

# MEASUREMENT OF FLUORESCENCE DECAY BY TEA uv NITROGEN LASER EXCITATION

By

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A simple and inexpensive pulse fluorometer based on transversely excited atmospheric nitrogen (TEA N<sub>2</sub>) laser of 1 ns pulse width is described, and the pulse fluorometric method is discussed. The fluorescence decay times of a series of solutions were measured using this arrangement. Correction for secondary fluorescence is emphasized, if the quantum yield and the overlap of absorption and fluorescence spectra of the compounds are considerable, or the concentration and the thickness of the solutions are large.

## *Introduction*

Fluorescence decay investigations are important for both molecular luminescence and laser theory [1, 2]. The published decay time values are very different [3—7]. Earlier measurements were performed using phase and modulation fluorometers [5—8]. The disadvantage of these methods is that the decay process cannot be directly observed. Different pulse fluorometers have recently been applied for decay studies using either a single photon counting system with flash pulser [9—11] or a mode-locked laser system [12—17]. With these techniques the decay of the fluorescent solutions are recorded on the screen of an oscilloscope and a teletype, and so the decay process can be observed immediately. There are a few results with pulse nitrogen lasers, too [18, 19]. For fluorescence excitation we applied a transversely excited atmospheric nitrogen (TEA N<sub>2</sub>) laser, developed very recently in our institute [20]. In this paper we report the experimental arrangement and our first experiences; to evaluate the sensitivity and the accuracy of the system we measured a series of fluorescence decays.

## *Experimental set up*

1. *Excitation source*: The pulse of the excitation source (TEA N<sub>2</sub> laser  $\lambda_{ex} = 337$  nm) had a typical output power of approximately 250 kW and a half-width ( $\delta$ ) of 1.0 ns. The photon density near the output was  $3 \cdot 10^{15}$  cm<sup>-3</sup> and the maximum power flux density was about 5 MW/cm<sup>2</sup> without focusing, because of the laser beam geometry,  $3 \times 1$  mm<sup>2</sup>. The beam divergence was  $6 \times 2$  mrad. The repetition

rates were 10, 25 and 50 Hz. The observed laser pulse shape ( $I_p^{obs}(t)$ ) may be fitted well by a gaussian function (Fig. 1):

$$I_p^{cal}(t) = \exp[-(t/0.6)^2]. \quad (1)$$

It has to be taken into account with laser pulse excitation of very high power flux density, that perceptible gain ensues in the observed dye solution already with  $N_1/N_0=0.01$  (where  $N_1$  — the population of the excited,  $N_0$  — the ground states), and this values can be achieved with a power flux density of  $5 \text{ kW/cm}^2$  [2]. To eliminate the amplified spontaneous emission we enlarged the laser beam to  $1 \text{ cm}^2$  and reduced thereby its intensity to one tenth.

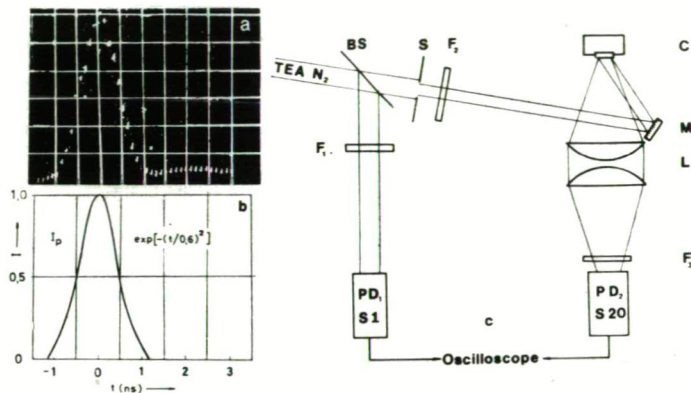


Fig. 1. Oscillogram of the TEA  $N_2$  nitrogen laser pulse —  $I_p^{obs}(t)$  (a). Time scale: 0.5 ns/div. Gaussian curve —  $I_p^{cal}(t) = \exp[-(t/0.6)^2]$  (b). Experimental arrangement for decay measurement (c): PD<sub>1</sub>, PD<sub>2</sub> — photodiodes, F<sub>1</sub>, F<sub>2</sub> — neutral reducing filters, F<sub>3</sub> — GG 18 filter, L — lenses, C — dye cell, M — mirror.

