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A set-up for precise steady-state fluorescence measurements *

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An experimental set-up for precise fluorescence intensity and anisotropy measurements is presented. Some results for singlet state rhodamine 6G quenching by cyclooctatetraene are reported.

1. Introduction

In spite of remarkable development in time-resolved spectroscopy, steady-state methods are still the basic ones, and great effort is made to increase the accuracy and reliability of steady-state measurements. From such measurements of fluorescence intensity, some information about radiationless transitions can be obtained. This is because the fluorescence quantum yield Φ depends also on rates of nonradiative processes from excited state

$$\Phi = k_F (k_F + k_{IC} + k_{ISC})^{-1}$$

where: k_F – rate of fluorescence, k_{IC} and k_{ISC} – internal conversion and intersystem crossing rates, respectively.

In applications, especially in flash lamp pumped dye lasers, an important role is played by singlet-triplet transition. This transition leads to the triplet state population, whereas triplet-triplet absorption causes the decreasing of dye laser efficiency. This is the reason for which the triplet state quenchers are added into active medium [1]. Internal conversion and intersystem crossing rates can vary under the influence of different external factors, so their changes upon the addition of some triplet quenchers can be expected and fluorescence intensity changes should be seen.

The interaction between fluorescent molecules and the quencher may be bimolecular, in some circumstances, however, aggregation is also possible. In the latter case, rotational reorientation time τ_{rot}, which is proportional to the volume of luminescence centre with solvation shell, increases and can be determined by fluorescence anisotropy measurements, based on Perrin's formula

$$r^{-1} = r_0^{-1}(1 + \tau/\tau_{rot})$$

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where: τ – mean lifetime, r_0 – fundamental anisotropy. Hence, the measurements of the fluorescence quantum yield and the fluorescence anisotropy can give information about quenching coefficients and, moreover, they can state whether aggregation in solution takes place or not. The quantum yield measurement can be replaced by measurement of fluorescence intensity for a given wavelength, assuming that the quencher does not affect the shape of the fluorescence spectrum. In this case quenching experiment is reduced to the fluorescence intensity measurements for the successively increasing quencher concentration. As thermodynamical equilibrium must be reached each time the quencher is added, experiment lasts for a long time, sometimes for several hours.

The purpose of this paper is to present an experimental set-up which can be used for precise, longlasting measurements of fluorescence spectra and emission anisotropy.

2. Fluorescence experiments

Figure 1 is a schematic diagram of two-channel apparatus in a version suitable for fluorescence quenching measurements. Fluorescence intensities I and I_{ref} are observed alternately. In this set-up high stability can be reached, because of elimination of the influence of excitation light instability, common detecting system for both channels and compensation of dye molecules adsorption on the cuvettes windows

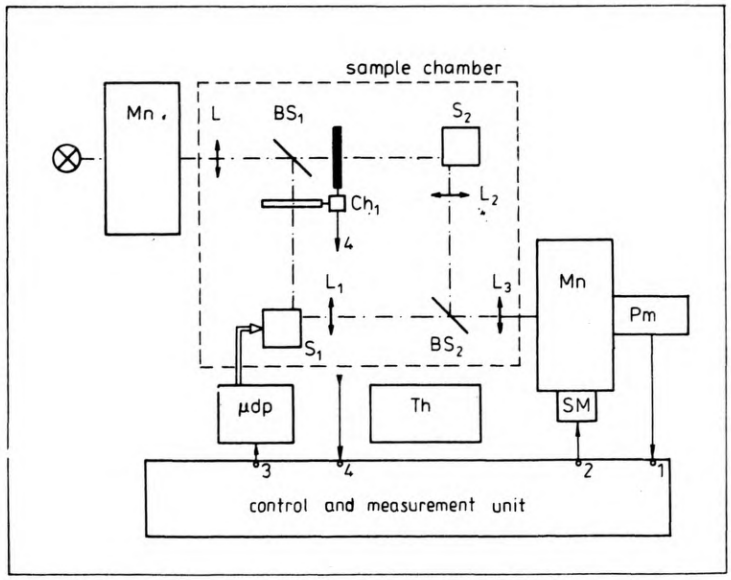

Fig. 1. Device for fluorescence-quenching measurements. LS – light source, Mn – monochromators, BS – beam splitters, Ch_1 – chopper, S_1 – measuring cuvette, S_2 – reference cuvette, L – lenses, Pm – photomultiplier, μdp – microdose pump, Th – thermostat, SM – step motor, 1–4 – control and measurement signals

[2]. The same temperature is maintained in both cuvettes. The apparatus consists of excitation light source (XBO 200), monochromators (SPM 2, Zeis, Jena), and sample chamber. Optical alignment can be easily modified. Chopper Ch_1 opens the excitation light in such a way that solutions in cuvettes S_1 and S_2 are excited alternately. The fluorescence light, after passing through the lenses L_1, L_2, L_3, beam splitter BS_2 and the monochromator, is detected by cooled photomultiplier (EMI 9863QB/350). Photon counting method is used for detection.

