Decay Times Study on the Fluorescence of 4-Methylumbelliferone

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Z. Naturforsch. **35a**, 946–950 (1980); received June 14, 1980

Phase, pulse and steady-state measurements of ethanolic solutions of 4-methylumbelliferone with various water and hydrochloric acid contents were performed. A kinetic model is proposed and some parameters are determined.

I. Introduction

The spectroscopic properties of the 4-methylumbelliferone (4-MU)-broadband fluorescence as well as the minimal overlap of the absorption and emission spectra-offer the possibility of achieving a laser emission over an exceptionally wide range, 385–575 nm [1].

It has been found that the excited molecule undergoes fast protolitic reactions to form other emitters, which the fluorescence spectra. After emission the products revert very rapidly to the neutral form and therefore do not contribute to the absorption spectrum. Several bands in the emission spectrum have been unequivocally assigned to molecular species: that at 390 nm to the neutral form, that at 445 nm to the anion and that at 412 nm to the cation. Despite this, the overall chemical reaction scheme is still under discussion. Different opinions exist about the origin of the broad long-wave band (480–525 nm), the number of species emitting in this region and their possible structures: tautomer [2], ionic pairs [3] or other products of degradation [4].

In this paper we adapt and develop the model of Trozzolo [5], since most of our experimental results are consistent with it. According to this model, in aqueous alcoholic solution of 4-MU over a very wide range of acidity and water content, a stable ground state exists only for neutral molecules of 4-MU (Figure 1). After excitation the aryl-OH group becomes more acidic and under favourable conditions (e.g. in presence of a little H_2O, a good proton acceptor) the molecule tends to become an anion. Re-neutralization of the anion can be realized by protonation of the aryl or the pyrone oxygens. In the first case the initial molecule will be recreated. However if the carbonyl group is more basic than the ionized hydroxyl group a tautomer will be formed.

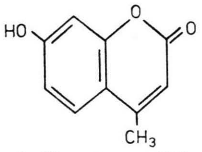

Fig. 1. Structure of the neutral form of 4-methylumbelliferone.

This model explains the simultaneous emission of at least three different excited-state species (neutral, anionic and tautomer).

Further addition of water to the alcoholic solutions leads to a large increase in the dielectric constant attended by an increase in the acidity of both the carbonyl and hydroxyl group. Thus after excitation the neutral molecule dissociates very fast and we can observe only the anion emission while the absorption spectrum remains that of the neutral form.

We intended to verify the above model by observation of the spectral and time response of the system after steady state, harmonically modulated or pulse excitation.

II. Experimental

Reagent grade 4-methylumbelliferone from Koch-Light was used. Absolute alcohol of spectrograde purity, hydrochloric acid and sodium chloride of reagent grade were supplied by POCh-Poland. The water was twice distilled.

The emission spectra were recorded using a home made spectrofluorimeter and were corrected for the sensitivity of the detecting system.

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Phase shift measurements of fluorescence decays were made with the fluorometer of Bauer-Rozwadowski [6]. The samples were excited by the 333 nm or 365 nm lines of a mercury lamp. The fluorescence bands at 390, 445, 480 and 525 nm were isolated by means of interference and absorption filter combinations. Care was taken to allow for wavelength dependent errors in the phase shifts measurements [7].

In the case of a monoexponential decay, $I(t) = I_0 \cdot \exp(-t/\tau)$ the tangens of the phase shift is directly related to the decay time $\mathrm{tg}\,\varphi = \omega\tau$ whereas in cases of multiexponential decay,

$$I(t) = \sum_k I_{0k} \exp(-t/\tau_k),$$

the phase shift is expressed by decay parameters in a more complex form:

$$\omega^{-1}\,\mathrm{tg}\,\varphi = \sum_k I_{0k}\tau_k^2(1+\omega^2\tau_k^2)^{-1} \Big/ \sum_k I_{0k}\tau_k(1+\omega^2\tau_k^2)^{-1}, \qquad (1)$$

where ω is the circular frequency of modulation of the excitation.