2. *Experimental arrangement*: The laser beam passed the experimental arrangement for the measurement of the fluorescence decay as shown in Fig. 1. The laser light was split into two beams by a quartz beamsplitter (BS). The reflected laser light passed through neutral reducing filters (F<sub>1</sub>) and was monitored by an ITL TF 50 M1 vacuum photodiode (PD<sub>1</sub>), which triggered an oscilloscope. The homogeneous middle part of the unreflected beam, cut by a slit (S) passed through reducing filters (F<sub>2</sub>) and was deflected by an aluminized flat mirror (M) to the dye cell (C). We applied "front"-surface geometry for excitation and observation of the solutions, which were in a cylindrical cuvette. The thickness of the sample ( $d$ ) was 0.200, 0.100 and 0.050 cm, and so the optical density of the solutions were less than 0.5 at the excitation wavelength ( $\lambda_{ex}$ ) ( $\alpha = k(\lambda_{ex}) \cdot d$ ) and less than 3.0 at the maximum of the absorption spectra ( $\gamma_{max} = k_{max}(\lambda) \cdot d$ ) (Table I). The emitted light (the total spectrum) was focused with two lenses (L) onto the ITL vacuum coaxial photodiode (HSD 1850 type, PD<sub>2</sub>). This photodiode was chosen because of its short rise time (0.1 ns). A GG 18 filter was applied for filtering out the scattered laser light (F<sub>3</sub>). We connected the photodiodes both to a real time oscilloscope (S7—10) and a sampling one (S7—8),

with an overall time resolution of 0.4 and 0.2 ns, respectively. For recording we photographed the screen of the oscilloscopes, 3000 shots with S7—10 (25 Hz, 2 min) and 50—300 shots with S7—8.

### Materials

To test our system we choosed perylene, quinine bisulphate and 9-amino-acridine, decay time of which are all well known; the measurements were relatively simply, well reproducible, and easily evaluable [5—7]. In addition we also studied the decay of a few dyes: rhodamine 6G (Rh 6G), fluorescein (Fl), rose bengal, rhodamine B (Rh B), acridine orange, acridine yellow and 7-diethylamino-4-methylcoumarin (7-Dea-4-MC). The solvents were ethanol (EtOH), methanol (MeOH), benzene, 1N H<sub>2</sub>SO<sub>4</sub>, and 0.1N NaOH in water. The concentrations were 10<sup>-4</sup> M/l or less in every case. We decreased the concentration of the solutions and the cuvette thickness to the lowest value possible.

### Evaluation method

To determine  $G(t)$ , the fluorescence response function of any molecular system is the aim of decay time measurements [21]. The observed intensity-time function of the laser pulse is  $I_P^{obs}(t)$ , the instrumental response function to the exciting light pulse. The observed decay (by the same detector)  $I_F^{obs}(t)$ , the response function of the luminescing molecular system, is modified by both the detector and the optical pumping, *i.e.* what we can record on the photographs is the *convolute* of the excitation shape with the emission decay [21], [22]:

$$I_F^{obs}(t) = \int_0^t I_P^{obs}(t')G(t-t')dt' \quad (2)$$

The evaluation method, *i.e.* the calculation of the decay time  $\tau$  from the decay data, was different according to relation of the exciting light profile —  $I_P^{obs}(t)$  to  $I_F^{obs}(t)$  [16]. If we investigate decays with first-order kinetics (*i.e.*  $I_F^{obs}(t)$  may be written by a single-exponential function:

$$G_F^{cal}(t) = I_{F0} \cdot \exp[-(t/\tau)], \quad (3)$$

where the  $\tau$  is the decay time and  $I_{F0}$  is the initial light intensity) we can classify the experimental decay data, and thus the evaluation method, on the basis of the  $\delta/\tau$  ratio and/or of the form of the  $\ln I_F^{obs}(t)$  function:

