SELF-ABSORPTION AND RE-EMISSION IN WAVELENGTH-DEPENDENT FLUORESCENCE DECAY

Jürg BAUMANN, Gion CALZAFERRI and Thomas HUGENTOBLER

Institute of Inorganic and Physical Chemistry, University of Bern, CH-3000 Bern 9, Switzerland

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The influence of fluorescence self-absorption and re-emission on the determination of the decay parameters of the excited state is reinvestigated. The jth re-emission step is treated as the convolution of step $j-1$ with the system's impulse response. For a single-exponential decay function, whose lifetime may depend on the emission wavelength, we show on what conditions the fluorescence re-emission alters the absolute value of the lifetime but not its wavelength dependence. The treatment can easily be extended to multiple exponentials. An explicit correction formula is given for phase fluorimetry experiments. Experimentally observed dual-exponential, wavelength-dependent, fluorescence decay of rhodamine 6G at different concentrations in ethanol is used to illustrate the theoretical results.

1. Introduction

It is well known that self-absorption and re-emission gives rise to a systematic lengthening of measured fluorescence lifetimes [1,2]. Recently we have observed that the luminescence decay parameters of rhodamine 6G and coumarin 535 in glycerol are not constants but depend on the emission wavelength [3]. We question whether self-absorption can affect this wavelength dependence and how re-emission has to be treated in modulation experiments. Our derivation is based on the assumption that the excitation intensity required is low and that the emission spectrum is independent of the excitation wavelength within the range of spectral overlap between the absorption and emission spectrum.

Spherical geometry of the dye cell is assumed. This situation is approached in our experiment since photons out of only a small solid angle are collected. Different geometries have been discussed by Hammond [1]. We further assume that the only concentration-dependent process that can affect the luminescence decay parameters with respect to infinite dilution is self-absorption/re-emission. First, we consider the spectral properties of the re-emitted photons in relation to the emission spectrum at infinite dilution. Second, the time evolution of the first re-emission is calculated for the case of a single-exponential decay, where the lifetime is first considered constant, and then allowed to vary across the emission spectrum.

Finally we compare the theoretical finding with experimental results in 10^{-5} to 10^{-7} molar alcoholic solutions of rhodamine 6G.

2. Re-emission influence on wavelength-dependent fluorescence decay

$E(\lambda)$ describes the normalized emission spectrum at the source of emission and $a(\lambda)$ the absorption probability between the source of emission and the detection:

$$a(\lambda) = 1 - 10^{-\epsilon(\lambda)cd}\,, \tag{1}$$

$\epsilon(\lambda)$ is the molar extinction coefficient, c the concentration and d the path length. The total fraction of fluores-

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cence photons absorbed in the solution is described by:

$$a = \int_{\lambda} E(\lambda) a(\lambda)\, \mathrm{d}\lambda \,. \tag{2}$$

Due to self-absorption, the spectrum of the primary emission at the point of detection $F_0(\lambda)$ is different from $E(\lambda)$,

$$F_0(\lambda) = [1 - a(\lambda)] E(\lambda) \,. \tag{3}$$

With η as fluorescence quantum yield, we obtain for the first re-emission at the point of detection:

$$F_1(\lambda) = \eta a [1 - a(\lambda)] E(\lambda) = \eta a F_0(\lambda) \,, \tag{4}$$

and for the jth re-emission step:

$$F_j(\lambda) = (\eta a)^j F_0(\lambda) \,. \tag{5}$$

The observed total emission $F_T(\lambda)$ is the sum of all contributions

$$F_T(\lambda) = F_0(\lambda) \sum_{i=0}^{n} (\eta a)^i = E(\lambda)[1 - a(\lambda)] \sum_{i=0}^{n} (\eta a)^i \,. \tag{6}$$

An important conclusion which can be drawn is that each emission step has the same spectrum $F_0(\lambda)$. The relative contribution v_j is therefore independent of wavelength and equal to:

$$v_j = F_j(\lambda)/F_T(\lambda) = (\eta a)^j / \sum_{i=0}^{n} (\eta a)^i \,. \tag{7}$$

During the last few years we have been developing a very precise multifrequency dual beam phase fluorometer [4]. We also refer to a review article written by Gratton et al. and to the book of Lakowicz [5]. What follows is a discussion of how self-absorption and re-emission affects this type of measurement in the case of a single-exponential decay. If necessary the calculations can be extended to multiple exponential functions.

