

THERMAL DEACTIVATION OF THE LOWEST SINGLET AND TRIPLET EXCITED STATES OF ACRIDINE DYES IN POLY(VINYL ALCOHOL) FILMS

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Summary

Measurements of the mean fluorescence lifetime τ_F , the lifetimes τ_{DF} of the delayed fluorescence, the phosphorescence lifetime τ_P , the fluorescence quantum yields ϕ_F , and the ratios of the phosphorescence and the delayed fluorescence quantum yields ϕ_P and ϕ_{DF} respectively, to the fluorescence quantum yield ϕ_F were carried out for three acridine dyes: acridine yellow, acridine orange and proflavine in poly(vinyl alcohol) films as a function of temperature. From these measurements, rate constants were determined for the radiative and radiationless transitions for both the lowest singlet excited state S_1 and the triplet state T_1 . The temperature dependence of the efficiency ϕ_T of the population of the triplet state was also examined.

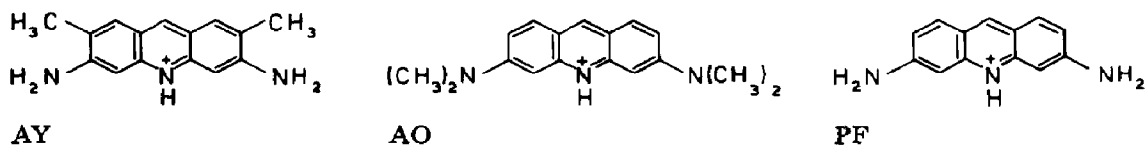
1. Introduction

In recent years acridine dyes and their cationic and dicationic forms have become the objects of numerous photophysical and photochemical investigations. The reason for the particular interest in this group of compounds is their ability to intercalate with biological macromolecules and their possible use as absorption and fluorescence probes. In particular, the photochemical reactions and the efficiencies ϕ_T of the population of the triplet state were investigated as a function of pH [1], as well as the acid-base equilibria in the ground state [2] and in the singlet and triplet excited states [3]. A report of the interaction of acridine dyes with DNA was included in refs. 4 - 8. For some acridine and anthracene dyes, the probabilities of the transitions $S_1 \rightsquigarrow T_1$ were investigated by the method of sensitized delayed fluorescence (Parker's method), and the values of ϕ_T were found to be 0.30 ± 0.05 , 0.45 ± 0.05 and 0.53 ± 0.07 for acridine orange

(AO), proflavine (PF) and acriflavine respectively (all in ethanol) [9]. The shifts [10] and the broadening [11] of the fluorescence and the delayed fluorescence spectra were studied in poly(vinyl alcohol) (PVA) films as a function of temperature. Recently, measurements of the linear dichroism of the cationic [12, 13], the neutral and the dicationic [14] forms were carried out for acridine dyes in oriented PVA films, which enabled the orientation coefficients and the directions of the electronic transition moments to be determined.

The detailed examination of the excited-state parameters of this group of molecules may prove to be very useful for their application to further biophysical and biochemical investigations of macromolecules. The parameters of the triplet state have recently been determined for AO [15].

The aim in the present paper is to investigate the radiative and radiationless transitions from both the singlet S_1 and the triplet T_1 states for AO, acridine yellow (AY) and PF in PVA films.



In our examination of the effect of temperature on the electronic transitions we assume that the rate constants k_F and k_P of the radiative transitions (Fig. 1) are much less affected by temperature than those of the radiationless transitions, which are expressed by

$$k_x = k_x^0 + A_x \exp\left(-\frac{E_A^*}{kT}\right) \quad (1)$$

where x denotes n, m, ST and TS as is appropriate (see below).

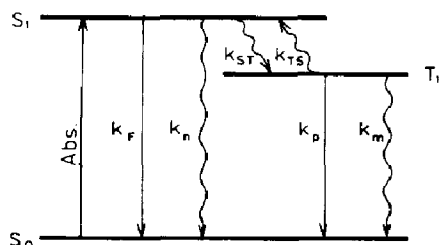


Fig. 1. Jablonski diagram.

