The Determination of Polarized Fluorescence Parameters of FAD in Water-Methanol Solutions

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INTRODUCTION

Fluorescence kinetics of the biological cofactor flavin adenine dinucleotide (FAD) in water-methanol solutions at 0%, 20%, 40%, 60% and 80% methanol concentrations have been investigated. **Fluorescence lifetimes, corresponding weighting coefficients, anisotropy, and rotational diffusion time were determined** from experiment through analysis of the polarized fluorescence decay excited by picosecond laser pulses. The dependence of the fluorescence parameters on solution polarity and viscosity has been analyzed.

METHOD

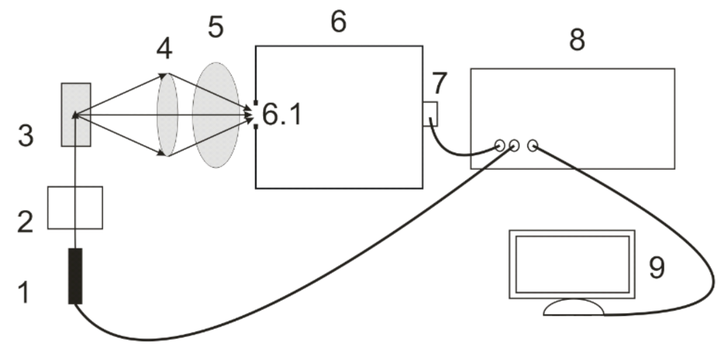

Fig. 1. Experimental setup. 1 - laser, 2 - Glan prism, 3 - cuvette with solution, 4 - lens, 5 - polarizer, 6 - monochromator, 6.1 - input slit of the monochromator, 7 - photomultiplier, 8 - TCSPC module, 9 - computer.

The setup was similar to those described in our previous studies, however had new features. In brief, a quartz cuvette containing FAD solution at the concentration of 60 μM was irradiated by a pulsed semiconductor laser at 452.5 nm. The fluorescence was detected at right angle to the laser beam and focused on the input slit of a monochromator. The polarized fluorescence parameters of FAD were previously shown to be independent of fluorescence wavelength within experimental error bars[1]. Therefore all experiments were conducted at 530 nm, close to the fluorescence intensity maximum of FAD in all solutions used. A thin-film dichroic polarizer was placed in front of the monochromator input slit to control the fluorescence polarization. The polarizer axis was alternately set to vertical and horizontal positions. The fluorescence intensity was detected by a photomultiplier operating in the photon counting mode and then processed by a time-correlated single photon counting (TCSPC) module.

RESULTS

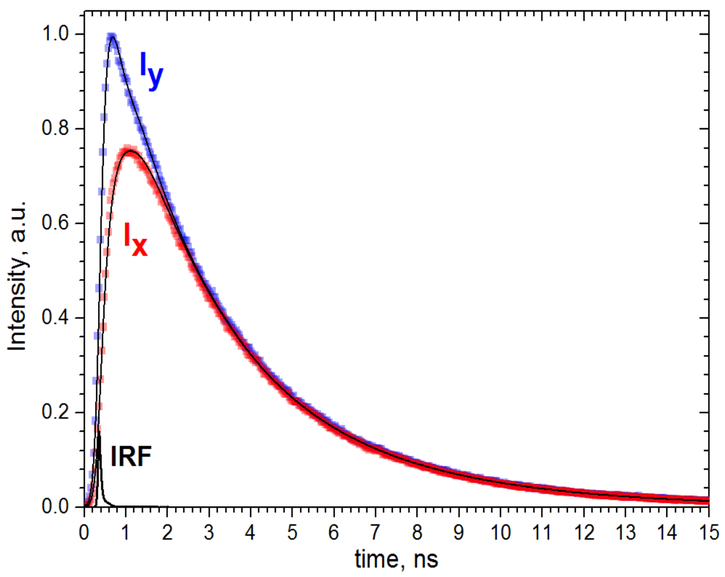

Fig. 2. Polarized fluorescence decay signals of FAD in aqueous solution at 530 nm. t = 0 is the excitation time. Symbols denote experimental data and solid curves are fits obtained with due regard to an instrument response function (IRF). The decay curves I_x and I_y are intensities of the horizontally and vertically polarized fluorescence components. The IRF shown in the left-down side has a full width at half maximum of about 320 ps.

