

Photochemistry of luteolinidin “Write-lock-read-unlock-erase” with a natural compound

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Abstract

In this work, the photochemistry of luteolinidin, a natural deoxyanthocyanidin, found essentially in mosses and ferns is described. In moderately acidic methanol/water (3:1) solutions the dominant species of luteolinidin is the photoactive *trans*-chalcone. The system was characterised by steady state irradiation and pulse light excitation experiments as well as by pH jumps. The use of this compound to carry out cycles able to *write-lock-read-unlock-erase*, is discussed. © 2000 Elsevier Science S.A. All rights reserved.

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1. Introduction

It is firmly established that the generality of the 2-phenyl-benzopyrylium derivatives, which include anthocyanins, anthocyanidins (sugar free analogues of anthocyanins), 3-deoxyanthocyanidins and synthetic flavylum salts, Scheme 1, give rise to a series of pH dependent chemical reactions, following the general pattern summarised in Scheme 2 for malvidin 3,5-diglucoside [1].

The flavylum cation, AH⁺, is the dominant species in very acidic solutions. With increasing pH a series of more or less reversible chemical reactions can occur: (i) proton transfer leading to the quinoidal base, A; (ii) hydration of the flavylum cation giving rise to the hemiacetal, B; (iii) tautomerization reaction responsible for ring opening, to give the *cis*-chalcone form, Cc; and finally (iv) *cis-trans* isomerization to form the *trans*-chalcone, Ct.

3-Deoxyanthocyanidins are yellow pigments found essentially in mosses and ferns, the most primitive of land plants

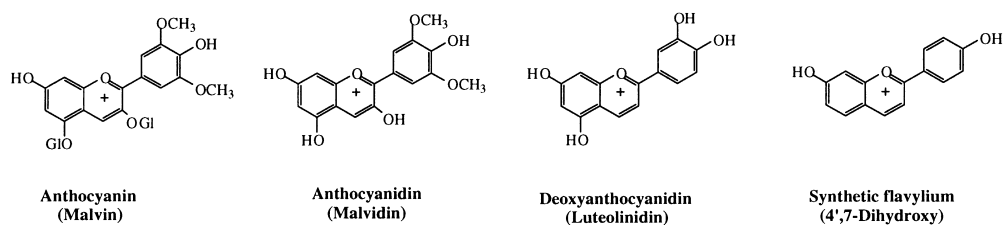
[2–4]. These molecules are considered the chemical ancestors of anthocyanins, the ubiquitous water-soluble pigments that are found in flowers and fruits and are responsible for their impressive blue and purple colors. In contrast with anthocyanins and anthocyanidins, the deoxyanthocyanidins do not bear a sugar (or a hydroxyl) at position 3 and thus their electronic absorption spectra are much less shifted to the visible. This fact suggests that hydroxylation at the position 3 was a crucial step that occurred early in the biochemical evolution of plants allowing the accessing of the blue and purple colors [2–6].

In the last few years, we have investigated in a systematic way the thermal and photochemical reactions of several synthetic flavylum salts [7–12] in order to emphasise the multistate/multifunctional character of the chemistry of these compounds.

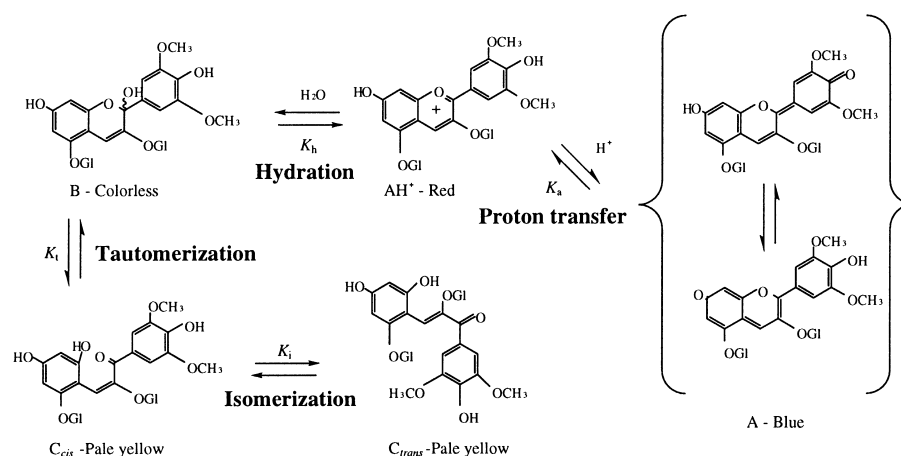
Our interest on 3-deoxyanthocyanidins derived from the fact that in a certain way they exhibit a chemical structure, which can be considered between anthocyanins and synthetic flavylum salts. In this work, we prove that 3-deoxyanthocyanidins exhibit chemical properties that are more similar to synthetic flavylum salts than to anthocyanins: for example in moderately acidic solutions they present a large amount of the photoactive *trans*-chalcone species, giving rise to an interesting photochemistry and to