2. Experimental details

AO and AY (Fluka AG) were purified by the method described in refs. 9 and 12; PF was purified in a column with active aluminium and then recrystallized from ethanol. The method of preparing PVA films was similar to

that reported by Tanizaki *et al.* [16]. Mean fluorescence lifetimes were measured with an accuracy of 10 ps by means of a phase fluorometer [17, 18]. The phosphorescence decay measurements were carried out using two coupled choppers. The values of the ratio ϕ_P/ϕ_F of the phosphorescence quantum yield to the fluorescence quantum yield and the values of the ratio ϕ_{DF}/ϕ_F of the delayed fluorescence quantum yield to the fluorescence quantum yield were measured by means of two synchronized choppers powered by a generator at a frequency of 40 Hz. The true values of ϕ_P/ϕ_F and ϕ_{DF}/ϕ_F were computed by Fourier analysis of the measured signals. The fluorescence quantum yields were measured by using a spectrofluorometer [19] which employed AO in ethanol as a standard ($\phi_F^S = 0.46$ [1]). A special optical cryostat enabled measurements to be made in the temperature range 80 - 300 K. The cell was thermostatted with an accuracy to ± 1 K and the quantum yields were measured with an accuracy to ± 0.002 .

3. Results and discussion

The results from the measurements of the temperature dependence of the mean phosphorescence and delayed fluorescence lifetimes τ_P and τ_{DF} respectively, and ϕ_P/ϕ_F and ϕ_{DF}/ϕ_F , are presented in Fig. 2. The temperature dependences ϕ_F , ϕ_T and ratios ϕ_{DF}/ϕ_P are given in Fig. 3. At low temperatures ($T < 130$ K), the values of the mean lifetimes and the quantum yields become constant for each of the substances investigated and are virtually independent of temperature. Equation (2) is fulfilled very accurately for each of the substances investigated at low temperatures.

$$\phi_P^0 \equiv \phi_T^0 = 1 - \phi_F^0 \quad (2)$$

The above implies that k_n^0 , k_m^0 and k_{TS}^0 in eqn. (1) are equal to zero, and that only k_{ST}^0 is non-zero.

3.1. Thermal deactivation of the singlet state S_1

Thermal deactivation of the singlet state S_1 (*cf.* the Jabłoński diagram, Fig. 1) may involve three processes: a radiative transition with a rate constant k_F and two radiationless processes with rate constants k_n and k_{ST} . Using the notation in Fig. 1 one obtains

$$k_F = \phi_F/\tau_F \quad (3)$$

The measurements of the fluorescence quantum yields and the mean fluorescence lifetimes at different temperatures indicate that the rate constant k_F is independent of temperature (*cf.* Table 1). The rate constants k_{ST} and k_n of the radiationless transitions can be expressed by the relations

$$k_{ST} = \frac{\phi_T}{\tau_F} = \frac{\phi_P}{\phi_F \tau_P} \frac{k_F}{k_P} \quad (4)$$