First, we simplify the calculations by assuming that the decay time τ does not depend on the emission wavelength. This restriction will be removed later. Within this approximation the normalized primary emission $V_0(t)$ after δ-pulse excitation equals the system's impulse response $T(t)$ at infinite dilution,

$$V_0(t) = \tau^{-1} \exp(-t/\tau) \,. \tag{8}$$

The time evolution of the first re-emission $V_1(t)$ is described by the convolution of the primary emission which acts as excitation source with the impulse response:

$$V_1(t) = a\eta\tau^{-2} \int_0^t \exp(-\theta/\tau) \exp[-(t - \theta)/\tau]\, \mathrm{d}\theta \,,$$

from which we get:

$$V_1(t) = a\eta\tau^{-2} t \exp(-t/\tau) \,. \tag{9}$$

$V_1(t)$ is the exciting function of the second re-emission $V_2(t)$. The time evolution of $V_2(t)$ is therefore:

$$V_2(t) = a^2\eta^2\tau^{-3} \int_0^t \theta \exp(-\theta/\tau) \exp[-(t - \theta)/\tau]\, \mathrm{d}\theta \,,$$

which leads to

$$V_2(t) = \tfrac{1}{2} a^2\eta^2\tau^{-3} t^2 \exp(-t/\tau) \,. \tag{10}$$

$V_3(t)$ and higher terms can be calculated the same way. We restrict the following discussion to situations in which the quadratic term $a^2\eta^2$ can be neglected. This corresponds to most practical situations. With this restriction we get from eqs. (3), (8) and (9) for $V_T(t)$:

$$V_T(t) = (1-a)[\tau^{-1}\exp(-t/\tau) + a\eta\tau^{-2}t\exp(-t/\tau)] \ . \tag{11}$$

In our experiment a harmonically modulated excitation source is used. The frequency response $T(\omega)$ is the Fourier transform of $V_T(t)$. Since only the phase shift is of interest for our purpose, we need not normalize $V_T(t)$ and can therefore omit the constant factor $(1-a)$ [3]:

$$T(\omega) = \int_0^\infty V_T(t)\exp(-j\omega t)\,\mathrm{d}t = 1/(1+j\omega\tau) + a\eta/(1+j\omega\tau)^2 \ ,$$

which gives after some rearrangement:

$$T(\omega) = [1/(1+\omega^2\tau^2)]\,[1 + a\eta(1-\omega^2\tau^2)/(1+\omega^2\tau^2)] - j\,[\omega\tau/(1+\omega^2\tau^2)]\,[1 + 2a\eta/(1+\omega^2\tau^2)] \ . \tag{12}$$

From $T(\omega)$ the phase shift $\Phi(\omega)$ between exciting and emitted light, from ref. [6], is

$$\tan\Phi(\omega) = \frac{\mathrm{Im}}{\mathrm{Re}} = \omega\tau\,\frac{1 + 2a\eta/(1+\omega^2\tau^2)}{1 + a\eta(1-\omega^2\tau^2)/(1+\omega^2\tau^2)} \ . \tag{13}$$

Eq. (13) can be arranged in powers of $a\eta$:

$$\tan\Phi = \omega\tau\{1 + a\eta - (a\eta)^2(1-\omega^2\tau^2)/[1+\omega^2\tau^2 + a\eta(1-\omega^2\tau^2)]\} \ . \tag{14}$$

Neglecting again second-order terms in $(a\eta)$ we obtain the simple result:

$$\tan\Phi = \omega\tau(1+a\eta) \ , \tag{15}$$

or

$$\tau(c\to 0) = \tau(c)/(1+a\eta) \ . \tag{16}$$

For small values of $a\eta$ we can write

$$\tau(c\to 0) \approx \tau(c)(1-a\eta) \ , \tag{17}$$

a formula which is often encountered in the literature [1,2]. Eq. (15) shows that the correction factor corr = $(1 + a\eta)$ does not depend on the modulation frequency, as long as quadratic terms in $(a\eta)$ can be neglected.

