

PROBLEMS ARISING DURING THE DETERMINATION OF THE LIFETIME OF ELECTRONICALLY EXCITED MOLECULES BY FLUORESCENCE INHIBITION TECHNIQUES UNDER CONTINUOUS EXCITATION

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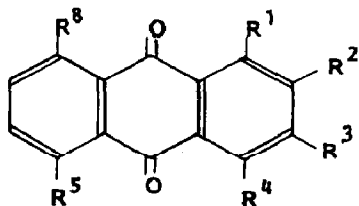
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Summary

Under the conditions at which the measurement of the lifetime τ of emissive electronic excited states becomes critical, a good estimation of τ can be determined from the inhibition of fluorescence under continuous excitation, the kinetics of which are diffusion controlled. However, this procedure requires that the inner quenching mechanism, the mutual diffusion coefficient D and the collision distance σ be known. These constants are sometimes difficult to evaluate, and several assumptions are then required. This method was used to determine the excited singlet lifetimes of a series of substituted anthraquinones which were compared with the values obtained by laser flash photolysis and single-photon counting measurements.

1. Introduction

When a compound fluoresces, it is possible to evaluate the lifetime of the electronically excited state which is the precursor of the fluorescence by using pulsed excitations such as single-photon counting [1] or laser excitation [2]. When the substances do not fit the excitation range of the coherent source or when their fluorescence quantum yield is weak, the fluorescence quenching technique can be used to measure the lifetime of the emitting species. This is the case for the series of substituted anthraquinones



- | | |
|-------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| 1, $R^1 \equiv \text{OH}$ | 9, $R^1 \equiv \text{NH}_2$ |
| 2, $R^1 \equiv R^2 \equiv \text{OH}$ | 10, $R^2 \equiv \text{NH}_2$ |
| 3, $R^1 \equiv R^2 \equiv \text{OH}, R^3 \equiv \text{CH}_2\text{N}(\text{COOH})_2$ | 11, $R^1 \equiv \text{NH}_2, R^4 \equiv \text{OH}$ |
| 4, $R^1 \equiv R^4 \equiv \text{OH}$ | 12, $R^1 \equiv \text{NH}_2, R^2 \equiv \text{OCH}_3, R^4 \equiv \text{OH}$ |
| 5, $R^1 \equiv R^5 \equiv \text{OH}$ | 13, $R^1 \equiv R^8 \equiv \text{NH}_2$ |
| 6, $R^1 \equiv R^8 \equiv \text{OH}$ | 14, $R^1 \equiv R^4 \equiv \text{NH}_2$ |
| 7, $R^1 \equiv R^2 \equiv R^5 \equiv R^8 \equiv \text{OH}$ | 15, $R^1 \equiv \text{NH}_2, R^2 \equiv R^4 \equiv \text{Br}$ |
| 8, $R^1 \equiv R^2 \equiv \text{OC}_2\text{H}_5$ | 16, $R^1 \equiv R^5 \equiv \text{N}(\text{C}_2\text{H}_5)(\text{C}_6\text{H}_5)$ |

in which the unspecified Rs are hydrogens.

An understanding of the photochemical behaviour of quinones is important because these compounds are believed to act as electron acceptors in biological electron transport chains [3]. Consequently, it is important to understand better the way in which electron donors interact with quinones. The formation of a charge transfer complex between electron donors and quinones in the triplet excited state has been well documented [4, 5]. Interactions involving an excited singlet state have been reported in a few papers [5, 6], and, to our knowledge, all of them are concerned with the quenching of the excited singlet state of the donor by a quinone. When the quenching involves the formation of an intermediate exciplex, the rate constant of the quenching process is the same whatever counterpart is excited. It is therefore of interest to know the lifetime of the singlet excited state of the quinones that interact with electron-donating molecules to form an intermediate exciplex. This has been done for a series of amino-anthraquinones (9 - 16) interacting with electron donors [7], for which a knowledge of the lifetime of the singlet excited state was necessary to check the validity of Weller's theory [8]. These compounds emit weakly in a wavelength region where photomultipliers become slightly insensitive (see Table 1).

