Polarized fluorescence parameters of FAD excited at 355 and 450 nm in water-propylene glycol solutions*

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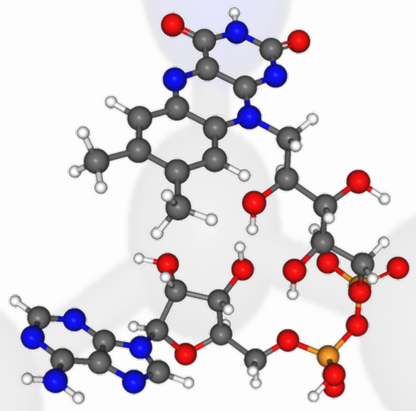
Introduction

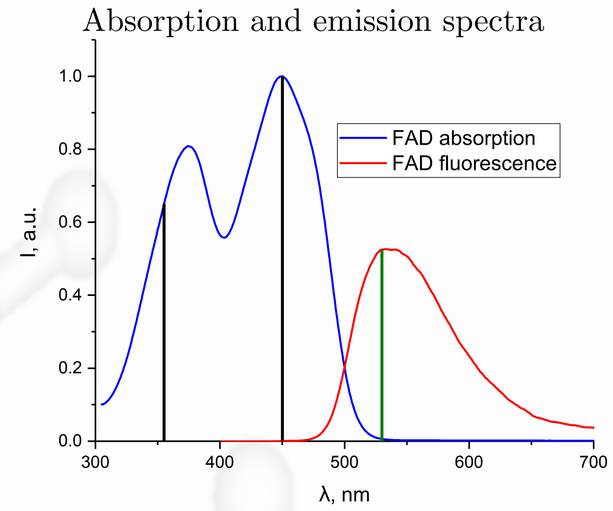
Flavin-adenine-dinucleotide (FAD) is an important biological coenzyme involved in the regulation of redox reactions in living cells that is widely used nowadays as a natural fluorescent biomarker for investigation of cellular metabolism. As known, in solutions FAD can exist in two conformations: "open", where the isoalloxazine and adenine moieties are well separated, and "folded", where they are located close to each other and the π -π stacking interaction occurs. In aqueous solution, FAD is believed to exist predominantly in its folded conformation; however, the addition of an alcohol breaks π -π interactions and results in the majority of open conformations. We present the study of fluorescence anisotropic decay in FAD in water-propylene glycol (PG) solutions. The fluorescence anisotropy and rotational diffusion times were determined from experiment for two excitatation wavelengh and analyzed.

