Phase Fluorometer Study of the Excited State Reactions of 4-Methylumbelliferone

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The fluorescence spectra and decay times (the phase differences between the modulated exciting light and the fluorescence) of 4-methylumbelliferone in ethanol-water-acid solutions were investigated. The excited state reaction rates and lifetimes of three forms of the studied dye were evaluated.

I. Introduction

Various spectroscopic properties of 4-methylumbelliferone (4-MU) in different solvents viz. absorption, fluorescence and gain spectra were investigated by many authors [1–9].

It was established that five different species of 4-MU (neutral, cationic, anionic and two tautomeric forms) can be assigned to fluorescence maxima at the respective wavelengths: 390 nm, 415 nm, 450 nm, 480 nm, and 520 nm [1]. Some of these (neutral, cationic, anionic) can exist both in the ground and excited states, while the tautomeric forms do not have a stable ground state. Under suitable conditions a very wide-band fluorescent emission from a single solution containing the excited neutral, anionic and tautomeric molecules of 4-MU is observed (Figure 1). In order to elucidate this fact Trozzolo et al. [10] have proposed the following excited state reaction scheme: After excitation the phenolic group becomes more acidic, whereas the carbonyl group becomes more basic relative to the ground state. Thus under favourable conditions the neutral molecule tends to lose the proton and to become an anion. The latter can pick up a proton from the solvent and a neutral or tautomeric molecule is formed, depending on the proponation site.

In earlier investigations it was found that results from decay time measurements cannot be exactly explained in terms of reaction schemes based on emission spectra measurements. The rate constants for the exciplex formation calculated from spectral and decay time measurements on ground of Dienes' [1] model differ by more than an order of magnitude. The aim of this paper is therefore to modify existing models in order to decrease as much as possible the above mentioned discrepancy and to explain the formation of tautomeric forms in solvents with negligible proton concentration [7, 8].

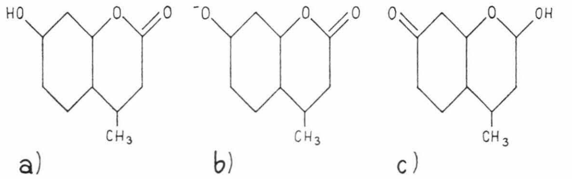

Fig. 1. a) Neutral, b) anionic, c) tautomeric species of 4-methylumbelliferone.

The presented model of excited state reactions of 4-MU is based on the schemes given by Trozzolo [10] and Dienes [1]. Since the acidity of the solutions was fairly low, we assume that the cationic form of 4-MU does not occur at all [1, 3]. Two of the remaining forms are tautomers. Dienes et al. [1] have suggested that the observed fluorescence maxima at 480 nm and 520 nm can be assigned to these tautomeric species. In the present fluorometric measurements the broad-band fluorescence emission of these two tautomeric forms could not yet be separated from each other [11]. Thus for simplicity the existence of only one tautomeric form was taken into account. As a result our model considers the system of the three main excited forms of 4-MU as presented in Figure 2.

The excited neutral molecule transforms into the excited anion with the rate k_1 depending on water content. The excited anion undergoes reactions with diffusion controlled rates k_2 and k_3, both depending on the proton concentration. It seems however reasonable to introduce an additional rate constant k_4 which describes the probability of tautomer forma-

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tion by reaction of the anion with the previously lost proton remaining in the solvent shell ("internal protonation"). τ_N^{-1}, τ_A^{-1}, τ_T^{-1} are representing the rates of deactivation of the respective excited species along the remaining pathways. In order to verify the proposed reaction model, spectroscopic investigations as well as phase fluorometer measurements have been carried out.

II. Experimental

The absorption spectra of the 4-MU solutions were measured with a SPECORD UV VIS recording spectrophotometer. The emission spectra were recorded by means of a calibrated spectrofluorimeter consisting of two monochromators SPM-2, a high pressure mercury arc, a photomultiplier EMI 9558 QBM and G1B1 recorder. The mentioned instruments were supplied by Zeiss-Jena. The optical density of the samples was less than 0.1.