In a more general study of the reactivity of the compounds of the present series of anthraquinones towards various electron donors [7], we investigated the possibility of measuring the lifetime of the excited singlet state by using fluorescence quenching experiments, the kinetics of which are diffusion controlled. The results of this study are reported in this paper. The details of sample purification and lifetime measurement are given in the Appendixes.

2. Diffusion-controlled reactions

Diffusion-controlled reactions have received considerable attention in recent decades, particularly those based on a continuum model introduced for the first time by Sroluchowski [9 - 14].

TABLE 1

Fluorescence data of anthraquinones 1 - 16 in acetonitrile solution

| Compound | Excitation wavelength ^a (nm) | Fluorescence maximum (nm) | Fluorescence quantum yield $\Phi_f \times 10^2$ |
|----------|--------------------------------------------|------------------------------|----------------------------------------------------|
| 1 | 420 | 608 | 1.3 |
| 2 | 435 | 660 | 0.4 |
| 3 | 440 | 660 | 0.3 |
| 4 | 487 | 575 | 12.6 |
| 5 | 435 | 585 | 1.9 |
| 6 | 435 | 585 | 1.7 |
| 7 | 490 | 590 | 2.5 |
| 8 | 490 | 646 | 0.9 |
| 9 | 468 | 600 | 3.4 |
| 10 | 430 | 620 | 1.9 |
| 11 | 530 | 630 | 4.1 |
| 12 | 475 | 616 | 11.8 |
| 13 | 495 | 596 | 3.2 |
| 14 | 585 | 660 | 3.0 |
| 15 | 465 | 594 | 0.3 |
| 16 | 540 | 630 | 1.3 |

^a At the maximum of the longest wavelength absorption band.

If σ is the true collisional distance between two reactive molecules A^* and B (A^* is an electronically excited species) and D is the sum of the diffusion coefficients of A^* and B, then the classical theory leads to the following well-known analytical expression for the apparent rate constant of the reaction:

$$k_a(t) = 4\pi N\sigma D \left\{ 1 + \frac{\sigma}{(\pi Dt)^{1/2}} \right\} \quad (1)$$

where N is the Avogadro number and t is the time. When the interaction between A^* and B is almost totally diffusion controlled, the experimental determination of the reaction distance σ' leads to values greater than the collisional distance σ which can be calculated using Edward's theories [15, 16]. Thus, if the reaction occurs at a distance greater than σ , several possibilities can be considered for the trapping of molecules B at the critical distance σ' : (i) overlap between the orbitals of A^* and B; (ii) attractive potential; (iii) solvent cage effect.

A kinetic model which takes this effect into account has recently been developed and leads to the following relations [14] for the apparent reaction rate constant $k_a(t)$ and the ratio Φ^0/Φ of the fluorescence quantum yields of A^* in the absence and the presence of B, all other variables being unchanged:

$$k_a(t) = k_0 \left\{ 1 + \frac{\sigma'}{(\pi Dt)^{1/2}} \right\} \quad (2)$$

where $k_0 = 4\pi N\sigma'D$ and

$$\frac{\Phi^0}{\Phi} = \frac{\exp(NV_1[B])(1 + k_0\tau[B])}{I(\lambda)} \quad (3)$$

The relation

$$k_{sv} = k_0 \left\{ 1 + \frac{\sigma}{(\pi D\tau)^{1/2}} \right\} \tau + NV_1 \quad (4)$$

is also obtained. τ is the lifetime of the excited species A^* ,

$$\lambda = \frac{2}{\pi^{1/2}} \frac{k_0\tau[B]}{(1 + k_0\tau[B])^{1/2}} \frac{\sigma'}{(D\tau)^{1/2}} \quad (5)$$

$$NV_1 = \frac{4}{3} \pi N(\sigma'^3 - \sigma^3) \quad (6)$$

$$I(\lambda) = 1 - \frac{\lambda}{2} \pi^{1/2} \exp\left\{ \left(\frac{\lambda}{2} \right)^2 \right\} \operatorname{erfc}\left(\frac{\lambda}{2} \right) \quad (7)$$

and k_{sv} is the rate constant of the Stern–Volmer plot. Thus if the reaction mechanism, σ , σ' and D are known, we can in principle estimate τ by a fluorescence quenching method. The determination of σ and σ' is sometimes difficult and is often based on semiempirical methods [15, 16] and simulation calculations to obtain the curves of best fit (see Section 4). The error can reach a factor of almost 2.

