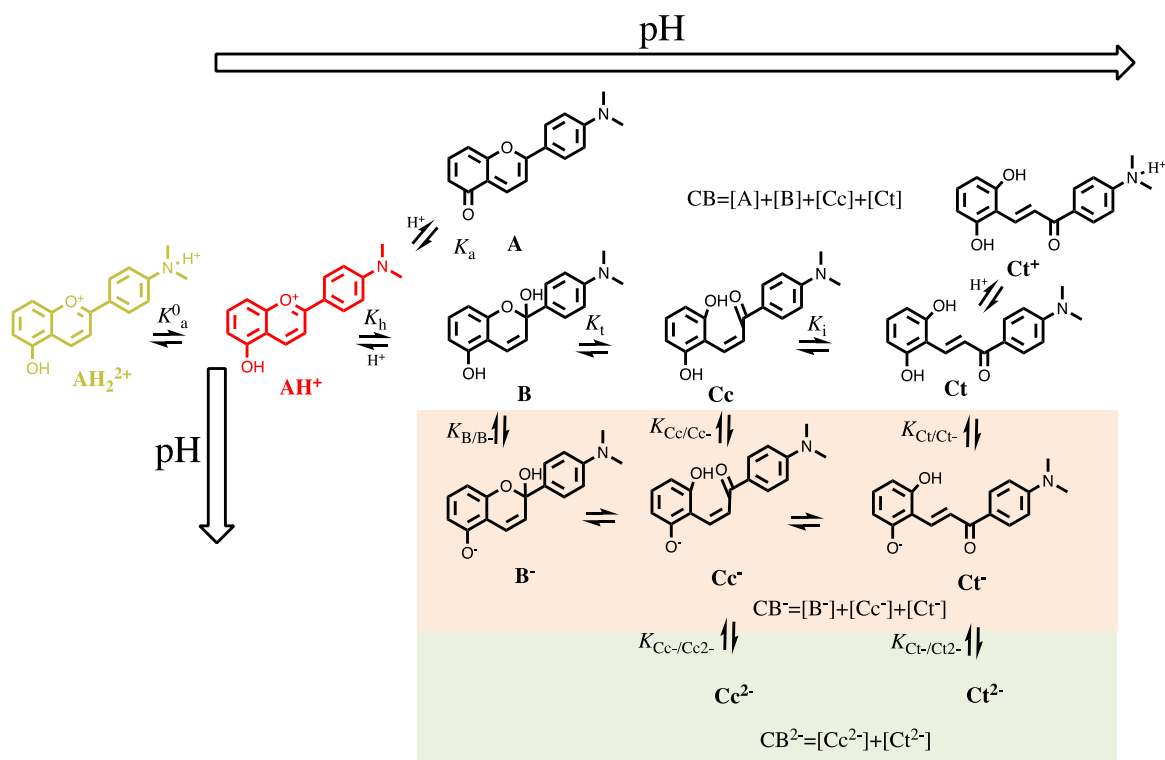
Keywords: Photochromism, Aminoflavylum, Quinoidal base, *trans*-Chalcones, Meta-stability, Anthocyanins

Graphical abstract



Introduction

Based on the study of many anthocyanins and related compounds it is possible to predict for any flavylium structure the chemical species that will be generated by increasing the pH.^{[1],[2],[3],[4],[5]} In the case of the newly synthesized 5-hydroxy-4'-dimethylaminoflavylium (5OH4'NMe₂) they are shown in **Scheme 1**. In the experimental conditions used within this work (**SDS micelles**) no evidence for the dianionic species was achieved and the protonated flavylium cation was only observed at extremely acidic conditions.



Scheme 1. Multistate of all possible species of 5-hydroxy-4'-dimethylaminoflavylium (5OH4'NMe2).

The flavylium cation (**AH**⁺) gives the name to the multistate and is the only species present at sufficiently acidic medium.^a The system is conveniently studied by addition of base to equilibrated solutions of the flavylium cation (direct pH jumps)[6]:[5] or by addition of acid to equilibrated solutions at higher pH values,[4, 5] in the present work the anionic *trans*-chalcone (reverse pH jumps). Upon a direct pH jump at sufficiently high pH values the quinoidal base (**A**) is formed in sub-microseconds.[7] In anthocyanins and related compounds, the evolution of the system towards the equilibrium takes place from the hydration of the flavylium cation to give hemiketal (**B**) and not from the quinoidal base, which only in basic medium is attacked by OH⁻.
Error! Bookmark not defined.[2] This is a breakthrough discovery made by Brouillard and Dubois and it is essential to the comprehension of the system, in particular to explain why in acidic to moderately acidic medium the hydration rate decreases by pH increasing.**Error! Bookmark not defined.** The hemiketal (**B**) is generally in fast equilibrium (seconds to sub-seconds) with *cis*-chalcone (**Cc**) through a ring opening/closure process (tautomerization) and *trans*-chalcone (**Ct**) is formed by an isomerization process from *cis*-chalcone.

^a Depending on the system, the pH could be more or less acid.