The fluorescence phase shifts of modulation were measured by means of the Bauer-Rozwadowski phase-fluorometer [12] with the modulation frequency of 11.4 MHz.

The samples were irradiated through a 325 nm interference filter combined with an absorption filter. The neutral and tautomer emissions were isolated by means of interference and absorption filter combinations. The accuracy of the phase shift measurements corresponded to $5 \cdot 10^{-11}$ s.

Reagent grade 4-MU was supplied by Pfaltz and Bauer Inc., 99.8% analytical grade ethanol, twice distilled water and spectroscopic grade HCl and $HClO_4$ were used. Three series of $5 \cdot 10^{-5}$ M/l ethanolic solutions of 4-MU were prepared. The water content varied from 0.1 to 8.89 M/l, whereas the acid concentration was kept constant at 0.0 M/l ($p_H = 5$), 10^{-3} M/l and 10^{-1} M/l, for the three series respectively.

III. Theory

The rate equations for the excited state reactions presented in Fig. 2 read as follows:

$$\mathrm{d}N^*/\mathrm{d}t = -(\tau_N^{-1} + k_1) N^* + k_2 A^* + I\,, \qquad (1)$$

$$\mathrm{d}A^*/\mathrm{d}t = -(\tau_A^{-1} + k_3 + k_2) A^* + k_1 N^*\,, \qquad (2)$$

$$\mathrm{d}T^*/\mathrm{d}t = -\tau_T^{-1} T^* + k_3 A^*\,, \qquad (3)$$

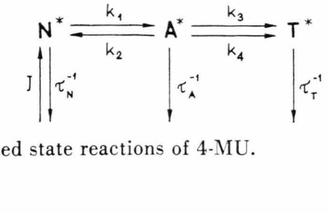

Fig. 2. Excited state reactions of 4-MU.

here N^*, A^*, T^* are the concentrations of the excited neutral, anionic and tautomeric forms, respectively, I is the rate of absorption of light by neutral molecules. The low intensity excitation results in $N \gg N^*$, A^*, T^*, where N is the concentration of the dye.

Two modes of excitation will be considered.

a) If the intensity of the exciting light is harmonically modulated with frequency ω

$$I = A_0 + B_0 \exp\{i\,\omega\,t\}\,, \qquad (4)$$

then the intensity of the fluorescent emission of the neutral, anionic and tautomeric species will also be modulated with frequency ω, but phases and degrees of modulation will differ from that of the exciting light:

$$\begin{aligned} I_N &= A_N + B_N \exp\{i(\omega t - \varphi_N)\}\,, \\ I_A &= A_A + B_A \exp\{i(\omega t - \varphi_A)\}\,, \\ I_T &= A_T + B_T \exp\{i(\omega t - \varphi_T)\}\,. \end{aligned} \qquad (5)$$

From Eqs. (1) to (5) the phase shifts may be calculated as

$$\varphi_N = \operatorname{arctg} \omega \frac{\omega^2 + a^2 + k_1 k_2}{n\,\omega^2 + n\,a^2 - a\,k_1 k_2}\,, \qquad (6)$$

$$\varphi_A = \operatorname{arctg} \omega (n + a) / (a\,n - \omega^2 - k_1 k_2)\,, \qquad (7)$$

$$\varphi_T = \operatorname{arctg} \omega \frac{(n + t) a + n\,t - \omega^2 - k_1 k_2}{a(n\,t - \omega^2) - \omega^2 (n + t) - k_1 k_2 t}\,, \qquad (8)$$

where $n = \tau_N^{-1} + k_1$, $a = \tau_A^{-1} + k_2 + k_3 + k_4$, $t = \tau_T^{-1}$.

b) If the exciting light intensity is constant, a steady state will be established and

$$\mathrm{d}N^*/\mathrm{d}t = \mathrm{d}A^*/\mathrm{d}t = \mathrm{d}T^*/\mathrm{d}t = 0\,.$$

For that case the fluorescence quantum yields of respective species are described by the following expressions:

$$\Phi_N = k_{fN} N^*/I = k_{fN}\, a / (n\,a - k_1 k_2)\,, \qquad (9)$$

$$\Phi_A = k_{fA} A^*/I = k_{fA}\, k_1 / (n\,a - k_1 k_2)\,, \qquad (10)$$

$$\Phi_T = k_{fT} T^*/I = k_{fT}\, k_1 (k_3 + k_4) / t (a\,n - k_1 k_2)\,, \qquad (11)$$

where k_{fN}, k_{fA}, k_{fT} are the rates of the radiative transitions.