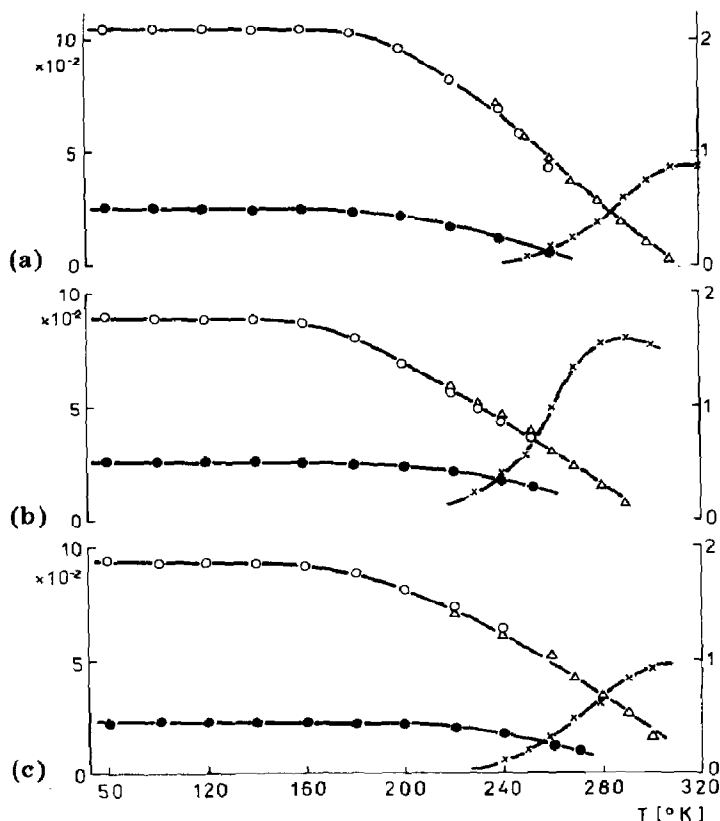


Fig. 2. Effect of temperature on the values of the quantum yield ratios ϕ_{DF}/ϕ_F (x) and ϕ_P/ϕ_F (●) and on the mean lifetimes τ_P (○) and τ_{DF} (△) for acridine dyes in PVA films: (a) AY; (b) AO; (c) PF.

$$k_n = \left(\frac{1}{\phi_F} - 1 - \frac{k_{ST}}{k_F} \right) k_F \quad (5)$$

From eqn. (1)

$$k_{ST} - k_{ST}^0 = \frac{\phi_P}{\phi_F} \frac{k_F}{\tau_P k_P} - \left(\frac{\phi_P}{\phi_F} \right)_0 \frac{k_F}{\tau_P^0 k_P} = A_{ST} \exp\left(- \frac{E_A^{ST}}{kT} \right) \quad (6)$$

$$k_n = \left(\frac{1}{\phi_F} - 1 - \frac{k_{ST}}{k_F} \right) k_F = A_n \exp\left(- \frac{E_A^n}{kT} \right) \quad (7)$$

The values of the parameters A_{ST} , A_n , E_A^{ST} and E_A^n can be found immediately from a logarithmic plot of relations (6) and (7). The parameters for the thermal deactivation of the lowest singlet excited state S_1 of the acridine dyes in PVA films determined from the linear plots of eqns. (6) and (7) are listed in Table 1.