We now want to calculate how the emission-wavelength dependence of τ influences this result. The impulse response in case of a single exponential decay is

$$T(t,\lambda) = \tau^{-1}(\lambda)\exp[-t/\tau(\lambda)]$$

and $V_0(t,\lambda_0)$ is the decay function of the primary emission at λ_0. $V_1(t,\lambda_1;\lambda_0)$ describes the time evolution of the first re-emission at λ_1 which was excited by primary emission photons of wavelength λ_0,

$$V_1(t,\lambda_1;\lambda_0) = a(\lambda_0)E(\lambda_0)\eta\int_0^t V_0(\theta,\lambda_0)T(t-\theta,\lambda_1)\,\mathrm{d}\theta \ ,$$

which can easily be calculated:

$$V_1(t,\lambda_1;\lambda_0) = \{a(\lambda_0)E(\lambda_0)\eta/[\tau(\lambda_0)-\tau(\lambda_1)]\}\{\exp[-t/\tau(\lambda_0)] - \exp[-t/\tau(\lambda_1)]\} \ . \tag{18}$$

To discuss the consequences of this equation it is useful to introduce

$$X = \tau(\lambda_0) - \tau(\lambda_1)$$

which leads to

$$V_1(t, \lambda_1; \lambda_0) = a(\lambda_0)E(\lambda_0)\eta \exp[-t/\tau(\lambda_1)]\{\exp[tX/\tau(\lambda_0)\tau(\lambda_1)] - 1\}/X . \tag{19}$$

For $X \to 0$, eq. (19) becomes identical with eq. (9). For other cases we develop (19) in powers of X:

$$V_1(t, \lambda_1; \lambda_0) = a(\lambda_0)E(\lambda_0)\eta \exp[-t/\tau_1(\lambda_1)]\,[t/\tau(\lambda_1)^2] \times \left(\frac{1}{1 + X/\tau(\lambda_1)} + \frac{1}{2}\,\frac{tX}{\tau(\lambda_1)^2[1 + X/\tau(\lambda_1)]^2} \ldots\right) . \tag{20}$$

For simplicity we abbreviate $\tau(\lambda_1)$ by τ_1. If $X/\tau_1 \ll 1$ the approximation $1/(1 + X/\tau_1)n \approx 1 - nX/\tau_1$ can be applied. Eq. (20) then becomes

$$V_1(t, \lambda_1; \lambda_0) = a(\lambda_0)E(\lambda_0)\eta \exp(-t/\tau_1)(t/\tau_1^2)[1 - X/\tau_1 + \tfrac{1}{2}(t/\tau_1)X/\tau_1] . \tag{21}$$

Eq. (21) must be integrated over the spectral overlap region:

$$V_1(t, \lambda_1) = \int_\lambda V(t, \lambda_1, \lambda_0)\, d\lambda_0 ,$$

which leads to

$$V_1(t, \lambda_1) = a\eta \exp(-t/\tau_1)(t/\tau_1^2)\left[1 - (1/\tau_1 + \tfrac{1}{2}t/\tau_1^2)\left(a^{-1}\int_\lambda a(\lambda_0)E(\lambda_0)\tau(\lambda_0)\, d\lambda_0 - \tau(\lambda_1)\right)\right] .$$

As long as $\tau(\lambda_0)$ is a slowly varying function in the spectral overlap region we can take the mean value $\bar{\tau}(\lambda_0)$ outside the integral. Replacing X by $\bar{X} = \bar{\tau}(\lambda_0) - \tau(\lambda_1)$ leads to:

$$V_1(t, \lambda_1) = a\eta \exp(-t/\tau_1)(t/\tau_1^2)[1 - \bar{X}/\tau_1 + \tfrac{1}{2}(t/\tau_1)\bar{X}/\tau_1] . \tag{21a}$$

With the substitutions $\alpha_1 = 1 - \bar{X}/\tau_1$, $\alpha_2 = \frac{1}{2}\bar{X}/\tau_1$ we obtain for V_T instead of eq. (11):

$$V_T(t, \lambda_1) = (1 - a)\tau_1^{-1} \exp(-t/\tau_1) + a\eta[(\alpha_1/\tau_1^2)t \exp(-t/\tau) + (\alpha_2/\tau_1^3)t^2 \exp(-t/\tau_1)] . \tag{22}$$