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Scheme 1.



Scheme 2.

the possibility of examining these systems from the point of view of a molecular-level-device capable of mimicking an elementary memory.

2. Experimental

Luteolinidin was purchased from Extrasynthese. All other chemicals used were of analytical grade. The experiments were carried out in a mixture of solvents methanol/water (3:1) at 25°C, instead of pure water because the amount of the photo-reactive Ct species at the equilibrium is largely increased by addition of methanol. The pH of the solutions was adjusted by addition of HCl (pH < 2) or NaOH and buffer², and measured by a Metrohm 713 pH meter. For ¹H NMR spectroscopy experiments, luteolinidin was dissolved in acid (DCl) d-methanol. When required the pH was changed by addition of small aliquots of NaOD 1 M or 0.1 M. pH measurements were made in the NMR tube using an Ingold glass electrode and the reported pH values, are direct readings without correction for the isotope effect [13], or for the fact that the experiments were carried out in a solvent mixture water/methanol (1:3).

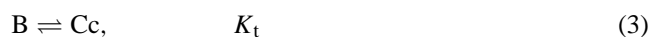
NMR spectroscopy [8,14] and flash photolysis, [15,16] experiments were performed as previously described. Absorption spectra were recorded on a Perkin-Elmer lambda

6 spectrophotometer. Photoexcitation in continuous irradiations experiments was performed by using a medium pressure mercury lamp, and interference filters (Oriel) to isolate the excitation bands. The incident light intensity was measured by ferrioxalate actinometry [17]. The estimated error on quantum yield values is ±10%.

3. Results and discussion

3.1. Processes in the dark

According to Scheme 2, Eqs. (1)–(4) account for the five species of luteolinidin occurring at acidic or neutral solutions,



These set of equations can be simplified in one single acid base equilibrium, Eq. (5) where the flavylum cation AH⁺ is in equilibrium with its conjugate base 'CB' constituted by the sum of the species A, B, Cc and Ct, Eq. (5) [18].



where $K'_a = K_a + K_h + K_h K_t + K_h K_t K_i$

² Universal buffer Theorell–Stenhagen, used in low concentration (0.05 M).

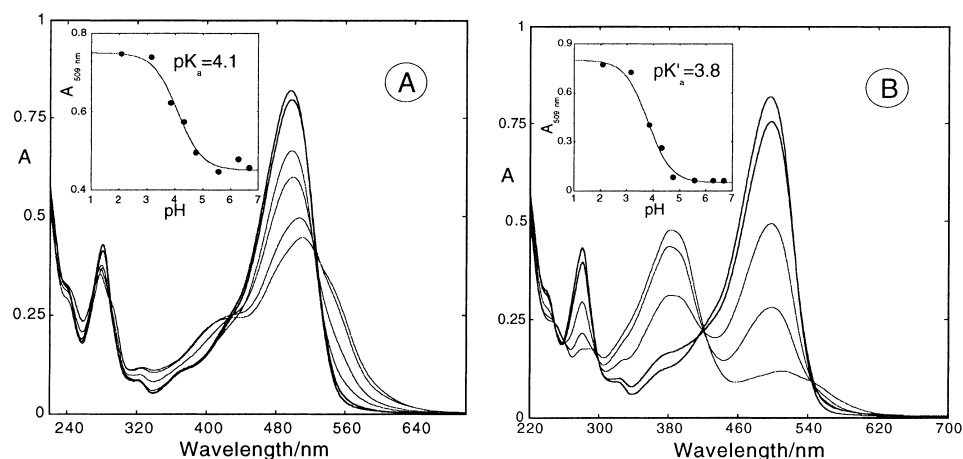


Fig. 1. (A) Absorption spectra of luteolinidin in methanol/water (3:1) immediately after the pH jump from 1 to 2.08, 3.16, 3.85, 4.32, 4.76, 5.57; (B) same as in (A) upon standing 2 days in the dark for pH 1, 2.08, 3.16, 3.85, 4.32, 4.76.

