Monomer and Excimer Fluorescence of 2-Phenylindole in p-Xylene at 20 °C Studied by Frequency-Domain Fluorometry

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The effect of 2-phenylindole (2PI) concentration in p-xylene upon the fluorescence decay time was investigated by frequency-domain fluorometry. The fluorescence decay of a dilute (10^{-5} M) 2PI solution in p-xylene at 20 °C is single exponential with the mean decay time $\tau = 1.87$ ns and practically independent of the observation wavelength (λ_{obs}) in the fluorescence band. The fluorescence decay in a highly concentrated solution (6×10^{-3} M) is heterogenic and depends on λ_{obs}. For a given modulation frequency (~ 200 Hz), the fluorescence phase shifts at the longwave observation (440–480 nm) distinctly exceed 90°, thus clearly indicating that the observed (excimer) fluorescence results from a process taking place in the excited state. The fluorescence decays were measured at seven values of λ_{obs} (360–480 nm), and all the results were analyzed comprehensively (τ_1 and τ_2 are equal for all λ_{obs}; the amplitudes are different). The lifetimes of the excimer state is relatively short (~ 3.5 ns). Radiative (τ_e) and non-radiative (τ_1) rate constants for monomer fluorescence of 2PI in p-xylene were determined using appropriate data from photo- and radioluminescence measurements.

1. Introduction

2-Phenylindole (2PI) is the chromophoric core of the DNA-binding probe 4,6-diamidino-2-phenylindole (DAPI) [1–4]. Kubista et al. [5] have shown that 2PI is a suitable model system for DAPI with regard to the almost total agreement between the spectral assignments of DAPI and 2PI. Theoretical conclusions about the electronic states of 2PI are applicable to DAPI. Based on experimental investigations and quantum chemical calculations, 2PI was found to have five distinct electronic transitions located between 200 and 350 nm [5]. The first absorption band (340–280 nm) consists of two electronic transitions separated from other higher energy transitions.

The molecule of 2PI is polar, and the electric dipole moments in the ground (μ_g) and excited (μ_e) states are oriented in the same direction. The values are: $\mu_g = 2.01$ D ($= 6.7 \times 10^{-30}$ C m) [6] and $\mu_e = 4.2$ D ($= 14 \times 10^{-30}$ C m) [7].

It was also found that 2PI forms excimers in p-xylene and n-butanol and the "isoemissive" points show a considerable solvent dependence [8]. Moreover, 2PI is

an efficient scintillator and the radioluminescence efficiency, I_{max}, is 0.274 [8, 9].

In this paper we report investigations on the decays of monomer and excimer 2PI fluorescence in p-xylene at 20 °C by frequency-domain fluorometry.

2. Experimental

2PI (Fluka AG, purity $> 99\%$) was used without further purification, p-xylene (Aldrich) was HPLC grade.

Frequency-domain measurements were performed on an instrument described previously [10, 11]. The light source was a synchronously pumped rhodamine 6G dye laser, cavity dumped at 3.76 MHz and frequency doubled to provide ultraviolet for the excitation of 2PI. Phase and modulation measurements could be carried out at any integer multiple of the pulse repetition rate, up to our current limit of 10 GHz. The detector was an externally cross-correlated Hamamatsu R2566 MCPPMT.

The frequency-domain data consist of phase angle, $\Phi_\omega(\lambda)$, and modulation values, $m_\omega(\lambda)$, each measured for a particular wavelength, λ_{obs}, and for a range of modulation frequencies ω. These values are used to

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obtain the best-fit parameters for the impulse response

$$I(\lambda, t) = \sum_i \alpha_i(\lambda) \, e^{-t/\tau_i}. \tag{1}$$

By using this equation we assume that the decay times, τ_i, are constant across the emission spectrum. The values of the parameters $\alpha_i(\lambda)$ and τ_i and the goodness-of-fit are determined from the minimum values of χ_R^2:

$$\chi_R^2 = \frac{1}{\nu} \sum_\omega \left[\frac{\Phi_\omega - \Phi_{c\omega}}{\delta\Phi} \right]^2 + \frac{1}{\nu} \sum_\omega \left[\frac{m_\omega - m_{c\omega}}{\delta m} \right]^2, \tag{2}$$

where ν is the number of degrees of freedom, and $\delta\Phi$ and δm are the experimental uncertainties in phase and modulation values, respectively. The subscript c is used to indicate calculated values for assumed values of α_i and τ_i.

The fluorescence quantum yield Φ_F was determined by comparison with that of a standard substance, quinine sulfate [12, 13], described in the previous papers [14, 15].