3. Diffusion limitation of the reaction rates

The fluorescence of the compounds under investigation can be quenched by a number of electron-donating molecules [7], and the most efficient quencher, *e.g.* *N,N*-dimethyl-*p*-toluidine, was chosen.

Figure 1 shows the decimal logarithm of the fluorescence quenching rate constant (measured using a standard Stern–Volmer plot) for anthraquinones 2, 9 and 11 *versus* the oxidation potential of a series of electron donors. The curves obtained for the other compounds lie between the two extreme limits. The location of the oxidation potential (0.65 V) of *N,N*-dimethyl-*p*-toluidine is included in Fig. 1.

The reaction kinetics are only partially limited by diffusion, and experiments in media of different viscosities have to be carried out to validate the postulated reaction mechanism [17]. Nevertheless, for the reactions considered here, which are sensitive not only to the viscosity η of the medium but also to its dielectric constant ϵ , the study of the effect of η is of significance only if ϵ is held constant during experiments at various viscosities. These mixtures were not adjusted and only diffusion-controlled kinetics can be assumed. As will be seen later, this assumption appears to be valid although it fails in one case.

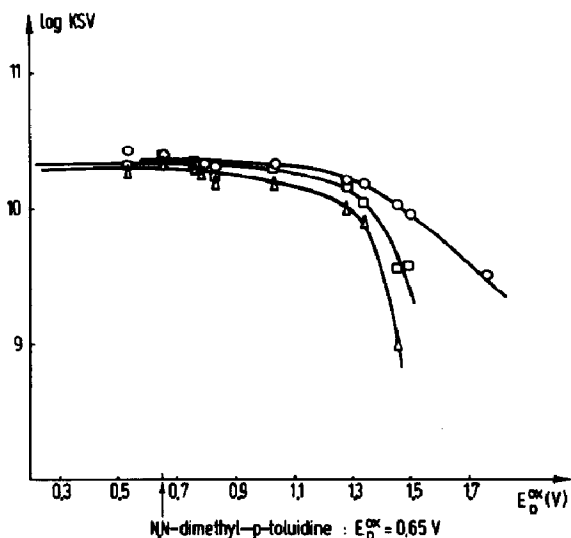


Fig. 1. Effect of the oxidation potentials of amines and methoxybenzenes on the fluorescence quenching of anthraquinones: \circ , anthraquinone 2; \square , anthraquinone 9; \triangle , anthraquinone 11.

4. Determination of σ and D

We require to determine optimal values of the parameters σ , σ' , D and τ in relations (2) and (3) from the results of appropriate fluorescence quenching experiments under steady state excitation. However, not all these parameters can be obtained from the Stern–Volmer curves, and the collisional distance σ was evaluated by semiempirical methods [15, 16]. A mean value of about 7 Å was obtained for the products investigated in this work.

If for simplicity we assume that $\sigma' = \sigma$, then the application of relation (3) to the variation in Φ^0/Φ with the quencher concentration $[Q]$ may result in varying values for the product τD which depend on the value assigned to σ . This approach was applied to the quenching of the fluorescence of anthraquinone 7 by *N,N*-dimethyl-*p*-toluidine and the values of best fit to τD were obtained for σ values of 6, 7 and 8 Å:

$$\begin{array}{ll} \sigma = 6 \text{ \AA} & \tau D = 4 \times 10^{-14} \text{ cm}^2 \\ \sigma = 7 \text{ \AA} & \tau D = 3 \times 10^{-14} \text{ cm}^2 \\ \sigma = 8 \text{ \AA} & \tau D = 2.2 \times 10^{-14} \text{ cm}^2 \end{array}$$

This behaviour results in an error which may be important in the determination of the lifetime τ . The difficulties encountered in adjusting the experimental results to the curves of best fit are shown in Fig. 2 for anthraquinone 7; in this case the assumption $\sigma = \sigma'$ cannot be made.