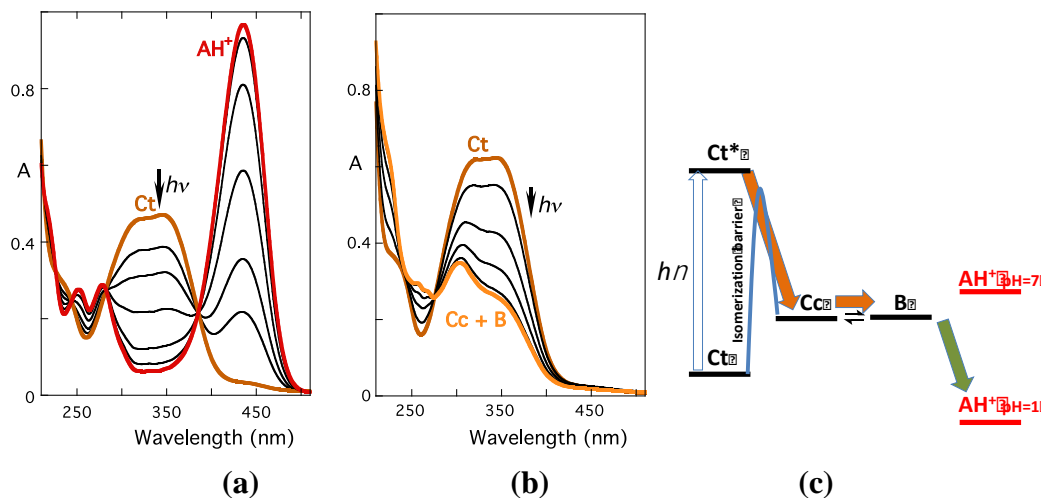


Figure 1. (a) Spectral variations of 4'-methoxyflavylium at pH=1.0, upon irradiation at 365 nm. (b) The same at pH=7.0 from the metastable *trans*-chalcone; (c) Qualitative energy level diagram to account for the photochromic system behaviour.

The photochemistry associated to the multistate of species originated by flavylum compounds has been subjected of interest since many years ago.[8].^{[9],[10],[11]} These systems have been used as models for optical memories capable of write-read-erase[12] and even mimic elementary properties of neurons.[13] The photochromic system is illustrated in **Fig. 1** for the compound 4'-methoxyflavylium. As shown in **Fig. 1c** the *trans*-chalcone is the stable species at higher pH values, but it is metastable at lower pHs as for example pH=1.0.^b When *trans*-chalcone is irradiated at pH=1, *cis*-chalcone is formed and equilibrates in seconds or sub-seconds^c with hemiketal. From this last species the system goes spontaneously to flavylium cation and no reversibility is observed, **Fig. 1a** (positive photochromism). Conversely, if the stable *trans*-chalcone is irradiated at pH=7, the flavylium cation is not

^b The anionic *trans*-chalcones are the stable species at very basic solutions (for example pH=13). The neutral and meta-stable *trans*-chalcone are obtained by protonation of the anionic species.

^c The tautomerization is catalyzed by H⁺ and OH⁻

thermodynamically accessible and the photoproducts are *cis*-chalcone in equilibrium with hemiketal, **Fig. 1b** (negative photochromism). In this case slow reversibility back to *trans*-chalcone is observed due the existence of the isomerization barrier.

The thermodynamic and kinetics of flavylum based systems as well as their photochromic properties are dramatically dependent on the nature and position of the substituents of the 2-phenylbenzopyrylium core. Anthocyanins are poorly photochromic as the *trans*-chalcone is a minor species at the equilibrium. In contrast, 3-deoxyanthocyanins such as luteolinidin, exhibit some photochromism since *trans*-chalcone (together with quinoidal base) are the main neutral species at moderately acidic medium.[14] The best photochromic systems based on the flavylum multistate of species have been described for simple synthetic flavylum compounds like 4'-methoxyflavylium (**Fig. 1**), 4'-hydroxyflavylium, 7-hydroxyflavylium or 4',7'-dihydroxyflavylium and similar compounds.^[15] However, they are limited in the colour pallet since the *trans*-chalcones (the ionized *trans*-chalcones generally lack of photochromic response) are almost colourless and the photoproduct is the yellow flavylum cation. Red shifted absorption bands can be achieved by introducing in the flavylum core amine substituents. This is the case of the compound 4'-diethylaminoflavylium previously reported.[16] The photochemical formation of the red flavylum cation was achieved upon irradiation of the metastable **Ct** and **Ct⁺** species.

The flavylum presented in this work (**Scheme 1**) has one hydroxyl group in carbon 5, allowing the formation of a quinoidal base exhibiting a slight red shifted of its maximum absorption in relation to flavylum cation, extending the colour pallet of the photochemical response. Moreover, micelles are known to modify the pH distribution of the multistate species and in particular **SDS** micelles extend the mole fraction

distribution of the flavylum cation and the quinoidal base to higher pH values.[17] Introduction of the hydroxyl substituent and the use of **SDS** micelles, maintains the meta-stability of *trans*-chalcones, as observed previously for dimethylaminoflavylum, but more significant the one of the quinoidal base, which becomes the photoproduct at more basic pH values. No negative photochromism is observed in the present system.

Material and methods

Reagents

2,6-dihydroxybenzaldehyde, 98% was obtained from Ark Pharm (USA), 4'-dimethylaminoacetophenone was purchased from Santa Cruz Biochemicals (Netherlands). Sodium dodecyl sulfate (SDS) was purchased Sigma-Aldrich (Madrid, Spain). A universal buffer of Theorell and Stenhagen[18] was prepared dissolving 2.25 mL of phosphoric acid 85% (w/w), 7.00 g of monohydrated citric acid, 3.54 g of boric acid and 343 mL of a 1 M NaOH solution in Millipore water until 1L.