3. Results and Discussion

3.1. Decays of Monomer and Excimer Fluorescence

The mean lifetimes of 2PI in dilute p-xylene solution (10^{-5} M) is 1.87 ns. It is homogeneous and independent of the observation wavelength, λ_{obs}, in the fluorescence band. The fluorescence spectrum of 2PI in p-xylene distinctly depends on the concentration (Figure 1). At high 2PI concentrations (above 10^{-3} M), a structureless excimer fluorescence band appears on the longwave side. In the case of the highest concentrations that could practically be achieved (7.8×10^{-2} M) for 2PI in p-xylene, two overlapping fluorescence bands, monomer and excimer, with comparable intensities at the emission maximum occur. The fluorescence decay of a concentrated solution (6×10^{-3} M) in p-xylene is heterogeneous and depends on λ_{obs}. Therefore the decays were measured every 20 nm from 360 to 480 nm. The observation of the phase shift (Table 1) enables the conclusion to be drawn that the process dealt with occurs in the excited state when the final state (R) is pumped through the initially excited state (F). For a fixed modulation frequency (~ 200 MHz, Table 1) the fluorescence phase shifts at the longwave observation considerably exceed $90°$, which clearly indicates that the observed fluorescence results from a process occurring in the excited state. Figure 2
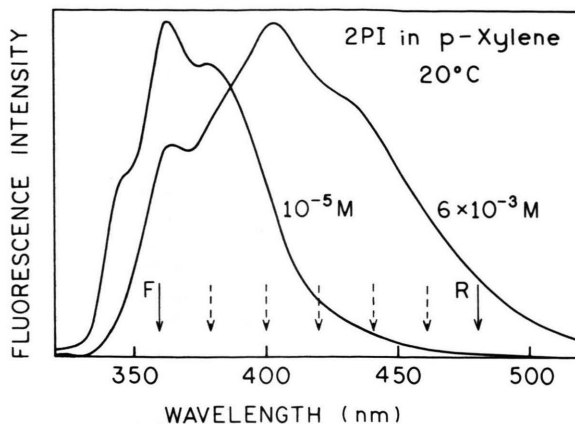

Fig. 1. Fluorescence spectra of 2-phenylindole in p-xylene at 20 °C. The arrows indicate the observation wavelength (λ_{obs}).

Table 1. Phases and modulations observed for 2PI ($C = 6 \times 10^{-3}$ M) fluorescence in p-xylene at 20 °C. The modulation frequency was 197.34 MHz.

λ_{obs} (nm)	Phase (degree)	Modulation
360	49.2	0.608
380	49.6	0.589
400	57.3	0.394
420	78.4	0.224
440	91.7	0.184
460	101.3	0.167
480	109.5	0.153

shows schematically the decay runs for processes occurring in the excited state (e.g. excimers) in the time- and frequency-domain. The phase and modulation runs in a concentrated 2PI solution (6×10^{-3} M) are shown in Fig. 3 for a short- (360 nm) and longwave (480 nm) observation. The wavelength dependence of the intensity decay in the fluorescence band of 2PI ($c = 6 \times 10^{-3}$ M) in p-xylene at 20°C is shown in Table 2. The intensity decay results obtained for each observation wavelength, λ_{obs}, were fitted simultaneously with τ_1 and τ_2 as global parameters. χ_R^2 of this global fit is 3.9 (the uncertainties of phase and modulation measurements are $\delta\Phi = 0.3°$ and $\delta m = 0.007$, respectively).

The double exponential model, where τ_1 and τ_2 are common for all λ_{obs}, was found to properly describe the fluorescence of 2PI. The single exponential model yields in this case ($c = 6 \times 10^{-3}$ M) a very poor fit ($\chi_R^2 = 1965$).

In the case of the shortwave observation, the monomer fluorescence is almost exclusively recorded
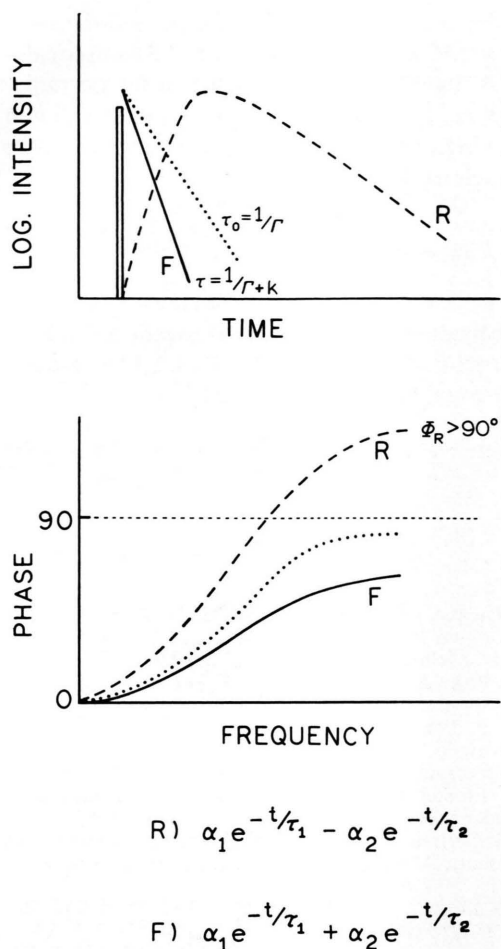
Fig. 2. Schematic description of the excited state reactions in pulse (top) and phase (bottom) fluorometry. The equations describe the intensity decays of the initially excited (F) and reacted (R) states. A phase shift exceeding 90° forces the occurrence of a negative pre-exponential factor in the fluorescence decay.