In the case of compounds of similar molecular weight, values of the order of $10^{-5} \text{ cm}^2 \text{ s}^{-1}$ have been obtained [17] for D by taking $\eta = 1 \text{ cP}$.

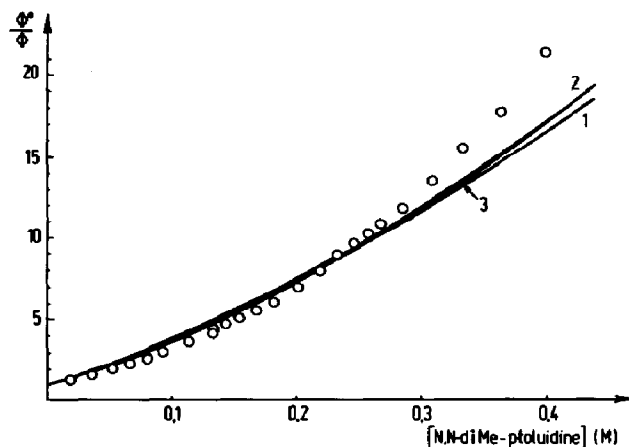


Fig. 2. Effect of σ on the determination of τ ($\sigma' = \sigma$) for anthraquinone 7: curve 1, $\sigma = 6 \text{ \AA}$; curve 2, $\sigma = 7 \text{ \AA}$; curve 3, $\sigma = 8 \text{ \AA}$.

Since the viscosity of the solvent used in the present study is less than 1 cP ($\eta = 0.44 \text{ cP}$ at $20 \text{ }^\circ\text{C}$ for acetonitrile), a value of the order of $1.5 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ is obtained for D by using the empirical relation [17, 18]

$$D = k/\eta^{0.7} \quad (8)$$

This is the second source of error in the treatment reported below in which the product $D\tau$ is calculated. Nevertheless, despite these drawbacks the values of τ obtained from fluorescence quenching under steady state excitation are satisfactory.

5. Effect of the static quenching process on the determination and the accuracy of τ

In the model used here [15] the variation in the fluorescence quantum yield Φ was determined as a function of the concentration of species B by taking into account that at $t = 0$ (excitation of A^*) there is a probability P of finding a B molecule inside the chemical reaction volume V_1 . Under these conditions we observe (i) almost instantaneous deactivation of A^* by at least one B molecule inside the volume V_1 and (ii) deactivation of A^* by a B molecule which has diffused through to a distance σ' in the medium.

Contribution (i) is a static quenching phenomenon; an increase in this effect leads to a rather good agreement of the experimental results with the curve of best fit (Fig. 3). The static quenching contribution explains the curvature in the plot of Φ^0/Φ versus $[Q]$.

The lifetime τ is plotted against the reaction distance σ' in Fig. 4. When the accuracy of determination of the concentrations and of the Φ^0/Φ values is taken into account this figure displays an acceptable region for the application of the least-squares method leading to a medium quality ($\pm 15\%$) estimation of the lifetime τ . Thus a third source of error is identified.

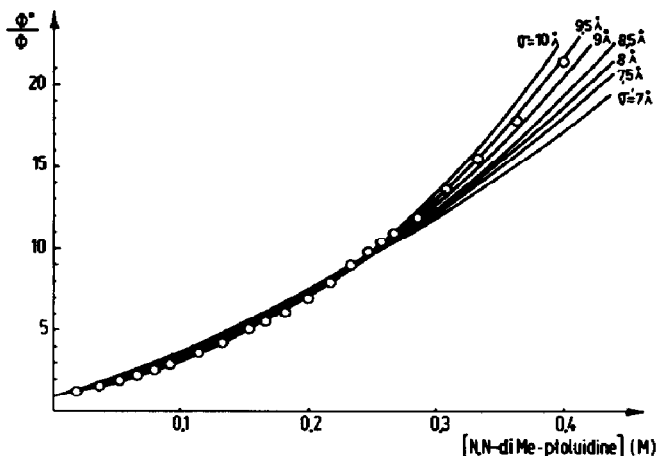


Fig. 3. Effect of the static quenching contribution on the parametric adjustment for anthraquinone 7 ($\sigma = 7 \text{ \AA}$).

