

Exercício feito na lousa:

Assumindo somente efeitos gerais do solvente, calcule a diferença entre os módulos dos dipolos elétricos do estado fundamental e do estado excitado de uma molécula com as características dadas abaixo, em solução aquosa:

$$\lambda_{\text{abs}} = 364 \text{ nm}$$

$$\lambda_{\text{em}} = 531 \text{ nm}$$

Raio estimado do fluoróforo $\approx 4.2 \text{ \AA}$

Dados: $\epsilon_{\text{água}} = 78.3$; $n_{\text{água}} = 1.33$

Resp: 14 D

Exemplos: Eq. Lippert

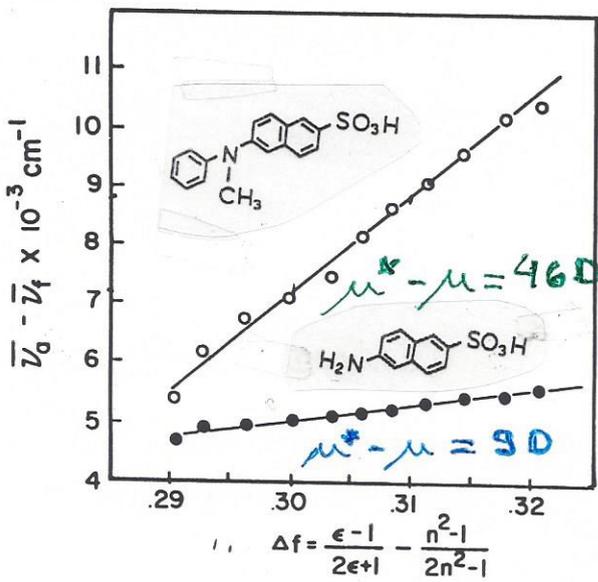


Figure 7.6. Lippert plots for two naphthylamine derivatives. Data are shown for N-phenyl-N-methyl-2-aminonaphthalene-6-sulfonate (○) and 2-aminonaphthalene-6-sulfonate (●). (From Ref. 9.)

→ avalia a sensibilidade do fluoróforo ao solvente

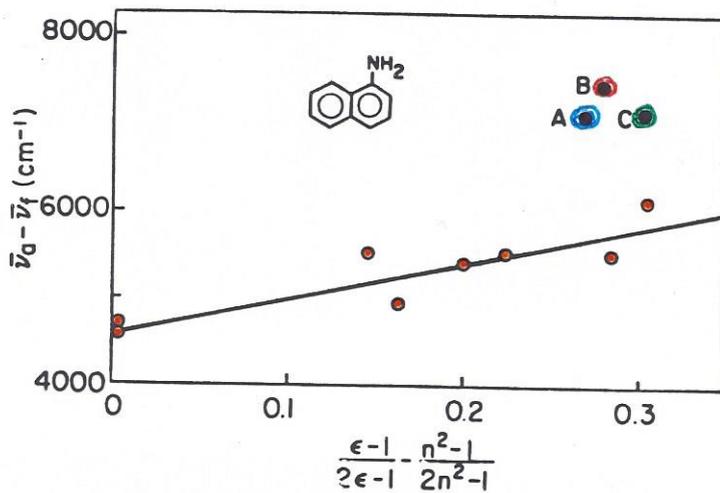
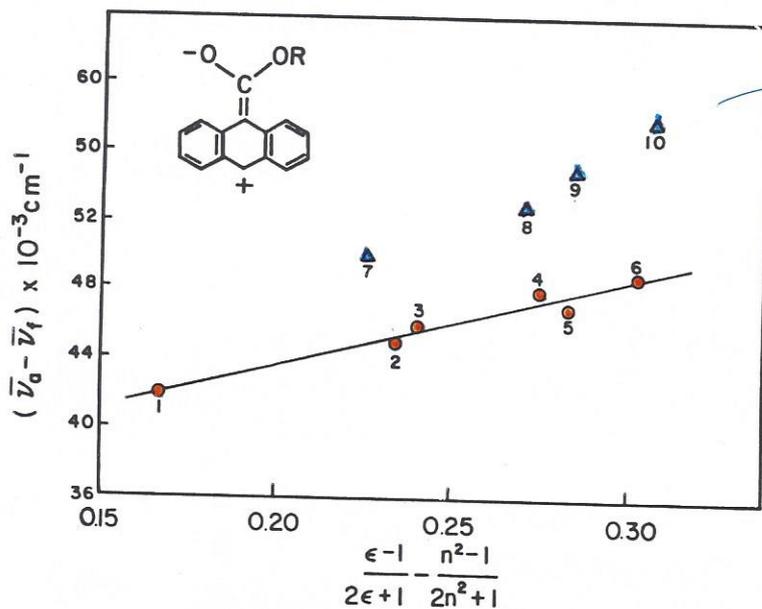


