Supplementary Material

Flavylium Based Dual Photochromism. Addressing *Cis-trans* Isomerization and Ring Opening-Closure by Different Light Inputs.

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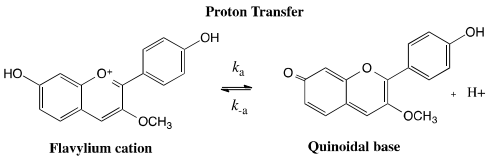
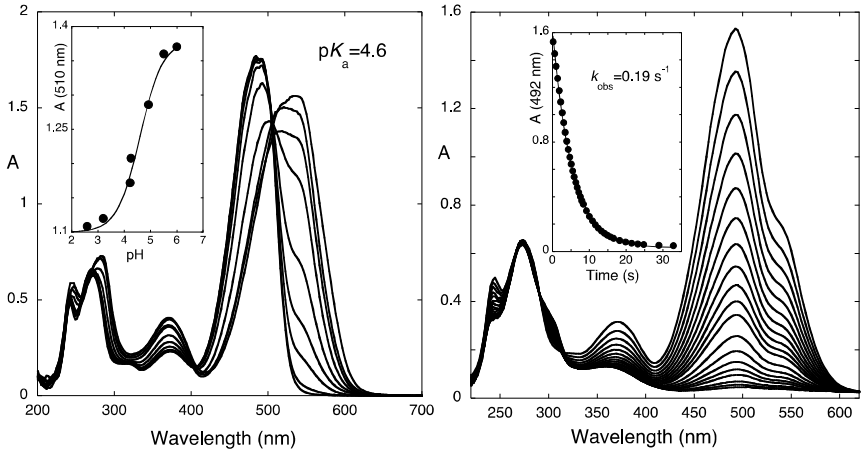
The spectral variations of the UV-vis absorption spectra of the compound 4',7-dihydroxy-3-methoxyflavylium measured by stopped flow 10 ms upon a pH jump from equilibrated solutions at pH=1 to higher pH values (direct pH jump) is shown in Fig. 1(a). The data indicates formation of quinoidal base during the mixing time of the stopped flow, which is by far the fastest kinetic process of the network, eq.(1)



$$pK_a = 4.6 \qquad (1)$$

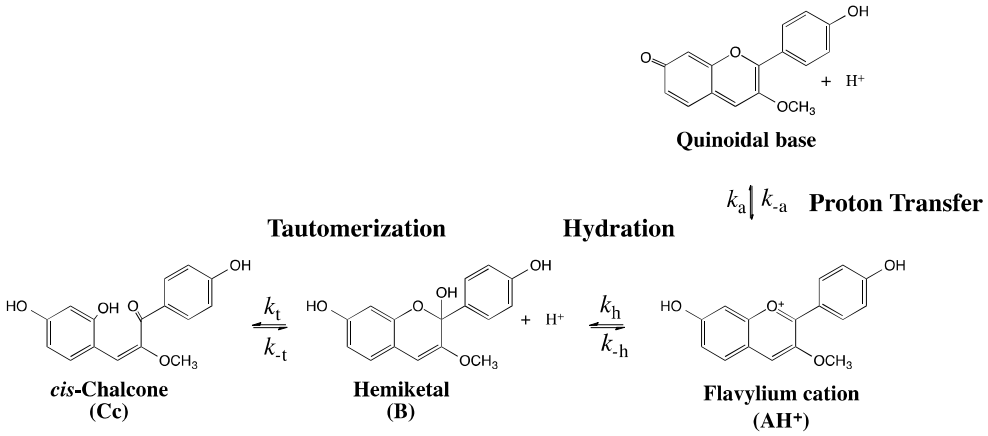


(a) **(b)**

Figure 1(a)-Spectral variations of the compound 3-methoxy-4',7-dihydroxyflavylium as a function of pH taken immediately after a pH jump from equilibrated solutions at pH=1.0 to higher pH values, followed by stopped flow. 6.7×10^{-5} M **(b)**-Kinetics of the quinoidal base disappearance upon a pH jump from 1.0 to 4.9 follow by stopped flow.