1) The decay times can be determined by a *least-square fit* for long-lived emission, when  $\delta/\tau < 0.5$ .

a) If the points of  $\ln I_F^{obs}(t)$  *vs.*  $t$ , represented in diagram give a straight line from the maximum of  $I_F^{obs}(t)$ , the decay is called *undistorted*. In this case we can calculate the  $\tau$ -value from the slope of the straight line. We get undistorted decay if  $\delta/\tau < 0.1$ . We observed such a decay function with quinine bisulphate in 1N H<sub>2</sub>SO<sub>4</sub> (Fig. 2a).

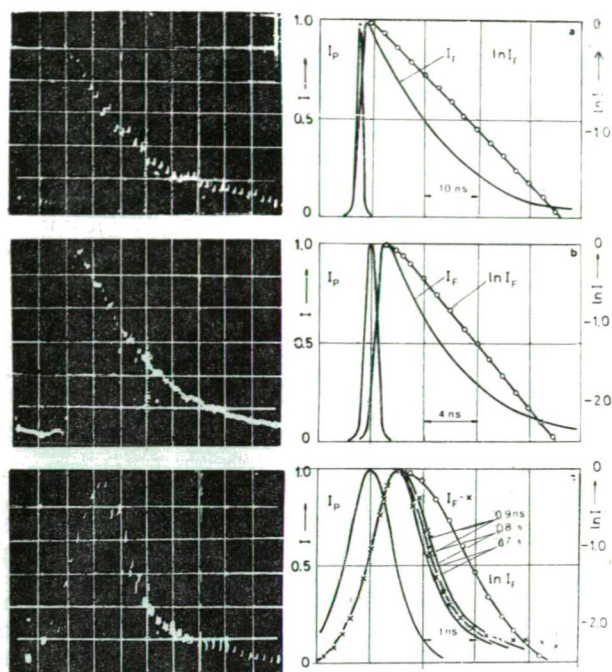


Fig. 2. Observed decay "curves" —  $I_F^{\text{obs}}(t)$  (left side) and their evaluation diagrams (right side).  $I_p$  — observed laser pulse,  $I_f$  — observed decay curve of compounds. a —  $10^{-4}$  M/l quinine bisulphate in 1N  $\text{H}_2\text{SO}_4$ , b —  $10^{-4}$  M/l perylene in benzene, c —  $10^{-4}$  M/l rose bengal in ethanol; the curves are convolutes of the calculated excitation shape ( $I_F^{\text{cal}}(t)$ ) with the single-exponential emission decay functions of  $\tau = 0.7, 0.8, 0.9$  ns values

b) If the decay is more rapid than in the former case, the  $\ln I_F^{\text{obs}}(t)$  vs.  $t$  plot gives straight line only at that part of the plot where effect of excitation can be neglected. We calculated the  $\tau$ -value only from these data points. This decay is called *partially distorted*, and is typically present when  $0.1 < \delta/\tau < 0.5$ . Fig. 2b shows the decay profile of perylene in benzene.

In these two cases we determined  $\tau$  not only geometrically from the figures, but by a least-squares fit of  $I_F^{\text{obs}}(t)$  vs.  $t$  to an exponential curve by means of a computer program, too.

2) When  $\ln I_F^{\text{obs}}(t)$  is not a straight line at all, *i.e.* for short-lived emission ( $0.5 < \delta/\tau < 2$ ), the previous procedures cannot be applied. This is the case of the *totally distorted* decay; we received such decay with rose bengal in ethanol (Fig. 2c). To process the decay data a convolute- and compare procedure was employed using the following equation:

$$I_F^{\text{cal}}(t) = \int_0^t I_p^{\text{cal}}(t') G_F^{\text{cal}}(t-t') dt' \quad (4)$$

We calculated the convolutions changing the value of  $\tau$  from 0.1 ns to 2.0 ns, in 0.01 ns steps. We compared the normalized  $I_F^{obs}(t)$  plot with the series of calculated  $I_F^{cal}(t)$  simulations and derived the  $\tau$ -value by visual superposition of the projected oscillograph upon a computer plot. This is a simple *iterative convolution method* [18, 23].