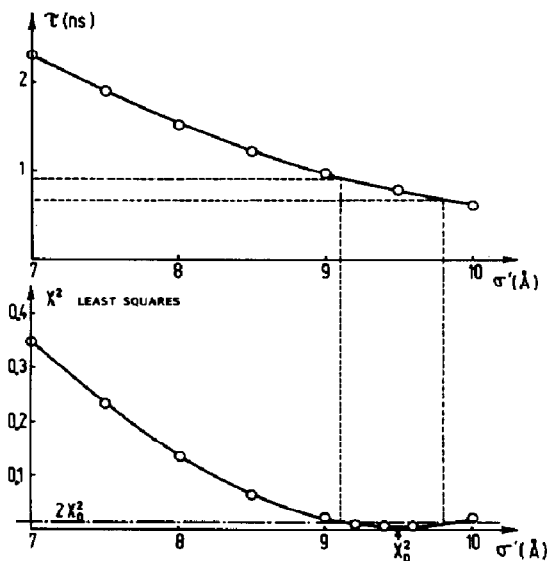


Fig. 4. Effect of σ' on the determination of τ for anthraquinone 7.

6. Results: comparison with direct measurements

The good agreement between the experimental results and the calculated curves for Φ^0/Φ versus $[Q]$ is shown in Figs. 5 and 6 for anthraquinones 13 and 3 respectively.

The best values of the lifetime τ and the reaction distance σ' calculated by the least-squares method are collected in Table 2. The lifetimes determined by fluorescence decay after excitation with a neodymium laser and by single-photon counting experiments are also included in this table. The

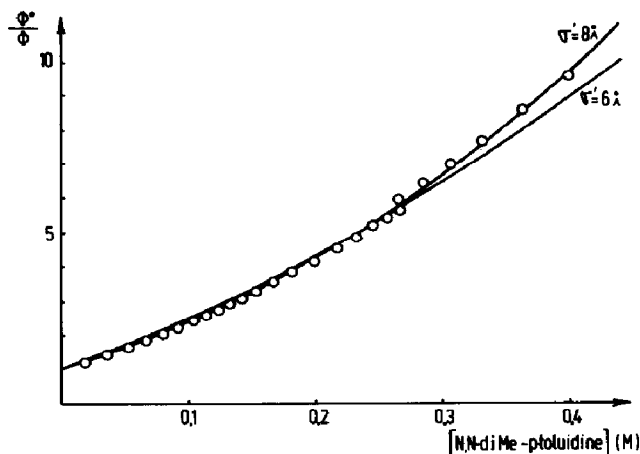


Fig. 5. Determination of τ for anthraquinone 13: $\tau = 0.45 \text{ ns}$; $\sigma' = 8 \text{ \AA}$.

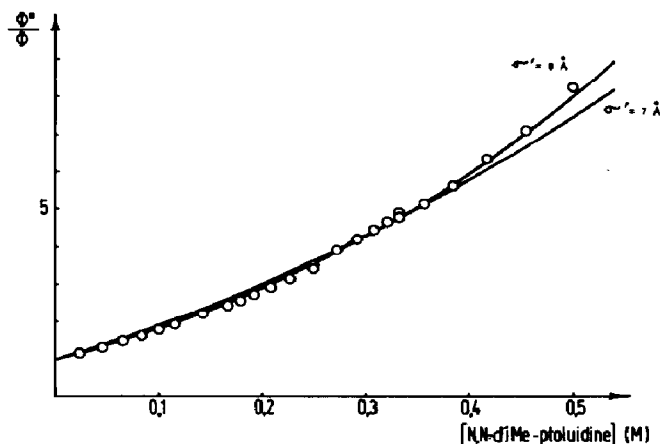


Fig. 6. Determination of τ for anthraquinone 3: $\tau = 0.25 \text{ ns}$; $\sigma' = 8 \text{ \AA}$.

single-photon counting data were obtained by using a low intensity hydrogen lamp at the appropriate wavelength to excite the anthraquinones. A monochromator was used to separate the excitation radiation from the emission analysis radiation. However, the photomultiplier was rather insensitive at long wavelengths, the fluorescence quantum yields were weak, long counting times were required during which fluctuations of the flash source could occur and the fluorescence lifetimes were short compared with the time profile of the flash. Nevertheless, in all the cases studied there was reasonable agreement between the different measurements based on pulsed excitation.