IV. Results

The phase shifts φ_N, φ_T were measured for 4-MU solutions described in the experimental section. φ_A was not measured, because it was yet impossible to isolate the anionic emission in these solutions. The

Table 1. $\omega^{-1}\,\mathrm{tg}\,\varphi_N\,(ns)$ values for different water and acid concentrations.

		$[H_2O]$ M/l					
		0.1	0.5	1.1	2.2	4.4	8.9
[HCl] M/l	0	1.3	1.3	1.25	1.15	1.0	0.8
	10^{-3}	1.3	1.3	1.25	1.15	1.0	0.8
	10^{-1}		1.0	1.0	0.9	0.8	0.75

Table 2. $\omega^{-1}\,\mathrm{tg}\,\varphi_T\,(ns)$ values for different water and acid concentrations.

		$[H_2O]$ M/l					
		0.1	0.5	1.1	2.2	4.4	8.9
[HCl] M/l	0	6.05	6.45	6.45	6.45	6.3	6.15
	10^{-3}	6.05	6.45	6.45	6.45	6.35	6.2
	10^{-1}		6.0	6.15	6.2	6.15	6.05

values of $\omega^{-1}\,\mathrm{tg}\,\varphi_N$ and $\omega^{-1}\,\mathrm{tg}\,\varphi_T$ are listed in Tables 1 and 2.

One can notice that $\omega^{-1}\,\mathrm{tg}\,\varphi_N$ values decrease with the increase of water content. An evident influence of the acid in solution on the phase shifts one observes only for the highest (10^{-1} M/l) acid concentration. It seems reasonable to assume that the rates k_1, k_2, k_3 are proportional to the water $[H_2O]$ and proton $[H^+]$ concentrations in solution, that is $k_1 = k_1'[H_2O]$, $k_2 = k_2'[H^+]$, $k_3 = k_3'[H^+]$. If $[H^+] \to 0$ then the rates k_2 and k_3 can be neglected in Eqs. (1) – (11) and the phase shift (6) will reduce to

$$\omega^{-1}\,\mathrm{tg}\,\varphi_N = \tau_N/(1+\tau_N k_1'[H_2O])\,. \tag{12}$$

Figure 3 presents the plot of $\omega\,\mathrm{ctg}\,\varphi_N$ versus water content, from which $k_1' = 6\cdot 10^7$ l/Ms can be obtained as the slope and $\tau_N = 1.35$ ns is the reciprocal of its intercept. The experimental values shown in Fig. 3 are independent of acid concentration up to 10^{-3} M/l. An attempt was made to estimate k_1' from the fluorescence spectra. From Eq. (9) it follows that the ratio of quantum yields of the neutral form versus the water concentration is:

$$\frac{\Phi_N([H_2O]=0.556\,\mathrm{M/l})}{\Phi_N([H_2O])} = \frac{\tau_N^{-1}(\tau_A^{-1}+k_2+k_3+k_4)+k_1'[H_2O](\tau_A^{-1}+k_3+k_4)}{\tau_N^{-1}(\tau_A^{-1}+k_2+k_3+k_4)+k_1'\cdot 0.556\,\mathrm{M/l}\,(\tau_A^{-1}+k_3+k_4)}\,, \tag{13}$$

where the concentration 0.556 M/l was arbitrarily chosen. This ratio should be a linear function of the water concentration. The slope/intercept of this straight line is equal to

$$R = \tau_N k_1'[1-k_2/(\tau_A^{-1}+k_2+k_3+k_4)]\,. \tag{14}$$

The experimental values of the quantum yields ratio of Eq. (13) are plotted in Figure 4. From this plot R is found to be 0.18 l/M. Since the above determined value of τ_N is equal 1.35 ns and the bracket in Eq. (14) is smaller than unity, one gets a lower