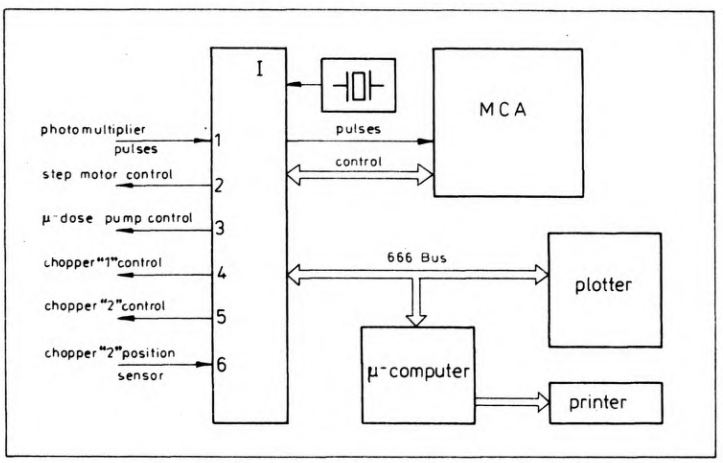

Fig. 2. Control and measurement unit

Control-measurement unit (C&MA, Fig. 2) contains a block I, a microcomputer (666B, EMG, Hungary), a multichannel analyser (NTA 1024, EMG, Hungary) and peripherals: plotter, printer.

Interface and control block I consists of the following parts: a) photon counting time circuit controlled by quartz generator, b) circuit for chopper control setting programmed number of pairs I and I_{ref}, c) microcomputer-multichannel analyser interface (adapted NN 7001, EMG, Hungary), d) circuit for μ-dose pump and step motor control. The multichannel analyser (MCA) collects the numbers of counts for both channels, separately. Further processing, like calculation of the I/I_{ref} ratio for every pair, averaging and error estimation, is performed by the computer.

To check the accuracy and stability of the apparatus in the course of the quenching experiment the following test we performed. To the measuring cuvette with dye solution (Rh 6G in EtOH) the same solution (without quencher) was added gradually. The intensities I and I_{ref} were measured 50 times for 4 s each, with light intensity of about 10^5 photon counts/s. In such conditions the estimated experimental error of the ratio I/I_{ref} was 0.08%. Instrument stability during 4 hour experiment was better than 0.1% per hour

$$1 > \frac{I/I_{ref}(t = 4\,\mathrm{h})}{I/I_{ref}(t = 0)} > 0.997.$$

The observed small drift seems to be connected with the change of liquid level in the cuvette resulting from the solution addition.

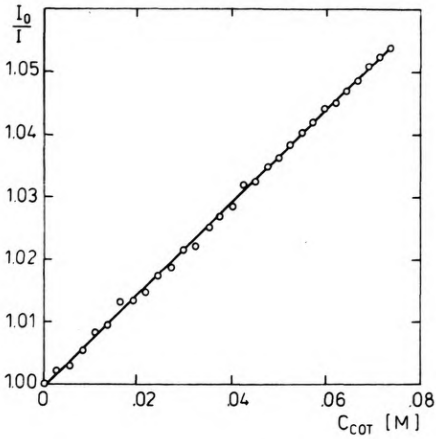

Fig. 3. Stern–Volmer plot for solution of Rh 6G in ethanol with different coencentration of COT

Representative result of the quenching experiment is presented in Fig. 3. Cyclooctatetraene (COT, C_8H_8) was added to Rh 6G ethanol solution ($c = 10^{-5}$ M). Apparently, the singlet state of the dye is quenched. Decrease of the fluorescence intensity vs quencher concentration may be described by the following formula:

$$I_{\mathrm{COT}=0}/I_{\mathrm{COT}} = 1+\tau_0 Kc$$

where: τ_0 mean lifetime when $c_{\mathrm{COT}} = 0$, c – COT concentration, K – 2nd order quenching parameter [3]. The least squares fit gives $\tau_0 K = 0.74 \pm 0.2\ \mathrm{M}^{-1}$ and $K = 1.76\ 10^8\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$ at $\tau_0 = 4.2$ ns. It should be noticed that the plot in Fig. 3 is, in a very good approximation, a straight line even for high COT concentration.

3. Polarization experiment

Figure 4 presents schematically an instrument for anisotropy measurements. The lack of movable optical elements, the automation of measurements and application of photon-counting detection technique allow us to measure the emission anisotropy with accuracy better than 1%. Linear polarized light is used for excitation. The fluorescence beam impinges upon Wollaston prism which splits in two components $I_\perp$ and $I_\parallel$. Due to the chopper Ch_2 these components are directed to the photomultiplier separately and alternately. The experiment was under control of C&MU, described above. Sensor ChS examines the chopper position in data aquisition process. The thermostat and microdose pump (as in Fig. 1) can be used. The microcomputer calculates emission anisotropy

$$r = (1 - I_\perp/I_\parallel)/(1 + 2I_\perp/I_\parallel),$$

averaging over a given number of measurements. The accuracy of r depends

obviously on their value, and for $r \simeq 0.02$ (case of Rh 6G in EtOH) $\Delta r/r = 0.02$. Knowing the fluorescence lifetime for different COT concentrations, effective reorientation times τ_{rot} can be determined from Perrin's formula.