Time-resolved spectra and decay curves were measured by means of a sampling pulse fluorometer. The excitation source was a nitrogen laser of subnanosecond pulse duration [8]. The detection system comprised a SPM-3 (Zeiss-Jena) monochromator, an ITT F4032 photomultiplier and a sampling oscilloscope (Schlumberger OCTV 557 A with SA 5891 sampling unit). The data were collected and averaged by a multichannel analyser (NTA-1024-Orion, Hungary) set in its "digital oscilloscope" mode.

The observed decay curves are convolutions of the observed lamp profile with the impulse response (true decay) of the fluorescence of the sample. This impulse response was obtained by deconvolution using a nonlinear least-squares procedure assuming a mono- or bi-exponential model of the true fluorescence decay [9].

III. Results and Conclusions

Acidified ethanol water solutions of 4-MU were studied. The water content varied from 1 to 9% v/v and the hydrochloric acid concentration was changed from 0 to $5 \cdot 10^{-2}$ M.

Time-resolved fluorescence spectra are depicted in Figure 2. At least three fluorescence bands are observed during emission of neutral and acidified aqueous alcoholic solution of 4-MU. In contrast to the water content the acid concentration has little effect on the shape of the spectra. It only reduces the anionic band (450 nm).

Immediately after excitation the shortwave emission from neutral molecules dominates, while after a few nanoseconds the longwave fluorescence prevails. Therefore the molecules emitting in the longwave region of the emission spectrum are photoproducts.

Because of the strong overlap of the 450, 480 and 525 nm bands we cannot decide whether there are one or two species emitting in the 480–525 nm region.

The steady state emission spectra of the solutions are presented in Figure 3. The ratio of the fluorescence intensities at 480 and 390 nm depends linearly on the water content, the slope being independent of the acid concentration (Figure 4).

Since the 480 nm peak is superimposed by the 390 nm band we calculated the true emission at 480 nm as $I(480) - f\,I(390)$, where f is the relative spectral intensity of the 390 band.

Since chemical equilibria can only be independent of the acid concentration in the case of uncharged molecular species, we conclude that in our case the molecules emitting at 480 nm are neutral. We therefore assume, like other authors [2, 4], that this species is a neutral tautomer. Quantum-mechanical calculations [10] support this assignment in that a batochromic shift of tautomer emission compared with the neutral 4-MU molecule is predicted, as observed. From these calculations it appears also that the ground state energy of a 4-MU tautomer is about 40.65 kcal above that of the neutral form.

Pulse and phase measurements could be performed with sufficient reliability only on the short wavelength side of the 390 emission band. In this region we can certainly extract the emission of a single species, the neutral one. The values of $(\mathrm{tg}\,\varphi_N)/\omega$ and τ_N calculated from phase and pulse measurements are presented in Table 1. The two methods yield the same results within experimental error. Monoexponential functions convoluted with the excitation response curve fit well to the experimental data. A biexponential function of course gives a slightly better fit. The parameters of a two-exponential analysis may be used in (1) to calculate $(\mathrm{tg}\,\varphi_N)/\omega$. The values thus calculated, however, differed considerably from the results of the phase

