Resolved Fluorescence Emission Spectra of PRODAN in Ethanol/Buffer Solvents

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The fluorescence steady-state emission spectra of lipophilic fluorescence probe PRODAN in ethanol/buffer solvents of different concentrations (0.3, 0.9, 3 mol L^{-1} ethanol) were extensively studied and analytically described. The complex experimental spectra, corrected for background effects, were fitted by two Gaussian curves. The energy separation of two maxima, (0.147±0.002) eV at 37 °C and (0.143±0.003) eV at 25 °C, was independent of ethanol concentration. The blue shifts observed for both maxima were linearly dependent on solvent polarity. The linear dependences of fluorescence's intensities on PRODAN concentration in all ethanol/buffer solvents indicate that no PRODAN self-quenching takes place even at the highest measured PRODAN concentrations.

1. INTRODUCTION

The great interest in introducing molecular probe 6-propanoyl-2-dimethylaminonaphthalene, PRODAN, into investigations of membrane and biomolecular structures was supported by the strong dependence of its fluorescence behavior on the environmental polarity. This well recognized lipophilic fluorescence probe (Figure 1) is used mainly in investigations of physical and chemical properties of membranes and biological macromolecules.[1−5]

Because of the 2-dimethylamino group PRODAN locates itself on the phospholipids−water boundary,[6] and that qualifies it as a suitable reporter for the changes in the structural and dynamical properties of the macromolecular interfaces. However, for the unambiguous interpretation of the results gained in complex systems of biological macromolecular solutions the behavior of the probe molecule in the environment with well-defined characteristics must be known. Several detailed studies upon PRODAN fluorescence behavior[7,8] were preformed, and theory was argued[9,10] since PRODAN has been designed. The unanimous interpretation of PRODAN fluorescence behavior is not yet reached. According to A. Parusel[8] PRODAN in the ground state has a planar configuration with a determined dipole moment of about 5D. The absorption of energy introduces the charge transfer from dimethylamines nitrogen to the propanoylnaphthalene moiety and consequently increases the dipole moment to about 13D. His calculations predicted two excited states of the molecule, and both are of planar geometry but with induced intramolecular charge transfer. Furthermore, Parusel stated that the conformations from which PRODAN is relaxing has a twisted propanoyl group (O-TICT conformation). That conformation is specially stabilized in polar environments, and the low energy of the conformation explains a high red shift in the polar environment.[11]

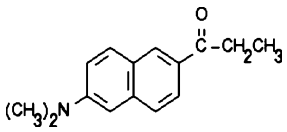

Figure 1. Structure of molecular probe PRODAN.

It is agreed that the dipole moment change upon PRODAN excitation introduces the pronounced solvent dipolar relaxation phenomena. The consequence is that PRODAN excited states energies are dependent on media polarity. For PRODAN in pure solvents it can be accurately measured by Stokes shift solvent polarity dependence.[12]

In their investigations of PRODAN relaxation T. Parasassi et al.[6] explained the complex excitation and emission spectra of PRODAN in solvents of different polarity and in phospholipids vesicles on the basis of dipolar relaxation of solvent molecules surrounding the fluorescent naphthalene moiety of the probe. They prefer this explanation to the charge transfer or rotation of side chains of the probe.

PRODAN is a very useful probe for membrane structure investigations although its steady-state spectra in the solutions of biological molecules are composed spectra. PRODAN expresses considerable partitioning and fluorescence activity in the lipid and in the buffer environment. Some authors have tried to resolve contributions from unlike environments, but mostly they have just compared the changes in the intensities and the shape of steady-state spectra. The only quantitative method we have found in the literature was the method of 3wGP by G. K. Krasnowska et al.[5] The method is based on the assessment of emission intensities in steady-state spectra at three different wavelengths. The wavelengths are chosen as the representatives of each one of the dissimilar environments of PRODAN in the complex system. The assumption which must be satisfied is that the contributions of all other environments are negligible at that wavelength.