The values of τ determined using the fluorescence quenching technique are mostly in agreement with those obtained using the pulsed excitation technique. Of all the compounds studied, only anthraquinones 9 and 12 displayed a net disagreement between the two types of experiments and had lifetimes τ for fluorescence quenching under steady state excitation about 30% higher than those obtained from the pulse technique. This deviation can

TABLE 2

Comparison of the lifetimes of anthraquinones 1 - 16 determined by three different techniques

| Compound | τ (ns) | | σ' (\AA) | τ (ns) | |
|----------|---------------------------|---------------------------|-------------------------------|---------------------------|---------------------------------------|
| | Single-photon counting | Laser flash photolysis | | ($\sigma \neq \sigma'$) | ($\sigma = \sigma' = 7 \text{\AA}$) |
| 1 | 0.6 | — | 9.4 | 0.5 | — |
| 2 | — | — | 8.6 | 0.25 | 0.6 |
| 3 | — | — | 8.0 | 0.3 | 0.6 |
| 4 | 2.8 | 2.8 | 9.4 | 3.1 | 6.3 |
| 5 | 0.5 | — | 9.9 | 0.5 | 1.7 |
| 6 | 0.8 | — | 9.4 | 0.55 | 1.5 |
| 7 | — | 0.7 | 9.6 | 0.7 | 1.6 |
| 8 | — | — | 9.0 | 0.3 | 0.9 |
| 9 | 0.7 | 0.9 | 7.6 | 1.4 | 1.7 |
| 10 | 0.7 | — | 8.2 | 0.7 | 1.1 |
| 11 | 1.0 | 1.0 | 7.6 | 1.3 | 1.7 |
| 12 | 1.9 | 2.3 | 7.0 | 3.1 | 3.1 |
| 13 | 0.8 | 0.7 | 8.0 | 0.65 | 1.0 |
| 14 | — | 0.6 | 7.0 | 0.4 | 0.4 |
| 15 | — | 0.3 | 8.5 | 0.2 | 0.4 |
| 16 | — | — | 7.5 | 0.35 | 0.4 |

be interpreted in terms of a chemical reaction rate constant between A^* and B which is not infinite with respect to k_0 , so that there is competition between the chemical process and diffusion.

In conclusion, the singlet lifetime τ of excited molecules can be measured by using a reaction such as electron exchange with an electron-donating molecule. These measurements are made using fluorescence quenching under continuous excitation which, in contrast with other techniques, is a simple method to operate and yields satisfactory results. However, the parameters σ and D must be known if acceptable results are to be obtained, and this is sometimes difficult to achieve. Semiempirical methods are required to evaluate σ and thus a systematic error is introduced. Despite this difficulty, the method described here may also be valid for compounds which form a charge transfer complex with electron-accepting molecules.

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thraquinones 1, 2, 4 - 7 and 9 - 15, and Dr. R. Loutfy, Xerox Company, Canada, for supplying a sample of anthraquinone 16.

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Appendix A

Materials

Anthraquinones 1, 2, 4 - 7 and 9 - 15 were obtained from Produits Chimique Ugine-Kuhlman and anthraquinone 16 was obtained from Dr. R. Loutfy, Xerox Company, Canada. Anthraquinone 3 was purchased from Eastman Kodak.

Anthraquinone 8 was prepared by adding 4 g of ethyl bromide to a solution of 1 g of anthraquinone 2 in 50 ml of ethanol containing 2 g NaOH and refluxing for 24 h. After cooling, 4 g of ethyl bromide and 2 g of NaOH were added to the solution which was refluxed for a further 24 h. The solvent was evaporated and the residue was extracted four times by 30 ml of benzene. The organic solution was washed twice in 10 ml of water and dried over Na_2SO_4 . The product was purified by thin layer chromatography using a silica gel column and eluting with benzene to give anthraquinone 8 (melting point, 160 °C).