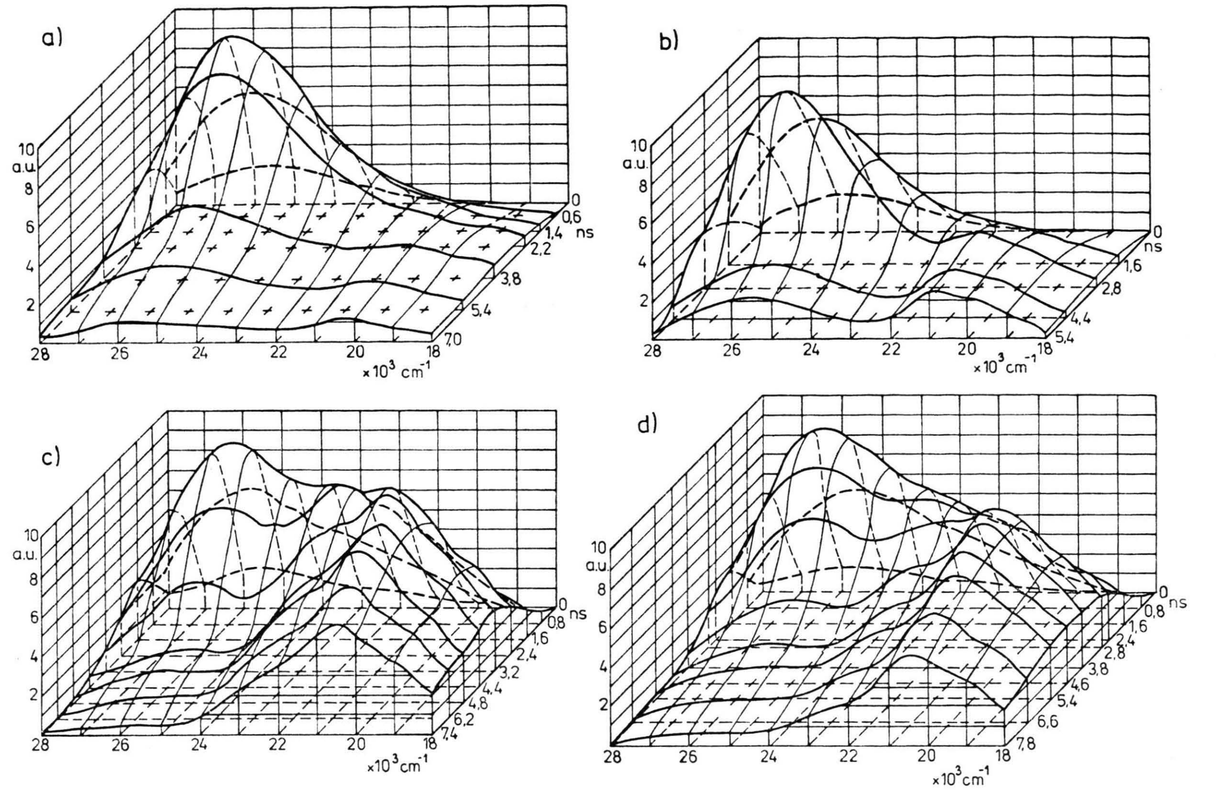


Fig. 2. Time-resolved spectra of acidified /right/ and neutral /left/ aqueous alcoholic solutions of 4-MU, a, b — 1% v/v H_2O; c, d — 17% v/v H_2O.

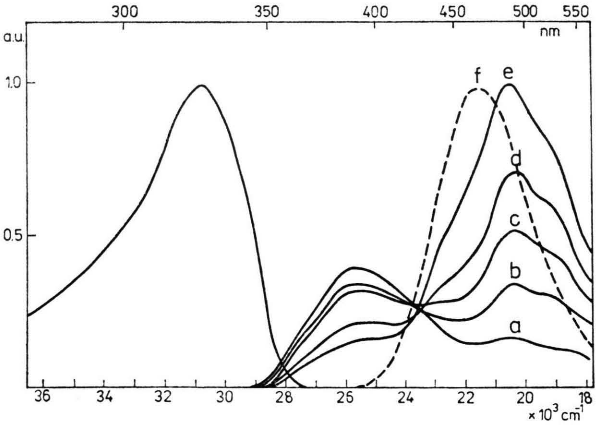


Fig. 3. Steady state absorption and luminescence spectra of aqueous alcoholic solutions of 4-MU solutions: a — 1% b — 3%, c — 5%, d — 9%, e — 17%, f — 99% v/v H_2O.