Our goal was to follow and precisely define the fluorescent behavior of PRODAN in ethanol/buffer solvents of different polarity. The idea behind was the intention to use PRODAN

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as the extrinsic probe for following the structural and dynamical changes introduced in lipoprotein particles by different ethanol concentrations. The problem we had to face in such a complex system is that PRODAN will participate in both environments, particle and solution. In each of the environments it will be fluorescence active but probably with distinct properties. By the method of G. K. Krasnowska et al.[5] the resolution was only partially achieved because of the overlapping of spectra from two environments. Our intention was to define the parameters of the fluorescence spectrum of PRODAN in pure ethanol/buffer solvents, which could be more reliable for distinguishing the changes in the PRODAN environment. Therefore, the primary goal of the presented study was not to discuss the processes of relaxation but to determine the reliable procedure for the interpretation of spectra in structured lipoprotein solutions. Once determined, that method should be applicable to all similar biological systems.

2. MATERIALS AND METHODS

The samples of the fluorescent lipophilic membrane probe PRODAN were prepared in solutions of ethanol in 0.01 mol L^{-1} phosphate buffer with 0.2 mol L^{-1} NaCl. PRODAN dissolved in organic solvent *N*,*N*-dimethylformamide, DMF, was gradually added to the buffer until the final concentrations of 0.25; 0.5; 1.5; 2, and 3 μmol L^{-1} were achieved. The ethanol concentrations in measured samples were 0.3, 0.9, and 3 mol L^{-1}. The spectra in buffer solution without ethanol and in a 100% ethanol solution were measured to support the discussion about solvent polarity importance. The same reason was for measurements of PRODAN dissolved in DMF. All samples were prepared immediately prior to measurements and thermostated for 10 min in the prethermostated cell compartment. The PRODAN probe was used as purchased from "Molecular Probe" (Eugene, OR) without further purification. All other chemicals were purchased from "Kemika" (Zagreb, Croatia) and were p.a. grade.

The steady-state fluorescence spectra were recorded by a Carry Eclipse, Varian spectrofluorimeter. The wavelength of excitation was 360 nm for all measured samples. The emission spectra were recorded in the wavelength interval from 370 to 700 nm. Excitation and emission monochromator band-passes were kept constant at 5 nm. The amplifier voltage in all measurements was 650 V except for the absolute ethanol measurements where 600 V was applied. The excitation spectra were recorded for an emission wavelength of 520 nm in the wavelength interval from 300 to 470 nm (Figure 2). Absorption values at wavelength 360 nm, checked on Carry-Varian spectrophotometer, were lower than 0.05 for all samples, indicating that no correction for inner filter effect was necessary.[13]

The temperature of the sample compartment was kept constant within ± 0.1 °C by circulating a water bath. Because of further investigations measurements were preformed at two temperatures 25 °C and 37 °C.

The refractive indexes of used solvents were determined by standard refractometer Zeiss Opton equipped with a Na-lamp, $\lambda = 550$ nm, at a constant temperature of 25 °C. The dielectric responses of all investigated solvents were measured with a homemade capacitive chamber in conjunction with an Agilent 4294A precision impedance analyzer from 40 to 100 MHz at a temperature of 25 °C.

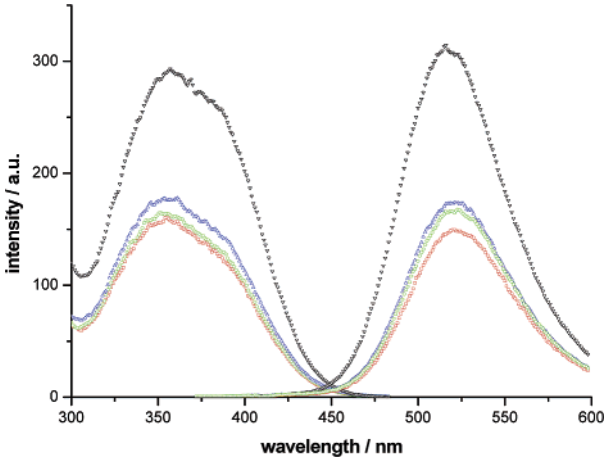

Figure 2. Excitation/emission spectra of PRODAN in ethanol/buffer solvents. Excitation spectra measured for $\lambda_{em} = 520$ nm; emission spectra for $\lambda_{ex} = 360$ nm in 0.01 mol L^{-1} phosphate buffer (red), 0.3 mol L^{-1} (green), 0.9 mol L^{-1} (blue), and 3 mol L^{-1} ethanol (black); temperature 25 °C. $c_{prodan} = 0.5$ μmol L^{-1}.

