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ORIGINAL PAPER

Analytical resolution of the reaction rates of flavylium network by Laplace transform

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Abstract A complete resolution of time evolution for all species of flavylium chemical reactions network in acidic to neutral media was obtained applying Laplace transform methods, Vieta's formulae and the general partial fraction theorem. Analyses of particular systems under direct pH-jump, reverse pH-jump, stopped flow and flash photolysis experiments have been performed. The deduced formulas cover all possibilities of flavylium and anthocyanins compounds—with or without quinoidal base and with or without *cis–trans* isomerization barrier. The expressions for the observed rate constants in different type of experiments are quite similar. This allows creation of global procedure, based on fitting of one single set of expressions with data-set from different experiments. The mathematical approach allows easy and versatile programming.

Keywords Kinetics · Flavylium network · Model · Chalcones · pH jump · Flash photolysis

1 Introduction

2-Phenyl-1-benzopyrilum constitutes an important family of compounds which comprise anthocyanins the ubiquitous colorants of most flowers and fruits [1]. The first 2-phenyl-1-benzopyrilum compounds described in literature were 4-methyl-7-hydrox-

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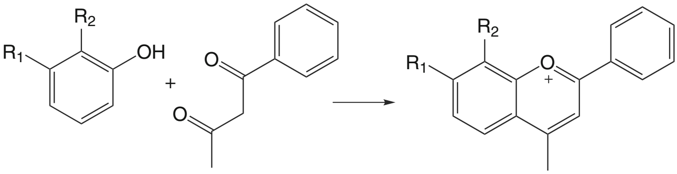
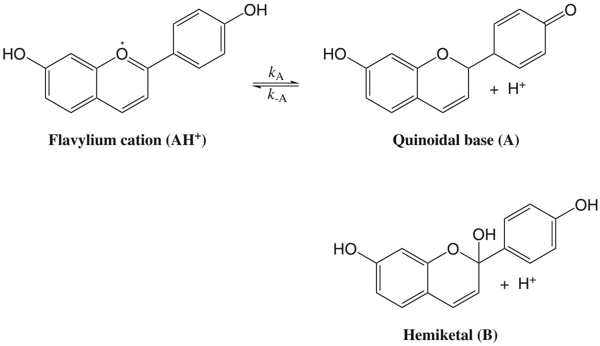


Scheme 1 Bülow synthetic flavylium compounds, $R_1 = OH$, $R_2 = H$ and $R_1 = OH$, $R_2 = OH$



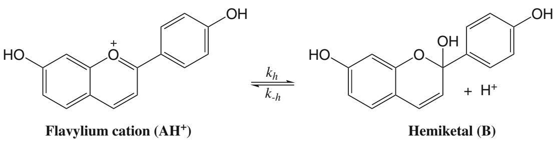
(proton transfer) (1)

Scheme 2 Quinoidal base (*A*) and hemiketal (*B*) species

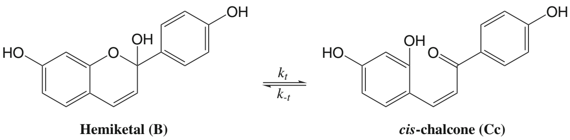
yflavylium and 4-methyl-7,8-dihydroxyflavylium, synthesized by Bülow in 1901 [2] Scheme 1.

The early researchers observed the formation of the quinoidal base, Eq. (1) and identified the hemiketal species (*B*), Scheme 2 [3].

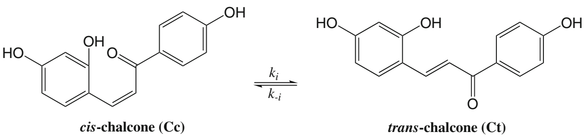
Latter on, the formation of chalcone species was reported [4,5] and the sequence $AH^+ \rightleftharpoons A \rightleftharpoons B \rightleftharpoons Cc \rightleftharpoons Ct$ was used to explain the photochemistry of 7,4′ dihydroxyflavylium [6]. A more realistic sequence of chemical reactions was firmly established after the work of Dubois and Brouillard, (anthocyanins) [7] as well as Mc Clelland [8], (synthetic flavylium compounds), as follows:



(hydration) (2)



(tautomerization) (3)



(isomerization) (4)

Proton transfer and hydration take place from flavylium cation. These two parallel reactions occur in different scales of time: proton transfer in the micro-second and hydration in seconds to sub-seconds, depending on pH. By consequence, A appears as a kinetic product that usually fades with time, being not the most stable species at the equilibrium.

The hemiketal (chromene) is involved in a tautomeric process that leads to the ring opening (and closure) with formation of *cis*-chalcone. This reaction usually occurs in the sub-second scale of time.

Finally the *cis*-chalcone isomerizes and gives the *trans*-chalcone, in a time scale of seconds to weeks, depending on the *cis–trans* isomerization barrier.

The complete solution for the sequence $A \rightleftharpoons AH^+ \rightleftharpoons B \rightleftharpoons C$ was reported by Dubois and Brouillard [9]. However, these authors have not considered the existence of the two chalcones *cis–trans* [10].

In cases where the *cis–trans* isomerization barrier is very high the system can be separated into the sequence of reactions $A \rightleftharpoons AH^+ \rightleftharpoons B \rightleftharpoons Cc$ on one hand and $Cc \rightleftharpoons Ct$ on the other, as in the case of anthocyanins, and the approach previously reported by these authors can be applied [7,11]. However, in some synthetic flavylium compounds the *cis–trans* isomerization occurs in a scale of time comparable with hydration or even tautomerization, and Dubois–Brouillard model is inapplicable.

In this work we are describing a mathematical treatment that allows the complete resolution of the flavylium network independently of the *cis–trans* isomerization barrier. The resolution here reported is not restricted to the flavylium compounds and can be applied to any sequence of consecutive and reversible reactions, as those occurring in some biochemical reactions [12].

2 Theory

The resolution of the kinetic Scheme 3 for direct pH jumps, reverse pH jumps and flash photolysis experiments is based on the Laplace transformation, general partial fraction theorem and Vieta's formulae.

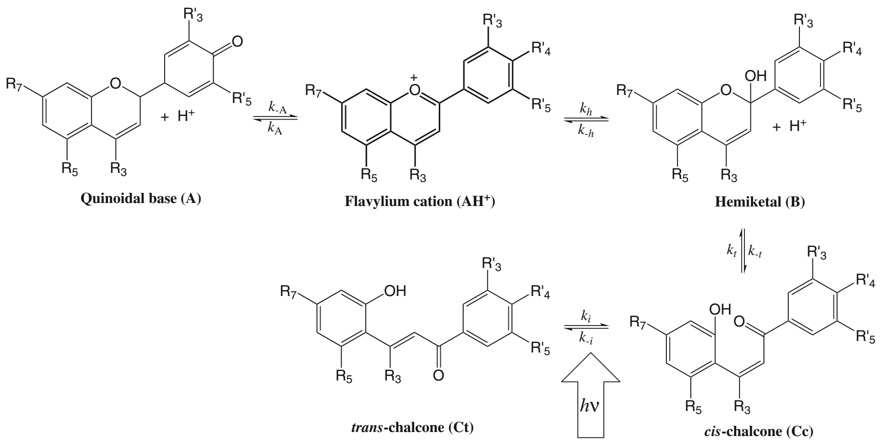
3 Direct pH jumps

This kind of experiments are generally carried out by adding base to a stock solution equilibrated at pH = 1, (AH^+), and following the absorbance variations by means of a common spectrophotometer or through a stopped flow apparatus if the rates involved are very fast.