### Results and discussion

We examined first the influence the power flux density ( $F$ ) of the laser pulse on the measured decay data with  $1 \cdot 10^{-4}$  M/l Rh 6G. There was no change in decay when  $F$  was less than  $10 \text{ kW/cm}^2$ , but the decay time decreased with increasing  $F$ .

Several decay time results obtained under different experimental circumstances are summarized in Table I indicating the name of the fluorescing compound and its solvent, the concentration ( $c$ ), the cuvette thickness ( $d$ ), the optical density of the solution at excitation wavelength ( $\alpha = k(\lambda_{ex}) \cdot d$ ), the absorption maximum ( $\gamma_{max} = k_{max}(\lambda) \cdot d$ ), and the absolute quantum yield ( $\eta$ ). In column  $\tau_{ph}^{cor}$  the experimental values obtained with a phase fluorometer in our institute are given (these values were corrected for secondary fluorescence [8, 25]). The  $\tau_p^{obs}$  values are our experimental results without correction and in the last column we have collected the decay data from the literature.

For discussion we divide Table I into three parts, to the first part of which those substances belong (1—4 in Table I) which are proposed as fluorescence standard [7], and they are thus suitable to evaluate the accuracy of the system. Quinine bisulphate in 1N sulphuric acid is a stable solution under prolonged irradiation and there is a very small overlap between the absorption and fluorescence spectra. Perylene and 9-aminoacridine have a high quantum yield near 1.0, but there is a partial overlap between their absorption and emission spectra. Rose bengal has a very little  $\eta$  ( $< 0.1$ ). Table I indicates that our experimental data are in good agreement with the literature data.

Fluorescein and rhodamine 6G belong to the second group of Table I. They are the most popular dyes for both molecular luminescence and dye laser studies. There is a large overlap between their absorption and fluorescence spectra, the fluorescence quantum yields are not far from 1.0. The obtained decay data were smaller (the difference was more than 1.0 ns), when the thickness was decreased from 2.00 mm to 0.50 mm ( $10^{-4}$  M/l). There was a similarly significant decrease in observed time ( $\sim 1.5$  ns with F1, and  $\sim 2.2$  ns with Rh 6G) when the dye concentrations were lowered from  $10^{-4}$  M/l to  $5 \cdot 10^{-6}$  M/l. These data can be considered as indication of strong *self-absorption* and *secondary* fluorescence, an effect which increases the duration of the observed decay [8, 13, 17, 24, 25]. It is very likely that the large deviation in literature data obtained in different experimental circumstance without correction for secondary fluorescence can be attributed to this effect.

Budó *et al.* [24] have treated mathematically the effect of self-absorption and secondary fluorescence on the fluorescence decay time, and derived formula for phase fluorometers. A simpler form of the mentioned correction for secondary fluorescence was given in [25], and we applied this method to correct the observed data. The calculated decay times were  $\sim 4.2$  ns (Rh 6G) and  $\sim 4.0$  ns (F1). These were higher than those reported in [8, 25]. Our experimental circumstances