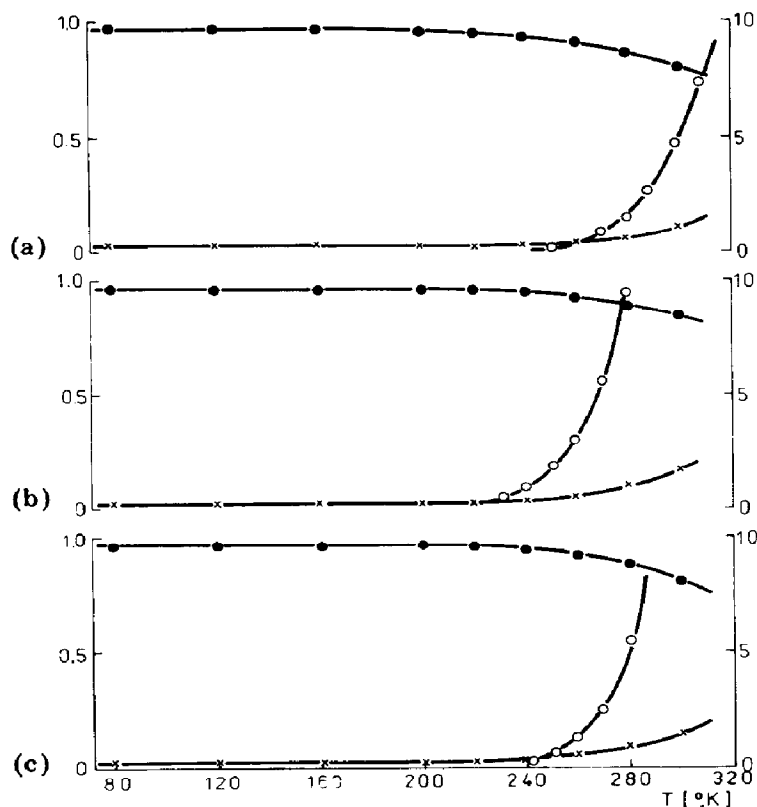


Fig. 3. The fluorescence quantum yield ϕ_F (\bullet), the triplet state population quantum yield ϕ_T (\times) and the value of the ratio ϕ_{DF}/ϕ_P (\circ) as functions of temperature for acridine dyes in PVA films: (a) AY; (b) AO; (c) PF.

3.2. Thermal deactivation of the triplet state T_1

Deactivation of the triplet state T_1 involves the following processes in the case of acridine dyes (Fig. 1): a radiative transition $S_0 \leftarrow T_1$ (phosphorescence), a radiationless transition with a rate constant k_m and a transition which is the reverse of intersystem crossing, proceeding with a rate constant k_{TS} . Using the notation in the Jabłoński diagram, one obtains the relation

$$\phi_T = \left(\frac{\phi_P}{\phi_F} \right) \phi_F \frac{1}{k_P} \frac{1}{\tau_P} = \frac{\phi_{DF}}{\phi_F} \frac{1}{k_{TS}} \frac{1}{\tau_{DF}} \quad (8)$$

where

$$\tau_{DF} \approx \tau_P = (k_P + k_m + k_{TS})^{-1} \quad (9)$$

The measured values of the mean phosphorescence and the delayed fluorescence lifetimes τ_P and τ_{DF} respectively, and the values of the ratios ϕ_{DF}/ϕ_F , ϕ_{DF}/ϕ_P and ϕ_P/ϕ_F are given in Figs. 2 and 3. A characteristic temperature dependence is displayed by the delayed fluorescence. The values ϕ_{DF}/ϕ_F at low temperatures are negligibly small, grow from 240 K (but not monoton-

TABLE 1

Parameters for the deactivation of the lowest singlet excited state S_1 of acridine dyes in PVA films^a

Compound	$\phi_F^0(77\text{ K})$	$\phi_F(293\text{ K})$	$\tau_F^0(77\text{ K})$ (ns)	$\tau_F(293\text{ K})$ (ns)	k_F ($\times 10^9\text{ s}^{-1}$)	E_A^{ST} (cm^{-1})	A_{ST} ($\times 10^4\text{ s}^{-1}$)	E_A^n (cm^{-1})	A_n ($\times 10^8\text{ s}^{-1}$)
AY	0.974	0.821	7.34	6.18	0.133	3243 (101)	23.0 (1.1)	851 (27)	10.5 (0.3)
AO	0.975	0.848	6.02	5.24	0.162	2468 (54)	1.4 (0.1)	757 (12)	3.4 (0.2)
PF	0.978	0.824	5.64	4.73	0.174	2569 (36)	1.6 (0.1)	704 (7)	2.5 (0.1)

^aThe parameters E_A^{ST} , E_A^n , A_{ST} and A_n and their standard deviations (in parentheses) were determined from the fit of the experimental points to the straight lines from eqns. (6) and (7) by the least-squares analysis [20].

TABLE 2

Parameters for deactivation of the triplet state T_1 of acridine dyes in PVA films^a

Compound	ϕ_P^0	τ_P^0 (s)	k_P (s^{-1})	E_A^{TS} (cm^{-1})	A_{TS} ($\times 10^6\text{ s}^{-1}$)	E_A^m (cm^{-1})	A_m (s^{-1})
AY	0.026	2.08	0.48	3260 (106)	34.1 (0.3)	958 (10)	77.9 (0.7)
AO ^b	0.025	1.78	0.56	2568 (57)	3.5 (0.1)	937 (14)	147.0 (1.1)
PF	0.022	1.93	0.52	3098 (43)	23.8 (0.2)	1004 (26)	129.8 (0.9)

^aThe parameters E_A^{TS} , E_A^m , A_{TS} and A_m and their standard deviations (in parentheses) were determined from the fit of the experimental points to the straight lines from eqns. (10) and (11) by least-squares analysis [20].