Two general cases will be considered in this work: **I**— compounds possessing high barrier and **II**—compounds with low *cis–trans* isomerization barrier.

I. Flavilium system in the acidic to neutral pH values, with quinoidal base and exhibiting very high barrier: pH $<$ 7; [Ct] = 0; [Cc^-] = 0 [13].

The general equation describing the network of reaction occurring after direct pH jump is presented by the Eq. (5)



Scheme 3 Most common pattern of substitution in 2-phenyl-1-benzopyrilium. The quinoidal base, **A**, only appears when at least one of the substituents is an hydroxyl group. In the case of anthocyanins the position 3 (mono glucoside) or 3,5 (di glucoside) are occupied by a sugar

$$\mathrm{A} + \mathrm{H}^+ \overset{K_a}{\rightleftharpoons} \mathrm{AH}^+ \underset{k_{-h}}{\overset{k_h}{\rightleftharpoons}} \mathrm{B} + \mathrm{H} \underset{k_{-t}}{\overset{k_t}{\rightleftharpoons}} \mathrm{Cc} \tag{5}$$

$$\mathrm{C}_0 = [A] + \left[AH^+\right] + [B] + [Cc] \tag{6}$$

$$K_a = \frac{[A][H^+]}{[AH^+]} \tag{7}$$

$$K_h = \frac{[B][H^+]}{[AH^+]} \tag{8}$$

$$K_t = \frac{[Cc]}{[B]} \tag{9}$$

where C_0 is a total concentration and $K_h = k_h/k_{-h,} K_t = k_t/k_{-t}$ are the thermodynamic constants of the hydration and tautomerization, respectively.

Carrying out the mass balance of the system Eq. (6), and using the equilibrium constants, Eqs. (7)–(9), the following formulas for the mole fraction distribution of the species at the equilibrium, Eqs. (10)–(13) are deduced.

$$\chi_A = \frac{K_A}{[H^+] + K_a + K_h + K_h K_t} \tag{10}$$

$$\chi_{AH^+} = \frac{[H^+]}{[H^+] + K_a + K_h + K_h K_t} \tag{11}$$

$$\chi_B = \frac{K_h}{[H^+] + K_a + K_h + K_h K_t} \tag{12}$$

$$\chi_{Cc} = \frac{K_h K_t}{[H^+] + K_a + K_h + K_h K_t} \tag{13}$$

3.1 Kinetics behavior

Formation of the base (A) is at least 10^3–10^4 times faster [14] that any other kinetic process. Therefore, it will be assumed that (A) is formed instantly, and by consequence AH^+ and A can be treated like one single species [7].

Regarding the kinetic behavior of the system, the following differential equations can be written

$$\begin{aligned}\frac{d[AH^+ + A]}{dt} &= -k_h\frac{[H^+]}{[H^+]+K_a}[AH^+ + A] + k_{-h}[H^+][B]\\ &= -k_h\frac{[H^+]}{[H^+]+K_a}(1-[B]-[Cc]) + k_{-h}[H^+][B] \qquad (14)\end{aligned}$$

$$\begin{aligned}\frac{d[B]}{dt} &= k_h\frac{[H^+]}{[H^+]+K_a}[AH^+ + A] - (k_{-h}[H^+]+k_t)[B] + k_{-t}[Cc]\\ &= \frac{k_h[H^+](1-[B]-[Cc])}{[H^+]+K_a} - (k_{-h}[H^+]+k_t)[B] + k_{-t}[Cc] \qquad (15)\end{aligned}$$

$$\frac{d[Cc]}{dt} = k_t[B] - k_{-t}[Cc] \qquad (16)$$

From the mass balance: $[A]+[AH^+] = C_0 - [B] - [Cc]$; this was used in Eqs. (14) and (15) to eliminate $[A + AH^+]$ [15].

For a typical direct pH jump from very acidic to less acidic or neutral pH, the initial conditions are:

$$[AH^+]_0 = \frac{C_0[H^+]}{[H^+]+K_a}; \quad [A]_0 = \frac{C_0K_a}{[H^+]+K_a}; \quad [B]_0 = 0; \quad [Cc]_0 = 0 \qquad (17)$$

After Laplace transformation of Eqs. (15) and (16) (see Appendix 1)

$$s.b = \frac{k_h[H^+]}{[H^+]+K_a}(1/s - b - cc) - (k_{-h}[H^+]+k_t)b + k_{-t}cc \qquad (18)$$

$$s.cc = k_tb - k_{-t}cc \qquad (19)$$

where b and cc are Laplace transforms of $[B](t)$ and $[Cc](t)$, s is the Laplace variable, $[H^+]$ is the proton concentration. Here for simplicity we will use $C_0 = 1$ and the equations express the molar fractions of the components.

Equation (19) is solved for cc and substituted into Eq. (18), which is solved for b [16]:

$$\begin{aligned}b &= \frac{\frac{k_h[H^+]}{[H^+]+K_a}(k_{-t}+s)}{s\left(\frac{k_h[H^+]}{[H^+]+K_a}(k_t+k_{-t}) + k_{-h}[H^+]k_{-t} + s\left(\frac{k_h[H^+]}{[H^+]+K_a} + k_{-h}[H^+] + k_t + k_{-t}\right) + s^2\right)}\\ &= \frac{\frac{k_h[H^+]}{[H^+]+K_a}(k_{-t}+s)}{s\left(s^2+Bs+C\right)} \qquad (20)\end{aligned}$$

Equation (20) can be rewritten as in Eq. (21).

$$b = \frac{\frac{k_h[H^+]}{[H^+]+K_a}(k_{-t}+s)}{s\left((s+\alpha)(s+\beta)\right)} \tag{21}$$

where $-\alpha$ and $-\beta$ are the roots of the second order equation in s inside the brackets.

According to the Vieta's formulae (see Appendix 2) B and C are defined by Eqs. (20), (22) and (23):

$$B = \alpha + \beta = \left(k_h \frac{[H^+]}{[H^+]+K_a} + k_{-h}[H^+] + k_t + k_{-t}\right) \tag{22}$$

$$C = \alpha\beta = k_h \frac{[H^+]}{[H^+]+K_a}(k_t + k_{-t}) + k_{-h}[H^+]k_{-t} \tag{23}$$

It is important to note that α and β are positive real numbers and they are in fact the observed rate constants characteristic for the system Eq. (5).

