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Kinetics of ultra-fast excited state proton transfer from 7-hydroxy-4-methylflavylium chloride to water

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Abstract

Excited state proton transfer from 7-hydroxy-4-methylflavylium chloride to water is reported. From a modified analysis of picosecond time-resolved fluorescence data (not using the lifetime of a parent compound), all rate constants were determined: the deprotonation rate constant of the flavylium cation, $k_d = 1.4 \times 10^{11}$ s^{-1}, the protonation rate constant of the base form, $k_p = 2.3 \times 10^{10}$ l mol^{-1} s^{-1} and the reciprocal fluorescence lifetimes of these species, $k_{AH^+} = 7.8 \times 10^9$ s^{-1} ($\tau_{AH^+} = 128$ ps) and $k_A = 7.6 \times 10^9$ s^{-1} ($\tau_A = 132$ ps), in water, at 20°C. The value of k_d is the largest measured value for an intermolecular proton transfer (to water). © 1998 Elsevier Science B.V. All rights reserved.

1. Introduction

Synthetic salts of 2-phenylbenzopyrilium (flavylium) [1–8] have been shown to be of great usefulness for understanding the rather complex multiequilibria of the more heavily substituted natural flavylium salts: the reddish or bluish anthocyanins, found in most flowers and fruits [9,10]. As an example, with 7-hydroxy-4-methylflavylium chloride (HMF) only two species (out of the five species in multiequilibria, usually found with natural anthocyanins in aqueous solution at moderately acidic pH values) [9] are detected in acidic pH: the flavylium cation (AH^+) and the quinonoidal base (A) [11]. With this simplification, the acid–base equilibrium ($AH^+ \rightleftharpoons A + H^+$) can be analysed separately from the others which is particularly useful for studies in the excited state.

Some of the multiequilibria of anthocyanins and synthetic flavylium salts are strongly affected by light absorption [1,12,13], which has important consequences on their photochemistry/photodegradation. Only in one case [14] have excited state rate constants have been determined (deprotonation and back protonation of the *cis*-chalcone form of malvidin 3,5-diglucoside).

Here, we report a full kinetic analysis of the excited state proton exchange between the flavylium cation of HMF and water, and the largest value, to the best of our knowledge, found for a deprotonation rate constant (to water).

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2. Experimental

HMF was prepared according to the published procedure [7,8]. The parent compound 4-methyl-7-methoxyflavylium chloride (MMF) was synthesised from 4-methyl-7-methoxycoumarin and the appropriate Grignard reagent (C_6H_5MgBr) in diethylether. The solution turns yellow and the reaction was complete after 4 h. The excess of Grignard reagent was hydrolysed with an aqueous solution of $HClO_4$ (20% v/v), the solid was filtered and dissolved in methanol to precipitate the inorganic salts. The methanolic solution was concentrated and purified by high-performance liquid chromatography (HPLC) to separate the excess of the unreacted coumarin. The eluent was evaporated and the solid was dissolved in a 0.1 M HCl solution and then precipitated by evaporation. Twice distilled and deionized water was used for the preparation of the solutions, and the pH values were adjusted by addition of HCl and NaOH: for pH values < 2, analytical concentrations of H^+ where used and for pH values > 2, the pH was measured at 20°C with a Crison micropH 2002. UV–Vis absorption spectra were recorded on a Beckman DU-70 spectrophotometer. Fluorescence spectra were recorded on a Spex Fluorolog F2121 and all spectra where corrected for monochromator and photomultiplier response curves.

Time-resolved fluorescence was measured using the time-correlated single-photon counting technique employing an excitation source consisting of a mode-locked Ti:sapphire laser (Spectra-Physics Tsunami) pumped by an argon ion (Spectra-Physics) laser running on multiline. The pulse repetition rate at the output of the Tsunami (82 MHz) was reduced to 800 kHz with an opto-acoustic modulator (Spectra-Physics Pulse Selector Model 3980). The light frequency of the Tsunami output was doubled using a second-harmonic generator crystal (LBO). The fundamental signal was monitored with a photodiode, filtered in a constant fraction discriminator (Canberra 2126) and used as start signal in the time-to-amplitude converter (TAC Canberra 2145).