In 2-phenyl-benzopyrylium derivatives, the relative amounts of the four species constituting the conjugate base 'CB' are dependent on the substituents, and thus one important information regarding the characterization of luteolinidin is the composition of 'CB'.

The electronic absorption spectrum of luteolinidin in water/methanol (1:3) is pH and time dependent, as demonstrated by Fig. 1. Fig. 1A shows the spectra immediately after a pH jump to the desired pH value, from a solution previously equilibrated at pH=1.0; Fig. 1B shows the spectra of the same solutions upon standing in the dark for 2 days in order to reach the final equilibrium. Inspection of Fig. 1A clearly shows that the characteristic absorption band of the flavylium cation AH^+ at 494 nm disappears by increasing pH; at pH>5 the new absorption spectra is compatible with the formation of A. These results were confirmed by 1H NMR, through a series of spectra of solutions carried from pH=1 to 6. The peaks corresponding to the flavylium cation were initially shifted as a consequence of the pH jump; then the area of the shifted peaks decreased to ca. 30% of the initial value; simultaneously a new set of peaks appears whose area corresponds to the remaining 70%. This behaviour is compatible with the initial transformation (within the mixing time) of AH^+ into A; then at longer time scale an equilibrium is attained where A and Ct coexist (30% of A and 70% of Ct). On the other hand the shape and position of the absorption bands reported in Fig. 1B are in agreement with the formation (at the thermodynamic equilibrium) of a mixture of Ct and A as AH^+ disappears. From NMR and UV-VIS experiments, no spectral evidence was obtained for the formation of B and Cc, most probably because they are formed in very low concentration as transient species (do not accumulate).

The absorption spectra of the three species that appear at the thermodynamic equilibrium are represented in Fig. 2: **a** is the spectrum of AH^+ obtained at pH=2; **b** is the spectrum of A obtained immediately after a pH jump from 1 to 5; **c** is

the spectrum of Ct obtained upon subtraction of 30% of the spectrum of A to the spectrum of the solution at pH=5, 2 days after the pH jump (containing a mixture of Ct and A).

Finally, using a method reported elsewhere, [16] from the equilibrium concentration of A at pH=6 and the value of K'_a taken from Fig. 1B we can calculate $K_a=8.0 \times 10^{-5}$. This value is in good agreement with the pK_a obtained from Fig. 1A, confirming the existence of a pseudo-equilibrium between AH^+ and A.

The chemical behaviour of luteolinidin can be summarized in Fig. 3 [16]. The left-hand side of this figure shows a semiquantitative description of the energy level of the different species at some selected pH values; the right part shows the pH dependence of the mole fraction distribution of the three main species either in pseudo-equilibrium or equilibrium conditions.

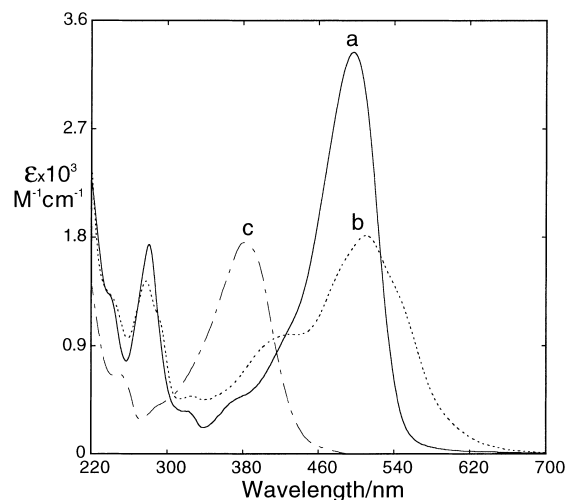


Fig. 2. Absorption spectra of the species derived from luteolinidin ion in methanol/water (3:1) solution at 25°C: (—) AH^+ ; (.....) A; (---) Ct.