The absorption spectra of the quinoidal base reported in Fig. 1 (a) evolves in a few seconds to an absorption band that is compatible with the presence of hemiketal and

cis-chalcone, see Fig. 1 (b) for the spectral variations followed by stopped flow after a direct pH jump from pH=1 to 4.9. This behavior is very similar to the one observed in anthocyanins. The flavylium cation and the quinoidal base are in fast equilibrium and the same can be considered for the species hemiketal and *cis*-chalcone. The rate determining step of this process is thus the hydration reaction, see eq.(2) and (3). After finishing this kinetic step the species $\mathbf{AH^+}$ and **A**, **B** and **Cc** are in a pseudo equilibrium defined by the pseudo constant $K^{\wedge}_a$, eq.(2)



Scheme 1.

$$\mathbf{AH^+} + H_2O \rightleftharpoons \mathbf{CB^{\wedge}} + H_3O^+ \qquad K^{\wedge}_a = K_a + K_h + K_hK_t \tag{2}$$

$$[\mathbf{CB^{\wedge}}] = [\mathbf{A}] + [\mathbf{B}] + [\mathbf{Cc}] \tag{3}$$

Representation of the rate constant of this process as a function of pH is shown in Fig. 2. Fitting can be achieved by means of eq.(4), for pK_a=4.6; k_h=0.3 s^{-1}; k_{-h}=75 $M^{-1}s^{-1}$.[1]

$$k_{hydration} = \frac{[H^+]}{[H^+]+K_a} k_h + \frac{1}{1+K_t} k_{-h}[H^+] \tag{4}$$

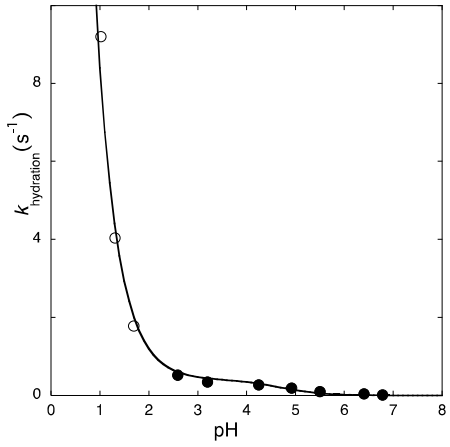
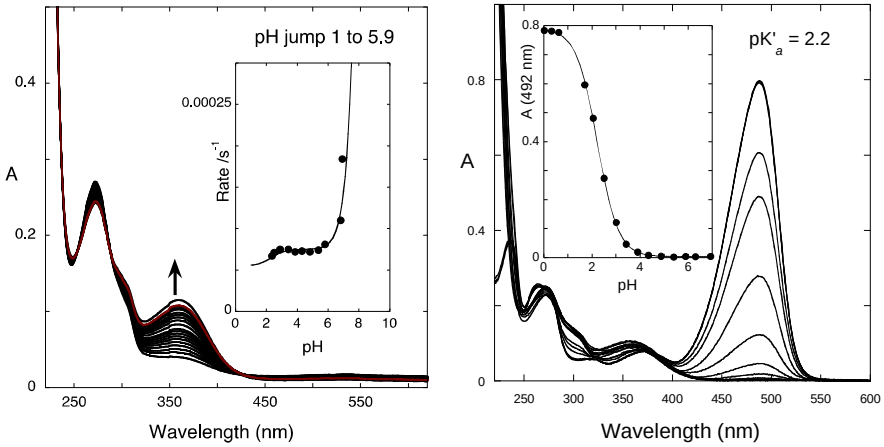


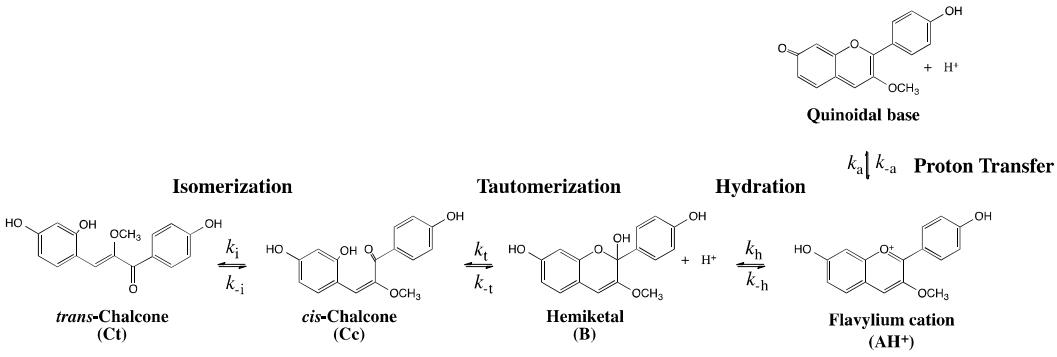
Figure 2. pH dependence of the kinetic process controlled by the hydration reaction followed by stopped flow: (●) direct pH jumps; Fitting was achieved with eq.(5) for pK_a=4.6; k_h=0.3 s^{-1}; k_{-h}=75 $M^{-1}s^{-1}$. (○) reverse pH jumps, fitting was achieved with eq.(8) which is coincident with eq.(5) within experimental error due to the low value of K_t=0.05, see below for more details.