The samples where measured with excitation at 413 nm [the excitation light is depolarized (Oriel depolarizer ref. 28110)], and the emissions at 480 and 600 nm were collected at 90° geometry with a polarizer at 35.3° (Spindler and Hoyer Glan-laser prism polarizer). The emission passed through an analyzing monochromator (Jobin-Yvon H20 Vis) and was detected with a microchannel plate photomultiplier (Hamamatsu R3809u-50). The signal from the photomultiplier was filtered in a constant fraction discriminator (Canberra 2126) and used as stop signal in the time-to-amplitude converter (TAC Canberra 2145). The signal from the TAC was sent to an ADC (Canberra 8213) and stored in a multichannel card (Canberra AccuSpec) installed in a PC. Alternate measurements (10^3 counts at the maximum per cycle) of the excitation pulse profile and sample emissions were made until a typical value of 5×10^3 counts at maximum.

3. Results

3.1. Absorption spectra

Absorption spectra of HMF in aqueous solution, at several pH values, are presented in Fig. 1. In the pH range from 1.3 to 3.0, the flavylium cation (spectra 1 and 2) is essentially the sole species present in solution. As the pH increases from 3.0 up to 6.9, the characteristic flavylium cation (AH^+) absorption band ($\lambda_{max} = 416.5$ nm, $\varepsilon = 42900$ l mol^{-1} cm^{-1}) decreases, while the absorption band

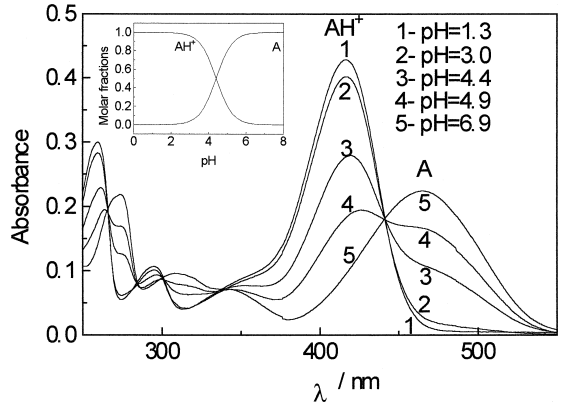


Fig. 1. Absorption spectra of 7-hydroxy-4-methylflavylium chloride ($\sim 5 \times 10^{-5}$ M) in aqueous solutions as a function of pH: 1 = pH 1.3; 2 = pH 2.2; 3 = pH 3.0; 4 = pH 4.8; 5 = pH 5.8; 6 = pH 6.1; 7 = pH 6.9. The inset of the figure represents the molar fractions of AH^+ and A as a function of the pH.

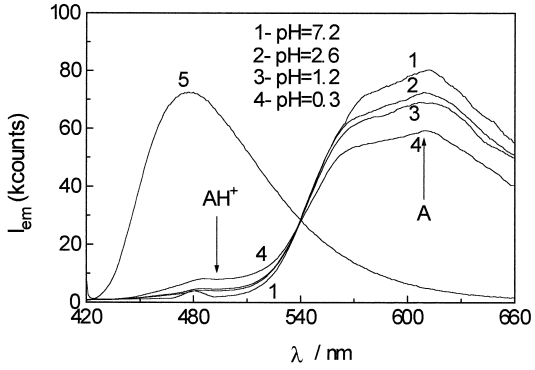


Fig. 2. Emission spectra of 7-hydroxy-4-methylflavylium chloride solution at: 1 = pH 7.2, 2 = pH 2.6, 3 = pH 1.2, 4 = pH 0.3; $\lambda_{exc} = 415$ nm. The spectrum at pH 7.2 (line 1) was multiplied by 4 for comparison purposes. The fluorescence spectrum of the parent compound (line 5) at pH 1 at the same excitation wavelength is also plotted.

at $\lambda_{max} = 464$ nm ($\varepsilon = 27700$ l mol^{-1} cm^{-1}) attributed to the quinonoidal base (A) (neutral form) increases (Fig. 1, curves 3–5). The value of the $pK_a = 4.4$ is in agreement with the published values [7,11].

Other species, usually formed with natural anthocyanins (hemiacetals and chalcones) [9,15] were not observed. The isosbestic point at $\lambda = 440$ nm also indicates that only the flavylium cation (AH^+) and the quinonoidal base (A) are in equilibrium within this pH range.