3. SPECTRA ANALYSIS

The PRODAN fluorescence emission spectra were corrected for background contribution and the Raman band at 410 nm which is measurable in water for 360 nm excitation. The spectra of adequate solvents without PRODAN were subtracted from experimental PRODAN solution spectra using a simple subtraction program, a part of the Microcal Origin 6.1 professional.

The PRODAN steady-state emission spectra retained the complex structure after correction. Therefore, the steady-state emission spectra of PRODAN were analyzed on asymmetry by a simple method. Two rates at half-height of the spectra were compared: $|(\lambda_{max} - \lambda_1)/\Delta\lambda|$ and $|(\lambda_{max} - \lambda_2/\Delta\lambda|$, where λ_{max} determines the wavelength of the center of gravity, λ_1 and λ_2 the end wavelengths of line at the half-height, and $\Delta\lambda = |\lambda_2 - \lambda_1|$ at the line width. The difference of the two was greater than 10% for all recorded spectra. That proves the asymmetry of the line. Based on that finding and on data found in the literature about two transitions in absorption spectra,[9] we simulated the steady-state emission spectra with two Gaussian curves. The theoretical simulation is presented in Figure 3. The accuracy of this simulation was checked by calculating the fit parameter for all measured samples.[14] If the value of

$$\text{fit} = 1 - \frac{\sum (f_{ex} - f_t)^2}{\sum (f_t - \bar{f})^2}$$

equals 1 the model is supported. The f_{ex} and f_t are measured and calculated intensities, respectively, and $\bar{f}$ is a mean intensity value. The requirement for the good simulation is satisfied within 2% for all measured samples.

According to this result the model of two fluorescence transitions, described by two Gaussian curves, was applied in further analysis of the PRODAN spectra. The experimental data were fitted with the sum of two Gaussian functions

$$Y = A_1 e^{-(\lambda-\lambda_1)^2/2\omega_1^2} + A_2 e^{-(\lambda-\lambda_2)^2/2\omega_2^2} \qquad (1)$$

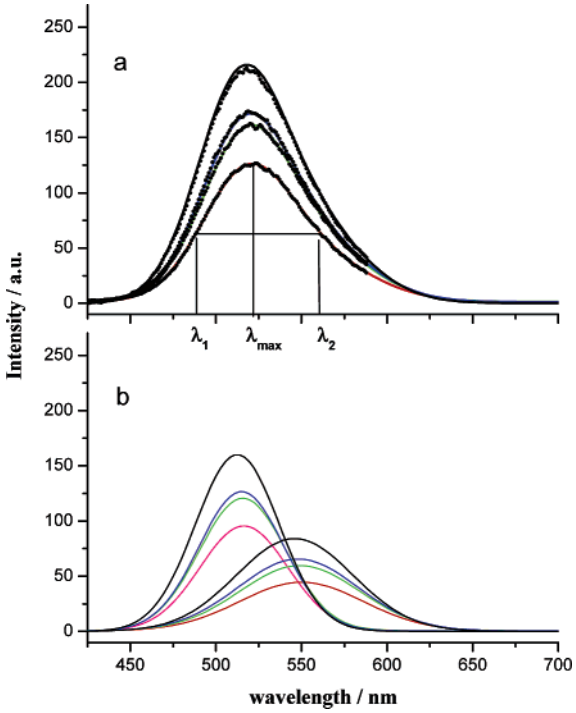

Figure 3. Steady-state spectra of PRODAN in ethanol/buffer solvents. $c_{prodan} = 0.5\ \mu$mol L^{-1} (a) theoretical simulations according to eq 1 of corrected experimental data for PRODAN in 0 mol L^{-1} (red), 0.3 mol L^{-1} (green), 0.9 mol L^{-1} (blue), and 3 mol L^{-1} (black) ethanol at 37 °C and (b) the spectra resolved into two Gaussian curves achieved by theoretical analysis of experimental data (the same kind of presentation is preserved).

Table 1. Polarity, Δf, Calculated for Ethanol/Buffer Solvents

$c_{ethanol}$/mol L^{-1}	n	ϵ	Δf
0	1.3323	77.85	0.3200
0.3	1.3331	76.03	0.3195
0.9	1.3346	74.68	0.3188
3	1.3418	69.87	0.3153
17.7	1.3604	24.33	0.2887

where A_1, A_2 and λ_1, λ_2 are maximal fluorescence intensities and corresponding wavelengths of two maxima, respectively. ω_1 and ω_2 are related to the half-width $\omega(1)$ and $\omega(2)$ of curves by

$$\omega_i = \frac{\omega(i)}{2\sqrt{\ln 4}} \tag{2}$$

The fitting procedure was performed by adjusted computer program Microcal Origin 6.1 Professional.

