Journal of Fluorescence, Vol. 7, No. 1, 1997 (Supplement)

Radiative Migration of Electronic Excitation Energy: Theory and Experiment

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Received September 27, 1995

Calculations according to a recent model of radiative migration (or radiative transport) of electronic excitation energy are presented and tested against experimental results. The theoretical analysis yields the fluorescence decay and macroscopic quantum yield, and also the time-resolved and steady-state fluorescence anisotropies. These observables are in general a function of the excitation and emission wavelengths, concentration, and excitation and detection geometries, and can be computed from known molecular spectroscopic properties. Comparison between theoretical and experimental fluorescence decays of rhodamine 101 in ethanol shows good agreement.

KEY WORDS: Fluorescence; energy transfer; radiative transfer; rhodamine 101.

INTRODUCTION

In an atomic or molecular system the photons emitted by electronically excited species may be reabsorbed and reemitted several times before they leave the sample. This phenomenon is known as radiation imprisonment, radiative migration or radiative transport [1]. Its importance depends on many factors: extent of spectral overlap between absorption and emission, fluorescence quantum yield, concentration, cell size and shape, geometry of detection, etc. It is particularly important in solutions of highly fluorescent substances with a reasonable overlap between absorption and emission, whether concentrated or in large volumes. When present, it may significantly distort the fluorescence decay law and the fluorescence spectrum, as well as the fluorescence anisotropy. These observables become then a function of the excitation and emission wavelengths, concentration, and excitation and detection geometries.

A general stochastic theory of radiative transport was recently developed [2]. The theoretical analysis yields expressions for the fluorescence decay and macroscopic quantum yield, and for the time-resolved and steady state fluorescence anisotropies, having as parameters only known molecular spectroscopic properties.

In this report we present the results of theoretical calculations and experimental results for rhodamine 101 in ethanol, at room temperature. The agreement is in general quite good.

EXPERIMENTAL

Acidified ethanol (ca. 10^{-2} M in HCl) was used as a solvent in order to ensure complete protonation of rhodamine 101 at all concentrations. Measurements of time-resolved fluorescence were made by the single photon timing technique using a Coherent mode-locked synchronously pumped and cavity dumped dye laser system whose output was frequency doubled to give excitation pulses with a pulse width of 6 ps at a repetition rate of 3.4 MHz. The experiments were performed using a resolution of 56 ps per channel. A total of 20,000 (right-angle) or 50,000 (front-face) counts was typically accumulated in the maximum channel.

THEORY

Consider a given volume, of convex but otherwise arbitrary shape, containing a macroscopically uniform

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0035-0509/97/0300-119S$12.50/0

distribution of identical molecules. The time evolution of the intensity of the fluorescence emitted by the molecular ensemble along a given direction Ω, in response to excitation by an instantaneous external pulse of essentially monochromatic light, is given by [2]

$$\rho^{\Omega}(\lambda_{exc},\lambda_{em},t)=\sum_{i=1}^{\infty} p_n^{\Omega}(\lambda_{exc},\lambda_{em})\rho_n(t) \quad (1)$$

where $\rho^{\Omega}(\lambda_{exc},\lambda_{em},t)$ is the probability that a photon of wavelength λ_{em} will leave the sample along Ω between t and t + dt, given that another photon of wavelength λ_{exc} was absorbed at time t = 0, and where $p_n^{\Omega}(\lambda_{exc},\lambda_{em})$ is the escape probability along Ω for a photon of wavelength λ_{em} after exactly n absorption events [2]. Finally, $\rho_n(t)$ is the probability that a n-th generation molecule will emit a photon between t and t + dt, given that it will emit one, and is given by

$$\rho_n(t)=\frac{1}{\tau_o}\frac{\left(\frac{t}{\tau_o}\right)^{n-1}}{(n-1)!}\exp\left(-\frac{t}{\tau_o}\right) \quad (2)$$

where τ_o is the molecular lifetime.