3.2. Fluorescence emission and excitation spectra

Fluorescence emission spectra of HMF in water, as a function of pH, are shown in Fig. 2. The emission spectrum of the parent compound 7-methoxy-4-methylflavylium chloride at pH = 1 is also shown for comparison (spectrum 5). Excitation at 415 nm (flavylium cation absorption band), gives rise to two emission bands, one at $\lambda_{max} = 484$ nm (very weak emission), and the other at $\lambda_{max} = 610$ nm (strong emission), the last one with a shoulder at 580 nm. Between pH = 0.3 and 2.6, where there is no significant change in the flavylium cation concentration (AH^+ is essentially the only species in the ground state), the intensity of the 480 nm emission band slightly decreases as the 610 nm emission intensity increases.

The emission band at 610 nm matches the emission obtained by direct excitation of the quinonoidal base at pH = 7.2 (where A is the only species in the

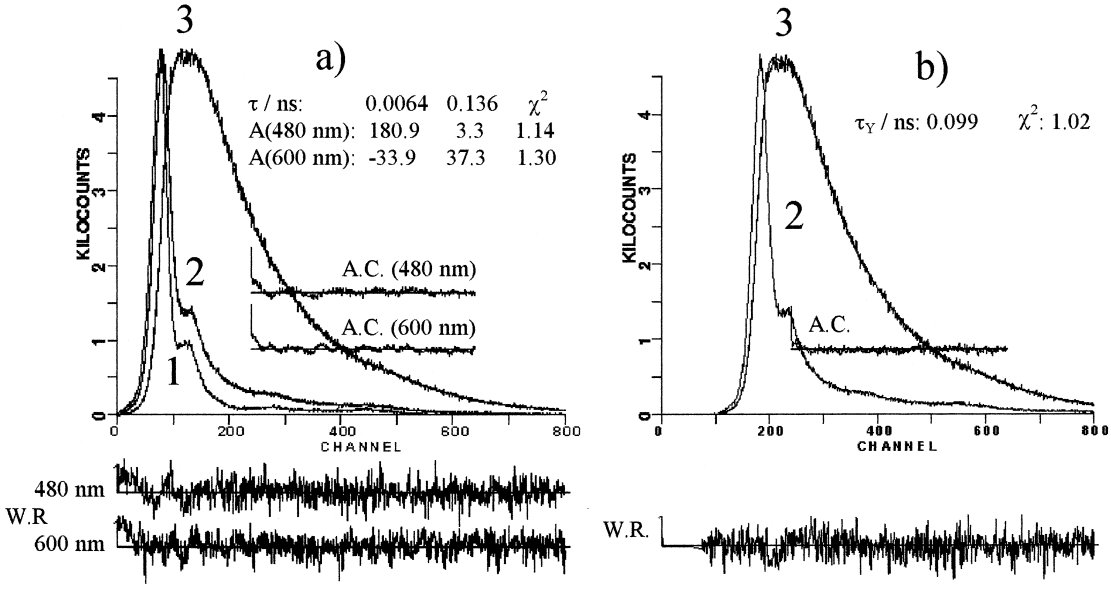


Fig. 3. Fluorescence decays of 7-hydroxy 4-methylflavylium chloride in water at pH 1, 20°C, excitation at 413 nm, collecting at 480 nm (flavylium emission) and 600 nm (base emission). (a) Global analysis; (b) deconvolution of the base decay with the flavylium cation decay.

ground state). Note that this emission is still observed for very low pH values (pH < 1), at which pH the base is not present in the ground-state and only the flavylium cation absorbs.

The fluorescence excitation spectrum from emission at $\lambda_{em} = 484$ nm is identical to the absorption spectrum of AH^+, at any pH value, indicating that the 484 nm band is due to the emission of that species (AH^+). Also, the excitation spectra at $\lambda_{em} =$ 610 nm matches the absorption of AH^+ for pH values where this is the sole species in the ground state. Therefore, the fluorescence emission band at $\lambda_{max} = 484$ nm is assigned to the flavylium cation (AH^+) and the emission band at $\lambda_{max} = 610$ nm is assigned to the quinonoidal base (A) form. The results are characteristic of an acid–base system where the excited state pK_a^* is lower than the ground state pK_a, as found in phenols [16] and many other molecules [16,17].