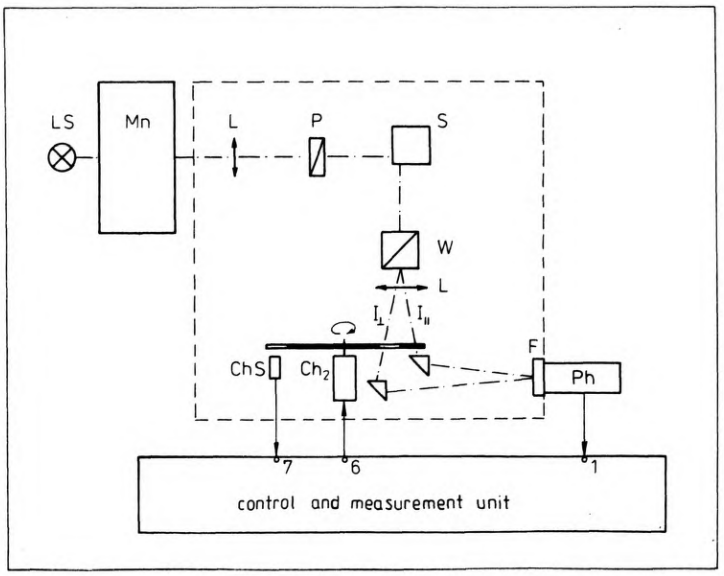

Fig. 4. Device for polarization measurements. LS – light source, L – lenses, P – polarizers, S – sample, W – Wollaston prism, Ch_2 – chopper, ChS – chopper sensor, F – interference filter, Ph – photomultiplier, 1, 6, 7 – control and measurements signals

As examples, results for fluorescence anisotropy and τ_{rot} vs COT concentration are presented in Fig. 5**a** and 5**b**, respectively. Rh 6G in ethanol solution was investigated ($c = 10^{-5}$ M). The estimated experimental error for τ_{rot} determination

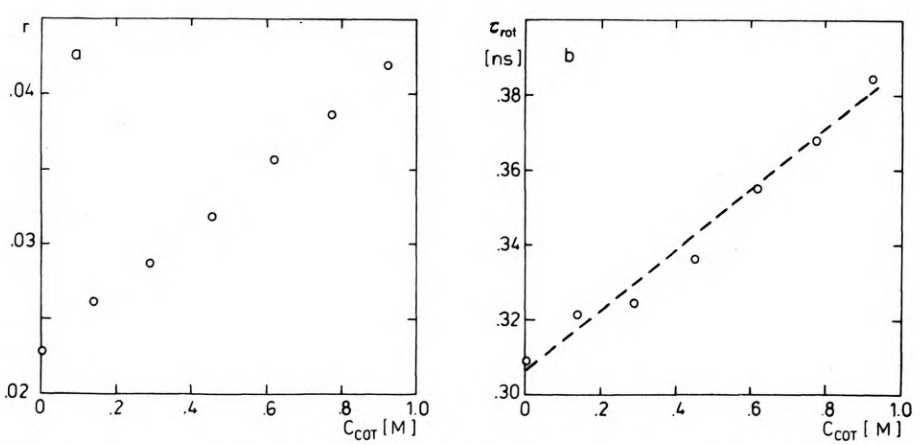

Fig. 5. Polarization measurements of RH 6G solution in EtOH vs COT concentration: **a** – fluorescence anisotropy, **b** – effective reorientation time τ_{rot}

was $\Delta\tau_{rot}/\tau_{rot} = 0.03$. Increase of τ_{rot} indicates the increase of the volume of luminescence centre with a solvation shell. The viscosity of the solution with different COT contents was the same within the experimental error. This and similar results for tetramethylrhodamine [4], indicate the that quenching of the singlet state of dyes by COT may to some extend be caused by aggregation in the solution. Results obtained for other rhodamines will be published elsewhere [5].

4. Summary

All the performed tests and measurements indicate that the presented apparatus is very accurate and stable (better than 0.1% per hour), thus that it is useful for measurements of small changes in the fluorescence intesity.

From the fluorescence anisotropy measurements, $r = 0.0229 \pm 0.0004$ and $\tau_{rot} = 0.31 \pm 0.01$ ns for Rh 6G in EtOH ($c = 10^{-5}$ M) were obtained. These results are consistent with the one obtained in a different way [6].

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Устройство для прецизионных измерений флуоресценции

В работе представлено устройство для точных измерений флуоресценции и анизотропии испускания. Представлены результаты измерения тушения люминесценции родамина 6Ж в этаноле циклооктатетраэном (ЦОТ). Измерения анизотропии излучения родамина 6Ж в этаноле с добавкой ЦОТ показали, что в растворе создаются агрегаты.