Synthesis of 5-Hydroxy-4'-Dimethylaminoflavylum

2,6-dihydroxybenzaldehyde (1.4×10^{-3} mol, 200 mg) and 4'-dimethylaminoacetophenone (1.4×10^{-3} mol, 235 mg) were weighted in a round bottom flask and dissolved in 10 mL of a mixture of ethyl acetate: methanol (2:1). Then, chlorotrimethylsilane (TMSCl; 5 equiv) was added and the mixture was left to react at room temperature during 5 hours under stirring. To stop the reaction, 30 mL of water were added and then, the mixture was purified through liquid-liquid extraction using ethyl acetate. The aqueous phase containing mainly the 5OH4'NMe₂ was later purified by column chromatography using LiChroprep[®] RP-18 (40-63 μ m, Merck, Germany). The flavylum compound was recovered with 40% (v/v) methanol acidified with 2% HCl, presenting a pink colour. Methanol was evaporated in a rotary evaporator at 38°C, and the purified 5OH4'NMe₂ pigment was freeze-dried and stored at -18°C until further analysis.

The formation of the new pigment was followed by HPLC-DAD (Merck), in a reversed-phase C18 column (Agilent) with 250 \times 4.6 mm i.d., particle size 2.7 μ m and at 25°C. The eluents used were (A) 1% (v/v) formic acid in water and (B) 0.5 %

(v/v) formic acid in 80% (v/v) acetonitrile and the elution gradient was performed from 40 to 85% B during 50 min at a flow rate of 0.4 mL/ min. After 50 min, the column was washed with 100% B during 10 min and then it was stabilized with the initial conditions for more 10 min.

The identity and purity of the new flavylum compound was determined by 1D and 2D NMR experiments (^1H , COSY, HSQC and HMBC) in CD_3OD in a Bruker – Avance 400 spectrometer.[19]

Determination of the Thermodynamic and Kinetic parameters

A stock solution (8.0×10^{-4} M) of 5OH 4 NMe $_2$ in ethanol acidulated with 0.1 M HCl; and a SDS 0.35 M stock solution in Millipore water were prepared. HCl, NaOH or Universal buffer were used to adjust the pH of all the solutions then prepared.

A set of 5OH 4 NMe $_2$ solutions in the presence of 0.1 M SDS were prepared in individual 10 mm pathlength plastic cuvettes for different pH values along the pH scale. The pigment was added at the end, and after mixing; the direct pH jumps kinetics were followed registering the spectral variations in a Varian-Cary 100 Bio and/or 5000 spectrophotometers (Palo Alto, CA, USA) always protecting the solutions from external light sources. This experiment was performed at room temperature and 60°C. The pH of the solutions was measured after their preparation in a Radiometer Copenhagen PHM240 pH/77 ion meter.

The determination of the $\text{p}K_a$ was performed from representation of the absorption taken immediately after a direct pH jump versus pH.

Once the 60°C solutions reached the equilibrium (5 days after), a reverse pH jump was performed adding enough amount of HCl to reach pH=1. The kinetics were followed in the same way as the direct pH jumps.

Titration of trans-chalcones species

A 2.5×10^{-5} M pigment solution in the presence of 0.1 M SDS was prepared in NaOH 0.1 M (anionic trans-chalcone). Once equilibrated at room temperature (15 minutes after) the solution was titrated with successive additions of small volumes of HCl and after, the pH and the spectra of the solution were recorded, always protecting the solutions from external light sources.

Photochemistry assay

A solution of 5OH4'NMe2 (2.53×10^{-5} M) in the presence of 0.1 M SDS was prepared in NaOH 0.1 M. Once equilibrated, the solution was placed in a quartz cell (10 mm pathlength) and then concentrated HCl, water and universal buffer were added to obtain solutions at pH 1, 8 and 10. The pH of the final solutions was measured, their initial spectrum was recorded and then each solution was irradiated at 365 nm under stirring during short periods of time, between each period a spectrum of the solution was recorded, always protecting from external light sources.

Flash Photolysis experiments

A solution of anionic *trans*-chalcone of 5OH4'NMe2 (4.5×10^{-5} M) in NaOH 0.1 M with 0.177 M of SDS was prepared. Once equilibrated, the solution was adjusted to pH 1, 3, 5, 7, 9 and 11 using HCl 10 M and universal buffer (at desired pH). The flash photolysis experiments were performed on a Varian Cary 5000 spectrophotometer equipped with a Harrick fibre-mate (Dunedin, FL, USA) coupled to an Oceanic Optics 4-way cuvette holder (Dunedin, FL, USA). The compartment was isolated from external light sources, the solution placed in a 2 mm quartz cell and a commercial Achiever 630AF camera flash (Hong Kong, China) was used directly in

contact with the cell. The kinetics after flash were monitored at the maximum absorption of the flavylum cation or the quinoidal base and their respective chalcones depending on the solution pH.