3.3. Fluorescence decays

The fluorescence decays of HMF in water, with excitation at 413 nm, at pH = 1 (only flavylium cation present in the ground state), collected at 480 nm (flavylium emission) and 600 nm (base emission) are shown in Fig. 3a). The decays can be fitted with the sum of two exponentials with extremely short lifetimes which are identical at the two emission wavelengths (Eqs. (1) and (2) below). At 600 nm, the shortest time (τ_2) is a rise-time (negative pre-exponential) and the sum of the two pre-exponential factors approaches zero, which means that the fluorescence intensity of the base is zero at time zero. When the concentration of H^+ is decreased, the two decay times do not appreciably change (Fig. 4 a), but the weight of the longest time (τ_1) in the decay at 480 nm (AH^+ emission) decreases and tends to zero at H^+ concentrations $< 10^{-3}$ M (the 480 nm decays become single exponential). This is shown in Fig. 4b where the ratio of pre-exponential factors ($R = a_2/a_1$) is plotted as a function of [H^+].

3.3.1. Kinetic analysis

The previous results are consistent with a simple acid–base process in the excited state, as represented in Scheme 1 where k_d and k_p are the deprotonation and protonation rate constants, respectively, and k_{AH^+} and k_A are the reciprocal fluorescence lifetimes of AH^+ and A, respectively.

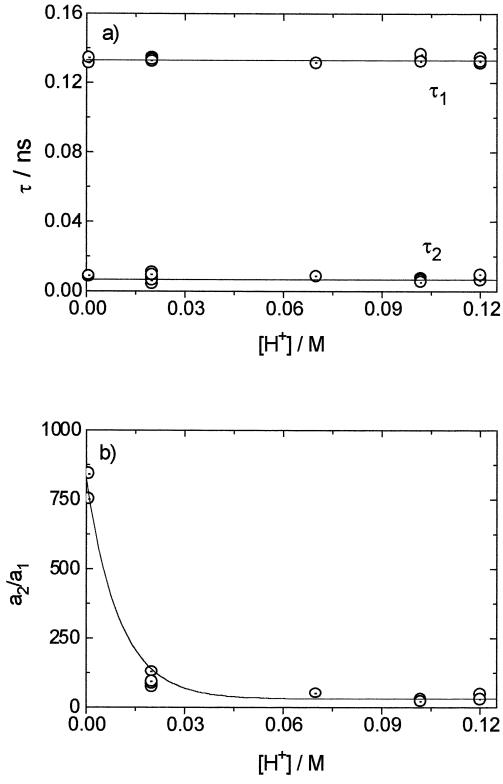


Fig. 4. (a) Decay times obtained from global analysis of the flavylium cation and base decays, plotted as a function of [H^+] and (b) ratio of pre-exponential factors ($R = a_2 / a_1$) plotted as a function of [H^+].

The time evolution of the concentration/fluorescence of the two species obey the well-known equations [18]:

$$I_{AH^+}(t) = a_1 e^{-\lambda_1 t} + a_2 e^{-\lambda_2 t}, \quad (1)$$

$$I_A(t) = a(e^{-\lambda_1 t} - e^{-\lambda_2 t}), \quad (2)$$

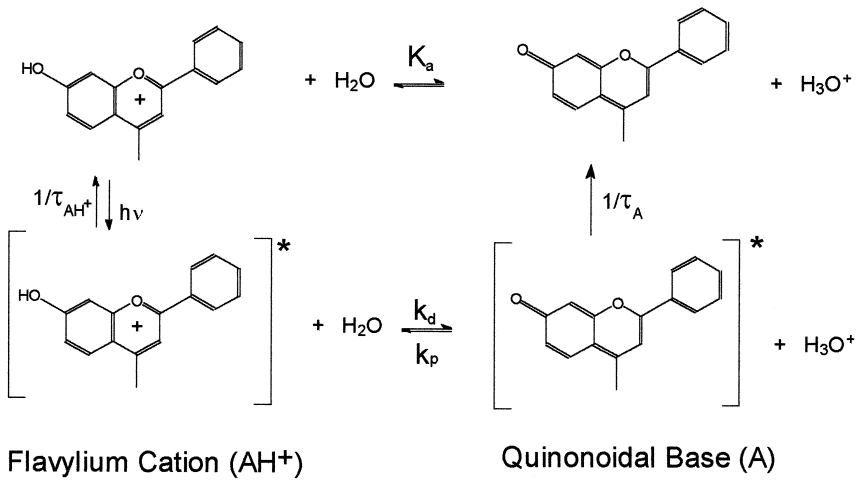
where λ_2 and λ_1 are the reciprocals of the shortest (τ_2) and the longest (τ_1) decay times, respectively, given by:

$$\lambda_{2,1} = \frac{X + Y \pm \sqrt{(X - Y)^2 + 4k_d k_p [H^+]}}{2}, \quad (3)$$

with

$$X = k_d + k_{AH^+}, \quad (4)$$

$$Y = k_p [H^+] + k_A, \quad (5)$$



Scheme 1.

