Short Communication

Determination of subnanosecond fluorescence decays of chlorobenzene, tryptophan and the benzene–triethylamine exciplex using a nanosecond flashlamp

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1. Introduction

Compared with lasers, flashlamps have a low cost and are continuously tunable over a wide spectral range. However, the determination of fast fluorescence decays, which interfere with the decay characteristics of the exciting lamp pulse, requires a stable flashlamp with sufficient full width at half-maximum and also a high intensity output since short fluorescence lifetimes often correspond to very low fluorescence yields caused by the radiationless deactivation of the first excited singlet states. The measurements presented here show that the separation of decays down to 200 ps from nanosecond lamp pulses and even from additional much more intense slower decays is possible.

2. Results and discussion

2.1. Monoexponential fluorescence decay of chlorobenzene

The quantum yield and the lifetime of the fluorescence of chlorobenzene were estimated to be less than about 10^{-4} and 50 ps respectively [1] since no fluorescence was observed [1, 2]. This small fluorescence yield is probably due to the heavy atom effect of chlorine which enhances the radiationless deactivation of the S_1 state by amplifying the intersystem crossing rate as was shown for the halonaphthalene series [3].

Under the optimum conditions for the measurement of low intensity fluorescence [4] it is possible to obtain a fluorescence decay curve for chlorobenzene with a lifetime of 786 ± 12 ps (Fig. 1). The fluorescence curve is well separated from the lamp profile in its increasing and decreasing regions.

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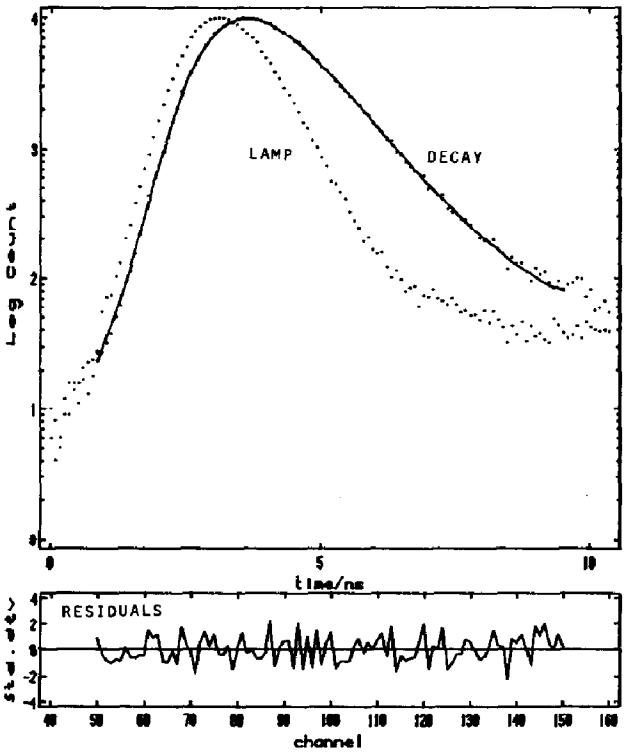

Fig. 1. Fluorescence decay of chlorobenzene in acetonitrile at 22 °C (c = 0.54 M; excitation at 265 nm; emission at 288 nm; fit from channel 50 to channel 150; τ = 786 ± 12 ps; χ^2 = 1.09; fitted background, 9.7 ± 1.6 counts): ···, experimental decay; ——, fitted curve.

2.2. Biexponential fluorescence decays of benzene and its exciplex in the presence of triethylamine

With the exception of the stationary state data for the S_1 benzene–triethylamine exciplex [4], no data for the intermolecular exciplex formation of S_1 benzene with amines in solution have been published [5]. The two emission maxima at 280 and 345 nm obtained for the benzene–triethylamine system in hexane [4] exhibit the decay characteristics shown in Fig. 2. The monomer emission is fitted by a sum of exponentials while the exciplex emission is described by the difference between the same exponentials. The fast decay with a lifetime of 470 ± 40 ps at 282 nm is well separated from the long-lived component although it contributes only 15% of the fluorescence intensity. The pre-exponential factors for the exciplex decay at 345 nm are not identical as demanded by theory [6], but the deviation is within the limits of the error of the fitted background.