Fig. 3. Frequency responses of the 2-phenylindole fluorescence decays at short ($\bullet$, $\lambda_{obs} = 360$ nm) and long (o, λ_{obs} = 480 nm) observation wavelengths. The lines represent the best double exponential fits to the data.

Table 2. Wavelength-dependent intensity decays of 2PI ($C = 6 \times 10^{-3}$ M) in p-xylene at 20 °C.

λ_{obs} [a] (nm)	α_1 $\tau_1 = 0.86$ ns	f_1 [b]	α_2 $\tau_2 = 3.42$ ns	f_2 [b]
360	0.967	0.879	0.033	0.121
380	0.951	0.831	0.049	0.069
400	0.731	0.405	0.269	0.595
420	−0.047	−0.012	0.953	0.988
440	−0.326	−0.108	0.674	0.829
460	−0.407	−0.147	0.593	0.853
480	−0.453	−0.172	0.547	0.828

[a] The intensity decay obtained for each λ_{obs} was fitted simultaneously with τ_1 and τ_2 as a global parameters. The χ_R^2 (2) of this global fit is 3.9.
[b] $f_i = \alpha_i \tau_i / \sum_i \alpha_i \tau_i$.

whereas the share of the second component is small. The excimer state lifetimes is relatively short: ~ 3.5 ns.

3.2. Radiative and Non-radiative Rate Constants for Monomer Fluorescence

The fluorescence quantum yield, Φ_F, given by the probability of a radiative transition divided by the total transition probability, is [16]

$$\Phi_F = \frac{1}{\tau_e} \left(\frac{1}{\tau_e} + \frac{1}{\tau_1} \right)^{-1} = \frac{\tau}{\tau_e}, \qquad (3)$$
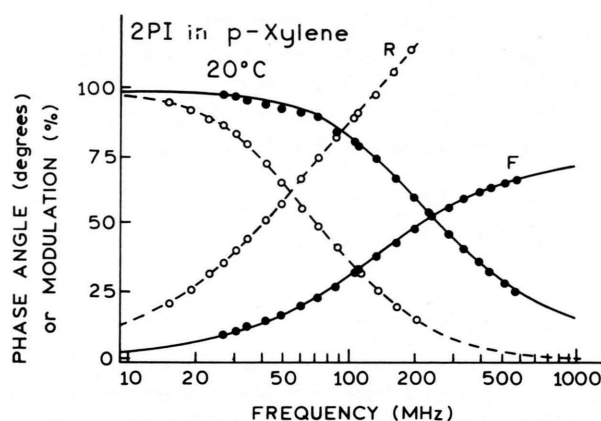
where τ_e and τ_1 are reciprocals of the transition probability for radiation and for a non-radiative process, respectively. Thus, the natural radiative lifetime, τ_e, can be determined by measuring the lifetime τ and the quantum yield Φ_F of the fluorescence. The non-radiative lifetimes, τ_1, can be calculated using the relationship

$$\tau_1 = \frac{\tau}{1 - \Phi_F}. \qquad (4)$$

The quantum yield of 2PI measured in p-xylene is 0.27 ($\lambda_{exc} = 318$ nm), which is markedly lower than that obtained in cyclohexane (according to Berlman [17], $\Phi_F = 0.86$). Based on the values of τ and Φ_F for 2PI in p-xylene, $\tau_e = 6.93$ ns and $\tau_1 = 2.97$ ns were obtained for the monomer fluorescence.

2PI in p-xylene displays radioluminescence proper-
ties [8, 9]. It was shown, based on the Kallman and
Furst theory [18, 19], that τ_e and τ_1 can be determined
using the following formulae involving the Kallmann
and Furst parameters, P and R [20]:

$$\tau_e = \frac{n_e \delta}{P/R} \tau,\qquad(5)$$

$$\tau_1 = \frac{n_e \delta}{n_e \delta - P/R} \tau\qquad(6)$$

(n_e denotes the number of electrons of the solvent
excited per unit time, capable of transferring their
energy to the solute, and δ is a constant parameter).

The following values were obtained for 2PI in
p-xylene: $n_e \delta = 0.85$, $P = 0.05020$ mole/l, $R = 0.1528$

mole/l [9]. Thus, for the above values and the mea-
sured $\tau = 1.87$ ns, $\tau_e = 4.84$ ns and $\tau_1 = 3.07$ ns are ob-
tained. A slight difference, in particular for the radia-
tive lifetime, τ_e, between the photoluminescence and
radioluminescence results is probably due to the effect
of the excimer fluorescence.

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