Results and Discussion

In spite of the complexity of the system reported in **Scheme 1**, it can be dramatically simplified considering flavylum cation a di-protic acid defined by eq.(1) and eq.(2), where CB, CB⁻ are defined as above.^d



Where:

$$[\text{CB}] = [\text{A}] + [\text{B}] + [\text{Cc}] + [\text{Ct}];$$

$$[\text{CB}^-] = [\text{B}^-] + [\text{Cc}^-] + [\text{Ct}^-]$$

$$K'_a = K_a + K_h(1 + K_t) + K_h K_t K_i \quad (3)$$

$$K''_a = \frac{K_{A/A^-} K_a + K_{B/B^-} K_h + K_{Cc/Cc^-} K_h K_t + K_{Ct/Ct^-} K_h K_t K_i}{K'_a} \quad (4)$$

5OH4'NMe2 in the presence of SDS micelles

In spite of the existence of a significant aggregation of this compound in pure water, even at low concentrations, see supplementary material, this phenomenon was not observed in the presence of SDS micelles.

The absorption spectra of 5OH4'NMe2 (2.56×10^{-5} M) in the presence of SDS micelles (0.1 M) taken immediately after direct pH jumps are shown in **Fig. 2**. There is an equilibrium between **AH⁺** and **A** with $pK_a=7.6$, **Fig. 2a**. These two species give rise to

^d In the present work the species Cc and B as well as the respective anionic forms are not observed at the equilibrium or in the metastable states. They are transient species.

a metastable state whose pH limits will be defined below. For very basic solutions it is necessary to heat the system at 60 °C in order to observe the kinetic processes towards the equilibrium, **Fig. 2b** and **Fig. 2c**. Only at very basic pH values, for example pH=13, the anionic form of the *trans*-chalcone is observed in a few minutes at room temperature, similarly to the behaviour of other flavylum systems bearing amine substituents.^{Error! Bookmark not defined.}

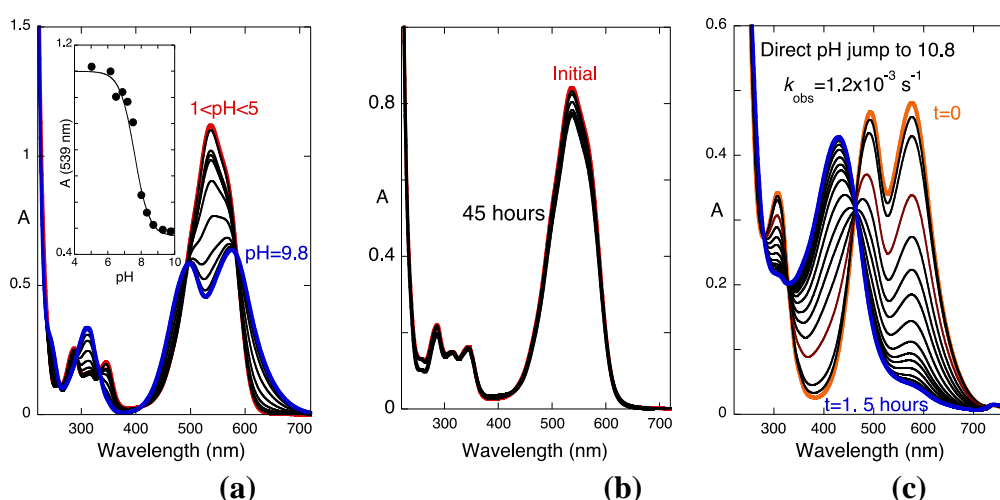


Figure 2. (a) Spectral variations of 5OH4'NMe2 (2.56×10^{-5} M) in the presence of SDS micelles (0.1 M) taken immediately after a direct pH jump. The results indicate that the flavylum cation is in equilibrium with the quinoidal base with $pK_a=7.6$; (b) Spectral variations at pH=6.8 at 60 °C after 45 hours; (c) Spectral variations upon a direct pH jump to pH=10.8 at 60 °C after 90 min.

More information regarding the meta-stability of the flavylum cation-quinoidal base system was achieved by carrying out a series of direct pH jumps at 60 °C, represented in **Fig. 3**. The absorbance of the flavylum cation after 5 days at 60 °C shows an apparent $pK_a = 7.5$. The quinoidal base completely disappears and at higher pH values the absorption spectra identified as *trans*-chalcones are dominant (see below). After 5 days at 60 °C some decomposition occurs. The pH dependent rate of the quinoidal base disappearance, after the direct pH jumps to basic medium at 60 °C, is shown in **Fig. 3c**. Similarly, to what was observed for other flavylum systems the quinoidal

base is attacked by OH^- and the respective hydration rate is directly proportional the hydroxyl concentration, $2.5[\text{OH}^-] \text{ s}^{-1}$.