For the purposes of computing the effect of radiative transport on fluorescence anisotropy we consider only measurements made in directions contained in the horizontal plane (denoted ⊥, and including the front-face and right-angle geometries), for which the anisotropy of fluorescence takes the highest value. We further suppose that the molecular rotational motion is negligible during the lifetime, that the exciting photons carry vertical polarization and that depolarization due to nonradiative migration is negligible. The time dependent anisotropy is then given by

$$r(\lambda_{exc},\lambda_{em},t)=r_1(\lambda_{exc})\frac{\sum_{n=1}^{\infty}p_n^{\perp}(\lambda_{exc},\lambda_{em})\rho_n(t)\beta^{n-1}}{\sum_{n=1}^{\infty}p_n^{\perp}(\lambda_{exc},\lambda_{em})\rho_n(t)} \quad (3)$$

where $r_1(\lambda_{exc})$ is the anisotropy of fluorescence of directly excited molecules (the so-called fundamental anisotropy), and β is the depolarization factor for radiative transfer (β=0.28 [2]).

RESULTS AND DISCUSSION

The absorption and fluorescence spectra of rhodamine 101 in acidified ethanol at room temperature are depicted in Fig. 1 for two concentrations: 5×10^{-7} M and 10^{-4} M. While the absorption does not change in this concentration range, the fluorescence spectrum becomes distorted at the higher concentration, owing to radiative migration. This phenomenon decreases the emission intensity in the blue part of the spectrum (overlap region) due to reabsorption and increases the intensity in the red part due to reemission, in other words, blue photons tend to end up as red photons (note that this increase is not correctly seen in Fig. 1 owing to the normalization procedure). However, unless the molecular quantum yield is unit, reemission does not always occur and therefore the macroscopic quantum yield (i.e., the ratio between the number of photons that leave the cell to those that are absorbed) will be lower than the molecular quantum yield.

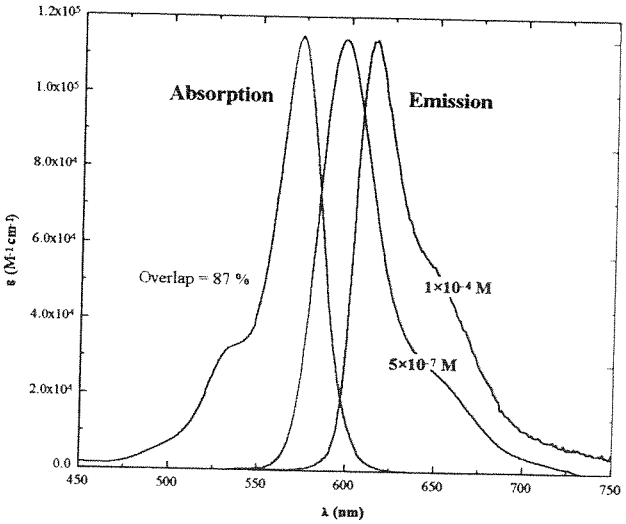

Fig.1. Absorption and fluorescence (right-angle geometry) spectra of rhodamine 101 in acidified ethanol. Fluorescence spectra are normalized at the maximum. Excitation wavelength was 530 nm.

The reabsorption-reemission process leads to a fluorescence decay that is slower than the intrinsic one, and thus to an increase of the average lifetime (Table I); On the other hand, the decay law becomes non-exponential (Eq. (1) and Fig. 2) and excitation and emission wavelength dependent. For the right-angle geometry, a risetime can even be observed (Fig. 3) if the optical density is so high as to prevent escape of radiation at the detection wavelength, and results from the finite time associated with the progression of the

Table I. Mean fluorescence lifetimes for the system rhodamine 101 in acidified ethanol viewed in a front-face geometry and with λ_{exc} = 294 nm. The intrinsic lifetime is 4.34 ns.

Concentration (M)	λ_{em} (nm)	$\langle\tau\rangle$ (ns) Monte-Carlo	$\langle\tau\rangle$ (ns) Experimental
10^{-4}	580	5.86	5.75
	600	6.25	6.18
	610	6.42	6.48
	630	6.48	6.65
10^{-3}	580	6.44	6.53
	600	7.36	7.23
	610	8.09	7.91
	630	8.67	8.65

excitation within the cell (initially concentrated away from the face where the observed photons come out).