2.3. Triple-exponential fluorescence decay of tryptophan

The fluorescence decay of tryptophan has been the subject of many recent investigations. Three different lifetimes ranging from 0.5 to 9 ns have

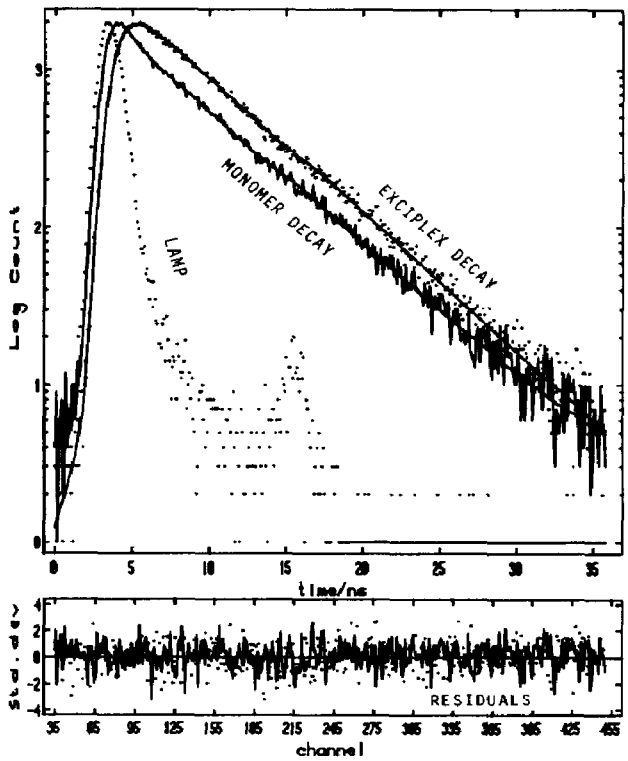

Fig. 2. Monomer and exciplex decay in the benzene–triethylamine (TEA) system in *n*-hexane at 22 °C: $c_{benzene}$ = 0.56 M; c_{TEA} = 0.39 M; excitation at 270 nm. Monomer decay at 282 nm: τ_1 = 4.9 ± 0.1 ns; τ_2 = 0.47 ± 0.04 ns; χ^2 = 1.09; fitted background, 1.6 ± 3 counts. Exciplex decay at 345 nm: τ_1 = 4.8 ± 0.06 ns; τ_2 = 0.52 ± 0.05 ns; χ^2 = 1.11; pre-exponential factors, B_1 = 0.083 and B_2 = −0.089; fitted background, 0.20 ± 0.70 counts. The fitted functions are smooth lines in the decay curves (residuals: ——, monomer; ···, exciplex).

been observed in aqueous solutions depending on the pH, the buffer composition and the emission wavelength [7 - 10]. Only one group of researchers [7] has succeeded in observing the three different lifetimes simultaneously, but in these experiments filters with a 305 nm cut-off were used on the emission side.

The observation of more than one distinct lifetime has been interpreted in terms of rotamers about the C(α)—C(β) bond of tryptophan [10]. Since the existence of these rotamers has been established by nuclear magnetic resonance in non-aqueous solvents [11], we measured the fluorescence decay of tryptophan in 2-propanol and obtained three superimposed lifetimes at each of the three emission wavelengths selected. The results are presented in Fig. 3 and Table 1.

The deconvolution of three lifetimes has been questioned on theoretical grounds [12]. However, it is obvious from the non-random distribution of the residuals in the time range of the lamp pulse (Fig. 3) that proper deconvolution cannot be achieved with two exponentials. All three lifetimes are slightly shortened compared with those in the aqueous solution.