Figure 7.10. Effects of solvent polarity on the Stokes' shift of 1-aminonaphthalene. The solid dots, A, B, and C, are for n-propanol, formamide, and methanol, respectively. The other solvents are nonprotic. (From Ref. 20.)

solventes não-proticos (aproticos) seguem a Eq. Lippert

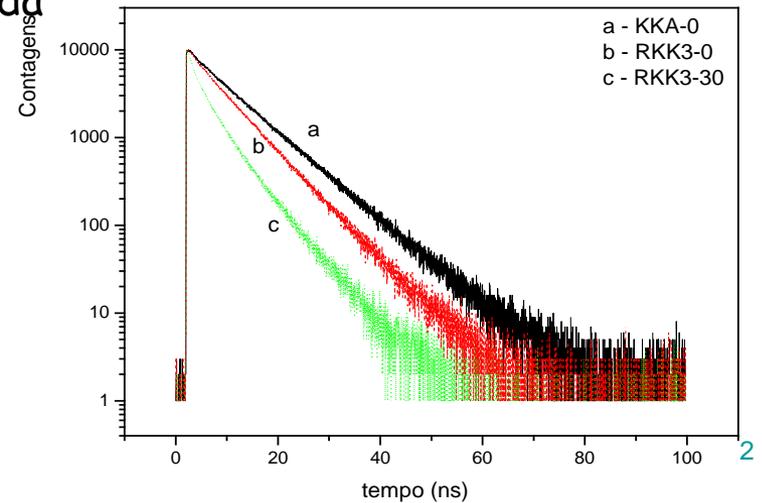
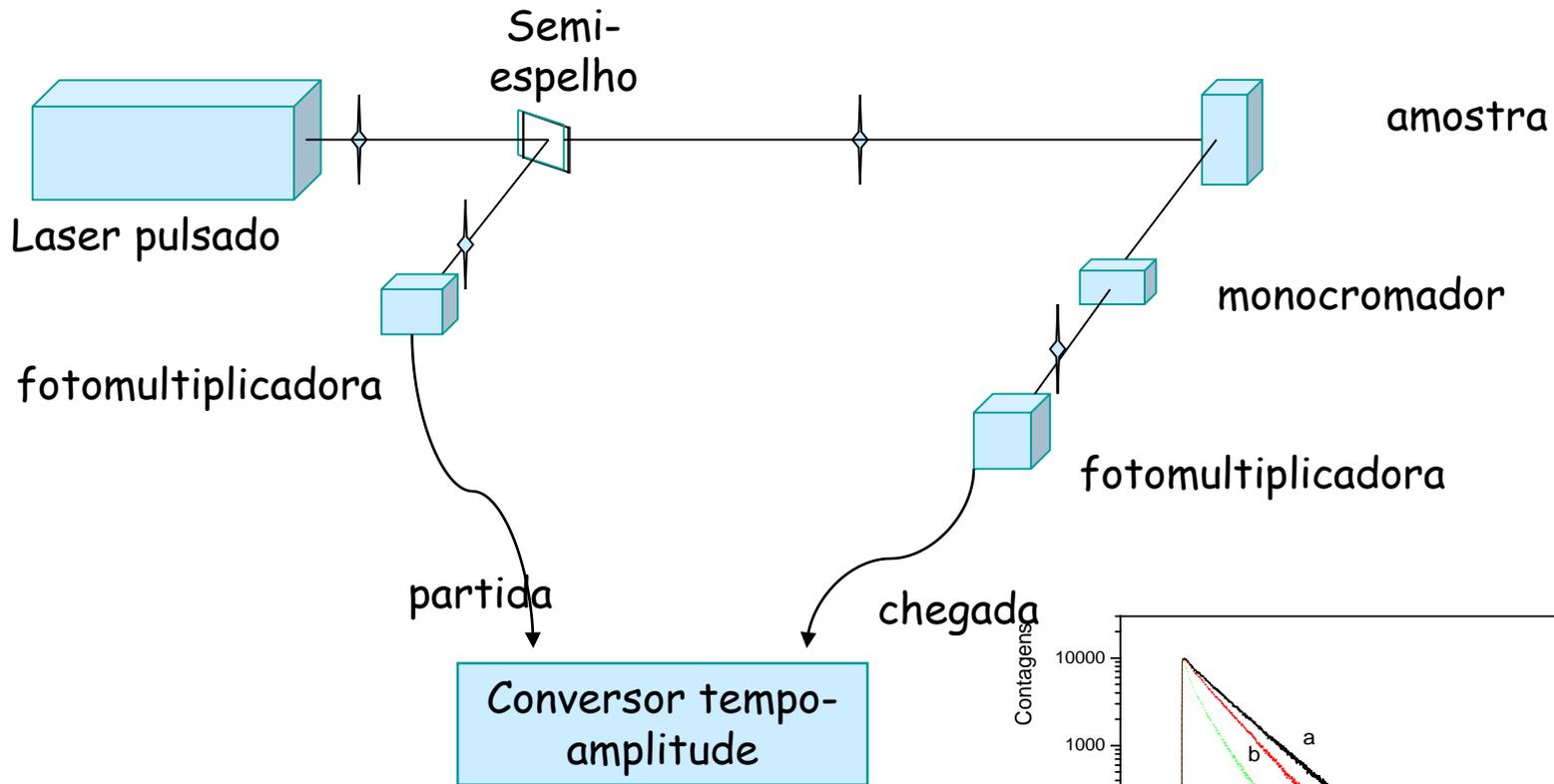


