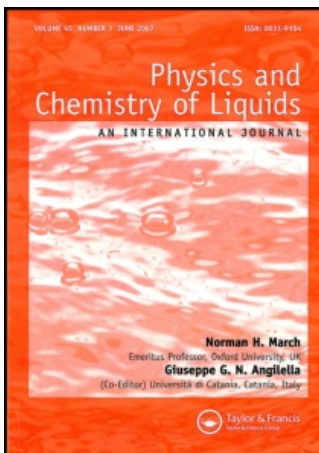


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L. Cid^a; B. Ginzberg^a; P. A. Sorichetti^a

^a Facultad de Ingeniería, Laboratorio de Sistemas Líquidos, Universidad de Buenos Aires, Argentina

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Analysis of fluorescence spectra of PRODAN in protic solvents as the superposition of two Gaussian functions

L. CID, B. GINZBERG and P. A. SORICHETTI*

Facultad de Ingeniería, Laboratorio de Sistemas Líquidos,
Universidad de Buenos Aires, Buenos Aires, Argentina

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The steady-state fluorescence emission spectra of 6-propionyl-2-dimethylaminonaphthalene (PRODAN) in aqueous solutions of methanol, ethanol and 1-propanol were studied in a wide range of concentrations. The fluorescence of PRODAN was also studied in aqueous solutions (from 3×10^{-3} to 21×10^{-3} mole L⁻¹) of dodecyltrimethylammonium bromide (DTAB), a micelle-forming ionic surfactant. In all cases, experimental data, as a function of wavelength, are very well fitted by the sum of two Gaussian functions. In alcohol solutions, the maximum of lower wavelength depends linearly both on concentration and polarizability. In DTAB solutions, the maximum of higher wavelength varies smoothly with concentration, but the lower wavelength maximum changes abruptly at the Critical Micellar Concentration. Results are explained by assuming that the fluorescence emission of PRODAN is originated in transitions from two excited states which are close in energy but have dissimilar interactions with the probe environment.

Keywords: PRODAN; Stokes shift; Emission spectra; Alcohol–water solutions; Amphiphile

1. Introduction

A thorough understanding of the interactions that exist among solvent molecules is a subject not fully solved neither by the condensed matter Physics nor by Biophysics. In fact, solvents can introduce significant changes in the geometric and electronic structure of solutes ('solvent effect').

Solvent polarity can even revert the stability of different configurations of solutes. Even in non-polar solvents, the reactivity in general and in particular the reactive sites of a molecule can be affected by the solute–solvent interactions.

Likewise, the kinetics of chemical reactions, the thermodynamic parameters, and also the organic and biochemical reactions and photo-physical processes generally depend on the solvent.

*Corresponding author. Tel.: 54 11 4343-0891 Ext: 214/216. Fax: 54 11 4331-1852, 54 11 4331-9877.
Email: psorich@fi.uba.ar

These interactions were studied by numerous authors, as it is described in textbooks of fluorescence [1], considering the solvent as a continuous dielectric medium without a specific structure. In this model solutes are embedded in a spherical cavity with a radius called 'radius of Onsager' and the non-specific interactions are characterized by two macroscopic properties of the solvent: the dielectric constant (ϵ) and the refractive index (n).

If the solute is an organic substance with photo-physical properties, also called a probe, when excited by photon absorption there is an increase in the dipole moment with respect to the fundamental (ground) state. The increase in the dipole moment disturbs the surrounding environment. The solvent responds to these changes by reorganizing its molecules around the probe (relaxation of the solvent). Therefore, if the emission from the solvent can be neglected, the emission spectrum of the probe can be used to measure the solvent polarity. Consequently, fluorescence spectroscopy is one of the most useful techniques to study solvent polarity and solution physical properties.