If the excitation light is polarized along Y axis, the fluorescence intensity along Z axis can be described by the expressions[2]:

$$I_y = G \cdot I_0(t)[1 + 2r(t)] \quad (1)$$

$$I_x = I_0(t)[1 - r(t)] \quad (2)$$

where I_x and I_y are orthogonal fluorescence polarization components along x and y axes, respectively, $I_0(t)$ is an isotropic fluorescence component, G is a sensitivity ratio in the x and y recording channels, and r(t) is a fluorescence anisotropy. The obtained experimental data were processed using the global fitting procedure implemented in MATLAB. Within this procedure eqs. (1) and (2) were convoluted with IRF. As found out, the temporal dependence of the isotropic component of fluorescence radiation under the conditions of our experiment was satisfactorily fitted by a double-exponential function:

$$I_0(t) = [a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2)] \quad (3)$$

where a_1 and a_2 are weighting coefficients, and τ_1, τ_2 are fluorescence decay times. The anisotropy parameter in eqs. (1) and (2) was described by a single-exponential function:

$$r(t) = r_0 \exp(-t/\tau_{rot}) \quad (4)$$

where r(t) is the anisotropy at the moment of excitation and τ_{rot} is the rotational diffusion time.

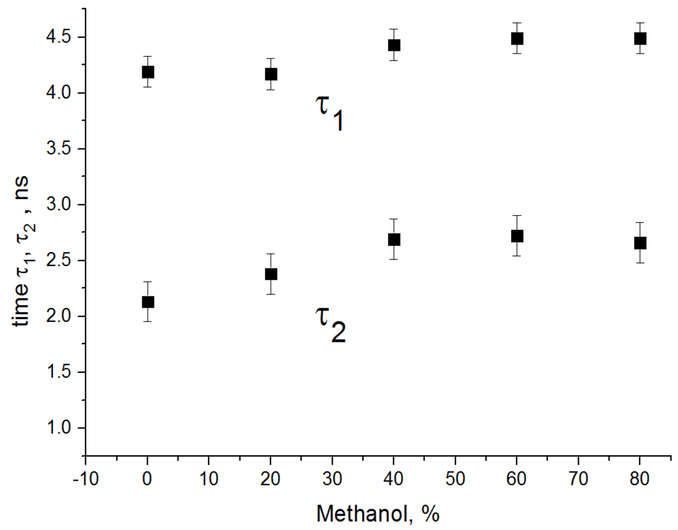

Fig. 3. FAD fluorescence decay times τ_1 and τ_2 as function of methanol concentration.

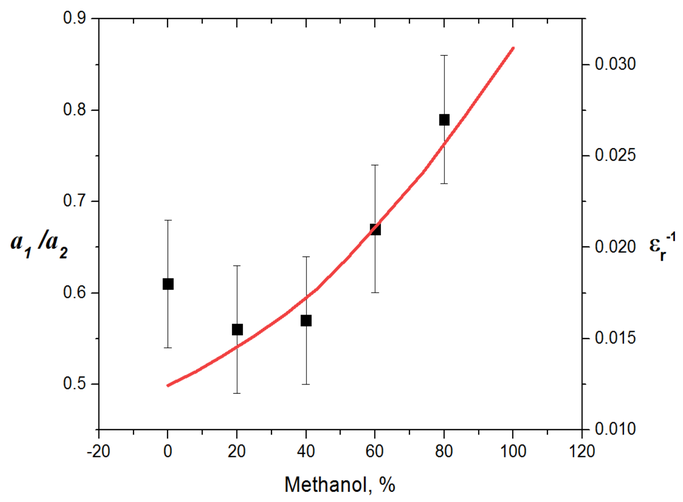

Fig. 4. Weighting coefficients ratio a_1/a_2 as function of methanol concentration. Red line shows the inverse dependence of relative dielectric permittivity ε_r on MeOH concentration.