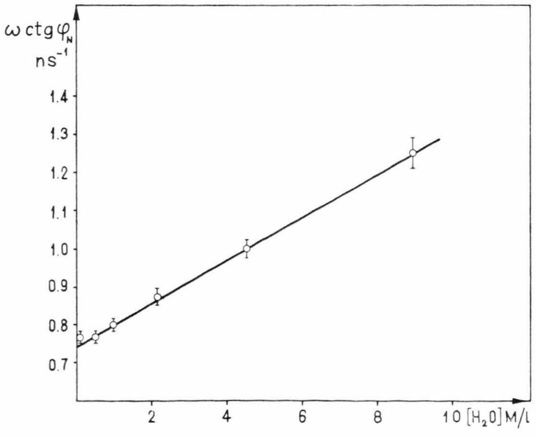

Fig. 3. Dependence of $\omega\,\mathrm{ctg}\,\varphi_N$ on water concentration in solution. Acid concentration $\leqq 10^{-3}$ M/l.

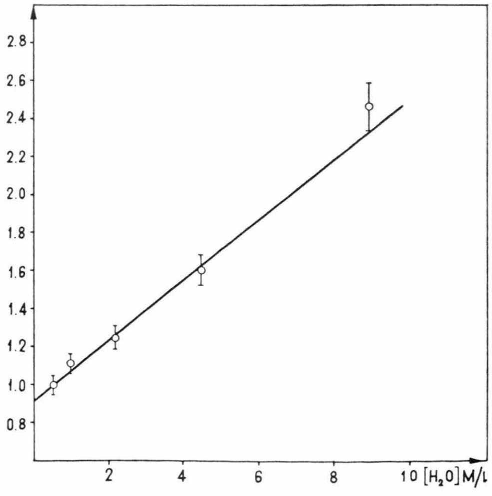

Fig. 4. Plot of $\Phi_N([H_2O]=0.556\,\mathrm{M/l})\,/\,\Phi_N([H_2O])$ versus water concentration. Acid concentration $\leqq 10^{-3}$ M/l.

limit for k_1', $k'_{1\,\text{min.}} = 13.3 \cdot 10^7$ l/Ms. To obtain a more accurate estimation for k_1' one has to find the value of the bracket i. e. the values of k_2, k_3, k_4 and τ_A.

$\tau_A = 4.1$ ns was measured directly in basic ethanolic solution of 4-MU, where the only light absorbing and emitting species was the anionic form of the dye.

The rates $k_2 = k_2'[\text{H}^+]$ and $k_3 = k_3'[\text{H}^+]$ depend on the proton concentration and determine the diffusion controlled reaction of protonation. At room temperature of the ethanolic solution k_2' and k_3' should be of the order of $10^9 \div 10^{11}$ l/Ms.

In order to evaluate k_4 let us examine the fluorescence quantum yield ratios of neutral and tautomeric species for two of the acid concentrations used. For this purpose a quantity U defined as

$$U = \frac{\Phi_T/\Phi_N\,([\text{H}^+] = 10^{-5}\,\text{M/l})}{\Phi_T/\Phi_N\,([\text{H}^+] = 10^{-3}\,\text{M/l})} \tag{15}$$

is introduced. Using Eqs. (9) and (11) one gets the formula

$$U = \frac{(k_4 + k_3' \cdot 10^{-5}\,\text{M/l})\,(\tau_A^{-1} + k_2' \cdot 10^{-3}\,\text{M/l} + k_2' \cdot 10^{-3}\,\text{M/l} + k_4)}{(k_4 + k_3' \cdot 10^{-3}\,\text{M/l})\,(\tau_A^{-1} + k_2' \cdot 10^{-5}\,\text{M/l} + k_3' \cdot 10^{-5}\,\text{M/l} + k_4)}\,, \tag{16}$$