The 'solvent effect' in the fluorescence spectra, on the basis of Onsager model, was described by Lippert and Mataga [2]. The Lippert–Mataga equation is widely used, since it describes satisfactorily many systems. However, it has certain limitations, specially with solvents, which form hydrogen bonds, since their treatment neglects the correlations between solvent molecules. It is for this reason that the solvent empirical scales of polarity [3] and semi empirical equations [4] were developed, to describe these situations.

The fluorescence emission spectra of the probe, 6-propionyl-2-dimethylamino-naphthalene (PRODAN) at room temperature shows a noticeable red shift with respect to the excitation radiation. This displacement, called Stokes shift, is the result of the energy loss of the probe (with respect to the absorbed energy) that takes place in the fluorescent emission [5]. This is produced by dynamic processes like vibrational energy dissipation, reorientation of solvent molecules around the excited dipole, redistribution of electrons in solvent molecules due to the dipole moment altered by the excited probe and to the probe–solvent interactions.

In this work, the PRODAN probe was selected for two reasons: (i) due to its donor–acceptor structure, it displays a significant Stokes shift in polar solvents and (ii) it shows a non-specific interaction with solvent molecules [6]. Therefore, it is considered a highly efficient fluorescent probe [7], widely used in biological studies [8, 9].

The molecular probe PRODAN is frequently used in biology as a polarity probe by measuring the shift of the wavelength corresponding to the observed maximum of the fluorescent emission spectrum. However, it is well-known that the observed values of the spectral shift in water and pure alcohols, as in other solvents with groups capable of forming hydrogen bonds, depart from the predictions of the Lippert–Mataga equation [2]. Finally, the fluorescence intensity, as a function of wavelength, is non-symmetrical with respect to the maximum, as it is shown by a careful analysis of the shape of the curve (in particular, the third-order moment).

It must be remarked that the possibility of fluorescent emission of PRODAN by two excited levels of close energies has been outlined by Balter [10] based on Lakowicz measurements. In this context, it is worth mentioning the computational results of Nowak *et al.* [11], by means of the CNDOS method and of Parusel *et al.* [12], with the DFT/SCI method and the AMI/CISD Hamiltonian. One of the two configurations of the excited state is more susceptible to 'hydrogen bond' interactions between solvent molecules [10]. Likewise, the paper of Raguz [13] reveals that the emission spectrum

of PRODAN in diluted ethanol–water solutions is very adequately described by a sum of two Gaussian functions.

In the present work, the steady-state fluorescent emission of PRODAN was studied in aqueous solutions of methanol, ethanol and 1-propanol, in a wide range of concentrations. It must be remarked that alcohol–water systems are particularly interesting since they may be considered the simplest prototype of biomolecular and micelle-forming systems [14].

The study was then extended to aqueous solutions of a more complex amphiphile, dodecyl trimethylammonium bromide (DTAB). This substance is a cationic surfactant used in many fields, such as isolation of membrane bound proteins, polymer solubilization and conformational changes of biopolymers [15]. The molecule of the surfactant is formed by a polar part, called head, and a hydrocarbon part named tail. It is important to remark that at a characteristic concentration around $16 \times 10^{-3} \text{ mole L}^{-1}$, indicated as the critical micellar concentration (CMC), DTAB molecules self-aggregate, forming structures known as micelles. This is an important difference with respect to the alcohol solutions, which are homogeneous at all the concentrations. As PRODAN is a polar molecule, it is located preferably near the surfactant head. Usually, the value of the maximum of fluorescent emission of PRODAN, λ_{max} , as a function of surfactant concentration is used to describe the evolution of the molecular aggregates, because its value diminishes steeply at the CMC.

For all the studied systems, the experimental values of the intensity of the fluorescence emission, as a function of wavelength, were fitted to the sum of two Gaussian functions, following the procedure of Raguz [13]. In all cases the fitting gave excellent results, as indicated by Chi-Square goodness-of-fit statistics. Moreover, the dependence of the wavelength of the maxima of the two Gaussian functions with respect to solvent properties was studied. Results show that there are significant differences in the behaviour of the two maxima, and that analysis of the PRODAN fluorescence spectra as the superposition of two Gaussian peaks provides a clearer understanding of its behaviour as a photo-physical probe.