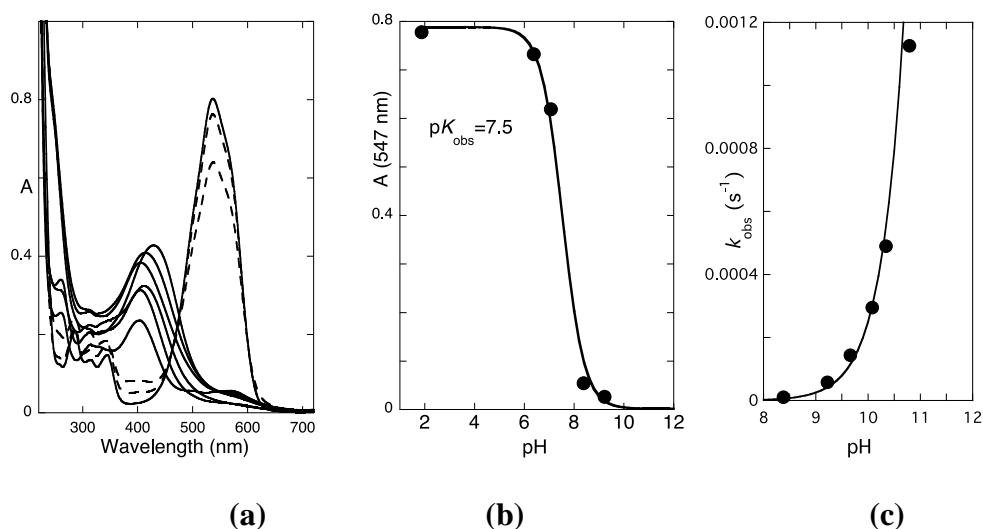


Figure 3. (a) Spectral variations of the compound 5OH4'NMe2 ($2.56 \times 10^{-5} \text{ M}$) after a direct pH jump (60°C after 5 days). The traced lines correspond to pH around 7 where stability of the solution was not yet achieved (traced lines); (b) Representation of the flavylum cation absorption in (a) at 547 nm as a function of pH; (c) Rate constants of the quinoidal base disappearance in basic medium at 60°C . Fitting was achieved for $2.5[\text{OH}^-] \text{ s}^{-1}$.

The anionic *trans*-chalcone was prepared at very basic pHs and from this species a series of reverse pH jumps were carried out, **Fig. 4**.^e Considering the behaviour of other flavylum compounds possessing amine substituents, the first protonation constant, $\text{p}K_{\text{Ct}^+/\text{Ct}} = 2.6 \pm 0.1$, can be attributed to the acid-base equilibrium between the protonated *trans*-chalcone and the neutral one. The protonated species is stabilized by the negatively charged surface of the **SDS** micelles. The second equilibrium, $\text{p}K_{\text{Ct}/\text{Ct}^-} = 10.7 \pm 0.1$, is attributed to the deprotonation of the *trans*-chalcone to give its anionic form. The formation of the negatively charged *trans*-chalcone, is not favoured by the negatively charge of the SDS micelles surface and thus takes place only at very basic

^e Special care should be taken to avoid interference of the photochemical reactions.

pH values. The *trans*-chalcones are meta-stable at room temperature unless for very basic solutions.

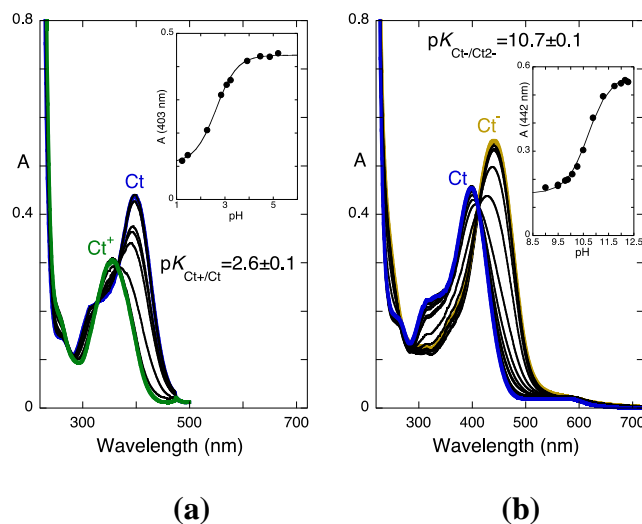


Figure 4. Titration of the *trans*-chalcones of the compound 5OH4'NMe2 from pH=13: (a) $1 < pH < 5$; (b) $8.5 < pH < 12.5$. The experiments were performed in the dark to prevent the photochromic response of the *trans*-chalcones. The solutions are meta-stable at room temperature unless for very basic pH values.

The limits of the meta-stability of the *trans*-chalcones was also studied, **Fig. 5**, by following the spectral variations of the reverse pH jumps shown in **Fig. 4** at 60 °C. The flavylum cation is formed and equilibrates with *trans*-chalcones (neutral and protonated), with $pK_{obs} = 4.1$.

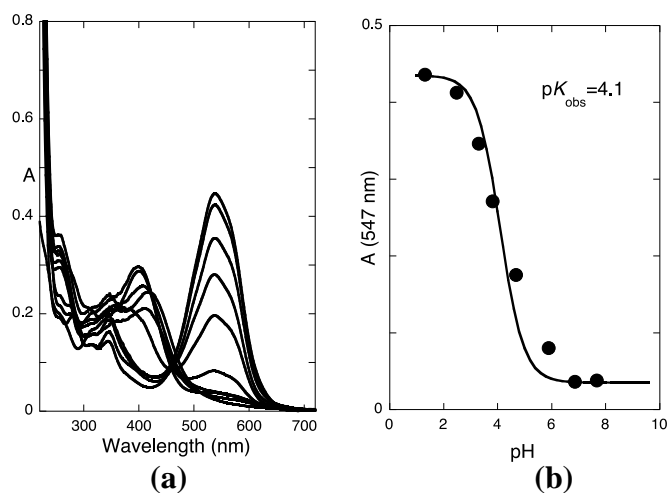
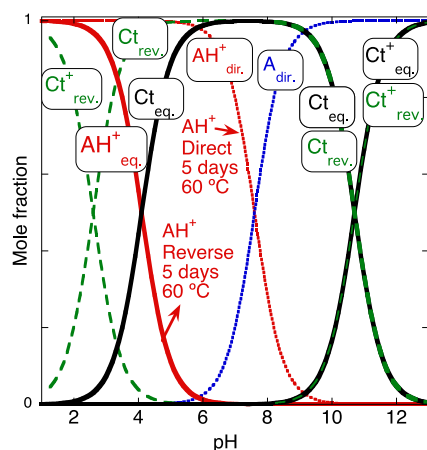