Applying inverse Laplace transformation (Table 1, Appendix 1-A.9 with $a = -\alpha$ and $b = -\beta$), b is transformed into $[B](t)$ and Eq. (21) becomes:

$$[B](t) = k_h \frac{[H^+]}{[H^+]+K_a}\left(\frac{k_{-t}}{\alpha\beta} + \frac{(k_{-t}-\alpha)}{\alpha(\alpha-\beta)}e^{-\alpha t} - \frac{(k_{-t}-\beta)}{\beta(\alpha-\beta)}e^{-\beta t}\right) \tag{24}$$

where

$$\alpha, \beta = \frac{B \pm \sqrt{B^2 - 4C}}{2} \tag{25}$$

B and C have been previously defined in Eq. (20)

Following the same procedure, the Eq. (16) could be solved for cc:

$$cc = \frac{\frac{k_h[H^+]}{[H^+]+K_a}k_t}{s\left(\frac{k_h[H^+]}{[H^+]+K_a}(k_{-t}+k_t) + k_{-h}[H^+]k_{-t} + s\left(\frac{k_h[H^+]}{[H^+]+K_a} + k_{-h}[H^+] + k_t + k_{-t}\right) + s^2\right)} = \frac{\frac{k_h[H^+]}{[H^+]+K_a}k_t}{s\left(s^2 + Bs + C\right)} \tag{26}$$

The denominators of Eqs. (20) and (26) are the same, and the same approach for Eq. (21) can be used. After inverse Laplace Transformation of Eq. (26):

$$[Cc](t) = \frac{k_h[H^+]}{[H^+]+K_a}k_t\left(\frac{1}{\alpha\beta} + \frac{1}{\alpha(\alpha-\beta)}e^{-\alpha t} - \frac{1}{\beta(\alpha-\beta)}e^{-\beta t}\right) \tag{27}$$

Finally from the mass balance Eqs. (6) and (7):

$$[AH^{+}](t) = [1 - [B](t) - [Cc](t)]\,\frac{[H^{+}]}{[H^{+}] + K_a} \tag{28}$$

$$[A](t) = [1 - [B](t) - [Cc](t)]\,\frac{K_a}{[H^{+}] + K_a} \tag{29}$$

or

$$[AH^{+}](t) = \frac{[H^{+}]}{[H^{+}] + K_a}\left(\frac{k_{-h}[H^{+}]k_{-t}}{\alpha\beta} + \frac{\frac{k_h[H^{+}]}{[H^{+}]+K_a}(\alpha - k_t - k_{-t})}{\alpha(\alpha-\beta)}e^{-at} + \frac{\frac{k_h[H^{+}]}{[H^{+}]+K_a}(\beta - k_t - k_{-t})}{\beta(\beta-\alpha)}e^{-bt}\right) \tag{30}$$

$$[A](t) = \frac{K_a}{[H^{+}] + K_a}\left(\frac{k_{-h}[H^{+}]k_{-t}}{\alpha\beta} + \frac{\frac{k_h[H^{+}]}{[H^{+}]+K_a}(\alpha - k_t - k_{-t})}{\alpha(\alpha-\beta)}e^{-at} + \frac{\frac{k_h[H^{+}]}{[H^{+}]+K_a}(\beta - k_t - k_{-t})}{\beta(\beta-\alpha)}e^{-bt}\right) \tag{31}$$

For verification of the model the following test could be done. If expressions for α and β are correct, at $t = \infty$ (equilibrium) a set of equations that predict the molar fractions of the species in equilibrium should be obtained. For example in the case

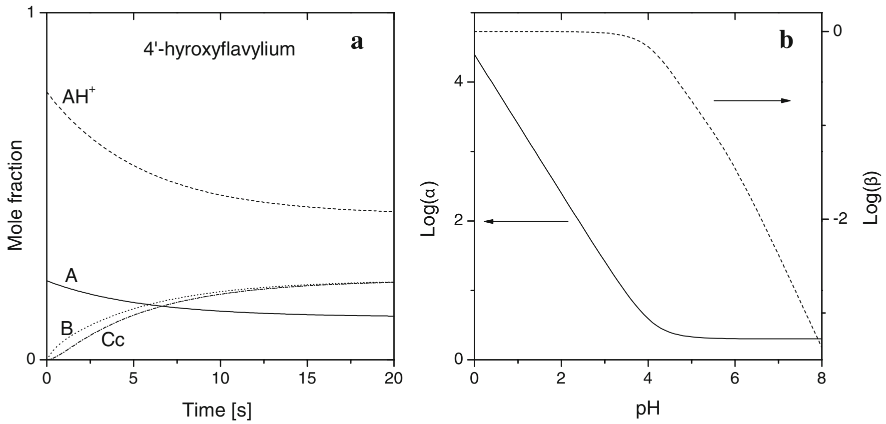


Figure 1 **a** Simulation of the mole fraction variation of A, AH^{+}, B and Cc as a function of time for 4′-hydroxyflavylium, using the rate constant values previously reported in literature [17]. As k_t and k_{-t} ($K_t = 1.0$) are not available a the simulation was made with both constants equal to $1\,s^{-1}$, which is in the order of magnitude expected for this compound in comparison with the constants observed in similar derivatives. **b** pH dependence of the rate constants

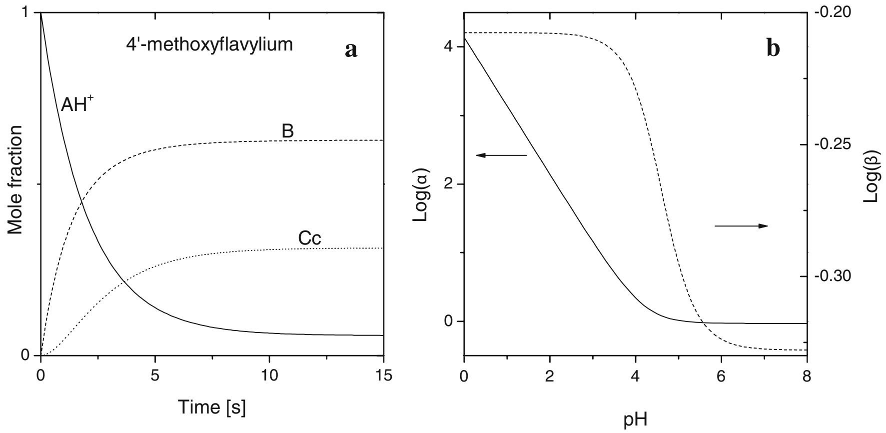


Figure 2 **a** Simulation of the mole fraction variation of AH^+, B and Cc as a function of time for 4′-methoxyflavylium, using the rate constant values previously reported in literature [8,18], **b** pH dependence of the rate constants

of Eq. (27) the molar fraction of Cc at the equilibrium, Eq. (32), is obtained, which is coincident with Eq. (13) above deduced (Fig. 1).

$$[Cc]_{eq} = \frac{\frac{k_h[H^+]}{[H^+]+K_a}k_t}{\alpha\beta} = \frac{\frac{k_h[H^+]}{[H^+]+K_a}k_t}{k_{-h}[H^+]k_{-t} + \frac{k_h[H^+]}{[H^+]+K_a}(k_t + k_{-t})}$$
$$= \frac{K_h K_t}{[H^+] + K_a + K_h + K_h K_t} \tag{32}$$

If none of the substituents R, R′ (Scheme 3) is a hydroxyl group, the quinoidal base, A, is not present and $K_a = 0$. As a consequence, in Eqs. (6)–(32) the term $k_h \frac{[H^+]}{[H^+]+K_a}$ in all formulas can be substituted by k_h and all deduced formulas Eqs. (10)–(32) are valid (Fig. 2).