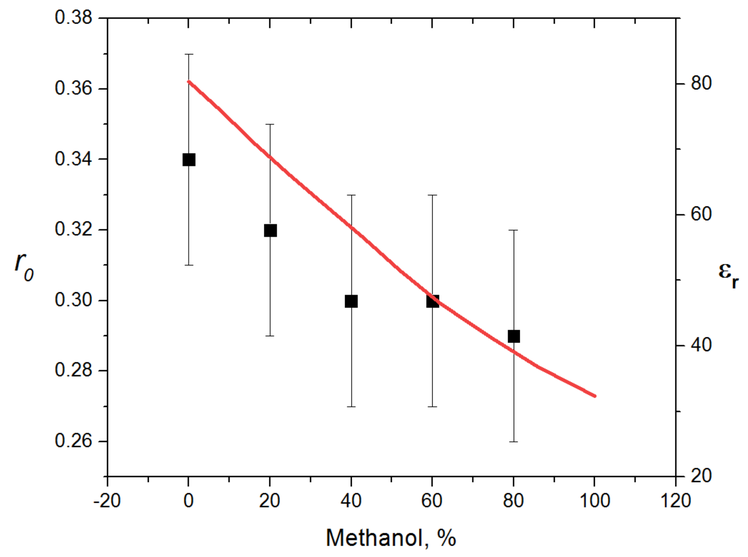

Fig. 5. FAD fluorescence anisotropy r_0 as function of MeOH concentration. Red line represents the dependence of relative dielectric permittivity ε_r of the solution on methanol concentration.

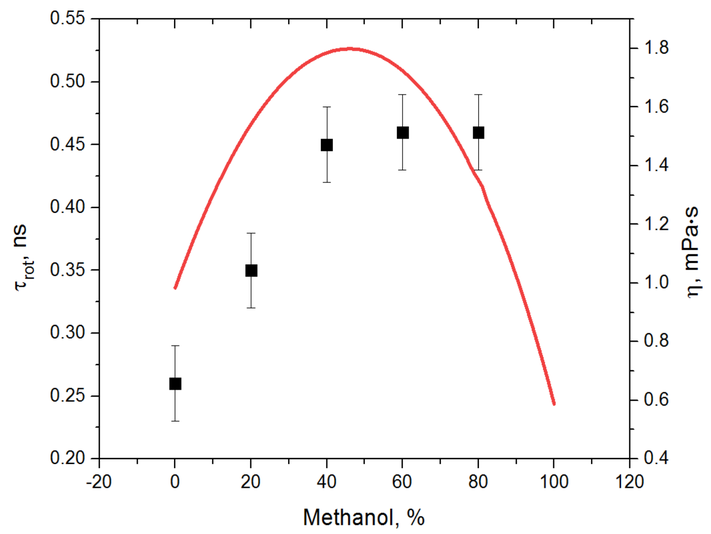

Fig. 6. FAD rotational diffusion time as function of MeOH concentration. Red curve presents solution viscosity as function of MeOH concentration.

CONCLUSIONS

1.The fluorescence kinetics demonstrated double-exponential decay providing two fluorescence lifetimes of about 2 and 4 μs.

2.Both fluorescence lifetimes demonstrated slight increase with methanol concentration up to 40% achieving plateaus at higher concentrations, while weighting coefficients ratio rose with methanol amount roughly in proportion to the inverse dielectric permittivity of the solution.

3.The fluorescence anisotropy was found to be proportional to the solution polarity within the whole range of methanol concentrations, that is likely due to the change of equilibrium nuclear configuration and an angle between the pump and fluorescence transition dipole moments depending on the methanol concentration.

4.The rotational diffusion time was proportional to the solution viscosity at lower methanol concentrations up to 40%, and reached a plateau at higher concentrations while the solution viscosity dropped down. This behavior can be due to the change of the shape factor f at high methanol concentrations.

REFERENCES

1. Krasnopevtseva M.K, Belik V.P., Semenova I.V., Smolin A.G., Bogdanov A.A. and Vasyutinskii O.S. Tech. Phys. Lett., 46, 6, 614-616 (2020).

2. Shternin P.S., Gericke K.-H., and Vasyutinskii O.S. Mol. Phys., 108(7-9):813-825 (2010).

The financial support from Russian Foundation for Basic Research under the grant #18-53-34001 is gratefully acknowledged