The spectral variations observed for the last and slowest kinetic process are shown in Fig. 3(a). Inspection of Fig. 3(a) shows the raising of a absorption band centered at 355 nm as expected upon formation of *trans*-chalcone. The isomerization is the slowest process of the network and by consequence the species **AH**$^+$, **A**, **B** and **Cc** equilibrate (pseudo-equilibrium) prior to observed the isomerization eq.(4)



(a) **(b)**

Figure 3 (a). Spectral variations corresponding to the last and slowest process to reach the equilibrium after a pH jump from pH=1.0 to 5.9; 3x10^{-5} M ; **inset-** variation of the rate constant of *trans*-chalcone formation as a function of pH: **(b**) Spectra of the equilibrated solutions as a function of pH.



(5)

Scheme 2

According to the kinetic scheme reported in eq.(5), the first two terms of eq.(6) can be deduced. In equation (6) a third and fourth term was added to account for the isomerization taking place through the ionized *cis*-chalcone.

$$k_{isomerization} = \frac{K_t K_h}{[H^+] + K_a + (1+K_t)K_h} k_i + k_{-i} + \frac{a}{[H^+] + b} k'_i + k'_{-i} \quad (6)$$

The pH dependent spectral variations of the equilibrium are shown in Fig. 3(b) and can be fitted with pK'_a = 2.2. The kinetic data depicted in Fig. 3(a) can be fitted with equation (6) with the following parameters: $K_a+(1+K_t)K_h=10^{-2.3}$; $K_tK_hk_i=10^{-7}$ M^{-1} s^{-1}; $k_{-i}\approx 5x10^{-5}$ s^{-1}; $a=7x10^{-12}$; $b=10^{-10.2}$.

Reverse pH jump

The reverse pH jumps consists on the pH jump from a solution equilibrated at the moderately acidic medium (where CB is dominant) to lower pH values in order to form flavylium cation. When the final pH of the reverse pH jump is sufficiently acid the hydration reaction becomes faster than the tautomerization, change of regime. [1] In principle the reverse pH jump trace shows an initial absorption due to the conversion of **A** present at the initial pH into $\mathbf{AH^+}$, because protonation of the quinoidal base takes place during the mixing time of the stopped flow. However in the present experiment the initial fraction of **A** is circa only 0.4 %. The first kinetic corresponds to the conversion of **B** into $\mathbf{AH^+}$ and the second and slower to the conversion of **Cc** into more $\mathbf{AH^+}$ via **B**. Eqs. (7) and (8) account for these two steps. Moreover, the ratio of the amplitude of the slowest exponential by the faster is equal to K_t=[Cc]/[B].

$$k_{reverse1} = \frac{[H^+]}{[H^+] + K_a} k_h + k_{-h}[H^+] \quad (7)$$

$$k_{reverse2} = k_{-t} \quad (8)$$

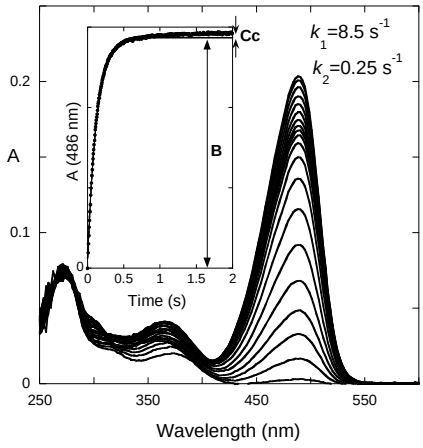


Figure 4. - Spectral variations upon a reverse pH jump of the compound 4',7-dihydroxy-3-methoxyflavylium 7.5×10^{-6} M, from equilibrated solutions at pH= 6 to 1.