Anthraquinones 1, 2, 3, 7, 15 and 16 were used without further purification after being checked by high pressure liquid chromatography using a Varian model 5020 apparatus. The other anthraquinones were purified by thin layer chromatography using various solvents for the elution: pure benzene for anthraquinones 5 and 6; benzene:ethyl acetate (90:10) for anthraquinone 11; benzene:ethyl acetate (85:15) for anthraquinones 4 and 9; benzene:ethyl acetate (80:20) for anthraquinone 14; benzene:diethyl ether (80:20) for anthraquinone 13; benzene:diethyl ether (50:50) for anthraquinones 10 and 12.

N,N-dimethyl-*p*-toluidine (Merck) was purified by distillation, and acetonitrile (Merck Uvasol) was used without further purification.

Appendix B

Experiments under steady state excitation

The optical qualities of the spectrofluorometers used (Perkin-Elmer MPF-44B and Jobin-Yvon JY3) enabled the fluorescence of the solution to be excited with a narrow parallel light beam. Fluorescence polarization was achieved by inserting a Glan prism before the outlet slit of the excitation monochromator or by inserting a Polaroid polarizer between the cell and the input slit of the analyser monochromator.

The fluorescence intensity I_F , which is proportional to the fluorescence quantum yield Φ , was determined from measurements of $I_{F\parallel}$, for which the analysing polarizer was set parallel to the excitation polarizer, and $I_{F\perp}$, for which the two polarizers were set perpendicular to each other. A correction coefficient α characteristic of the instrument [B1] was used to calculate the correct fluorescence intensity:

$$I_F = I_{F\parallel} + 2\alpha I_{F\perp}$$

The measurements were carried out at 20 °C in acetonitrile solution. The optical density of the anthraquinone solutions was adjusted to 0.2 at the excitation wavelength which was chosen to be the maximum of the absorption band at the longest wavelength.

The fluorescence quantum yields were determined using a Perkin-Elmer MPF-44B spectrofluorometer to which a DCSU-2 differential corrected spectra unit had been attached. The optical density of the solutions of the anthraquinones in acetonitrile was adjusted to a value of 0.2 at the excitation

wavelength (Table 1). Fluorescein was used as the standard under the following experimental conditions: buffer solution at pH 10; optical density 0.2 at an excitation wavelength of 436 nm (a value of 0.85 has been reported [B2] for the fluorescence quantum yield at this wavelength).

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Appendix C

Experiments with pulsed excitation

Single-photon counting measurements

The fluorescence decay measurements of each fluorescent compound (anthraquinones 1 - 16) used in this investigation were made using a single-photon counting apparatus constructed in our laboratory from Ortec electronic components and an Intertechnique multichannel analyser similar to that described by Ware and coworkers [C1, C2]. The emission lifetime was measured using a classical iterative reconvolution technique based on the least-squares method.

Laser flash experiment

The laser excitation of the anthraquinones was achieved using the experimental procedure described in ref. C3. Frequency doubling of the fundamental light (1064 nm) by a prism gave a 532 nm light beam which passed through a diaphragm before traversing a quartz cell of dimensions 1 cm × 1 cm which contained the sample in acetonitrile solution. The emitted light was received at right angles by the photocathode of a high speed photodiode (type S11; rise time, less than 150 ps). A gelatin filter (Kodak no. 21 or 22 or both) was inserted between the photocathode and the cell in order to eliminate Rayleigh diffusion. The photodiode signal was transmitted to a Tektronix 7104 oscilloscope with a rise time of about 350 ps and the oscilloscope trace was recorded on Polaroid film. A 30 ps laser pulse produced an electronic echo in the detection system about 1 ns after the signal; treatment of the data enabled this perturbation to be removed.

The light beam was split before passing through the cell and one part was focused on a Gen Tec pyroelectric detector; the signal obtained was proportional to the energy of the exciting light. A linear relation between the fluorescence intensity and the excitation energy was obtained for all the compounds studied. The power density at the sample was less than 1 MW cm⁻².

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