Table I

	Name of compounds	c (M) Solvent	d (mm)	$\alpha$	$\gamma_{\max}$	$\eta$	$\tau_{\text{ph}}^{\text{cor}}$	$\tau_{\text{p}}^{\text{obs}}$	Literature
1	Quinine bisulphate	$10^{-4}$ 1NH <sub>2</sub> SO <sub>4</sub>	2.00 0.50	0.40 0.10	2.55 0.64	0.54 [7]	20.2	20.5±0.5	20.1 [3] 19.4 [6] [7]
2	Perylene	$10^{-4}$ Benzene	2.00 0.50	0.05 0.01	1.88 0.47	0.89 [7]	3.5	5.0±0.2	4.79 [5] 5.02 [6] [7] 4.9 [3]
3	9-aminoacridine	$10^{-4}$ EtOH	2.00 0.50			0.99 [7]	14.2 14.2	15.5±0.5	13.9 [5] 15.1 [6] [7]
4	Rose bengal	$10^{-4}$ EtOH	2.00 0.50	0.08 0.02	1.00 0.25	0.3 [3]	0.8	0.8±0.1	0.93 [15]
5	Rhodamine 6G MeOH	$10^{-4}$	2.00 0.50	0.27 0.07	4.70 1.17	1.0 [14]	3.78	6.8±0.3 5.6±0.2	6.8 [13] 6.7 [16] 4.8 [14]
		$2 \cdot 10^{-5}$	2.00 0.50	0.05 0.01	0.94 0.24	1.0 [14]	3.78	5.1±0.2 4.7±0.2	4.1 [15] 6.0 [13] 3.1 5.8 [18][14]
		$5 \cdot 10^{-6}$	2.00 0.50	0.01	0.24 0.06	1.0 [14]	3.78	4.6±0.2	3.1 [14] 4.1 [15]
6	Fluorescein 0.01 N NaOH H <sub>2</sub> O	$10^{-4}$	2.00 0.50	0.24 0.06	3.88 0.97	0.93 [6]	3.1	5.8±0.2 4.7±0.2	4.5 [18] 6.8 [12] 7.0 6.8 [12]
		$2 \cdot 10^{-5}$	2.00 0.50	0.05 0.01	0.78 0.19	0.93 [6]	3.1	4.8±0.2	
		$5 \cdot 10^{-6}$	2.00	0.01	0.19	0.93 [6]	3.1	4.4±0.1	4.02 [5] 4.62 [5]
7	Rhodamine B	$10^{-4}$ EtOH	2.00 0.50	0.20 0.05	2.20 0.55	0.97 [5]	2.54	3.0±0.2	2.9 [15] 3.2 [3] 3.2 2.3 [10] [18]
8	7-Dea-4-MeC	$10^{-4}$ EtOH	2.00	0.40	1.60		2.14	3.6±0.15	
9	Acridine yellow	$10^{-4}$ EtOH	2.00	0.02	0.80		4.20	6.0±0.2	5.1 [3]
10	Acridine orange	$10^{-4}$ EtOH	2.00	0.03	0.64			3.0±0.2	4.7 [18]

for excitation and observation are a little different from that of the phase modulation technique, and for this reason very likely a modified correction will be needed for us. The application of these corrections in our evaluation procedure requires further studies. We hope to report these results in a forthcoming paper.

The third part of Table I contains decay times of further dye solutions.

In conclusion, we believe that the simplicity of fluorescence decay time measurements using TEA N<sub>2</sub> pulses recommends itself as a routine method.

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#### ИЗМЕРЕНИЕ ВРЕМЕНИ ЗАТУХАНИЯ ФЛУОРЕСЦЕНЦИИ, ВОЗБУЖДЕННОЙ «uv TEA N<sub>2</sub>» ЛАЗЕРОМ

К. Сюч, Б. Рау, Б. Немет, Л. Козма, И. Шанта и М. Хилберт

Описан простой и недорогой флуорометр, применяющий азотный лазер возбужденный поперечным разрядом в УФ области при давлении 760 торр (uv TEA N<sub>2</sub>), время импульсов этого лазера 1 нсек, и разъяснена пульсо-флуорометрическая методика. Этим аппаратом измерено время затухания флуоресценции серии растворов. Необходимо ввести коррекцию на вторичную флуоресценцию, когда квантовый выход большой и спектр поглощения значительно покрыт спектром флуоресценции соединений, или когда концентрация и толщина растворов тоже большие.