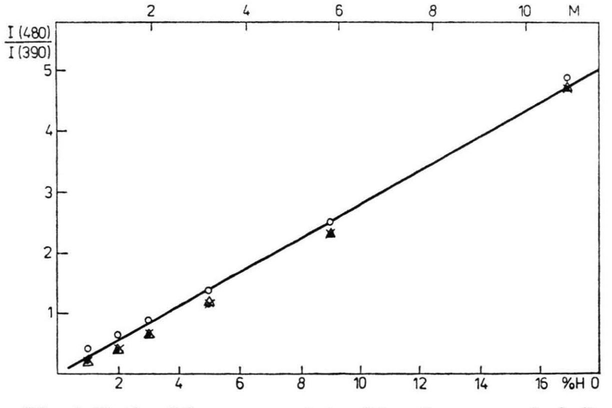


Fig. 4. Ratio of fluorescence intensities of aqueous alcoholic solutions of 4-MU at 480 nm and 390 nm versus water content; hydrochloric acid concentrations: ● — no acid added, Δ — 10^{-3} M, X — 10^{-2} M and 0 — 5×10^{-12} M.

fluorometer measurements. We came therefore to the conclusion that the fluorescence of neutral molecules decays monoexponentially.

The fact that the rate of the monoexponential fluorescence decay of the neutral form is practically independent of acid concentration indicates that:

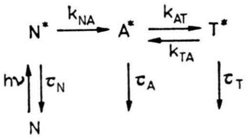
Table 1.

%H_2O	1	2	3	5	9	Method
0	1.4	1.3	1.2	1.1	0.9	pulse
HCl	1.4	1.3	1.2	1.1	1.0	phase
$5 \cdot 10^{-2}$	1.3	1.2	1.1	1.0	0.8	pulse
HCl	1.3	1.2	1.1	1.0	0.9	phase

a) backward reactions which regenerate neutral molecules are negligible in comparison with forward ones.

b) the anion A^- emits or recombines with H^+ ions so as to produce a new uncharged species rather than regenerating the original neutral form. The slight decrease of the decay times with increasing acidity is the result of chloride ion quenching.

In view of this monoexponential behaviour we propose the reaction kinetic scheme corresponding to the coupled kinetic equations



$$
\begin{aligned}
d[N^*]/dt &= -(k_{NA} + \tau_N^{-1})[N^*] + f(t),\\
d[A^*]/dt &= -(k_{AT} + \tau_A^{-1})[A^*]\\
&\quad + k_{NA}[N^*] + k_{TA}[T^*],\\
d[T^*]/dt &= -(k_{TA} + \tau_T^{-1})[T^*]\\
&\quad + k_{AT}[A^*], \qquad (2)
\end{aligned}
$$

where $[N^*]$, $[A^*]$, $[T^*]$ are the concentrations of neutral, anionic and tautomer molecules and $f(t)$ describes the excitation. Of particular interest are the cases where $f(t)$ is constant, varies harmonically or takes the form of a δ-pulse (effectively instantaneous.The initial conditions required to solve (2) depend on the kind of excitation.

This model may explain the water content dependence of the decay time of the neutral form. If it is assumed that k_{NA} and k_{TA} depend linearly on the water concentration:

$$k_{NA} = k'_{NA}[H_2O], \quad k_{TA} = k'_{TA}[H_2O], \qquad (3)$$

then from (2) with harmonically modulated excitation the phase shift of the fluorescence of the neutral form can be calculated:

$$\omega^{-1} \operatorname{tg} \varphi_N = \tau_N/(1 + \tau_N + k'_{NA}[H_2O]). \qquad (4)$$

Indeed $\omega \operatorname{ctg} \varphi_N$ depends linearly on $[H_2O]$ as shown in Figure 5. The deviation from strict linearity observed in presence of HCl may reflect the appearance of a small but significant rate of reversion to the original ground state at high enough acid concentrations. From the slope and the intercept there resulted $k'_{NA} = (6.5 \pm 0.5) \times 10^7\ M^{-1}\ s^{-1}$ and $\tau_N = 1.35$ ns Unfortunately, for water contents higher than 10% the measurements of φ_N are disturbed by the increasing influence of longwave fluorescence bands. For high water contents, although the absorption spectrum is still typical for neutral molecules, in the observed fluorescence spectrum only the anion emission band can be observed. In this case no tautomer fluorescence is detected and we can neglect k_{AT} and k_{TA}. This enables us to derive from (2) an expression for the phase shift of this fluorescence:

$$
\begin{aligned}
\omega^{-1} \operatorname{tg} \varphi_A = [1 + \tau_A(k_{NA} + \tau_N^{-1})]/\\
(k_{NA} + \tau_N{}^{-1} - \omega^2 \tau_A). \qquad (5)
\end{aligned}
$$