Figure 5. (a) Spectral variations of the compound 5OH4'NMe2 after a series of reverse pH jumps from the anionic *trans*-chalcone equilibrated at pH=13 after 5 days at 60 °C; **(b)** Absorbance of the flavylum compound at 547 nm versus pH. The inflection point occurs for $pK_{obs}=4.1$

The absorption spectra after 5 days at 60 °C obtained from direct pH jumps of equilibrated solutions at pH=1, **Fig. 3a**, and reverse pH jumps from anionic *trans*-chalcone, **Fig. 5a**, are similar but not coincident. The most interesting feature is however the observed pK_a of the flavylum cation: $pK_{obs}=4.1$ (**Fig. 5b**) that compares with $pK_{obs}=7.5$ (**Fig. 3b**). The question is which of the two values is closer to the putative equilibrium that even at 60 °C we are not able to define with precision. In both cases, the quinoidal base disappears completely, confirming its metastable state. Considering that the equilibrium between flavylum cation and the neutral species is given by eq.(3), the value $pK_{obs}=7.5$ would imply the flavylum cation and the quinoidal base as the major neutral species, *circa* 95% and lack of the others in particular the *trans*-chalcone, which is not the observed behaviour in **Fig. 3a**, see in supplementary material the respective mathematical demonstration. On the other hand, the value $pK_{obs}=4.1$ (0.2% of flavylum cation and quinoidal base) is compatible with a system where *trans*-chalcone is dominant and quinoidal base is negligible. For practical purposes we assign the value $pK_{obs}=4.1$ as representing the equilibrium, but it cannot be excluded that this mole fraction distribution is another meta-stable state reached from the *trans*-chalcones.



Scheme 2. Mole fraction distribution of the flavylum cation (red-pointed line) and quinoidal base (blue-pointed line) after a direct pH jump from equilibrated solutions at pH=1; mole fraction distribution after a reverse pH jump from the stable anionic *trans*-chalcone at pH=13 (green traced lines); flavylum cation (red-full line), *trans*-chalcone (black full line) and anionic *trans*-chalcone (black full line). For pH>9 the equilibrium distribution is coincident with the one of the reverse pH jumps.

Scheme 2 summarizes the behaviour of this compound. It can be observed that a meta-stable state is achieved after a direct pH jump from equilibrated solutions of the flavylum cation. Only two species are observed, the flavylum cation (red-pointed line) and the quinoidal base (blue-pointed line) with a $pK_a=7.5$. The extended pH range of the flavylum cation is attributed to the electrostatic effect that stabilizes the flavylum cation. (meter J. C. Lima, C. Vautier-Giongo, A.Lopes, E. Melo,

F. H. Quina, A. L. Maçanita, Color Stabilization of Anthocyanins: Effect of SDS Micelles on the Acid-Base and Hydration Kinetics of Malvidin 3-Glucoside (Oenin), J. Phys. Chem. A 2002, 106, 5851-5859).

Similarly to anthocyanins and other related compounds, the quinoidal base does not hydrate but reacts with OH^- at higher pH values. **Error! Bookmark not defined.** However, in the present case this reaction is extremely slow unless for very high pH values. The

titration of the anionic *trans*-chalcone (reverse pH jumps) in dark conditions leads to the other metastable system, represented in **Scheme 2** by the green traced lines.

The evolution of the absorption spectra of the metastable species resulting from direct pH jumps (AH^+ and A) as well as those from the reverse pH jumps (Ct^+ , Ct and Ct^-) after 5 days at 60 °C indicate that the quinoidal base disappears in the former to give the *trans*-chalcone or its anionic form (depending on pH) and the protonated *trans*-chalcone disappears to give the flavylum cation. The neutral *trans*-chalcone equilibrates with flavylum, $\text{p}K_{\text{obs}} = 4.1$, and it is the dominant equilibrium species in the pH interval $5 < \text{pH} < 9$, when starts to equilibrate with the anionic *trans*-chalcone at higher pH values. The pH domain of the flavylum cation at the equilibrium, Scheme 2, is reduced because *trans*-chalcone that is slowly formed is solubilized in the more hydrophobic region of the SDS micelles.

A final remark to mentioned that the double-charged flavylum cation was observed at 6M (HCL) (Paulinha confirmar) but not the dianionic *trans*-chalcone, probably due to the electrostatic repulsion of the negatively charged species.

The photochromic system

The spectral variations upon irradiation of the metastable *trans*-chalcones of the compound 5OH4'NMe2 (obtained from protonation of the stable anionic *trans*-chalcone at pH=13) for three representative pH values are shown in **Fig. 6**.