The polarity of all used ethanol solvents were calculated according to eq 3[13] after the refractive indexes and dielectric constants had been determined independently.

$$\Delta f = \frac{\epsilon - 1}{2\epsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \tag{3}$$

The results of calculations are presented in Table 1.

4. RESULTS AND DISCUSSION

In this work we investigated the sensitivity of PRODAN fluorescence emission spectra parameters to the microenvironment polarity in the ethanol/buffer solvents of different concentrations. The fluorescence characteristics of PRODAN in pure solvents are presented in Table 2. The result indicates a significant blue shift of the center of gravity with a decreasing polarity of the solvent which is in accordance with data from the literature.[6]

Figure 2 presents the excitation/emission spectra of PRODAN in 0.01 mol L^{-1} phosphate buffer of different ethanol concentrations indicating the influence of the increasing ethanol concentration on PRODAN fluorescence behavior. Two transitions are clearly visible in the absorption spectra and are more pronounced in a less polar environment. The asymmetry in the fluorescence emission spectra is less pronounced. The difference in the asymmetry of absorption and emission spectra could be argued in terms of different transitions involved in both processes, but it is beyond the present work. For both processes the titration data indicate that the center of gravity position is dependent on ethanol concentration which is in accordance with the data from the literature.[9]

Steady-state emission spectra were recorded as a function of ethanol and PRODAN concentrations at 25 °C and 37 °C. The decomposition of experimental emission data at 37 °C, corrected for background contribution, in terms of two Gaussian type spectral curves, eq 1, is presented in Figure 3 and in Table 3 at 25 °C and Table 4 at 37 °C. It has to be stressed that the theoretical curves simulate the experiment data precisely. The center of gravity position dependence on ethanol concentration is not dramatic. The red shift measured in buffer compared to the Stokes shift in 3 mol L^{-1} ethanol is 3−5 nm. The change in polarity of two solvents is only 1.5%, which would explain the observed behavior. The wavelengths of two calculated maxima are ethanol concentration dependent, but interestingly enough the difference between them is concentration independent. To rationalize that, it is reasonable to predict that the excited states of PRODAN would not change within the range of solvent polarity used in these investigations. In less polar solvents the complexity of the PRODAN spectra was more pronounced. The increase of intensity with a decrease of solvent polarity indicates the solution molecules influence on the excited state of PRODAN.

Table 2. Fluorescence Emission Parameters of PRODAN in Solvents of Different Polarity[c]

	emission maximum/nm		maximal intensity/a.u.		full width at half-maximum/nm		
solvent	25 °C	37 °C	25 °C	37 °C	25 °C	37 °C	polarity
0% ethanol	522.92	521.58	68.75	58.14	69.94	70.44	0.3200[a]
100% ethanol	488.55	486.48	713.9	678.92	68.33	68.21	0.2887[a]
DMF	458.89	459.85	979.82	907.05	60.14	61.34	0.2743[b]

[a] Data from Table 1. [b] Calculated according to the eq 3 with the data for the refractive index and dielectric constant from literature. [c] Excitation wavelength 360 nm.

Table 3. Fitting Parameters for the Emission Spectra of PRODAN in Solutions of Different Ethanol Concentrations at 25 °C

	experimental spectra			decomposed spectra					
sample	λ_{max}/nm	A_{max}/a.u.	w_{max}/nm	λ_1/nm	A_1/a.u.	w_1/nm	λ_2/nm	A_2/a.u.	w_2/nm
0 M ethanol	522.39	149.47	71.7	517.1 ± 0.2	110 ± 6	57.9 ± 0.7	550 ± 3	56 ± 4	82 ± 2
0.3 M ethanol	521.82	166.63	70.86	516.7 ± 0.2	122 ± 7	57.7 ± 0.7	549 ± 3	62 ± 5	82.4 ± 2
0.9 M ethanol	521.17	173.48	71.83	516 ± 0.2	126 ± 7	57.2 ± 0.7	548 ± 3	66 ± 4	82.4 ± 2
3 M ethanol	517.12	308.76	73.51	512 ± 0.2	230 ± 10	57.2 ± 0.7	545 ± 3	115 ± 7	81 ± 2

[a] In terms of two Gaussian type curves according to eq 1.