FAD

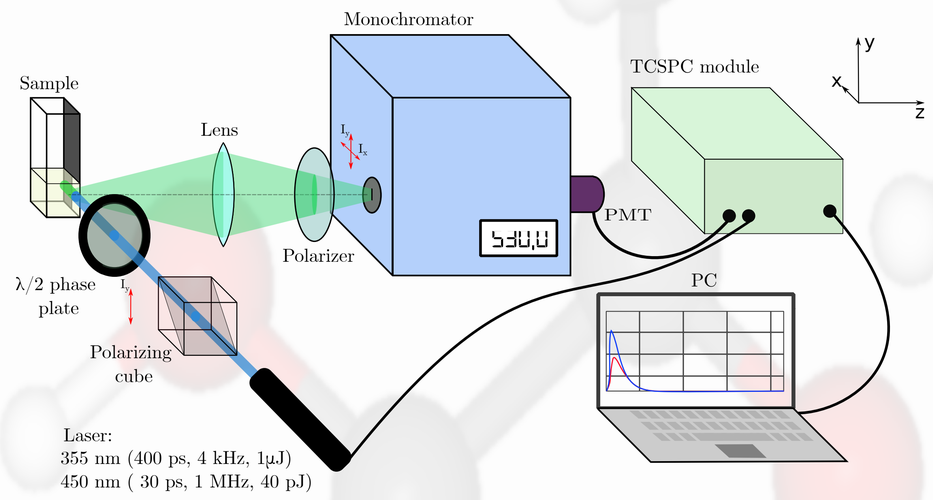
Molecule structure



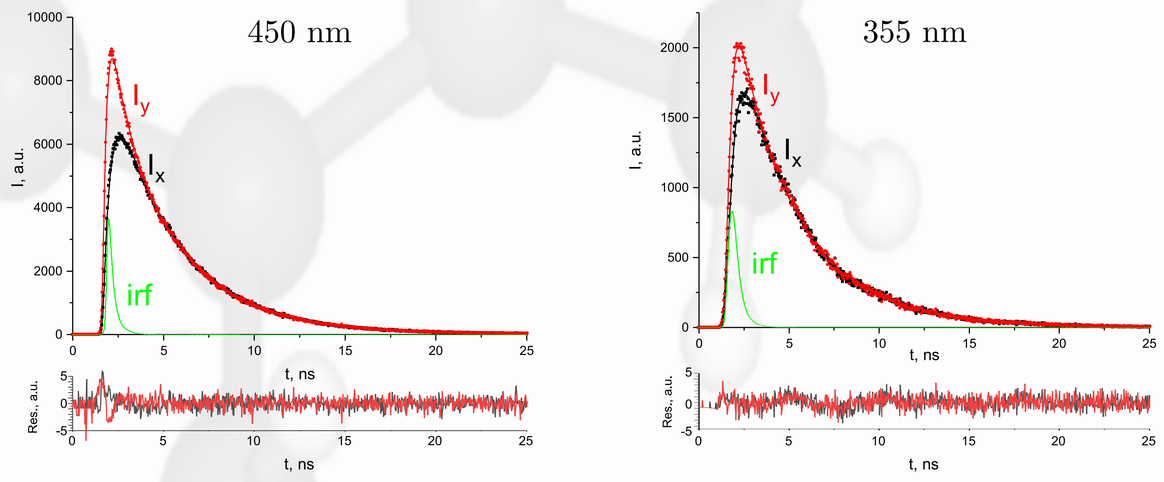
Absorption and emission spectra



Experimental setup



Typical expirimental signals



Results

Fitting formulas

$$I_y = GI_0\left(a_1e^{-t/\tau_1} + a_2e^{-t/\tau_2}\right)\left(1 + 2r_0e^{-t/\tau_{rot}}\right) * IRF(t)$$

$$I_x = I_0\left(a_1e^{-t/\tau_1} + a_2e^{-t/\tau_2}\right)\left(1 - r_0e^{-t/\tau_{rot}}\right) * IRF(t)$$

Fluorescence parameters

Table 1: Fluorescence decay parameters obtained under excitation at 355 nm

% PG	τ_1, ns (a_1)	τ_2, ns (a_2)	τ_{rot}, ns	r_0
0	4.26±0.31 (0.30)	2.17±0.21 (0.70)	0.21±0.05	0.24±0.02
20	3.93±0.36 (0.68)	2.43±0.19 (0.32)	0.38±0.09	0.24±0.02
40	4.37±0.28 (0.80)	2.23±0.22 (0.20)	0.75±0.18	0.23±0.02
60	4.77±0.35 (0.84)	2.15±0.18 (0.16)	1.79±0.29	0.22±0.02
80	5.10±0.34 (0.82)	2.35±0.19 (0.18)	3.41±0.35	0.23±0.02
98	5.57±0.37 (0.77)	1.90±0.18 (0.23)	7.95±0.59	0.22±0.02

Table 2: Fluorescence decay parameters obtained under excitation at 450 nm

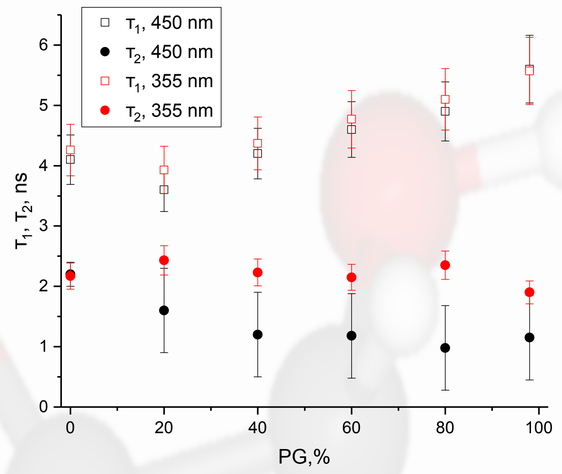
% PG	τ_1, ns (a_1)	τ_2, ns (a_2)	τ_{rot}, ns	r_0
0	4.12±0.32 (0.38)	2.21±0.15 (0.62)	0.25±0.05	0.35±0.03
20	3.65±0.35 (0.90)	1.65±0.64 (0.10)	0.48±0.08	0.34±0.03
40	4.21±0.31 (0.87)	1.21±0.66 (0.13)	0.89±0.19	0.34±0.03
60	4.62±0.38 (0.91)	1.18±0.62 (0.09)	1.70±0.25	0.35±0.03
80	4.93±0.41 (0.88)	0.98±0.71 (0.12)	3.72±0.21	0.35±0.03
98	5.61±0.45 (0.85)	1.15±0.68 (0.15)	9.10±0.52	0.33±0.03