At pH=1.5 it is the metastable protonated *trans*-chalcone that absorbs light and the photoproduct is the flavylum cation. At pH=8.4 and 10.1 it is essentially the neutral *trans*-chalcone, that absorbs light and give a mixture of flavylum cation and quinoidal base (at pH=8.4) and quinoidal base (at pH=10.1). The photoproduct quinoidal base is a metastable photoproduct.

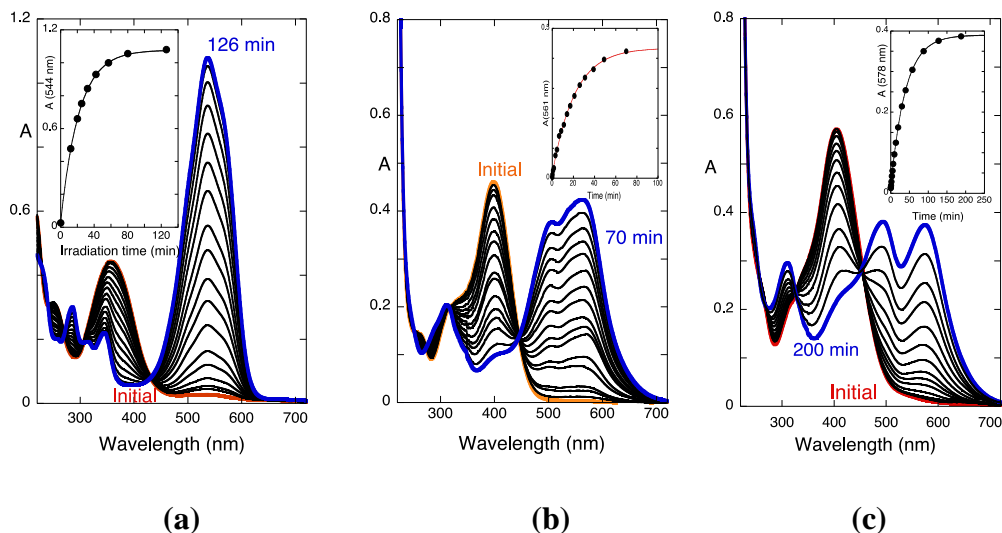


Figure 6. (a) Irradiation at 365 nm ($I_0=2.8 \times 10^{-7}$ Einstein min^{-1}) of the metastable *trans* chalcone at pH=1.5, 2.6×10^{-5} M; (b) the same at pH=8.4, 2.5×10^{-5} M; (c) the same at pH=10.1, 2.8×10^{-5} M.

Flash photolysis in the domain of seconds[20] was performed for representative pH values. The results at pH=3 and pH=10 are shown in **Fig.7a** and **Fig. 7b** respectively. In the pH interval $1 < \text{pH} < 9$, the behaviour is the same of the one at pH=3. In **Fig. 7a** two successive flashes were applied to the solution. At 540 nm (flavylium cation absorption) there is a sudden increasing of the absorption while at 390 nm (*trans*-chalcone absorption) a concomitant bleaching. This means that during the resolution time of this flash photolysis (0.1 s) the flavylium cation is already formed at the expenses of *trans*-chalcone. The light absorption promotes the photo-induced isomerization and the primary photoproduct (*cis*-chalcone) should give the flavylium cation via tautomerization reaction followed by the de-hydration, **Scheme 1**. These processes are concluded before 0.1 s. At pH=10 formation of the quinoidal base is relatively slow and the system reverts back thermally to the equilibrium. It is similar to the previous photochromic systems described in the introduction, **Fig. 1b**, but takes place at much higher pHs.

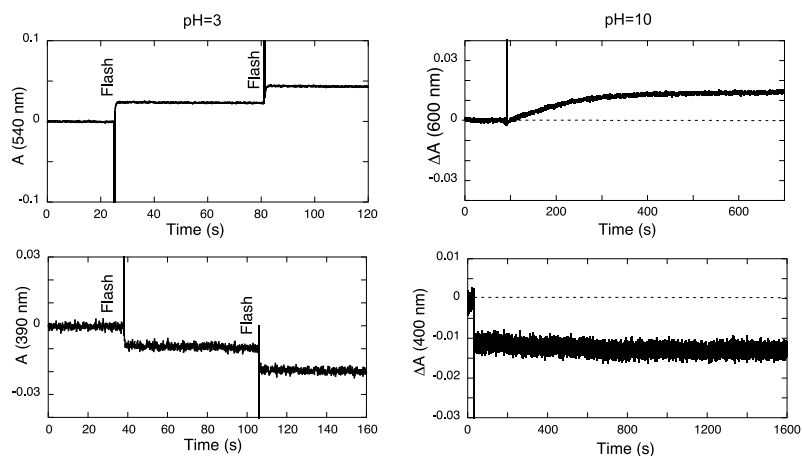
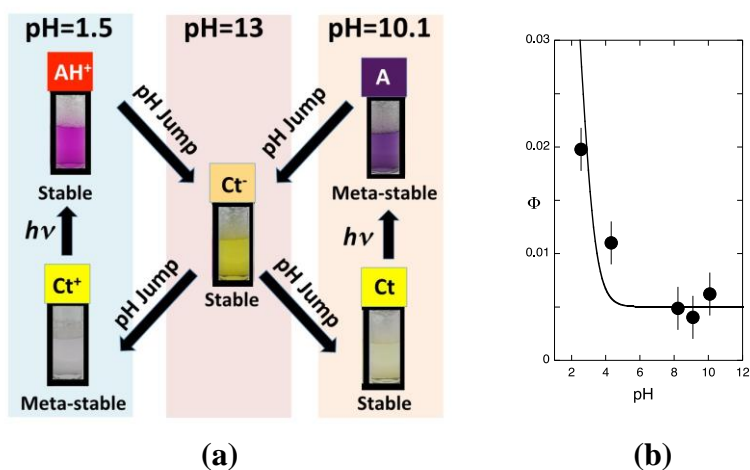