and the ratio of the pre-exponential factors $R = a_2/a_1$ is related to the kinetic constants by:

$$R = \frac{X - \lambda_1}{\lambda_2 - X}. \tag{6}$$

The four rate constants in Scheme 1 are usually evaluated from the three parameters obtainable from the above decays (λ_2, λ_1 and R) and from the fluorescence lifetime of the so-called parent/model compound of AH^+, the lifetime of which is assumed to be identical to that of AH^+ in the absence of the reaction process involved (in this case the deprotonation reaction). An obvious choice for HMF would be MMF, for which the deprotonation reaction cannot occur and which fluorescence lifetime in water at 20°C is $\tau_{AH^+} = 762$ ps. The standard procedure [18] would involve the successive determination of:

$$X = \frac{\lambda_2 R + \lambda_1}{R + 1}, \tag{7}$$

$$Y = \lambda_2 + \lambda_1 - X, \tag{8}$$

$$k_d = X - k_{AH^+} \tag{9}$$

and

$$k_p[H^+] = \frac{(\lambda_2 - \lambda_1)^2 - (X - Y)^2}{4k_d}. \tag{10}$$

There are two reasons to avoid this procedure for the present case: first, the value of the pre-exponential factors ratio (R) can be uncertain because even a very small contribution of emission from the base at 480 nm (note in Fig. 2 the low fluorescence quantum yield of AH^+ as compared to A) would cause significant changes to R (R is very large). Second, the choice of a model compound is generally problematic [19] and, in particular here, the assumption of identical photophysical properties for HMF and MMF, in the strongly interacting solvent water, is obviously dangerous.

An alternative to the above procedure (not using the pre-exponentials ratio or the fluorescence lifetime of the model compound) is based on the evaluation of Y by the deconvolution of the decay of the base (A) from the decay of the acid (AH^+) (Eq. (11) where the symbol $\otimes$ means convolution) [20].

$$I_A(t) = k_d\left[I_{AH^+}(t) \otimes e^{-Yt}\right]. \tag{11}$$

We have performed this kind of analysis and in fact obtained perfect single exponential fits (Fig. 3b). The values of the decay times (equal to $1/Y$) are clearly pH dependent (Fig. 5a), in contrast with the decay times obtained from global analysis (Fig. 4a).

After determination of Y, the evaluation of the four constants is straightforward. First, from the linear plot of Y vs. $[H^+]$ (Fig. 5a) the values of the two rate constants $k_A = (7.6 \pm 0.1) \times 10^9$ s^{-1} ($\tau_A = 132$ ps) and $k_p = (2.3 \pm 0.2) \times 10^{10}$ l mol^{-1} s^{-1} are determined from the zero intercept and slope, respectively (see Eq. (5)). The determination of the two remaining rate constants (k_d and k_{AH^+}) is accom-

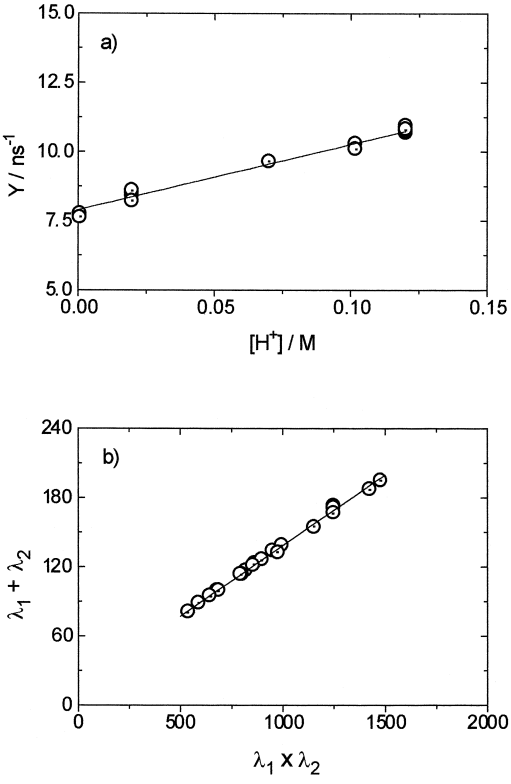


Fig. 5. (a) Plot of Y as a function of $[H^+]$ and (b) plot of $\lambda_1 + \lambda_2$ vs. $\lambda_1\lambda_2$.