→ misturas dioxano/água
7 - 20%
8 - 40%
9 - 60%
10 - 80%

Figure 7.11. Stokes' shifts of 9-methylanthroate in protic and nonprotic solvents. The numbers refer to the following solvents: 1, ethyl ether; 2, benzonitrile; 3, methyl formate; 4, N,N-dimethylformamide; 5, acetone; 6, acetonitrile; 7, 80% dioxane-20% water; 8, 60% dioxane-40% water; 9, 40% dioxane-60% water; 10, 20% dioxane-80% water. The inserted structure shows the proposed charge distribution of the excited state. (From Ref. 21.)

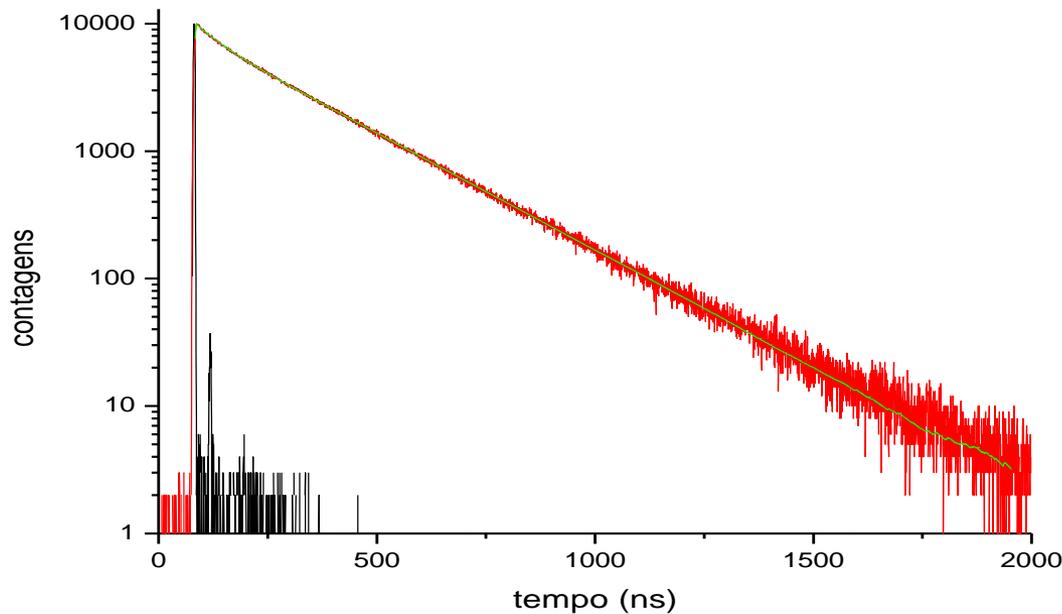
(fak.)