In this expression $(\operatorname{tg} \varphi_A)/\omega$ and τ_A are directly measured and τ_N found indirectly, thus enabling the calculation of k_{NA} for a very high water content. $\tau_A = 5.4 \pm 0.05$ ns was measured in basic aqueous solutions of 4-MU(pH = 13), where the only light absorbing and emitting species are anionic forms of the dye. With

$$
\begin{aligned}
(\operatorname{tg} \varphi_A)/\omega &= 5.7 \pm 0.05\,\text{ns},\\
\omega^2 &= 55.5 \times 10^{14}\,s^{-2}
\end{aligned}
$$

and $\tau_N = 1.35$ ns, one gets:

$$k_{NA} = (3.6 \pm 1.5) \times 19^9\,s^{-1}.$$

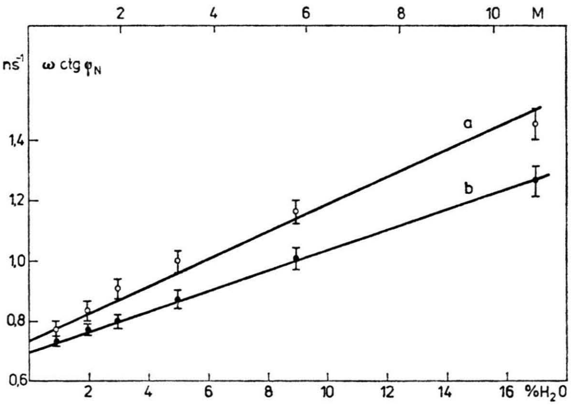


Fig. 5. Dependence of $\omega \operatorname{ctg} \varphi_N$ on water concentration in aqueous alcoholic solutions of 4-MU for: a -5×10^{-2} M HCl and b— no acid added.

On the other hand we can calculate k_{NA} under these conditions using the value obtained previously from decay measurements at low water concentrations:

$$\begin{aligned} k_{\mathrm{NA}} &= k'_{\mathrm{NA}}\,[\mathrm{H_2O}] \\ &= (6.5 \pm 0.5) \times 10^7\,\mathrm{M^{-1}\,s^{-1}} \times 55\,\mathrm{M} \\ &= (3.6 \pm 0.3) \times 10^9\,\mathrm{s^{-1}}. \end{aligned}$$

The values obtained by the two methods are in very good agreement, which strongly confirms our assumption that the dissociation rate is proportional to the water content. We also tried to find out how many species emit in the longwave region (480—525 nm) of the fluorescence spectrum by measuring the decay in that region. The results of phase measurements at 480 and 525 nm are presented in Table 2. These results cannot be interpreted in terms of our model because of the strong overlap of the observed bands. Nevertheless it is evident that the difference between the phase shifts at 480 and 525 nm correspond approximately to 1 ns.

Table 2.

% H_2O		2	3	5	9	17
OHCl	480 nm	4.7	4.8	4.5	4.5	4.4
	525 nm	4.9	4.8	4.7	4.7	4.5
$5 \cdot 10^{-2}$ H/l HCl	480 nm	5.4	5.4	5.5	5.3	5.3
	525 nm	6.4	6.1	6.1	6.2	6.2

The origin of this difference remains an open question.

Acknowledgements

The authors are greatly indebted to Dr. R. E. Dale for valuable discussions and proof reading of the manuscript. We also thank Dr. A. Szabo for providing us with a FORTRAN deconvolution programme. This work was carried on under the Research Project MR.I.5.

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