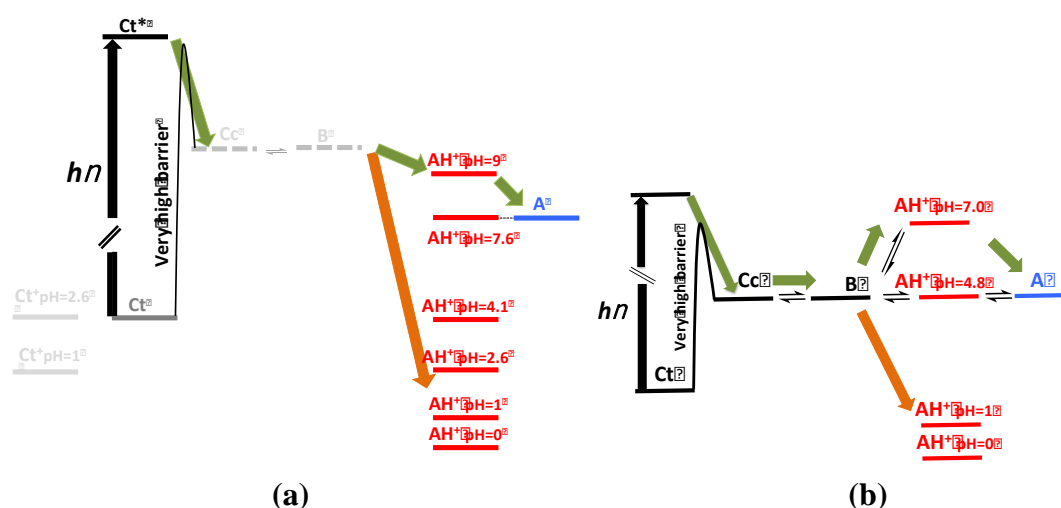
Figure 7. Flash photolysis in the domain of seconds of the compound 5OH4'NMe2: (a) at pH=3; (b) at pH=10.

In **Scheme 3** the photochromic system is summarized. At lower pH values it is the metastable protonated *trans*-chalcone that yields the flavylium cation as described in **Fig. 1a** for 4'-methoxyflavylium. At pH=10.1 *trans*-chalcones are still the dominant species and the photoproduct is the metastable quinoidal base.



Scheme 3. (a) A photochromic system from a metastable state to give a stable photoproduct can be prepared at pH=1.5. The contrary is obtained at pH=10.1. Ct⁻ is thermal and photochemical stable; (b) Quantum yields as a function of pH. The quantum yields are roughly fitted for pK=2.6 (Ct⁺/Ct) and Φ=0.05 and Φ=0.005 respectively for Ct⁺ and Ct.

Several years ago, we introduced the energy level diagram to account for the details of the flavylum based multistates.[5, 21] In **Scheme 4**, a semi-quantitative proposal is shown for 5OH4'NMe₂, because it was not possible to clearly define the equilibrium (in grey colour the species not defined with precision). The quantitative energy level of 4'-hydroxyflavylium is shown for comparison purposes.[22]



Scheme 4. (a) Qualitative energy level diagram of 5OH4'NMe₂ in the presence of SDS micelles; (b) Quantitative energy level diagram of 4'-hydroxyflavylium for comparison purposes.[22]

The interpretation for the unusual photochromic signal of the quinoidal base at basic pH values seems to be linked to the high energy level of **Cc** and **B**. As shown in **Scheme 4a**, after a direct pH jump to pH=9, the primary photoproduct gives spontaneously the quinoidal base, while in 4'-hydroxyflavylium, the primary photoproduct **Cc** equilibrates fast with **B** and more slowly with **A**. The diagram in **Scheme 4a** explains also the meta-stability of **A** upon a direct pH jump. **A** once formed to reach the more stable **Ct**, needs to overcome a high energy barrier, since the system should pass through **AH⁺**, **B** and **Cc**.

Conclusions

The photochromic systems based on the flavylum multistate of species are extremely versatile. The nature and position of the substituents can modify not only the kinetics and thermodynamic of the system but also the photochromic response. In particular the colour pallet of the photochromic products could be extended by the amine substituents. Moreover, the use of micelles and other hosts can dramatically modify the mole fraction distribution of the flavylum multistate species, in particular extending the pH domain of the photoactive *trans*-chalcone . In the present work the conjugation of the selected substituents and their position together with the use of **SDS** micelles allowed to define a new flavylum based photochromic system exhibiting positive photochromism in an extended pH region, $1 < \text{pH} < 10$. In spite of the low quantum yields of reaction, the performance of the system relatively to the appearance of the colour is good due to the very high molar absorption coefficients of the final photoproducts.

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