Fluorímetro com resolução temporal



Fluorímetro com resolução temporal

Cinética do decaimento do estado excitado

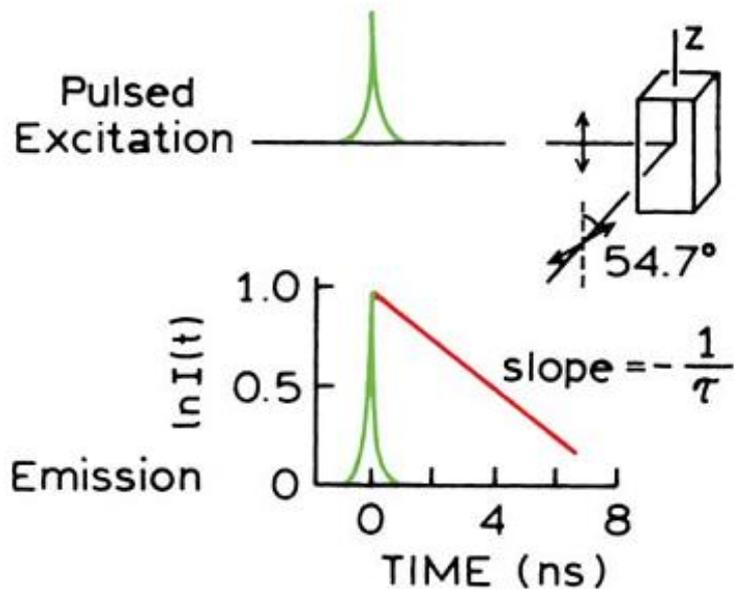


$I(t)$: função de decaimento, após excitação por um pulso de luz

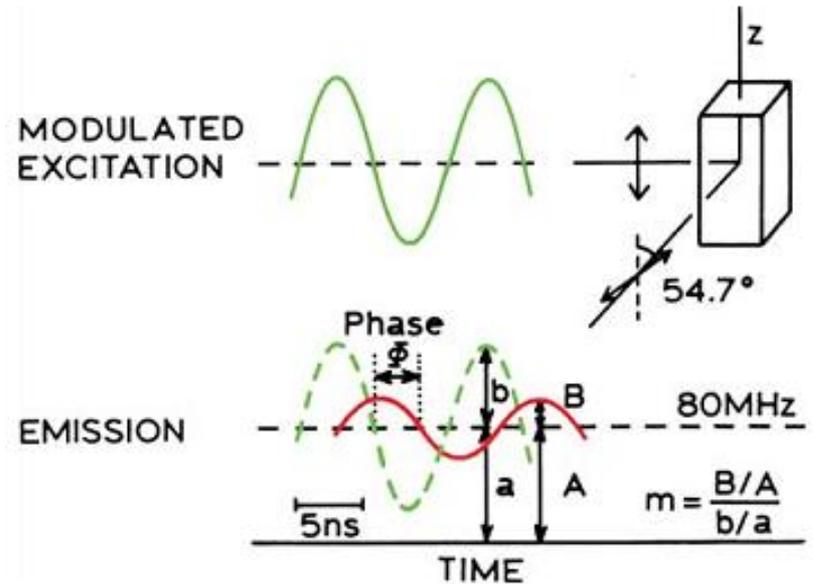
FLUORESCÊNCIA RESOLVIDA NO TEMPO

Tempos de vida de fluorescência \rightarrow da ordem de 10^{-8} s

DOMÍNIO DO TEMPO

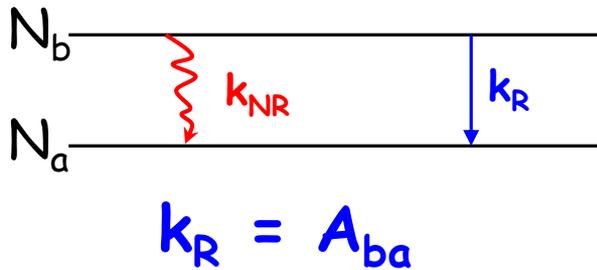
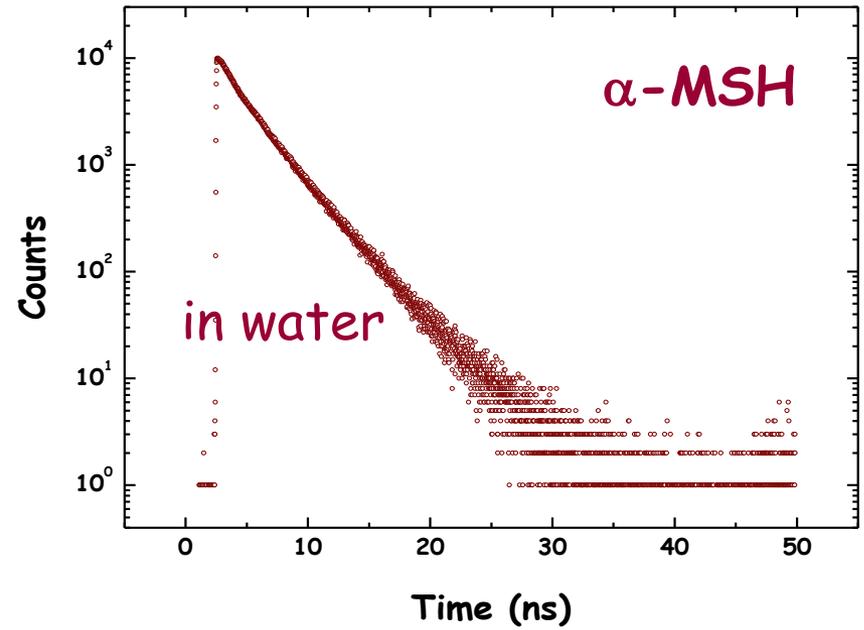


DOMÍNIO DA FREQUENCIA