II. Flavylium compounds possessing a hydroxyl substituent and lacking the *cis–trans* isomerization barrier.

$$\mathrm{A} + \mathrm{H}^+ \overset{K_a}{\rightleftharpoons} \mathrm{AH}^+ \underset{k_{-h}}{\overset{k_h}{\rightleftharpoons}} \mathrm{B} + \mathrm{H}^+ \underset{k_{-t}}{\overset{k_t}{\rightleftharpoons}} \mathrm{Cc} \underset{k_{-t}}{\overset{k_t}{\rightleftharpoons}} \mathrm{Ct} \tag{33}$$

$$K_i = \frac{[Ct]}{[Cc]} \tag{34}$$

Adding Eq. (34) to case **I**, using Eqs. (7)–(9) and (34) and the mass balance Eq. (35), the mole fractions distribution at the equilibrium are obtained Eqs. (36)–(40).

$$C_0 = [A] + [AH^+] + [B] + [Cc] + [Ct] \tag{35}$$
$$\chi_A = \frac{K_A}{[H^+] + K_a + K_h + K_h K_t + K_h K_t K_i} \tag{36}$$

$$\chi_{AH^+} = \frac{[H^+]}{[H^+] + K_a + K_h + K_h K_t + K_h K_t K_i} \tag{37}$$

$$\chi_B = \frac{K_h}{[H^+] + K_a + K_h + K_h K_t + K_h K_t K_i} \tag{38}$$

$$\chi_{Cc} = \frac{K_h K_t}{[H^+] + K_a + K_h + K_h K_t + K_h K_t K_i} \tag{39}$$

$$\chi_{Ct} = \frac{K_h K_t K_i}{[H^+] + K_a + K_h + K_h K_t + K_h K_t K_i} \tag{40}$$

3.2 Kinetic behavior

The differential rate equations for the system Eq. (33) are now given by Eqs. (41)–(44)

$$\frac{d[AH^+ + A]}{dt} = -k_h \frac{[H^+]}{[H^+] + K_a}[AH^+ + A] + k_{-h}[H^+][B] \tag{41}$$

$$\frac{d[B]}{dt} = k_h \frac{[H^+]}{[H^+] + K_a}[AH^+ + A] - (k_{-h}[H^+] + k_t)[B] + k_{-t}[Cc] \tag{42}$$

$$\frac{d[Cc]}{dt} = k_t[B] - (k_{-t} + k_i)[Cc] + k_{-i}[Ct] \tag{43}$$

$$\frac{d[Ct]}{dt} = k_i[Cc] - k_{-i}[Ct] \tag{44}$$

Considering again the case of a direct pH jump from very acidic to less acidic or neutral pH, the following initial conditions should be taken into account: $[AH^+]_0 + [A]_0 = C_0, [B]_0 = [Cc]_0 = [Ct]_0 = 0$.

After Laplace transform and excluding an equation for $[AH^+ + A]$ by using as previously the mass balance Eq. (35):

$$s.b = k_h \frac{[H^+]}{[H^+] + K_a}(1/s - b - cc - ct) + cck_{-t} - b\left(k_{-h}[H^+] + k_t\right) \tag{45}$$

$$s.cc = ctk_{-i} - cc(k_i + k_{-t}) + bk_t \tag{46}$$

$$s.ct = cck_i - ctk_{-i} \tag{47}$$

where *b, cc* and *ct* are the Laplace transforms of $[B](t)$, $[Cc](t)$, $[Ct](t)$ and s is the Laplace variable. Again, as in case **I** C_0 will be considered equal to 1.

The solution of linear system Eqs. (45)–(47) for *b, cc* and *ct* is

$$b = -\frac{k_h[H^+]}{[H^+] + K_a} \frac{(k_i s + (k_{-i} + s)(k_{-t} + s))}{s.P(s)} \tag{48}$$

$$cc = -\frac{k_h[H^+]}{[H^+] + K_a} \frac{k_t(k_{-i} + s)}{s.P(s)} \tag{49}$$

$$ct = -\frac{k_h[H^+]}{[H^+] + K_a} \frac{k_t k_i}{s.P(s)} \tag{50}$$

where:

$$P(s) = k_i \left((k_{-h}[H^+]k_{-i} + k_h \frac{[H^+]}{[H^+] + K_a}(k_{-i} - k_t) \right) + (k_{-i} + s)\left(k_t \left(\frac{-k_h[H^+]}{[H^+] + K_a} + k_{-t} \right) - (k_i + k_{-t} + s)\left(\frac{k_h[H^+]}{[H^+] + K_a} + k_{-h}[H^+] + k_t + s \right) \right) \tag{51}$$

As it was mentioned before, the roots of the polynomial $P(s)$ are the observed rate constants of the system. $P(s)$ could be presented in a form:

$$P(s) = (s^3 + Bs^2 + Cs + D) = (s + \alpha)(s + \beta)(s + \gamma) \tag{52}$$

where:

$$B = \left(\frac{[H^+]k_h}{[H^+] + K_a} + k_{-h}[H^+] + k_t + k_{-t} + k_i + k_{-i} \right) \tag{53}$$

$$C = \left(\frac{[H^+]k_h}{[H^+] + K_a}(k_i + k_{-i} + k_t + k_{-t}) + k_{-h}[H^+](k_i + k_{-i} + k_{-t}) + k_t(k_i + k_{-i}) + k_{-i}k_{-t} \right) \tag{54}$$

$$D = \left(\frac{[H^+]k_h}{[H^+] + K_a} + k_{-h}[H^+] \right) k_{-i}k_{-t} + \frac{[H^+]k_h}{[H^+] + K_a} k_t(k_i + k_{-i}) \tag{55}$$

Substituting Eq. (52) into Eqs. (48)–(50) and after inverse Laplace transformation:

$$[B](t) = \frac{[H^+]k_h}{[H^+] + K_a} \left(\frac{k_{-t}k_{-i}}{\alpha\beta\gamma} - \frac{(\alpha^2 - \alpha(k_i + k_{-i} + k_{-t}) + k_{-i}k_{-t})e^{-\alpha t}}{\alpha(\alpha - \beta)(\alpha - \gamma)} + \frac{(\beta^2 - \beta(k_i + k_{-i} + k_{-t}) + k_{-i}k_{-t})e^{-\beta t}}{\beta(\alpha - \beta)(\beta - \gamma)} - \frac{(\gamma^2 - \gamma(k_i + k_{-i} + k_{-t}) + k_{-i}k_{-t})e^{-\gamma t}}{\gamma(\gamma - \alpha)(\gamma - \beta)} \right) \tag{56}$$

$$[Cc](t) = \frac{[H^+]k_h k_t}{[H^+] + K_a} \left(\frac{k_{-i}}{\alpha\beta\gamma} + \frac{(\alpha - k_{-i})e^{-\alpha t}}{\alpha(\alpha - \beta)(\alpha - \gamma)} + \frac{(\beta - k_{-i})e^{-\beta t}}{\beta(\beta - \alpha)(\beta - \gamma)} + \frac{(\gamma - k_{-i})e^{-\gamma t}}{\gamma(\gamma - \alpha)(\gamma - \beta)} \right) \tag{57}$$