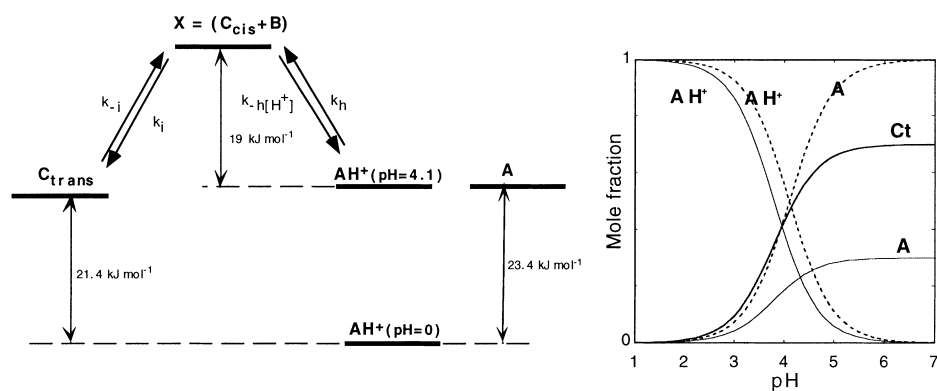


Fig. 3. Thermodynamic scheme of luteolinidin together with the mole fraction distribution of the AH^+ , A and Ct as a function of pH. (–) at the thermodynamic equilibrium; (---) at the pseudo equilibrium.

When the system is equilibrated at $pH=1.0$ the most stable form is the flavylum cation. Raising the pH, other species become more stable, and thus a kinetic process involving the disappearance of the flavylum cation can be observed. In previous cases [9–11] it was possible to distinguish three steps prior to reach the final equilibrium: (i) a very fast process (subseconds time domain) corresponding to the conversion of AH^+ into A , (ii) a fast process (seconds to minutes) in which the species AH^+ , A , B , and Cc pseudo-equilibrate, (iii) a slower process (hours or days) controlled by the *cis-trans* isomerization that allows the system to reach the final equilibrium.

In the present system (where we do not detect sizeable amount of B and Cc) only two processes were observed: (i) the first one that leads to the formation of A during the time of mixing the base added for the pH jump, (ii) the second one in which A disappears through a slow first order kinetic process leading to Ct . This behaviour is illustrated in Fig. 4. As it can be seen, immediately after the pH jump from dark equilibrated solutions at $pH 1$ to $pH 6.1$ the species AH^+ is completely converted into the species A . In a subsequent step

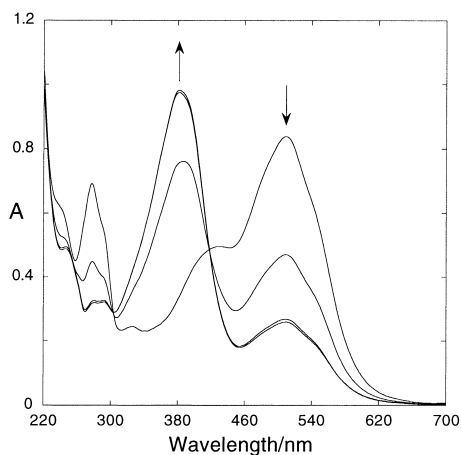
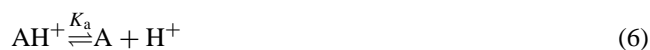


Fig. 4. Spectral modifications that occur upon a pH jump from 1 to 6.1, for the following times: 0, 261, 1318, 1718 min.

A is partially converted to Ct , by a first order process whose kinetic constant is $k_{obs}=7 \times 10^{-6} \text{ s}^{-1}$. The ratio between the initial (immediately after the pH jump (100% of A)) and the final (2 days later) absorbance at 509 nm, confirms the presence of ca. 30% of A at the final equilibrium, as observed by 1H NMR.