which shows that U should be independent of the water concentration. The experimental value of U for different water concentrations was found to be equal to unity (in the limits of the experimental error). Thus the value of k_4 was calculated to be $k_4 = \tau_A^{-1}(k_3'/k_2')$. k_4 is therefore not sensitive to the k_2' and k_3' values if one assumes a diffusion controlled process ($k_2' = k_3'$) and an equal probability of N^* and T^* forming via protonation of A^*. Since $\tau_A = 4.1$ ns, k_4 equals to $2.4 \cdot 10^8\,\text{s}^{-1}$. One sees now that the bracket in Eq. (14) even for a $[\text{H}^+]$ concentration of 10^{-3} M/l is at least equal 0.85, thus leading to a k_1' value of $15.6 \cdot 10^7$ l/Ms, what appears to be more than twice as much as estimated from phase shift measurements. There still exists a discrepancy between k_1' evaluated from two experimental methods which in our case may be due to the simplified assumption of equal probability of $A^* \rightarrow N^*$ and $A^* \rightarrow T^*$ reactions.

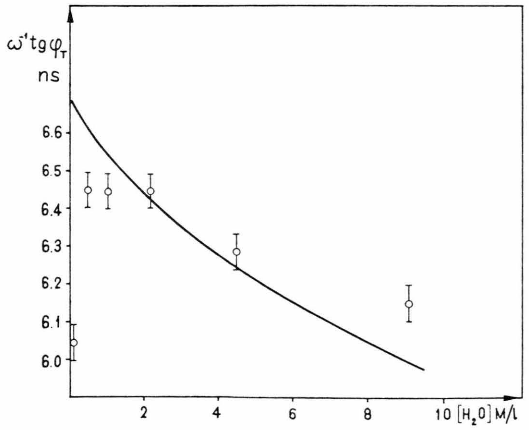

Fig. 5. Dependence of calculated (solid line) and directly measured values of $\omega^{-1}\,\text{tg}\,\varphi_T$ on water concentration. Acid concentration $\leqq 10^{-3}$ M/l.

In this way all the rates in our reaction scheme (Fig. 2) except τ_T are determined (at least their order of magnitude). To find τ_T let us substitute all the other rates (of which k_1 depends on the water concentration in the solution) into Eq. (8) and search for a value of τ_T, which will result in the best fit of the so calculated curve (solid line in Fig. 5) with the experimental values (Table 2) of $\omega^{-1}\,\text{tg}\,\varphi_T$. The value τ_T obtained in this way is equal to $2.9 \cdot 10^{-9}$ s *.

V. Conclusions

The presented model describes fairly well the behaviour of the excited state species of 4-MU for water contents ranging from 0.5 M/l to 10 M/l and acid concentrations below 0.1 M/l.

We believe that acid concentrations of 0.1 M/l and higher influence the fluorescence quenching and change the τ values considerably. In the proposed reaction scheme and its resulting expressions, for the sake of simplicity we do not take this quenching into account. It seems therefore that the decrease of measured phase shifts of fluorescence in solutions with 10^{-1} M/l acid content may be attributed to fluorescence quenching by Cl^- and ClO_4^- ions. All the obtained results are the same for both HCl and HClO_4 acids.

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* The discrepancy between experimental and calculated values in Fig. 5 is worst for lowest water content in solution what may be due to poor spectroscopic isolation of the strong and fast decaying neutral form fluorescent emission and the weak tautomer band (for higher water concentrations the tautomer emission prevails).

It is interesting to compare the estimated value of k_4 (i. e. the rate of tautomer formation due to "internal protonation") with generally accepted orders of magnitude of diffusion controlled process rates (k_2, k_3). These rates must change from $10^4\ s^{-1}$ (the lowest value for 10^{-5} M/l proton concentration) up to $10^8\ s^{-1}$ (the highest value for 10^{-3} M/l proton concentration). Since k_4 is equal $2.4 \cdot 10^8\ s^{-1}$, the proposed "internal protonation" process is fully justified.

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