^bData for the parameters of the triplet state T_1 deactivation of AO can be also found in ref. 15; however, the values of A_{TS} and A_m given were incorrect. The correct values are $3.47 \times 10^6\text{ s}^{-1}$ and 147.0 s^{-1} respectively.

ically), and attain a maximum value at about 290 K for AO and at 310 K for AY and PF. In all cases, at low temperatures ($T < 160$ K) the mean phosphorescence lifetime becomes constant and equal to the radiative phosphorescence lifetime ($\tau_p^0 = 1/k_p$). The values for the efficiency ϕ_T of the population of the triplet state at low temperatures determined using eqn. (8) do not exceed 3% and 15% at room temperature, whereas in liquid solutions the values of ϕ_T are two to three times as large [9]. The parameters for thermal deactivation of the triplet state T_1 can be determined from

$$k_{TS} = \left(\frac{\phi_{DF}}{\phi_P} \right) \frac{k_P}{\phi_F} = A_{TS} \exp\left(-\frac{E_A^{TS}}{kT}\right) \quad (10)$$

and by analogy

$$k_m = \frac{1}{\tau_P} - k_P - k_{TS} = A_m \exp\left(-\frac{E_A^m}{kT}\right) \quad (11)$$

Logarithmic linear plots of eqns. (10) and (11) enable the values E_A^{TS} , E_A^m , A_{TS} and A_m to be immediately determined. The values of the triplet state T_1 parameters of acridine dyes in PVA films have been listed in Table 2. The values of E_A^{TS} obtained for AY and PF (3150 and 3000 cm^{-1} respectively) are in good agreement with those determined from the fluorescence and phosphorescence spectra (the value $E_{S_1} - E_{T_1}$ is 3200 and 3100 cm^{-1} for AY and PF respectively). Such agreement has not been observed with AO for which $E_A^{TS} = 2550 \text{ cm}^{-1}$ but $E_{S_1} - E_{T_1} = 3000 \text{ cm}^{-1}$. The values of the activation energies for the radiationless processes $S_1 \rightsquigarrow T_1$ and $T_1 \rightsquigarrow S_1$ are almost identical, with the exception of PF (*cf.* Tables 1 and 2). The activation energies E_A^m which lead to the radiationless deactivation $T_1 \rightsquigarrow S_0$ (rate constant, k_m) are in each case higher by several hundred reciprocal centimetres than the activation energies for the series of aromatic compounds reported in ref. 21.

4. Conclusions

(1) For all acridine dyes investigated, the equation $\phi_P + \phi_F = 1$ has been found to be obeyed excellently at low temperatures. The rate constant k_F of the radiative transition $S_1 \rightarrow S_0$ was found to be independent of temperature from the fluorescence quantum yields ϕ_F and mean lifetimes τ_F measured as a function of temperature.

(2) It was found that the delayed fluorescence quantum yield at higher temperatures ($T > 300$ K) did not grow with increasing temperature. The additional thermal deactivation process (*e.g.* $T_1 \rightsquigarrow T_2$) might be one of the reasons for this.

(3) A relatively good agreement was found between the activation energies determined for the processes $S_1 \rightsquigarrow T_1$ and $T_1 \rightsquigarrow S_1$. It was also

found that the values of the activation energies of the processes $T_1 \rightsquigarrow S_0$ were markedly higher for acridine dyes than those obtained with other aromatic compounds.

(4) The efficiency ϕ_T of the population of the triplet state at room temperature was found to be two to three times lower in PVA films than in liquid solutions.

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