Table 4. Fitting Parameters for the Emission Spectra of PRODAN in Solutions of Different Ethanol Concentrations at 37 °C

	experimental spectra			decomposed spectra[a]					
sample	λ_{max}/nm	A_{max}/a.u.	w_{max}/nm	λ_1/nm	A_1/a.u.	w_1/nm	λ_2/nm	A_2/a.u.	w_2/nm
0 M ethanol	521.39	126.4	71.83	516.4 ± 0.3	95 ± 6	60.3 ± 0.9	550 ± 4	44 ± 4	85 ± 2
0.3 M ethanol	521.04	161.54	73.51	515.7 ± 0.3	120 ± 9	59.5 ± 0.9	549 ± 4	59 ± 6	82.4 ± 2
0.9 M ethanol	520.37	171.88	70.98	515 ± 0.3	126 ± 8	58.4 ± 0.9	548 ± 3	65 ± 5	82.4 ± 2
3 M ethanol	518.07	216.06	72.67	512.5 ± 0.4	160 ± 10	57.9 ± 0.9	546 ± 4	84 ± 8	80 ± 5

[a] In terms of two Gaussian type curves according to eq 1.

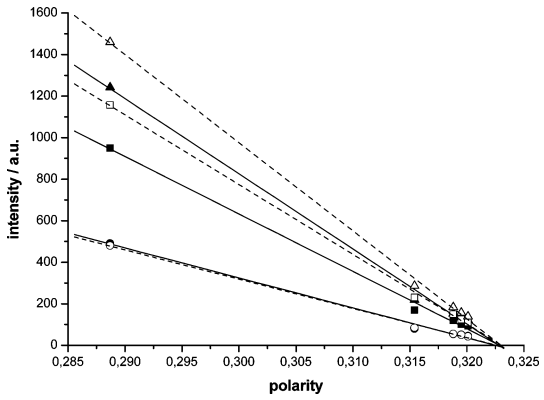

Figure 4. PRODAN fluorescence emission intensities of center of gravity (▲,△) and two calculated bands, λ_{em} = 517 nm (■,□) and λ_{em} = 550 nm (●,○), in dependence on solvent polarity, Δf, at 25 °C (△, □,○) and 37 °C (▲,■,●). Solid lines (25 °C) and dashed lines (37 °C) are calculated linear functions.

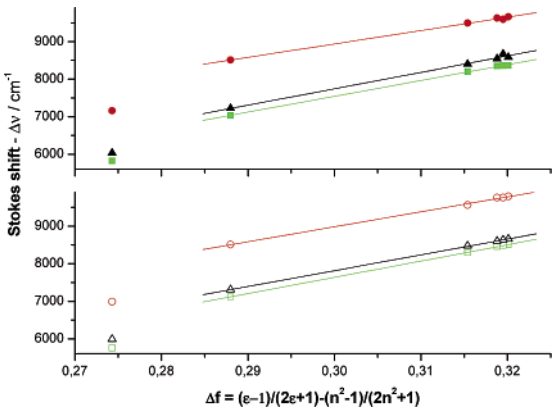

Figure 5. Stokes shift, $\Delta\nu$, of PRODAN for center of gravity (▲,△, black line) and two calculated maxima, λ_{em} = 517 nm (■,□, green line) and λ_{em} = 550 nm (●,○, red line), in dependence on solvent polarity, Δf, at 25 °C (△,□,○) and 37 °C (▲,■,●). c_{prodan} = 0.5 μmol L^{-1}.

In Tables 3 and 4 the parameters of the PRODAN spectra in solvents of different polarity on two temperatures are presented. The increase in temperature has a negligible influence on the transition energy (λ of the center of gravity changes by 1 nm) but decreases the intensity of the fluorescence. The decrease in solvent polarity induces the blue shift to both calculated maxima, but the energy difference between them stays constant. This energy difference is slightly temperature dependent (0.143±0.003) eV at 25 °C and (0.147±0.002) eV at 37 °C.

Further, the influence of ethanol concentration on the PRODAN fluorescence intensity was determined. The intensities of the center of gravity and the two calculated maxima as functions of solvent polarity, Δf, are presented in Figure 4.