On this basis the system can be simplified considering the following steps:



where X is the intermediate state constituted by B and Cc in rapid equilibrium. Because X does not accumulate, its formation must be slower than its disappearance. This is possible if (i) the formation of Ct from AH^+ and A is controlled by the hydration reaction leading to B and (ii) the formation of AH^+ and A from Ct is controlled by the *trans-cis* isomerization reaction (Scheme 2). In other words $k_h \ll k_{-h}[H^+]$ and $k_{-i} \ll k_i$. A kinetic treatment, based on the steady state approximation for the intermediate species X and considering that equilibrium 1 (or 6) is by far the fastest process of the system, leads to the following equation [16,19]

$$k_{obs} = \frac{[H^+]}{[H^+] + K_a} \frac{k_i k_h}{k_i + k_{-h}[H^+]} + \frac{k_{-i} k_{-h} [H^+]}{k_i + k_{-h}[H^+]} \quad (8)$$

According to Eq. (8) the plot of k_{obs} as a function of pH, can be used to fit the rate constants. However k_i and k_{-h} are calculated with much more accuracy by means of flash photolysis experiments, see below. For this reason the best strategy consists of using the experimental values for k_i and k_{-h} in Eq. (8) and then determine k_{-i} and k_h through a best fitting procedure. The results are reported in Table 1.

Table 1
Thermodynamic and kinetic constants of luteolinidin in methanol/water (3:1) and 4',7-dihydroxyflavylium in pure water taken from [19]

Compound	Luteolinidin	4',7-diOH
K'_a	$10^{-3.8}$	$10^{-3.05}$
K_a	$10^{-4.1}$	$10^{-4.0}$
K_h	3.3×10^{-8}	1.4×10^{-6}
K_i	6.5×10^3	1.4×10^3
k_h	$2.0 \times 10^{-3} \text{ s}^{-1} \text{ M}^{-1}$	$1.8 \times 10^{-2} \text{ s}^{-1} \text{ M}^{-1}$
k_{-h}	$6.1 \times 10^4 \text{ s}^{-1} \text{ M}^{-1}$	$1.3 \times 10^4 \text{ s}^{-1} \text{ M}^{-1}$
k_i	0.13 s^{-1}	0.26 s^{-1}
k_{-i}	$2.0 \times 10^{-5} \text{ s}^{-1}$	$1.8 \times 10^{-4} \text{ s}^{-1}$

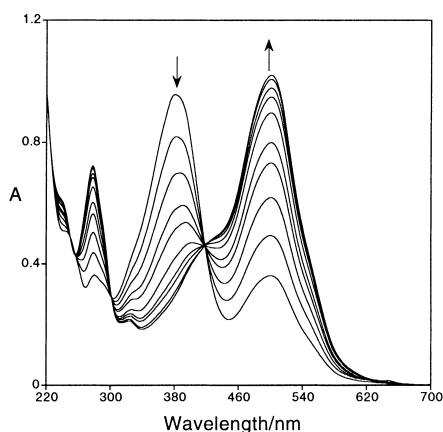


Fig. 5. Irradiation at 365 nm ($I_0=1.21 \times 10^{-6} \text{ einstein min}^{-1}$) of luteolinidin $5 \times 10^{-5} \text{ M}$ methanol/water (3:1) at pH=4.7, for the following irradiation times: 0, 5, 11, 17, 22, 32, 42, 52, 72, 102 s.

4. Photochemical processes

4.1. Continuous irradiation

Irradiation of dark equilibrated, acidic or neutral solutions of luteolinidin shows the simultaneous disappearance of Ct and formation of A (Fig. 5).

The quantum yield for the photochemical reaction is $\phi=0.28$, at pH=4.7.

The thermal back reaction is slow at 25°C $k_{\text{obs}}=4 \times 10^{-6} \text{ s}^{-1}$ but can be increased by raising the temperature, $k_{\text{obs}}=4 \times 10^{-4} \text{ s}^{-1}$ at 47°C. The absorption spectrum of a solution exposed to a cycle of irradiation, thermal recovery at 47°C and equilibration at room temperature was identical to that of a reference solution kept in the dark (or a solution before irradiation).