$$[Ct](t) = \frac{[H^+]k_h k_t k_i}{[H^+] + K_a} \left(\frac{1}{\alpha\beta\gamma} - \frac{e^{-\alpha t}}{\alpha(\alpha-\beta)(\alpha-\gamma)} - \frac{e^{-\beta t}}{\beta(\beta-\alpha)(\beta-\gamma)} - \frac{e^{-\gamma t}}{\gamma(\gamma-\alpha)(\gamma-\beta)} \right) \tag{58}$$

and

$$[AH^+](t) = \frac{[H^+]}{[H^+] + K_a}(1 - [B](t) - [Cc](t) - [Ct](t)) \tag{59}$$

$$[A](t) = \frac{K_a}{[H^+] + K_a}(1 - [B](t) - [Cc](t) - [Ct](t)) \tag{60}$$

A complete solution of the cubic equation appearing in Eq. (52) to express α, β, and γ in terms of the kinetic constants can be achieved using Cardano's formula [19]. However, the fully analytical expression is already very complicated and in fact we do not need it. It is necessary to solve the full cubic equation, only when the observed rate constants are of the same order of magnitude. In the anthocyanins and flavylium systems this is a situation that never occurs. Notwithstanding, fully analytical or numeric solution could be used especially in global optimization models. In present work instead of solving Eq. (52) some practical simplifications will be introduced:

Case 1 $(\alpha, \beta \gg \gamma)$:

This is a very common situation in flavylium networks: the slowest process is the *cis–trans* isomerization.

Applying Vieta's formulae leads to Eqs. (61)–(63)

$$\alpha + \beta + \gamma = B \tag{61}$$

$$\alpha\beta + \alpha\gamma + \beta\gamma = C \tag{62}$$

$$\alpha\beta\gamma = D \tag{63}$$

and can be simplified to

$$\alpha + \beta = B \tag{64}$$

$$\alpha\beta = C \tag{65}$$

$$\alpha\beta\gamma = D \tag{66}$$

where B, C and D are defined in Eqs. (53)–(55)

Solving Eqs. (64)–(66) for α, β, and γ :

$$\alpha, \beta = \frac{B \pm \sqrt{B^2 - 4C}}{2} \tag{67}$$

$$\gamma = \frac{D}{C} \tag{68}$$

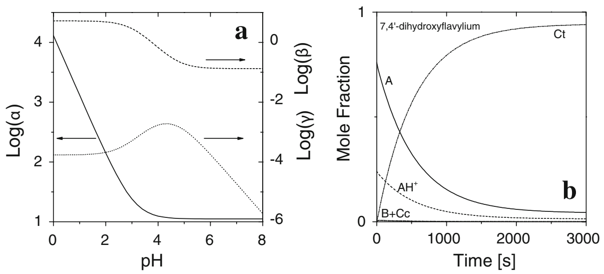


Figure 3 **a** Rates of the compound 7, 4′-Dihydroxyflavylium using the reported constants $k_h = 0.018\,\mathrm{s}^{-1}$; $k_{-h} = 1.3 \times 10^4\,\mathrm{M}^{-1}\mathrm{s}^{-1}$; $k_t = 5.9\,\mathrm{s}^{-1}$ and $k_{-t} = 5.0\,\mathrm{s}^{-1}$; $\mathrm{k}_i = 0.25\,\mathrm{s}^{-1}$and $k_{-i} = 1.8 \times 10^{-4}\,\mathrm{s}^{-1}$. **b** Simulation of the mole fraction variation of AH^+, A, B, Cc and *Ct*, as a function of time for pH= 4.5 **c** pH dependence of the rate determining process for the same parameters

The simulation for the compound 7, 4′-Dihydroxyflavylium is shown in Fig. 3. The rate determining step of the overall kinetics is γ as shown in Fig. 3a. In Fig. 3b the time dependence of the mole fractions of the several species of the flavylium network are represented at pH = 4.5. At this pH, $\alpha = 11.6$, $\beta = 0.32$ and $\gamma = 0.0018$ as required by the approximation of case 1. According to this figure the concentrations of *B* and *Cc* are very small ($< 0.5\%$) as observed experimentally. This result justifies the use of the steady state approach for *B* and *C*c, previously reported [20]. The curve in Fig. 3c, obtained from the γ root, is coincident with the rate constant deduced for the steady state approximation to hemiketal (*B*) and *cis*-chalcone (*Cc*) [20].

$$k_{obs} = \frac{\frac{[H^+]}{[H^+]+K_a} K_h K_t K_i + [H^+]}{\frac{K_i K_t}{k_{-h}} + [H^+]} \tag{69}$$

Case 2 Case 2 ($\alpha \gg \beta, \gamma$):

In this case, Vieta's formulae is transformed into:

$$\alpha = B \tag{70}$$

$$\alpha\beta + \alpha\gamma = C \tag{71}$$

$$\alpha\beta\gamma = D \tag{72}$$

Solving Eqs. (70)–(72) for α, β, and γ :

$$\alpha = B \tag{73}$$

$$\beta, \gamma = \frac{C \pm \sqrt{C^2 - 4BD}}{2B} \tag{74}$$

Case 2 can also be applied to 7,4′-Dihydroxyflavylium at this pH because it follows approximately its requirements.

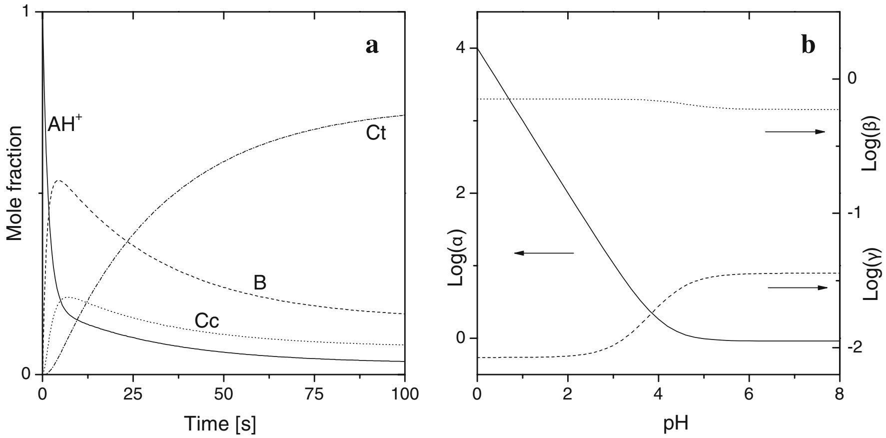


Figure 4 **a** Simulation of the mole fraction variation of AH^+, B, Cc and Ct, as a function of time for $k_h = 0.5\,\text{s}^{-1}$; $k_{-h} = 10^4\,\text{M}^{-1}\text{s}^{-1}$; $k_t = 0.3\,\text{s}^{-1}$ and $k_{-t} = 0.6\,\text{s}^{-1}$; $k_i = 10^{-1}\,\text{s}^{-1}$ and $k_{-i} = 10^{-2}\,\text{s}^{-1}$ for pH = 5. **b** pH dependence of the rate constants for the same parameters

Again, if none of the substituents R, R' (Scheme 3) is a hydroxyl group, the quinoidal base, A, is not present and $K_a = 0$. As a consequence the term $k_h \frac{[H^+]}{[H^+]+K_a}$ in all formulas can be substituted by k_h and the deduced formulas for case **II** are valid.