Stokes-Einstein-Debye equation:

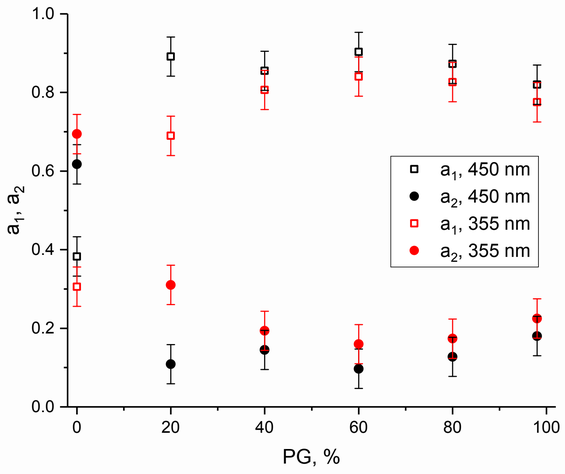
$$\tau_{rot} = fC\frac{\eta V}{kT}$$

where f is a form factor of the molecule, C is a boundary condition parameter, η is the solution viscosity, V is Van der Waals volume of the solute molecule, k is the Boltzmann constant, and T is the absolute temperature.

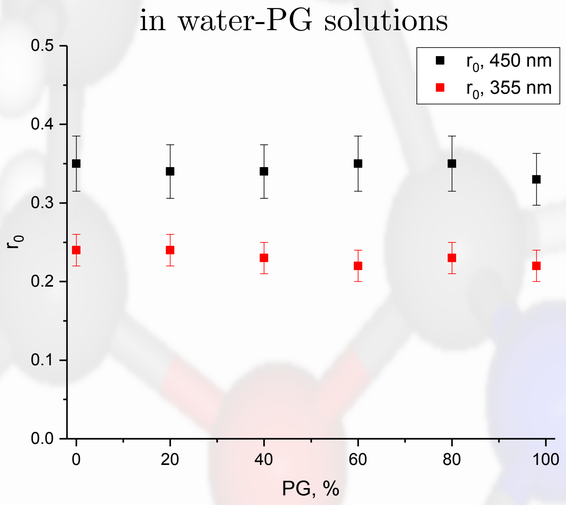
Decay times of FAD fluorescence in water-PG solutions



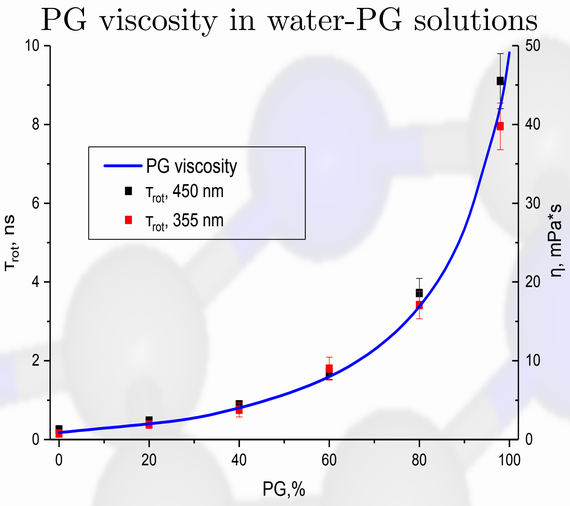
Weighting coefficient of FAD fluorescence in water-PG solutions



Anisotropy coefficient of FAD in water-PG solutions



Rotational diffusion time of FAD and PG viscosity in water-PG solutions



Conclusions

1. Fluorescence kinetics of FAD was well approximated by two exponents with characteristic fluorescence decay times τ_1 and τ_2 at all propylene glycol concentrations
2. With an increase in the concentration of PG, the decay time τ_1 increased, while the decay time τ_2 was constant within the experimental error bars. At the same time, the weight contribution of the decay time τ_1 increased.
3. The rotational diffusion time τ_{rot} was found to be directly proportional to solution viscosity in agreemint with the Stokes-Einstein-Debye equation.

* Beltukova, D. M., et al. "Polarised fluorescence in FAD excited at 355 and 450 nm in water–propylene glycol solutions." Molecular Physics (2022): e2118186.

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