In Fig.4 a solution equilibrated at pH=6.0 was acidified to pH=1.0 by means of a stopped flow apparatus. The traces at the maximum of the flavylium absorption in a short time scale (inset of Fig. 4) show that the initial absorption due to **A** is negligible as expected from the ratio of the constants K_a/K'_a=0.4% which gives the fraction of **A** into **CB**. The ratio between the two exponetials leads to K_t=0.05. The small vale of K_t makes eq.(4) and (7) undistinguishable within experimental error Fig. 2. In this last Figure the experimental rate constants of the reverse pH jumps experiments have also been included (open circles). The rate of the slowest step in Fig. 4 permits to calculate $k_t=1.25 \times 10^{-2}\ s^{-1}$.

Calculation of the equilibrium and rate constants

The calculation of the equilibrium constants is made considering the following experimental data

$$K'_a = K_a + K_h + K_h K_t + K_h K_t K_i = 10^{-2.2} \qquad \text{Fig. 3b} \qquad (9)$$

$$K_a = 10^{-4.6} \qquad \text{Fig. 1a} \qquad (10)$$

$$K_t = 0.05 \qquad (11)$$

$K_h = \frac{k_h}{k_{-h}}$ =0.3 s^{-1}/75 $M^{-1}s^{-1}$. Giving $K_h=4 \times 10^{-3}\ M^{-1}s^{-1}$.

Taking into account the value ok K_t=0.05 a value of K_i=10.4 is obtained, Table 1.

The remaining rate constants can be calculated from eqs.(8) and (6) and the respective equilibrium constants, see Table 2.

Table 1. Equilibrium constants of the compound 4',7-dihydroxy-3-methoxyflavylium.

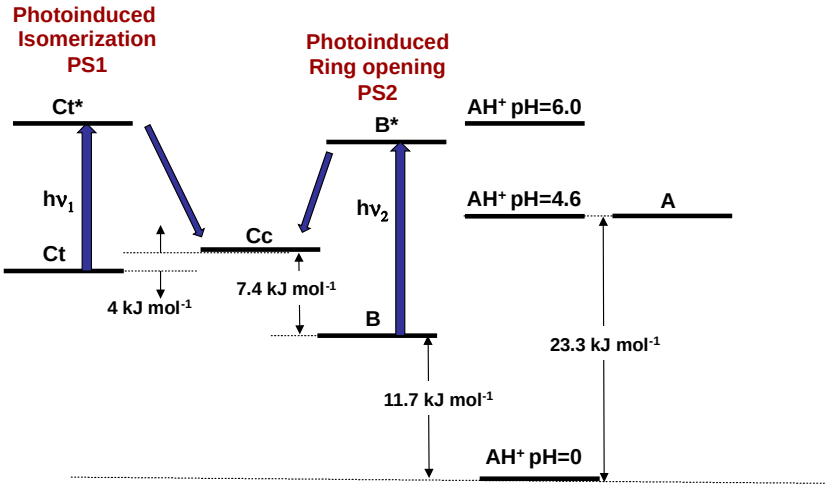
pK_a'	$pK^{\wedge}_a$	pK_a	K_h/ M^{-1}	K_t	K_i
2.2	2.37	4.6	4×10^{-3}	0.05	10.4

Estimated error 10%

Table 2. Rate constants of the compound 4',7-dihydroxy-3-methoxyflavylium

k_h $s^{-1(a)}$	k_{-h} M^{-1} $s^{-1(a)}$	k_t $s^{-1(a)}$	k_{-t} $s^{-1(a)}$	k_i $s^{-1(b)}$	k_{-i} $s^{-1(b)}$
0.3	75	1.25×10^{-2}	0.25	2.3×10^{-4}	2.2×10^{-5}

Estimated error 15%



Scheme 3. Energy level diagram of the compound 4',7-dihydroxy-3-methoxyflavylium at the equilibrium.

The mole fraction of the species at the equilibrium are given by K_a/K'_a=0.4%; K_h/K'_a=63.4%; K_hK_t/K'_a=3.2%; $K_hK_tK_i/K'_a$=33% respectively for **A**, **B**, **Cc** and **Ct**.[Erro! Marcador não definido.]