As seen in Fig. 4a, AH^+ disappears very fast in the beginning of the reaction to give B and Cc, and these last two species also equilibrate very fast and the slower process is the *cis–trans* isomerisation to give Ct.

4 Reverse pH jumps

In this kind of experiments a direct pH jump is carried out, and after pseudo-equilibration, before the formation of significant amounts of Ct (very easy to perform if the *cis–trans* isomerization barrier is high), the system reverts to low pH values by addition of acid. The pseudo equilibrium leads to the following mole fraction distribution:

$$AH_0^+ = \frac{[H^+]}{[H^+] + K_a + K_h + K_h K_t} \quad A_0 = \frac{K_a}{[H^+] + K_a + K_h + K_h K_t}$$
$$B_0 = \frac{K_h}{[H^+] + K_a + K_h + K_h K_t} \quad C_0 = \frac{K_h K_t}{[H^+] + K_a + K_h + K_h K_t}$$

The situation is similar to Section **I**, but now the initial conditions are different.

$$AH_0^+ + A_0 = 1 - B_0 - B_0 K_t; \quad Cc_0 = B_0 K_t; \quad \frac{AH_0^+}{A_0} = \frac{[H^+]}{K_a} \tag{75}$$

In Laplace space

$$ah = \frac{AH_0^+ s(k_{-t} + k_t + s) + [H^+]k_{-h}(k_{-t} + (AH_0^+ + B_0)s)}{s([H^+]k_{-h}(k_{-t} + s) + k_h(k_{-t} + k_t + s) + s(k_{-t} + k_t + s))} \tag{76}$$

$$b = \frac{s(k_{-t} - AH_0^+ k_{-t} + B_0 s) + k_h(k_{-t} + (AH_0^+ + B_0)s)}{s([H^+]k_{-h}(k_{-t} + s) + k_h(k_{-t} + k_t + s) + s(k_{-t} + k_t + s))} \tag{77}$$

After Inverse Laplace:

$$\begin{aligned}[AH^+](t) = & \frac{k_{-h}[H^+]k_{-t}}{\alpha\beta} \\ & + \frac{AH_0^+ \alpha(\alpha - k_{-t} - k_t) + k_{-h}[H^+](k_{-t} - (AH_0^+ + B_0)\alpha)}{\alpha(\alpha - \beta)} e^{-\alpha t} \\ & + \frac{AH_0^+ \beta(k_{-t} + k_t - \beta) + k_{-h}[H^+]((AH_0^+ + B_0)\beta - k_{-t})}{\alpha(\alpha - \beta)} e^{-\beta t}\end{aligned} \tag{78}$$

$$\begin{aligned}[B](t) = & \frac{k_h \frac{[H^+]}{[H^+]+K_a} k_{-t}}{\alpha\beta} \\ & + \frac{\alpha((AH_0^+ - 1)k_{-t} + B_0\alpha) + k_h \frac{[H^+]}{[H^+]+K_a}(k_{-t} - (AH_0^+ + B_0)\alpha)}{\alpha(\alpha - \beta)} \\ & - \frac{\beta((AH_0^+ - 1)k_{-t} + B_0\beta) + k_h \frac{[H^+]}{[H^+]+K_a}(k_{-t} - (AH_0^+ + B_0)\beta)}{\alpha(\alpha - \beta)}\end{aligned} \tag{79}$$

$$[Cc](t) = 1 - [B](t) - \left(1 + \frac{K_a}{[H^+]}\right)[AH^+](t) \tag{80}$$

where α, β are the same as in Eq. (25) (Fig. 5).

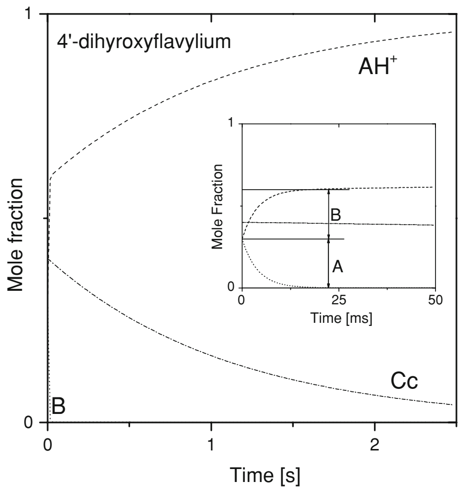


Figure 5 Simulation of the mole fraction variation of A, AH^+, B, and Cc, as a function of time. Rates constants of the compound 4′-hydroxyflavylium, pH = 2.0; $pK_a = 5.53$; $k_h = 0.089\, s^{-1}$; $k_{-h} = 2.5 \times 10^4\, M^{-1}s^{-1}$; $k_t = 5.9\, s^{-1}$ and $k_{-t} = 5\, s^{-1}$; $k_i = 0.25\, s^{-1}$ and $k_{-i} = 1.8 \times 10^{-4}\, s^{-1}$ $K_t = 0.9$ [17]; Inserted—scaled the fast process of hydration and mole fraction of A and B species

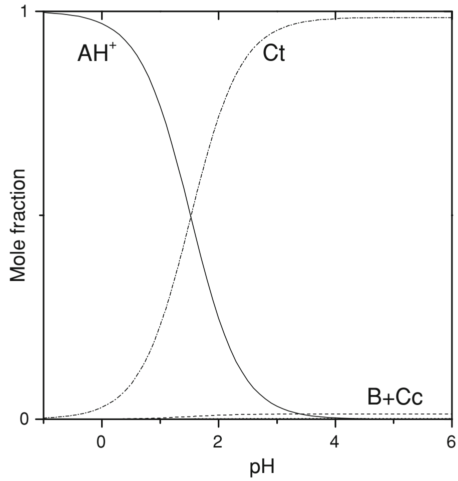


Figure 6 Equilibrium mole fraction distribution as a function of pH for 2′-methoxyflavylium compound [21]; $K_h = 4.0 \times 10^{-4}$, $K_t = 0.12$, $K_i = 600$

5 Flash photolysis

This experiment is ordinarily performed by irradiation with a light pulse in a solution where *Ct* is the dominant species which could be achieved by direct dissolution of previously synthesized *Ct* or by thermal equilibration of the solution at pH where the *Ct* is the dominant species (Fig. 6, pH > 4).

After the irradiation, *Ct* is transformed into *Cc* in less than a nanosecond. For simplification, it could be considered that the initial molar fraction of *Cc* is equal to 1 and the entire quantity of *Ct* is transformed into *Cc*. Thereby the following equations represent the evolution of mole fractions of the species in solution after irradiation. Two opposite processes occurs—formation of A/AH^+ trough *B* and recovery of *Ct*. These two processes could be represented as one reverse pH jump, and one direct pH jump, respectively. Recovery of *Ct* specie occurs from two sources—first from *Cc* and after from A/AH^+ formed from *Cc*.