Fluorescência resolvida no tempo

- k_R decaimento radiativo
- k_{CI} conversão interna
- k_{IS} cruzamento intersistemas
- $k_q(Q)$ supressão (quenching)
- ... outros processos



$$-\frac{dN_b}{dt} = \underbrace{(k_R + k_{CI} + k_{IS} + k_q)}_{k_T} N_b$$

$$N_b(t) = N_b(0)e^{-t/\tau_{exp}}$$

$$\tau_{exp} = 1/k_T$$

$$I(t) = \alpha e^{-t/\tau_{exp}}$$

$$k_R = A_{ba}$$

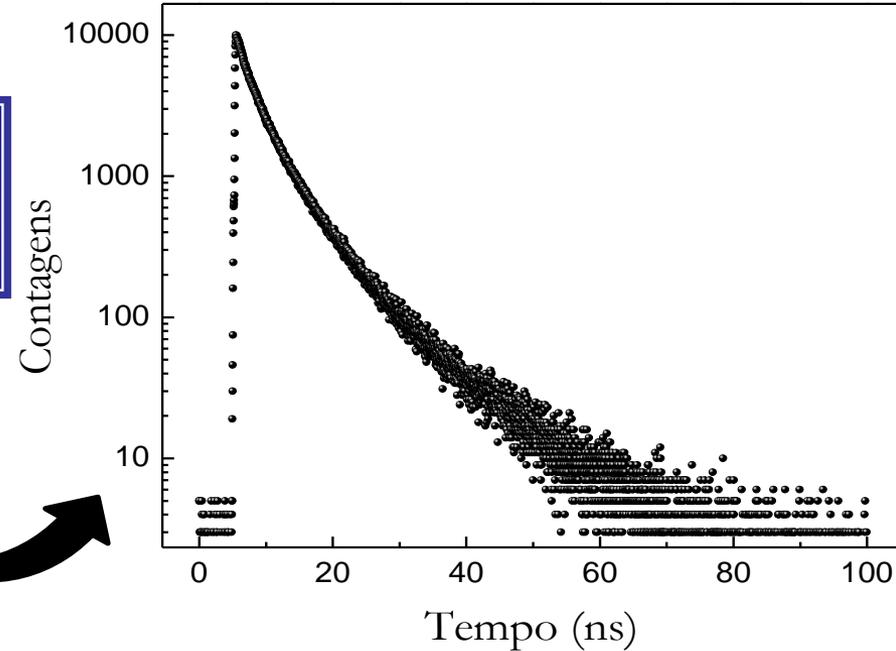
Coeficiente de Einstein para emissão espontânea

Discutido na lousa...