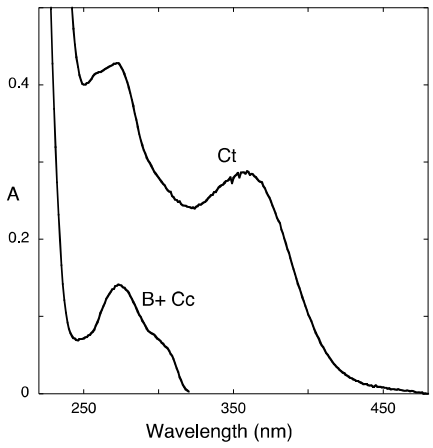


Figure 5. Absorption spectra of the species **Ct** and **B**+**Cc** obtained by mathematical decomposition.

At the pseudo-equilibrium as the last absorption spectra of Fig. 2 (a) the percentage of the species is: 1% **A**, 94% **B** and 5% **Cc**. While it is not possible to separate **B** from **Cc** the last recorded spectra of Fig.2 (a) is essentially due to the **B** absorption. At the equilibrium the spectra of **Ct** can be obtained by decomposition taking into account that B (and Cc) contribute circa 66%, and Ct 33%, Fig. 5 and Fig. 6.

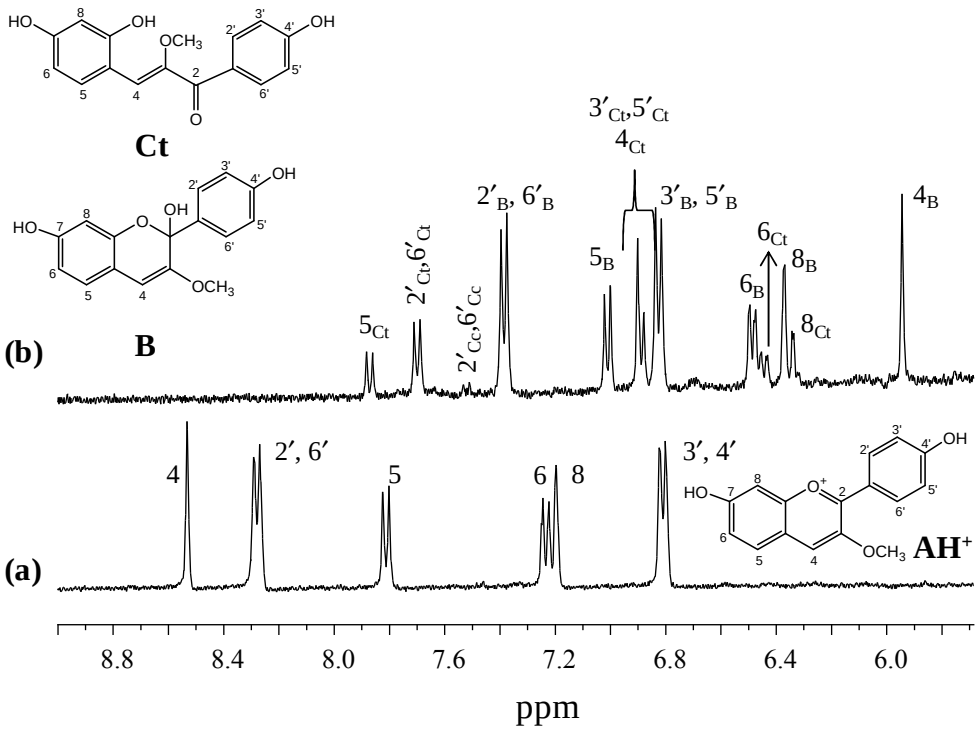


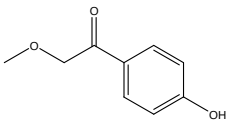
Figure 6: ^{1}H NMR spectra of 7,4′-dihydroxy-3-methoxyflavylium perchlorate (aromatic region) at room temperature in D_2O: a) flavylium cation AH^+, b) B+Ct+Cc (pD = 5.8).