Initial conditions are:

$$[Cc]_0 = 1, [Ct]_0 = [AH^+]_0 = [B]_0 = 0.$$

Taking into account mass balance Eqs. (35) and (41)–(44), excluding equation for *Cc*, the system in Laplace space is:

$$s.(ah + a) = -k_h \frac{[H^+]}{[H^+] + K_a}(ah + a) + k_{-h}[H^+]b \tag{81}$$

$$s.b = k_h \frac{[H^+]}{[H^+] + K_a}(ah + a) + k_{-t}(1/s - (ah + a) - b - ct) - (k_t + k_{-h}[H^+])b \tag{82}$$

$$s.ct = k_i(1/s - (ah + a) - b - ct) + k_{-i}ct \tag{83}$$

Solutions of Eqs. (81)–(83) for *ah, b, ct* are:

$$(ah + a) = \frac{k_{-h}[H^+]k_{-t}(k_{-i} + s)}{s.P(s)} \tag{84}$$

$$b = \frac{k_{-t}\left(k_h \frac{[H^+]}{[H^+]+K_a} + s\right)(k_{-i} + s)}{s.P(s)} \tag{85}$$

$$ct = \frac{k_i\left(k_h \frac{[H^+]}{[H^+]+K_a}(k_t + s) + s(k_{-h}[H^+] + k_t + s)\right)}{s.P(s)} \tag{86}$$

where $P(s)$ is defined in Eqs. (51) and (52).

After inverse Laplace transformations of Eqs. (84)–(86) and following the same model as in **II**:

$$[AH^+](t) = k_{-h}[H^+]k_{-t}\left(\frac{k_{-i}}{\alpha\beta\gamma} + \frac{e^{-\alpha t}(\alpha - k_{-i})}{\alpha(\alpha-\beta)(\alpha-\gamma)} + \frac{e^{-\beta t}(\beta - k_{-i})}{\beta(\beta-\alpha)(\beta-\gamma)} + \frac{e^{-\gamma t}(\gamma - k_{-i})}{\gamma(\gamma-\alpha)(\gamma-\beta)}\right) \times \frac{[H^+]}{[H^+] + K_a} \tag{87}$$

$$[A](t) = k_{-h}[H^+]k_{-t}\left(\frac{k_{-i}}{\alpha\beta\gamma} + \frac{e^{-\alpha t}(\alpha - k_{-i})}{\alpha(\alpha-\beta)(\alpha-\gamma)} + \frac{e^{-\beta t}(\beta - k_{-i})}{\beta(\beta-\alpha)(\beta-\gamma)} + \frac{e^{-\gamma t}(\gamma - k_{-i})}{\gamma(\gamma-\alpha)(\gamma-\beta)}\right) \times \frac{K_a}{[H^+] + K_a} \tag{88}$$

$$[B](t) = k_{-t}\left(\frac{k_h k_{-i}}{\alpha\beta\gamma} + \frac{e^{-\alpha t}(k_h - \alpha)(\alpha - k_{-i})}{\alpha(\alpha-\beta)(\alpha-\gamma)} + \frac{e^{-\beta t}(k_h - \beta)(\beta - k_{-i})}{\beta(\beta-\alpha)(\beta-\gamma)} + \frac{e^{-\gamma t}(k_h - \gamma)(\gamma - k_{-i})}{\gamma(\gamma-\alpha)(\gamma-\beta)}\right) \tag{89}$$

$$[Cc](t) = \left(1 - [A](t) - [AH^+](t) - [B](t) - [Ct](t)\right) \tag{90}$$

$$[Ct](t) = k_i\left(\frac{k_h k_t}{\alpha\beta\gamma} + \frac{e^{-\alpha t}(\alpha(k_{-h}[H^+] + k_t - \alpha) + k_h \frac{[H^+]}{[H^+]+K_a}(\alpha - k_t))}{\alpha(\alpha-\beta)(\alpha-\gamma)} + \frac{e^{-\beta t}(\beta(k_{-h}[H^+] + k_t - \beta) + k_h \frac{[H^+]}{[H^+]+K_a}(\beta - k_t))}{\beta(\beta-\alpha)(\beta-\gamma)} + \frac{e^{-\gamma t}(\gamma(k_{-h}[H^+] + k_t - \gamma) + k_h \frac{[H^+]}{[H^+]+K_a}(\gamma - k_t))}{\gamma(\gamma-\alpha)(\gamma-\beta)}\right) \tag{91}$$

where α, β and γ are the same as in Eqs. (67) and (68) (Fig. 7).

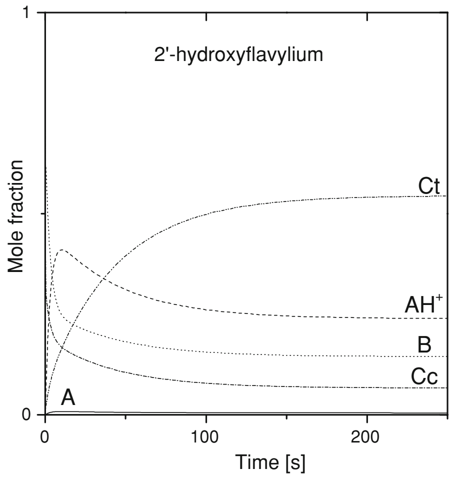


Figure 7 Simulated data for mole fraction evolution of A, AH^+, B, Cc and Ct species after flash photolysis experiment for 2′-hydroxyflavylium compound [22]; pH = 3.5; $K_a = 10^{-4.8}$, $k_h = 0.15$, $k_{-h} = 3.2 \times 10^2$, $k_t = 3.0$, $k_{-t} = 6.5$, $k_i = 0.1$, $k_{-i} = 1.2 \times 10^{-2}$

6 Conclusions

The resolution of the flavylium network was previously reported by Dubois and Brouillard [7]. These authors have clarified for the first time the mechanism of the flavylium network in a seminal paper [7].

They arrived to the important conclusion that hydration takes place from AH^+ to give B, and not from A [23]. In a subsequent paper Brouillard and Delaporte resolved the system for the species AH^+, B and C [24]. However, these authors did not considered the existence of two chalcones *cis–trans*. This situation is not limitative in the case of anthocyanins. The *cis–trans* isomerization is much slower than all the other kinetic processes. Therefore, the resolution can be applied considering **C** as the *cis*-chalcone, and treat separately the *cis–trans* isomerization. However this assumption is not valid in many flavylium networks, where the *cis–trans* isomerization can occur in the same time scale of the other kinetic processes. The present resolution is general, and the only restriction made for **II** is the occurrence of one kinetic process higher or lower than the others, which is a recurrent situation in the flavylium network. The deduced formulas are complicated, but cover all possibilities of flavylium and anthocyanins compounds—with or without quinoidal base and with or without *cis–trans* isomerization barrier. The clear mathematical apparatus of the approach allows an easy implementation of computer programs. Moreover, the fact that equations for the observed rate constant are equivalent for different type of experiments, allows creation of a global procedure, based on fitting of one single set of expressions with set of data from different type of experiments such as direct pH jumps, reverse pH jumps, stopped flow experiments and flash photolysis.