From

$$T(\omega, \lambda_1) = \int_0^\infty V_T \exp(-j\omega t)\, dt$$

we get, by omitting the constant factor $(1 - a)$,

$$T(\omega, \lambda_1) = 1/(1 + j\omega\tau_1) + a\eta\alpha_1/(1 + j\omega\tau_1)^2 + 2a\eta\alpha_2/(1 + j\omega\tau_1)^3 , \tag{23}$$

which after some rearrangement leads to:

$$T(\omega, \lambda_1) = [1/(1 + \omega^2\tau_1^2)]\{1 + a\eta\alpha_1[(1 - \omega^2\tau_1^2)/(1 + \omega^2\tau_1^2) + 2a\eta\alpha_2(1 - 3\omega^2\tau_1^2)/(1 + \omega^2\tau_1^2)^2]\} + j[\omega\tau_1/(1 + \omega^2\tau_1^2)]\,[1 + 2a\eta\alpha_1/(1 + \omega^2\tau_1^2) + 2a\eta\alpha_2(1 - 3\omega^2\tau_1^2)/(1 + \omega^2\tau_1^2)^2] . \tag{24}$$

Again the phase shift is the quantity of interest:

$$\tan\Phi = \omega\tau_1\,\frac{1 + 2a\eta/(1 + \omega^2\tau_1^2) - a\eta(\bar{X}/\tau_1)(1 - 3\omega^2\tau_1^2)/(1 + \omega^2\tau_1^2)^2}{1 + a\eta(1 - \omega^2\tau_1^2)/(1 + \omega^2\tau_1^2) + a\eta(\bar{X}/\tau_1)\omega^2\tau^2(\omega^2\tau_1^2 - 3)/(1 + \omega^2\tau_1^2)^2} .$$

This is the equivalent of eq. (13).

Because $a\eta$ and $\bar{X}/\tau_1$ have been assumed to be much smaller than 1 the terms in $a\eta(\bar{X}/\tau_1)$ can be neglected. This means that even in case that τ depends on the emission wavelength, eq. (15) is valid as long as $\bar{X}/\tau_1$ remains small.

3. Experimental comparison of the wavelength dependent decay of rhodamine 6G in 10^{-5} to 10^{-7} molar alcoholic solutions

Recently we have observed that the fluorescence decay of rhodamine 6G and of coumarin 535 in glycerol cannot be described by a single exponential decay and that the decay parameters show a significant wavelength dependence [3]. This was interpreted in terms of different excited state conformations which are stabilized by the highly viscous solvent.

The influence of intramolecular movements on the very strong viscosity dependence of the fluorescence quantum yield of methinecyanine dyes was discussed by us several years ago [7]. Two years later Grabowski et al. [8] explained the discovery of Lippert, Lüder and Boos [9], that *p*-cyano-N,N-dimethylaniline and its N,N-diethyl analogue exhibit in medium- or high-polarity fluid solvents two fluorescence bands, by assuming two different rotamer structures. In both cases experimental observation is already possible in static measurements. In contrast to this, the different excited state conformations of rhodamine 6G and coumarin 535 can only be observed in precise spectrally resolved luminescence decay measurements.

In section 2 of this paper we have proved that, at moderate concentrations, self-absorption and re-emission only influences the absolute value of decay parameters, but not their wavelength dependence. We have also shown under which conditions formula (15) can be applied in modulation measurements to correct decay data to infinite dilution.

Table 1
Rhodamine 6G, 10^{-5} mol/ℓ in ethanol with 5% water, degassed solution, observed at magic-angle polarization. τ_2 = 200 ps

Wavelength (nm)	Single exponential decay		Dual exponential decay		
	τ (ns)	red. χ^2	τ_1 (ns)	a_2	red. χ^2
525	4.20	1.29	4.23	0.07	1.25
535	4.23	0.7	4.24	0.02	0.78
550	4.26	1.6	4.24	0.02	0.78
565	4.29	3.52	4.23	−0.21	0.6
580	4.32	7.86	4.23	−0.26	1.1
595	4.35	9.87	4.21	−0.36	1.1

mean value of τ_1, corrected to infinite dilution: $\tau_1(c \to 0)$ = 3.82 ns

Table 2
Rhodamine 6G, 10^{-6} mol/ℓ in ethanol with 5% water, degassed solution, observed at magic angle polarization. τ_2 = 200 ps