5. Pulse irradiation

The pulse irradiation of dark equilibrated luteolinidin solution at pH 4.9 and 6.1 was performed according to a flash photolysis technique previously described [15,16].

In Fig. 6 the absorbance decay traces obtained at 380 nm (absorption maximum of Ct) and 501 nm (absorption maximum of A) are shown for the solutions at pH=4.9. Like in the case of 4',7-dihydroxyflavylium [15,16] the traces clearly show the presence of three consecutive kinetic processes. The first one occurs within the flash and is too fast to be monitored with our apparatus (instantaneous bleaching at 380 nm, Fig. 6a). It may be assigned to the reaction of Ct to give Cc in equilibrium with the hemiacetal B. The second process ends in a few seconds and can be assigned to the formation (from Cc and B) of A (and AH^+) (increase of absorbance in the visible region, Fig. 6b). The third process, not shown in Fig. 6, completely restores the initial absorbance (before irradiation); it falls into the time domain of hours and is assigned to the conversion of A (and AH^+) to Ct according to the molar fraction distribution of the system at this pH shown in Fig. 3. A kinetic treatment of the trace at 501 nm (Fig. 6b) shows that the formation of A (and AH^+) occurs via a first order process, with lifetime of 1.0 s. The same lifetime is obtained from the partial recovery of absorbance at 380 nm (15% recovery of Ct at pH=4.9, Fig. 7). The increase of the pH to 6.1 increases either the lifetime of the Cc species (to 5.6 s), or the amount of recovery of Ct (to 75%). These results show that the *cis*-chalcone, the first product of light excitation, is an unstable species which disappears (i) by a pH-controlled reaction to give quinoidal base and flavylium cation, and

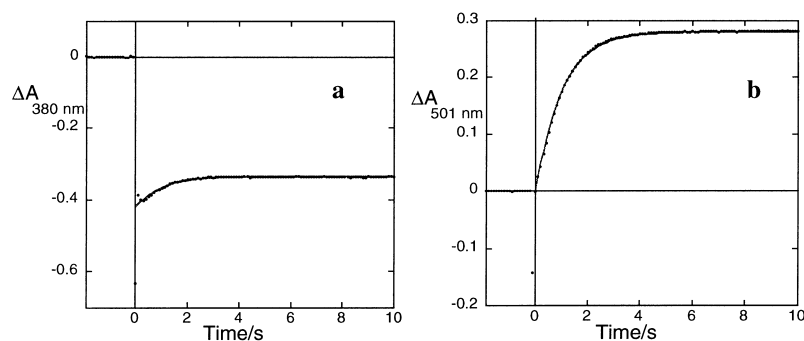


Fig. 6. Pulse irradiation of a previously dark equilibrated solution of Luteolinidin $5 \times 10^{-5} \text{ M}$, in water/methanol (1:3) at pH=4.9. Both kinetic processes, partial recovery of the absorbance at 380 nm (a), and increasing of the absorbance at 501 nm (b), exhibit the same lifetime of 1 s.

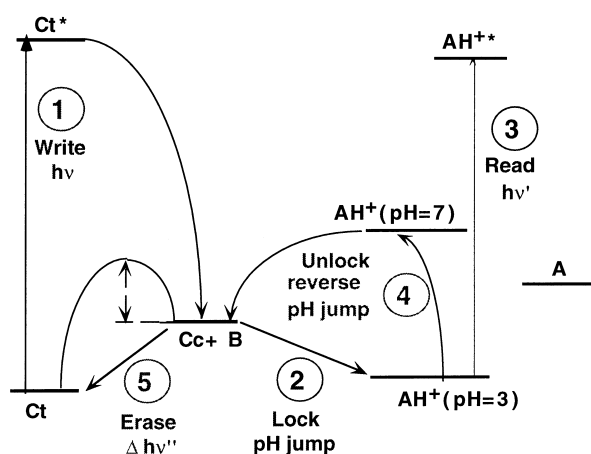


Fig. 7. A full cycle (write-lock-read-unlock-erase) for the 4'-methoxyflavilium ion.