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Table 1

No	Function $F(t)$, Y	Laplace transform image $f(s)$, y
A.3	a	a/s
A.4	t	$1/s^2$
A.5	e^{at}	$1/(s-a)$
A.6	te^{at}	$1/(s-a)^2$
A.7	$\frac{1}{a-b}(e^{at}-e^{bt})$	$\frac{1}{(s-a)(s-b)}$
A.8	$-\frac{(b-c)e^{at}+(c-a)e^{bt}+(a-b)e^{ct}}{(a-b)(b-c)(c-a)}$	$\frac{1}{(s-a)(s-b)(s-c)}$
A.9	$-\frac{(b-a)e^{bt}+(a-c)e^{ct}}{(b-c)}$	$\frac{(s-a)}{(s-b)(s-c)}$
A.10	$\frac{(b-a)e^{bt}}{(b-c)(b-d)}-\frac{(c-a)e^{ct}}{(b-c)(c-d)}+\frac{(d-a)e^{dt}}{(b-d)(c-d)}$	$\frac{(s-a)}{(s-b)(s-c)(s-d)}$
A.11	$\frac{dY}{dt}$	$sy-Y_0$

a, b, c and d are constant; Y_0 is the value of Y when $t=0$

Appendix 1: Laplace transform

Laplace transform is a mathematical technique very useful to solve a system of zero or first order differential equations with constant coefficients. If $Y=F(t)$ is a function of *time* the Laplace transform of $F(t)$ is defined by:

$$f(s)=\int_0^{\infty} F(t)e^{-st}dt \tag{A.1}$$

Here s is a Laplace transformation variable and $f(s)$ is a Laplace transform of $F(t)$. Several equivalent symbolic presentations of Laplace transform could be found in the literature.[1]

$$y=f(s)=L[F(t)]=L(Y)=\bar{y} \tag{A.2}$$

The physical meaning of y, is an area.

Applying the definition (or checking the literature), one can obtain the Laplace transformed image of a function. Several Laplace transformed functions needed in this paper are summarized in table below Table 1.

The definition (A.1) shows that Laplace transform is a linear operator and this property is presented with the equations below:

$$L[F_1(t)+F_2(t)]=L[F_1(t)]+L[F_2(t)]=y_1+y_2 \tag{A.12}$$

$$L[aF(t)]=aL[F(t)]=aL[Y]=ay \tag{A.13}$$

[1] Equivalent symbolic presentations of Laplace.

Example For the first order simple reaction ($Z \xrightarrow{k} P$), whose differential rate equation is given by:

$$\frac{d[Z]}{dt} = -k[Z] \tag{A.14}$$

Applying (A.11) and (A.13) to the left and right side of the equation provides Laplace transform

$$s.z - Z_0 = -k.z \tag{A.15}$$

or

$$z = Z_0 \frac{1}{s+k} \tag{A.16}$$

Where: s is a new variable; z is Laplace image of $[Z]$ and Z_0 is value of Z when $t = 0$—in this case Z_0 in an initial concentration of compound Z.

We now take the inverse Laplace transform, converting z into Z(into time space - t) (Table 1, A.5) with constant $a = -k$ and the result is:

$$[Z](t) = Z_0 e^{-kt} \tag{A.17}$$

This as we know [14] is a correct result.

Appendix 2: Vieta's formulae

This theorem gives a relation between coefficient (a_i) of the polynomial P(x) and its roots (r_i) if ($a_n \neq 0$):

$$\begin{aligned} If\, P(x) &= a_n x^n + a_{n-1} x^{n-1} + a_{n-2} x^{n-2} \cdots\ a_2 x^2 + a_1 x + a_0 = 0 \\ P(x) &= a_n (x - r_1)(x - r_2) \cdots (x - r_n) \\ &= a_n (x^n - S_1 x^{n-1} + S_2 x^{n-2} + \ldots + (-1)^n S_n) \\ S_i &= (-1)^i \frac{a_{n-1}}{a_n} \end{aligned} \tag{A.18}$$

in a general form

$$\sum_{1 \le i_1 < i_2 \cdots < i_k < n} r_{i_1} r_{i_2} \cdots r_{i_k} = (-1)^k \frac{a_{n-k}}{a_n} \tag{A.19}$$

For example: For $n = 2$; and

$$P(x) = a_2 x^2 + a_1 x + a_0 = a_2 (x - r_1)(x - r_2) = 0 \tag{A.20}$$

We have a relation:

$$r_1 + r_2 = -a_1/a_2 \tag{A.21}$$
$$r_1 r_2 = a_0/a_2 \tag{A.22}$$

For $P(x) = a_3x^3 + a_2x^2 + a_1x + a_0 = a_3(x - r_1)(x - r_2)(x - r_3) = 0$ (A.23)

We have:

$$\begin{aligned} r_1 + r_2 + r_3 &= -a_2/a_3 \\ r_1r_2 + r_1r_3 + r_2r_3 &= a_1/a_3 \end{aligned} \tag{A.24}$$
$$r_1r_2r_3 = -a_0/a_3 \tag{A.25}$$

In case of positive real roots, the formula could be transformed as follow:

$$\begin{aligned} P(x) &= a_n(x + r_1)(x + r_2)\ldots(x + r_n) \\ &= a_n(x^n + S_1x^{n-1} + S_2x^{n-2} + \cdots + S_n) \end{aligned} \tag{A.26}$$

For example:

$$(s + a)(s + b) = s^2 + s(a + b) + (ab) = s^2 + Bs + C \tag{A.27}$$

or

$$\begin{aligned} B &= a + b \\ C &= a.b \end{aligned} \tag{A.28}$$

For

$$\begin{aligned} (s + a)(s + b)(s + c) &= s^3 + s^2(a + b + c) + s(ab + ac + bc) + (abc) \\ &= s^3 + Bs^2 + Cs + D \end{aligned} \tag{A.29}$$

or

$$\begin{aligned} B &= a + b + c \\ C &= a.b + a.c + b.c \\ D &= a.b.c \end{aligned}$$

Appendix 3: General partial fraction theorem [25]

This theorem could be applied to the Laplace transform that could be written in a form:

$$y = \frac{F(s)}{G(s)} \tag{A.30}$$

Where: $F(s)$ and $G(s)$ are polynomials of s, and degree of G(s) is grater than $F(s)$ If G(s) could be written in a form:

$$G(s) = (s - a_1)(s - a_2)\cdots(s - a_n) \tag{A.31}$$

Where:

$$a_i \neq a_j, (i \neq j) \tag{A.32}$$

Then

$$y = \sum_{r=1}^{n} \frac{1}{(s - a_r)} \frac{F(a_r)}{(a_r - a_1)(a_r - a_2)\cdots(a_r - a_{r-1})(a_r - a_{r+1})\cdots(a_r - a_n)} \tag{A.33}$$

is a valid equation.

In this case, Reverse Laplace Transform of y is equal to:

$$Y = \sum_{r=1}^{n} \frac{F(a_r)e^{-a_r t}}{(a_r - a_1)(a_r - a_2)\cdots(a_r - a_{r-1})(a_r - a_{r+1})\cdots(a_r - a_n)} \tag{A.34}$$

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