Wavelength (nm)	Single exponential decay		Dual exponential decay		
	τ (ns)	red. χ^2	τ_1 (ns)	a_2	red. χ^2
525	3.85	1.08	3.84	−0.01	1.25
535	3.87	0.74	3.85	−0.08	0.24
550	3.92	8.5	3.84	−0.22	0.98
565	3.94	4.9	3.88	−0.19	1.54
580	3.95	13.9	3.82	−0.42	1.17
595	3.98	11.9	3.82	−0.5	0.84

mean value of τ_1, corrected to infinite dilution: $\tau_1(c \to 0)$ = 3.80 ns

Table 3
Rhodamine 6G at different concentrations in ethanol with 5% water, degassed solution, observed at 540 nm emission wavelength and under magic-angle polarization for τ_1. For τ_{rot} see eq. (23) [3]. The modulation frequency was 45 MHz

Concentration (mol/ℓ)	Single exponential τ_1 (ns)		Self-absorption a, eq. (2)	Rotational diffusion τ_{rot} (ps)
	measured	extrapolated $c \to 0$		
1.03×10^{-5}	4.281 ± 0.017	3.871	0.1136	283 ± 10
5.15×10^{-6}	4.054 ± 0.013	3.828	0.0633	280 ± 9
1.03×10^{-6}	3.884 ± 0.013	3.834	0.0139	280 ± 8
5.15×10^{-7}	3.869 ± 0.008	3.843	0.0070	284 ± 10
1.03×10^{-7}	3.853 ± 0.011	3.848	0.0014	263 ± 6

In this section we compare the theoretical results with measurements on 10^{-5} to 10^{-7} molar alcoholic solutions of rhodamine 6G. To get the data in tables 1 and 2 phase shifts for each experimental configuration have been measured at 7 different modulation frequencies, equally spaced between 20 and 50 MHz. Excitation was at 530.9 nm, the bandpass of the emission monochromator 4 nm and the temperature 25°C. Rhodamine 6G (Merck) was recrystallized from toluene/ethanol and further purified by column chromatography. The ethanol was Merck UVASOL for fluorescence spectroscopy. In the 10^{-5} molar solution, the decay kinetics at 525 nm to 550 nm, and in the 10^{-5} molar solution at 525 nm and 535 nm, can be described by a single exponential. At longer wavelength a dual exponential function is needed to fit the experimental data:

$$\exp(-t/\tau_1) + a_2 \exp(-t/\tau_2) .$$

As in case of rhodamine 6G in glycerol, a_2 and τ_2 are correlated for the accuracy of our data [3], but τ_1 is not correlated with a_2 and τ_2. The significant difference between τ_1 in the 10^{-5} molar solution and the 10^{-6} molar solution should be interpretable according to eq. (15) as due to self-absorption and re-emission.

To check the validity of this interpretation, we have measured τ_1 at 540 nm for five different concentrations, ranging from 1.03×10^{-5} mol/ℓ to 1.03×10^{-7} mol/ℓ. From the data reported in tables 1 and 2 we know that the decay kinetics at the emission wavelength of 540 nm can be described by a single exponential function.

The self-absorption factor a was calculated according to eq. (2) based on measurements of the fluorescence spectrum in a 5×10^{-7} molar solution and a path length of 0.33 cm. The fluorescence quantum yield $\eta = 0.93$ was taken from the literature [1]. $\tau_1(c)$ has been extrapolated to infinite dilution according to eq. (15). It follows from the data in table 3 that the extrapolation causes an uncertainty in the value of $\tau_1(c \to 0)$ which is not much larger than the precision of the measurements.

In the last column of table 3 we report the experimentally determined rotational diffusion constant τ_{rot}. In highly viscous solvents the experimentally observed τ_{rot} is expected to be influenced by depolarized re-emission. But in alcoholic solution the rotational diffusion of rhodamine 6G is so fast that this depolarisation effect cannot influence τ_{rot} within the investigated concentration range.

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