Fluorescência resolvida no tempo

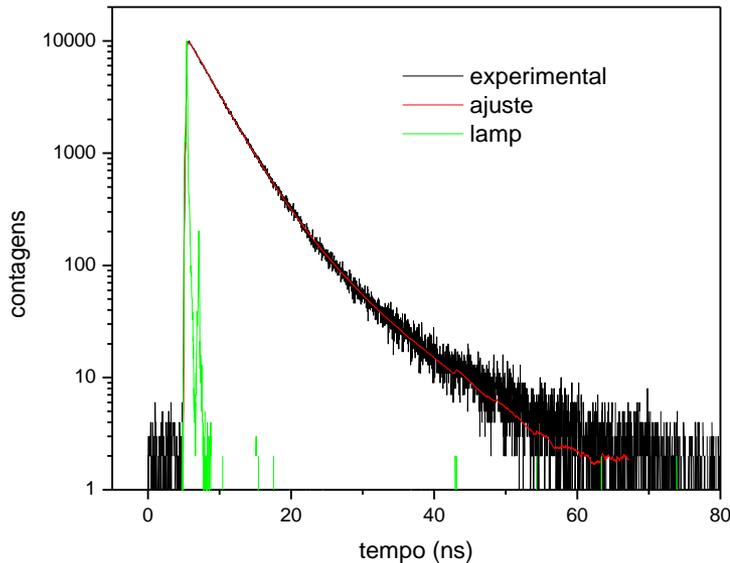
Decaimentos complexos:
multiexponencial

$$I(t) = A + \alpha_1 e^{-\frac{t}{\tau_1}} + \alpha_2 e^{-\frac{t}{\tau_2}} + \dots$$