plished using the values of λ_1 and λ_2 after appropriate re-arrangements of Eqs. (3)–(5) (Eqs. (12) and (13)).

$$\lambda_1 + \lambda_2 = k_d\left(1 - \frac{k_A}{k_{AH^+}}\right) + k_{AH^+} + \frac{1}{k_{AH^+}}\lambda_1\lambda_2 , \tag{12}$$

$$k_d = \lambda_1 + \lambda_2 - Y - k_{AH^+} . \tag{13}$$

From the slope of the linear plot of $\lambda_1 + \lambda_2$ vs. $\lambda_1\lambda_2$ (Fig. 5b), $k_{AH^+} = (7.8 \pm 0.1) \times 10^9$ s^{-1} ($\tau_{AH^+} = 128$ ps) is obtained and from Eq. (12), $k_d = (1.4 \pm 0.3) \times 10^{11}$ s^{-1}.

4. Discussion

4.1. Data analysis

First, note that the value of k_{AH^+} (7.8 ns^{-1}) is lower than the error in k_d (± 30 ns^{-1}) which makes its determination from $k_{AH^+} = X - k_d$ (Eq. (9)) impossible. However, the plot of $\lambda_1 + \lambda_2$ vs. $\lambda_1\lambda_2$ (Fig. 5b) allows a surprisingly accurate determination of k_{AH^+} due to cancellation of the errors arising from the uncertainty of the values of the shortest reciprocal decay time λ_2. Also, the value of the experimental zero intercept in Fig. 5b (11.4 ns^{-1}) is in excellent agreement with the one calculated using the values of k_d, k_A and k_{AH^+} (11.2 ns^{-1}).

Second, note that the value of the fluorescence lifetime of the flavylium cation of HMF ($\tau_{AH^+} = 128$ ps) is quite different from that of MMF ($\tau_{AH^+} = 762$ ps). This supports our concerns with respect to the use of data obtained with the parent compound.

4.2. Comparison with steady-state fluorescence data

From the pH dependence of fluorescence intensities of AH^+ and A, the value of the proton transfer yield η^* and the value of the apparent acidity constant in the excited state K^*_{ap} have been evaluated ($\eta^* = 0.95$ and $K^*_{ap} = 5.0$) [21]. Both values are consistent with those calculated with Eqs. (14) and (15) and time-resolved fluorescence: $\eta^* = 0.93$ and $K^*_{ap} = 4.9$.

$$\eta^* = k_d/(k_d + k_{AH^+}) , \tag{14}$$

$$K^*_{ap} = (k_d/k_p)(k_{AH^+}/k_A) . \tag{15}$$

4.3. Rate constants

The value of the deprotonation constant of HMF to water ($k_d = 1.4 \times 10^{11}$ s^{-1}) is extremely large (to the best of our knowledge, it is the largest value measured for an intermolecular proton transfer to water). Although this value does not exclude a diffusional mechanism ($[H_2O] = 55$ M), preliminary results obtained in methanolic solutions in presence of smaller concentrations of water indicate the presence of a static mechanism involving the hydrogen-bonded to water HMF molecule [22].

The value of the protonation rate constant ($k_p = 2.3 \times 10^{10}$ l mol^{-1} s^{-1}) is within the diffusion-control region but it may contain some proton migration contribution. Both problems are presently being addressed from the influence of water concentration and temperature on the fluorescence decays.

5. Conclusions

The excited state proton transfer reactions between HMF and water are very fast, leading to short decay times with the inherent measurement difficulties. Neither the parent compound lifetime nor the pre-exponentials ratio can be used for data analysis. The determination of all rate constants in Scheme 1 is possible by the use of non-standard methods of analysis of time-resolved fluorescence data. These are applicable under particular conditions, such as this one, where the kinetics depends on the proton concentration.

Acknowledgements

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