All emission intensities are linearily decreasing with increasing polarity. In the concentration range up to 3 mol L^{-1} of ethanol (which is the concentration of interest in biological investigations) the decrease in solvent polarity is only 1.5%. However, this small change doubles the intensity. The slope of the best-fit for the center of gravity and λ_1 = 516.4 nm at 25 °C is steeper than at 37 °C, while it is temperature independent for the band at 550 nm. At the same time the intensity change on 516.4 nm maximum (40%) is higher than for maximum at 550 nm (32.2%).

Figure 5 illustrates the linear relationship of the Stokes shift and solvent polarity. The behavior is the same at both temperatures. Furthermore, the dependence on temperature is less than 3%. The couple of linear functions representing the dependence of the Stokes shift of three maxima on solvents polarity at two temperatures are nearly identical. The difference in Stokes shifts of PRODAN in buffer and in absolute ethanol has the same blue shift of (1153±20) cm^{-1} for all three maxima. This fact is very strong evidence that the energy difference between the bands is independent of changes in solution polarity. That allows the conclusion that ethanol did not introduce changes in the structure of PRODAN molecule. The fact that the PRODAN structure is not affected by ethanol is in favor of the prediction that the PRODAN fluorescence emission is mainly governed by relaxation over the solvent molecules and not over the intramolecular charge transfer.[8] Therefore the Stokes shift could be a reliable parameter for determination of ethanol induced changes in apolar regions in lipoproteins.

The Stokes shift for apolar DMF does not satisfy the calculated linear function in Figure 5. A smaller value is in

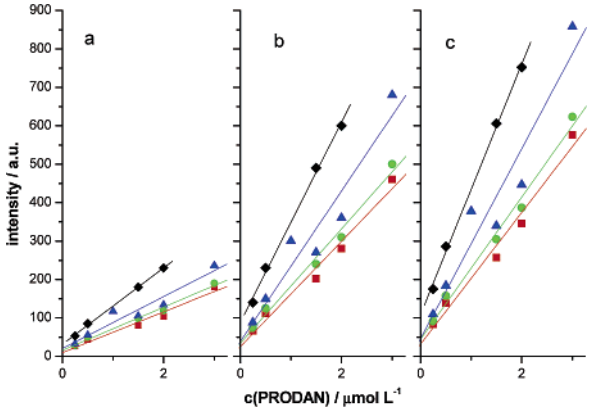

Figure 6. The dependence of fluorescence emission intensities on PRODAN concentration for (a) $\lambda_{em} = 519$ nm, (b) $\lambda_{em} = 550$ nm, and (c) center of gravity. Experimental points for PRODAN in 0 mol L^{-1} (■, red), 0.3 mol L^{-1} (●, green), 0.9 mol L^{-1} (▲, blue), and 3 mol L^{-1} (▼, black) ethanol solutions.

agreement with the results obtained from the Lippert−Mataga plot[8] of PRODAN.

Steady-state spectra for solutions of variable PRODAN concentrations, 0.25, 0.5, 1.5, 2, and 3 μmol L^{-1} in 0.3, 0.9, and 3 mol L^{-1} ethanol solvents, were measured (Figure 6). The wavelengths of all emission maxima are PRODAN concentration independent. The slope of linear functions, relating the dependence of fluorescence emission intensity to PRODAN concentration, increases with an increasing ethanol concentration (decreasing polarity). The linear dependence of the intensity upon the PRODAN concentration would support the conclusion that there was no PRODAN/PRODAN dipolar relaxation process, meaning no self-quenching is present. This is an important conclusion when deciding to use the dye in biological molecules research. Because of its lipophilicity, PRODAN will partition more in the hydrophobic milieu of the biological molecules and therefore increase the concentration in the membrane compared to one in the solvent.[15] It is absolutely necessary to avoid self-quenching of the PRODAN molecules to get a relevant conclusion from the changes in spectra intensities.[16]

The half-width of the spectral bands, $\omega_1 = (57.2 \pm 0.9)$ nm and $\omega_2 = (81 \pm 3)$ nm, are PRODAN concentration as well as ethanol concentration independent in the range of measured ethanol concentrations. There is only a 7% decrease from buffer to absolute ethanol solvent. The measured decrease of only 0.24% for 3 mol L^{-1} ethanol solution allows us to consider it constant for that range of solution polarity differences.