(ii) by an isomerization reaction yielding *trans*-chalcone. Therefore, the flash photolysis experiments can give information on the fast processes (dehydration and *cis*–*trans* isomerization) not accessible to the pH jump technique. Following the procedure described in a previous paper [16] and using the lifetimes values of Cc and the amount of Ct recovery in the two pH conditions it was possible to evaluate the rate constants for the *cis*–*trans* isomerization and the dehydration reactions ($k_i=0.13\text{ s}^{-1}$ and $k_{-h}=6.1\times 10^4\text{ s}^{-1}$) reported in Table 1. These values were then used in Eq. (8) in order to obtain the remaining rate constants k_{-i} and k_h , as well as the corresponding equilibrium constants.

Inspection of Table 1 shows that luteolinidin in water/methanol (3:1) behaves like 4',7-dihydroxyflavylium in pure water. The main difference concerns the dehydration reaction (k_{-h}) which is substantially higher in luteolinidin than in the synthetic flavylium salt.

5.1. Write-lock-read-unlock-erase cycles

In current computers, based on binary logic, the information processing operations rely on bistability condition, that is the possibility for a system to switch between two stable states when a given external stimulus is applied.

In chemistry, systems that can exist in two forms interconvertible by an external input are rather common. Typical examples are the photochromic compounds, that are molecules that change their color upon light excitation [20,21].

A memory device based on a photochromic system must have switching states indefinitely stable, to avoid spontaneous erasure, and insensitive to the reading action, to avoid loss of information while reading. In recent work, we proved that destructive reading does not occur combining two reversible processes that can be addressed by means of two different stimuli. This procedure can be illustrated in the case of the 4'-methoxyflavilium ion:

1. a stable form (Ct) can be photochemically converted by irradiation with 365 nm light (*write*) into a form (Cc) that can be reconverted back either thermally or on optical reading;
2. by a second stimulus (addition of acid), Cc can be converted into a kinetically inert form AH^+ (*lock*);
3. the AH^+ form shows a spectrum clearly distinct from that of Ct and is photochemically inactive, so that it can be optically detected (*read*) without being erased;
4. by addition of base, AH^+ can be reconverted into Cc (*unlock*);
5. Cc can be thermally reconverted into the initial Ct form (*erase*).

In the case of 4'-methoxyflavilium ion, lock and unlock processes can be avoided working at a given acidic pH (*autolock* pH) without perturbing the behaviour of the system. This is due to the high activation energy associated to the isomerization reaction that slow down the *cis*–*trans* and *trans*–*cis* conversion making sufficiently stable the two switching states.

In the case of luteolinidin lacking any kinetic barrier in the *trans*–*cis* isomerization reaction it is impossible to find working conditions at a single (*autolock*) pH and thus a full cycle (*write-lock-read-unlock-erase*) has to be performed. The writing step is performed by light irradiation at 365 nm of the Ct/A mixture (70/30) at pH 5 (or 6); the light converts Ct into A which being photochemically stable can be read (at 500 nm) without erasing. However, since A is not thermally stable a pH jump to $\text{pH}\leq 2$ is needed to lock the system (and the information) in the form of the thermally and photochemically stable flavylium cation (AH^+). Moreover, the absence of a kinetic barrier in the isomerization reaction could reduce the efficiency of the writing step because part of the Cc formed during the irradiation is immediately (time scale of seconds, see Fig. 7) back converted to Ct, unless the locking action (addition of acid) is performed in the millisecond time scale. The unlock step (pH jump to 6) followed by a thermal reaction (*erase*) gives back the initial Ct/A mixture. Increasing the temperature the deleting process can be accelerated. While the system is quite stable in a single erasing step at 47°C, some decomposition was detected at room temperature in the dark after 1 month.

6. Conclusions

Through this work we have shown that luteolinidin does not exhibit better properties in comparison with synthetic flavylium salts substituted in 4' position which concerns its use as optical memory devices [7]. As we have seen, this is due to the fact that in the title compounds lacking any kinetic barrier the thermal *cis*–*trans* isomerization reaction is quite efficient, like in 7-substituted synthetic flavylium compounds [16].

Additional interesting features of luteolinidin are: (i) the absorption spectrum of the Ct form red shifted in comparison with the synthetic analogs and (ii) the chemical (and photochemical) behaviour similar to that of synthetic flavylum salts, even though luteolinidin is a natural compound.

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