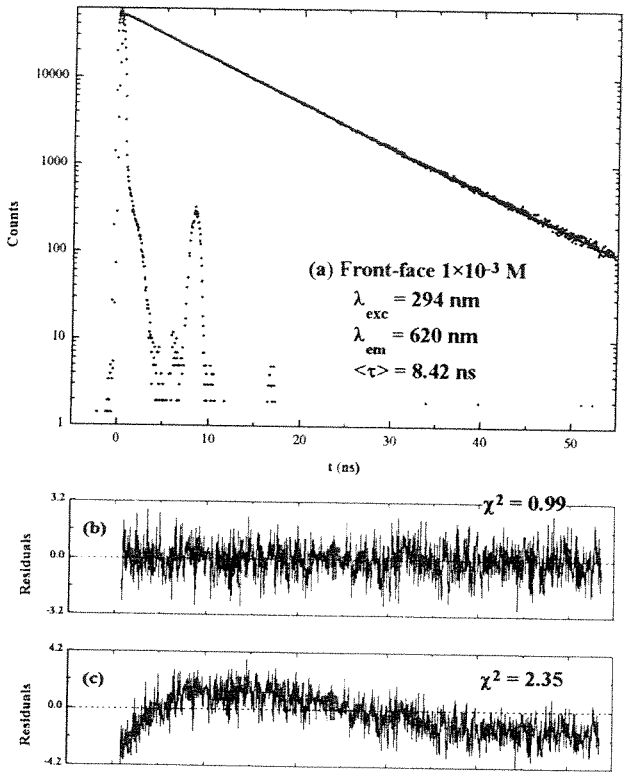

Fig. 2. (a) Fluorescence decay of a 10^{-3} M solution of rhodamine 101 in ethanol. Front-face geometry. (b) Residuals plot for a fit to Eq. (1). (c) Residuals plot for a fit to a single exponential.

As can be seen from Figs. 2 and 3, the fluorescence decay of concentrated solutions cannot be fitted to a single exponential. In right-angle conditions (Fig. 3) a risetime is even observed. Eq. (1) gives good fits to experimental data in all cases, the recovered parameters being in close agreement with the theoretical ones.

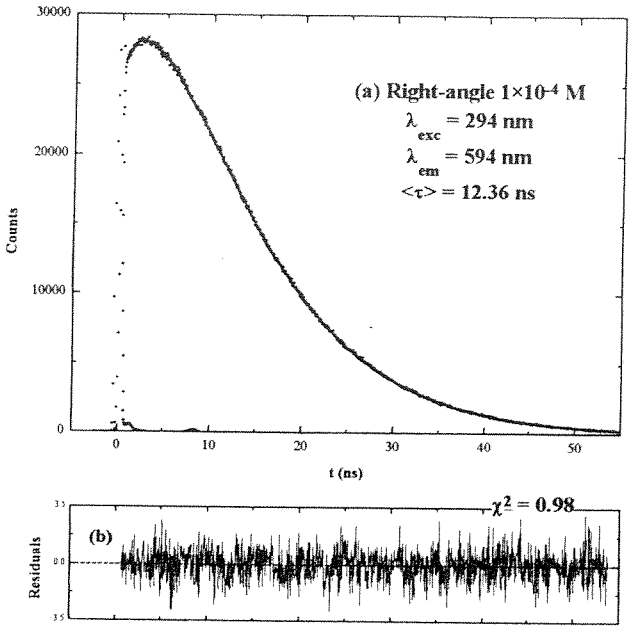

Fig. 3. (a) Fluorescence decay of a 10^{-4} M solution of rhodamine 101 in ethanol. Right-angle geometry . (b) Residuals plot for a fit to Eq. (1).

ACKNOWLEDGMENT

This work was supported by JNICT (Junta Nacional de Investigação Científica e Tecnológica, Portugal) and FEDER (Fundo Europeu para o Desenvolvimento Regional, União Europeia) through research contract STRDA/C/CEN/421/92.

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