Experimental Section

General: All reagents and solvents were of analytical grade. ^{1}H NMR spectra were recorded at 400.13 MHz with a Bruker AMX400. Elemental analysis was performed in a Thermofinnigan Flash EA 112 series. Mass spectra were run on an Applied Biosystems Voyager-DE™ PRO. Spectroscopic experiments were carried out in buffered water solvent.[2] pH values were adjusted by the addition of a 0.1 M NaOH aqueous solution or a 0.1 M HCl aqueous solution, and measured with a MeterLab

pHM240 pH meter from Radiometer Copenhagen. For solutions with HCl concentration above 0.1 M, pH was calculated from –log [HCl]. UV/Vis absorption spectra were recorded with a Varian-Cary 100 Bio spectrophotometer or in a Shimadzu VC2501-PC. The stopped flow experiments were conducted in an Applied Photophysics SX20 stopped-flow spectrometer provided with a PDA.1/UV photodiode array detector. Irradiation experiments were carried out on a spectrofluorimeter Spex Fluorolog 1681 at the wavelengths 275 nm and 365 nm without slits. Light intensity was measured by ferrioxalate actinometry.[3] Quantum yields were measured based on the total absorbed light. Flash photolysis experiments were run on a LKS.60 ns laser photolysis spectrometer from Applied Photophysics, with a Brilliant QSwitch Nd:YAG laser from Quantel, using the fourth harmonic (λexc =266 nm, laser pulse half-width equal to 6 ns).

2.3.1. Synthesis of 2-methoxy-4'-hydroxyacetophenone[4]



A solution of 2-Bromo-4'-hydroxyacetophenone (2.0 g, 9.3 mmol) in methanol (10 mL) was added droppwise to a solution of NaOMe (2.0 g of Na in 40 mL of methanol) under stirring and nitrogen. The yellow mixture was refluxed for 40 min, cooled and the solvent removed by rotary evaporator. The reaction was monitored by TLC in silica (ethyl acetate:hexane, 3:2) and after 40 minutes the reagent was completely consumed. The residue was suspended in water (20 mL), pH was adjusted with concentrated HCl to 6.5 and the product extracted with ethyl acetate (3×20 mL). The organic phase was dried with Na_2SO_4 and evaporated. An yellow oil was obtained and yellow needles started to precipitate. This product was dried under vacuum.

Yield: 0.75 g, 49 %. ^{1}H-RMN (C_2D_6CO, 400.13 MHz, 298 K) δ (ppm): 7.9 (2H, d, H3 and H5, ^3J = 8.0 Hz), 6.95 (2H, d, H2 and H6, ^3J = 8.0 Hz), 4.65 (2H, s, COCH_2), 3.41 (3H, s, OCH_3).

Synthesis of 4',7-dihydroxy-3-methoxyflavylium perchlorate

A solution of 2-methoxy-4'-hydroxyacetophenone (0.6 g, 3.6 mmol) with one equivalent of 2,4-dihydroxybenzaldehyde (0.50 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 purple mixture but a precipitate in oil form was obtained. After removal of solvent, the product was recrystallized from ethanol solution with addition of perchloric acid and water to precipitate as powder. A dark purple solid was filtered, washed several times with diethyl ether and dried under vacuum. Yield: 0.86 g, 65%. ^{1}H-RMN (D_2O + DCl, 400.13 MHz, 298 K) δ (ppm): 8.53 (1H, H4, s), 8.28 (2H, H2', H6', d, 3J = 8.8 Hz), 7.23 (1H, H6, d, 3J = 8.8 Hz), 7.20 (1H, H8, s), 6.81 (2H, H3', H4', t, 3J = 8.4 Hz), 4.03 (CH_3O, s). (MALDI-TOF/MS: m/z (%): calcd for $C_{16}H_{13}O_4^+$ (AH^+ species), 269.08; found, 269.08 [M+] (100%). Elemental analysis (%) calcd for $C_{16}H_{13}O_4.ClO_4.0.5H_2O$ (Mr=377.73): C, 50.88; H, 3.74; Found: C, 51.17; H, 3.58.

References

1-F. Pina, J. Agric. Food Chem., 2014, **62**, 6885.

2- F. W. Küster, A. Thiel, "Tabelle per le Analisi Chimiche e Chimico-Fisiche", 12nd ed, 1982, Hoepli, Milano, p.157-160. The universal buffer used was prepared in the following way: 2.3 cm^3 of 85% (w/w) phosphoric acid, 7.00 g of monohydrated citric acid, and 3.54 g of boric acid are dissolved in water; 343 mL of 1 M NaOH is then added, and the solution diluted to 1 dm^3 with water.

3- C. G. Hatchard, C. A. Parker, *Proc. R. Soc. London Ser. A*, 1956, **235**, 518.

4- H.U. Shetty, W.L. Nelson, J. Med. Chem., 1988, **31**, 55.