Critério de ajuste: χ^2

Ajustes de decaimento da fluorescência



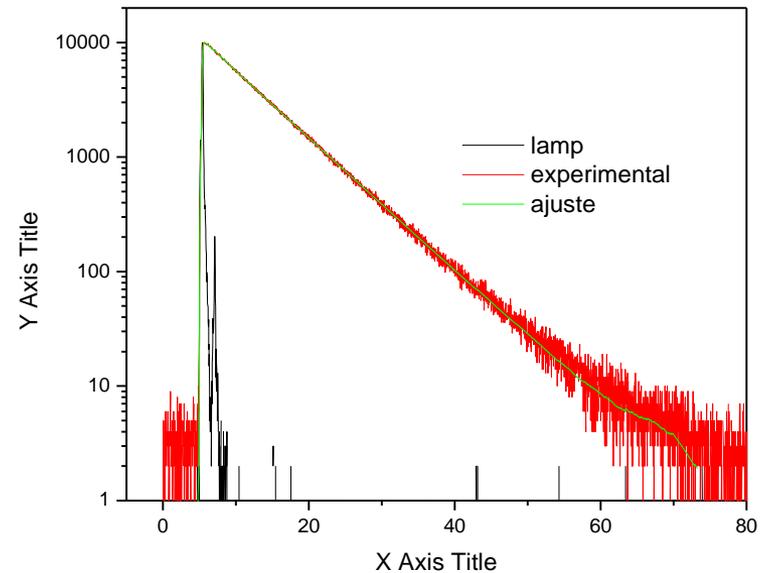
AbzNH2 diclorometano

$$f(t) = b_1 e^{-t/\tau_1} + b_2 e^{-t/\tau_2}$$

$$\tau_1 = 8,66 \text{ ns}; b_1 = 0,06 ; \%_1 = 14,6$$

$$\tau_2 = 3,58 \text{ ns}; b_2 = 0,94; \%_2 = 85,4$$

$$\chi^2 = 1,164$$



AbzNH2 metanol

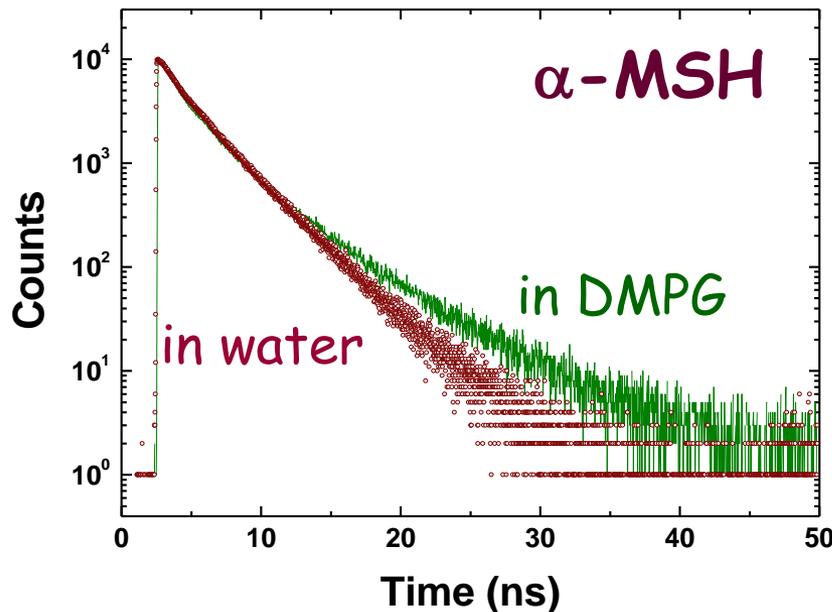
$$\tau_1 = 7,48 \text{ ns}; b_1 = 0,93 ; \%_1 = 98,1$$

$$\tau_2 = 2,04 \text{ ns}; b_2 = 0,07; \%_2 = 1,9$$

$$\chi^2 = 1,156$$

Interação Trp/membrana também pode ser monitorada pelo aumento do tempo de vida do estado excitado do fluoróforo

fluorescência
resolvida no tempo



$$I(t) = \sum_i \alpha_i e^{-t/\tau_i}$$

$$\tau_{\text{exp}} = 1/k_T \quad k_T = (k_R + k_{CI} + k_{IS} + k_q)$$

Maior taxa de desexcitação não radiativa do fluoróforo (conversão interna, k_{CI}) por interação com moléculas de H_2O

	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)
em H_2O	3.68	2.28	0.54
em DMPG	5.34	2.45	0.62

Em DMPG, maior τ_{exp} e maior rendimento quântico

$$\phi_F = I_{\text{em}}/I_{\text{abs}} = k_R/k_T = \tau_{\text{exp}}/\tau_R$$

Discussão na lousa sobre rendimento quântico:

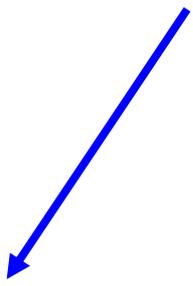
$$k_T = (k_R + k_{CI} + k_{IS} + k_q + \dots)$$

$$\tau_{\text{exp}} = 1/k_T$$

$$N_b(t) = N_b(0)e^{-t/\tau_{\text{exp}}}$$

$$I(t) = I_o e^{-t/\tau_{\text{exp}}}$$

Em determinado λ_{em}



$$I_{em} = \int_{t=0}^{t=\infty} I_o e^{-t/\tau_{\text{exp}}} dt = I_o \tau_{\text{exp}}$$

Se $\tau_{\text{exp}} = \tau_R \Rightarrow I_{em} = I_{abs} = I_o \tau_R$

$$\Phi_R \equiv \frac{I_{em}}{I_{abs}} = \frac{I_o \tau_{\text{exp}}}{I_o \tau_R} = \frac{k_R}{k_T}$$

Bibliografia

"Physics of Atoms and Molecules"

B.H. Bransden, C.J. Joachain

"Principles of Fluorescence Spectroscopy"

J.R. Lakowicz

"Molecular Fluorescence: Principles and Applications"

B. Valeur

- .

Série dos livros de "Topics in Fluorescence Spectroscopy"

Ed. J.R. Lakowicz