The line width of measured steady-state emission spectra are wide which is expected when the spectral relaxation is the process of emission.[13] The increase of half-width is the consequence of the emission from the partly relaxed state.[17]

The purpose of this research to quantify the steady-state emission spectra of PRODAN and to provide reliable parameters for the decomposition of complex spectra of PRODAN in biological molecules solutions is fulfilled. The linearity in dependence of all measured parameters with the increase of ethanol concentration (decrease of solution polarity) would justify the subtraction of the PRODAN spectra in solvents from spectra in biological molecules solutions. The calculated spectra would then illustrate the behavior of PRODAN in a lipid environment and could be used to identify the changes in the lipid molecular structure.

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REFERENCES AND NOTES

(1) Mcgregor, R. B.; Weber, G. Estimation of the polarity of the protein interior by optical spectroscopy. *Nature* **1986**, *319*, 70−73.
(2) Chong, P. L. G. Effects of Hydrostatic Pressure on the Location of PRODAN in Lipid Bilayers and Cellular Membranes. *Biochemistry* **1988**, *27*, 399−404.
(3) Hutterer, R.; Parusel, A. B. J.; Hof, M. Solvent Relaxation of Prodan and Patman; A Useful Tool for the Determination of Polarity and Rigidity Changes in Membranes. *J. Fluoresc*. **1998**, *8*, 389−393.
(4) Hutterer, R.; Schneider, F. W.; Hermens, W. Th.; Wagenvoord, R.; Hof, M. Binding of prothrombin and its fragment 1 to phospholipid membranes studied by the solvent relaxation technique. *Biochim. Biophys. Acta* **1998**, *1414*, 155−164.
(5) Krasnowska, E. K.; Bagatolli, L. A.; Gratton, E.; Parasassi, T. Surface properties of cholesterol-containing membranes detected by Prodan fluorescence. *Biochim. Biophys. Acta* **2001**, *1511*, 330−340.
(6) Parasassi, T.; Krasnowska, E. K.; Bagatolli, L.; Gratton, E. Laurdan and Prodan as Polarity-Sensitive Fluorescent Membrane Probes. *J. Fluoresc.* **1998**, *8*, 365−373.
(7) Ilich, P.; Prendergast, F. G. Singlet Adiabatic States of Solvated PRODAN: A Semiempirical Molecular Orbital Study. *J. Phys. Chem.* **1989**, *93*, 4441−4447.
(8) Parusel, A. Semienpirical studies of solvent effects on the intramolecular charge transfer to the fluorescence probe PRODAN. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 2923−2927.
(9) Parusel, A. B. J.; Nowak, W.; Grimme, S.; Köhler G. Comparative Theoretical Study on Charge-Transfer Fluorescence Probes: 6-Propanoyl-2-(N, N-dimethylamino)naphthalene and Derivatives. *J. Phys. Chem*. **1998**, *102*, 7149−7156.
(10) Parusel, A. B. J.; Schneider, F. W.; Köhler, G. An ab initio study on excited and ground-state properties of the organic fluorescence probe PRODAN. *J. Mol. Struct. (THEOCHEM)* **1997**, *398*−*399*, 341−346.
(11) Sengupta, B.; Guharay, J.; Sengupta, P. K. Characterization of the fluorescence emission properties of prodan in different reverse micellar environments. *Spectrochim. Acta, Part A* **2000**, *56*, 1433−1441.
(12) Kawski, A. Ground- and Excited-State Dipole moments of 6-Propionyl-2-(dimethylamino)naphthalene Determined from Solvatochromic Shifts. *Naturforsch.* **1999**, *54a*, 379−381.
(13) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic/Plenum Press: New York, 1999.
(14) Pompei, F.; Wilson, R. Age distribution of cancer: the incidence turnover at old age. *Hum. Ecol. Risk Assess.* **2001**, *7* (6), 1619−1650.
(15) Krasnowska, E. K.; Gratton, E.; Parasassi, T. Prodan as a Membrane Surface Fluorescence Probe: Partitioning between Water and Phospholipid Phases. *Biophys. J.* **1998**, *74*, 1984−1993.
(16) Al-Hassan, K. A.; Khanfer, M. F. Fluorescence Probes for Cyclodextrin Interiors. *J. Fluoresc*. **1998**, *8*, 139−152.
(17) Samanta, A.; Fessenden, R. W. Excited-State Dipole Moment of PRODAN as Determined from Transient Dielectric Loss Measurements. *J. Phys. Chem. A* **2000**, *104*, 8972−8975.

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