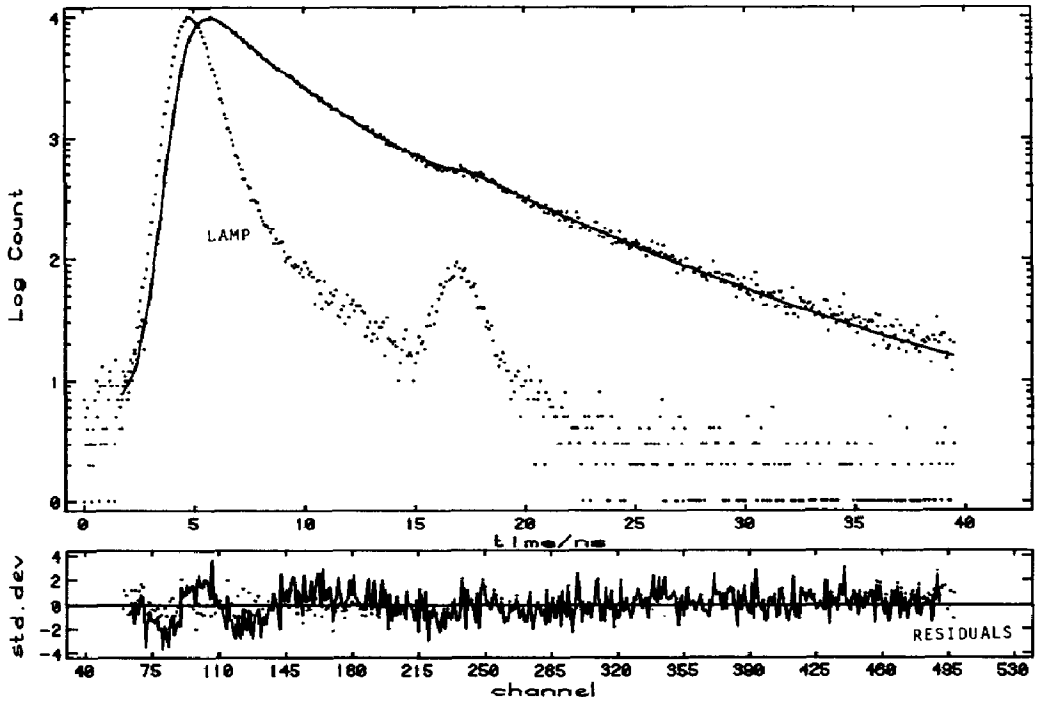

Fig. 3. Fluorescence decay of tryptophan in 2-propanol (emission at 335 nm (see Table 1); background, 5 counts (not fitted)): ···, experimental decay; ———, fitted curve. Residuals: ———, two-exponential fit; ···, three-exponential fit.

TABLE 1

Lifetimes of tryptophan fluorescence at 315, 335 and 367 nm in 2-propanol excited at 282 nm, and at 340 nm in 0.1 M sodium tetraborate buffer (pH 8.5) excited at 280 nm

λ_{em} (nm)	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	χ^2
315	0.20 ± 0.04	2.11 ± 0.04	6.08 ± 0.27	1.06
335	0.39 ± 0.10	2.06 ± 0.05	6.02 ± 0.18	1.14
335[a]	—	1.81 ± 0.10	5.85 ± 0.15	1.56
367	0.85 ± 0.28	2.60 ± 0.10	6.89 ± 0.62	1.16
340[b]	0.48 ± 0.04	3.08 ± 0.01	7.70 ± 0.07	1.09

λ_{em}, wavelength of emission.
[a] Biexponential fit.
[b] Tetraborate buffer solution ($c = 9.8 \times 10^{-4}$ M).

Solvent effects cannot be neglected in the interpretation of the change in the observed lifetimes with the emission wavelength. Indoles for example undergo a charge transfer interaction with polar solvents in the ground [13] and excited [14] states. The decrease in the subnanosecond lifetime observed at short emission wavelengths may be due to a dynamic solvent–solute interaction which produces an additional decrease in the fluorescence lifetime in the shorter wavelength region of the fluorescence spectrum [15].

A more thorough understanding of the changes in the fluorescence lifetimes requires an investigation of the effects produced by a number of solvents and this work is now in progress.

3. Experimental details

The fluorescence decays were measured and calculated using an Edinburgh Instruments 199 M fluorescence lifetime spectrometer system with a nanosecond hydrogen flashlamp as described by Birch and Imhof [16]. The spectral bandwidth of the excitation and emission monochromator was 5 nm for tryptophan in the tetraborate buffer and 10 nm in all other cases.

The fitted values were obtained by a least-squares reconvolution using a non-linear χ^2 minimization technique [17, 18]. The errors quoted are standard deviations. The triple-exponential fits were carried out as follows. First it was shown that two biexponential fits covering the short and the long time ranges of the decay curve led to four lifetimes, two of which coincided for both time ranges. In the subsequent triple-exponential fit each lifetime in turn together with the background was fixed at various values near those obtained in the biexponential fits until a minimum value of χ^2 was found.

The fluorescence spectra were measured using a Perkin–Elmer MPF-3 spectrometer (chlorobenzene and benzene–triethylamine) and a FICA-55 spectrometer (tryptophan). The shapes of the excitation spectra were identical for monomer and exciplex emission (benzene–triethylamine) and also for the three monitored emission wavelengths of tryptophan.

Benzene (Uvasol), *n*-hexane (Uvasol), acetonitrile (Uvasol) and 2-propanol (fluorescence grade) were obtained from Merck and were used as received. L-tryptophan (Serva) was recrystallized from a water–ethanol solution and was checked for the absence of oxidation products using the procedure described by Truong [19]. The absorbance of the tryptophan solutions was adjusted to below 0.02.

The fluorescence of chlorobenzene and benzene–triethylamine was measured in a triangular fluorescence cuvette as described elsewhere [4], and that of tryptophan was measured in a standard 1 cm fluorescence cell (Hellma).

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