2. Experimental

2.1. Materials

The alcohol–water solutions were prepared by weight from methanol and ethanol (Merck, pro analysis) and 1-propanol (Mallinckrodt, Analytical Grade). In all cases, deionized water was used (conductivity below $0.1 \mu\text{S cm}^{-1}$, filtered with a Millipore® filter). The aqueous solutions were prepared with mass concentrations of 0, 20, 40, 60, 80 and 100% w/w of each alcohol.

The aqueous solutions of the cationic surfactant have been prepared by weighting the appropriate amounts of DTAB, Sigma and adding the necessary amount of deionized water. DTAB ($M_w = 308.35 \text{ g mol}^{-1}$) was used, without further purification. The solutions were prepared in a wide range of concentrations: 3, 6, 9, 10, 12, 14, 15, 17, 19 and 21 mM ($10^{-3} \text{ mole L}^{-1}$).

The fluorescence probe, PRODAN, was used as received.

All measurements were made at a temperature $25^\circ\text{C} \pm 0.1^\circ\text{C}$ unless otherwise indicated.

2.2. Methods

2.2.1. Steady-state fluorescence spectroscopy. Fluorescence measurements were carried out on a PTI fluorimeter in a 90° configuration. PRODAN was added to the different alcohol–water systems immediately before measurements. In all cases the probe concentration was adjusted to achieve an absorbance value of 0.05 in order to avoid the formation of dimers and self-absorption. Emission spectra were taken with excitation wavelengths of 360 nm for PRODAN.

2.2.2. UV-visible absorption spectroscopy. The absorption spectra were taken by a double-beam Shimadzu spectrophotometer UV 2401 PC.

2.2.3. Index of refraction. The refractive indexes of the systems were measured by means of an Abbe Refractometer Type 1T, 4T Atago. The estimated relative uncertainty of the experimental values is less than 0.2%.

2.2.4. Static permittivity measurements. The static permittivity values at 25°C was obtained from measurements in the range from 100–15,700 Hz with a RLC meter Topward 5100. We have used a measurement cell with Platinum electrodes (Parsec, Argentina), calibrated with reference liquids (methanol, carbon tetrachloride). The estimated relative uncertainty of the static permittivity is less than 2%.

3. Treatment of experimental data

The PRODAN steady-state emission spectra are usually analyzed taking into account that the wavelength corresponding to the observed maximum emission, $\lambda_{I\max}$.

On the basis of the previous research performed by Raguz [13] for ethanol–water solutions at low concentrations, the model of two fluorescence transitions described by two Gaussian curves was applied in the analysis of the PRODAN spectra. The experimental data of intensity as a function of wavelength, $I(\lambda)$, were fitted with the sum of two Gaussian functions according to the following expression:

$$I(\lambda) = A_1 e^{-\left[\frac{\lambda - \lambda_{p1}}{2\omega_1}\right]^2} + A_2 e^{-\left[\frac{\lambda - \lambda_{p2}}{2\omega_2}\right]^2}. \quad (1)$$

The fitting parameters are the wavelengths of the maxima (λ_{p1} and λ_{p2}), the half-swifths (ω_1 and ω_2) and the peak intensities (A_1 and A_2). The maximum of lower wavelength is indicated as λ_{p1} .

The fitting procedure was performed for all the systems studied in this work, using the computer program Microcal Origin 5.0. As an example, the experimental data, together with the fitting, are given by equation (1) and the two Gaussian functions used in the fitting, for a solution of 60% w/w of ethanol, are shown in figure 1. It is easy to see that there is a noticeable difference between the wavelength corresponding to the maximum of the experimental data ($\lambda_{I\max}$) and the wavelength of the maxima of the Gaussian functions (λ_{p1} and λ_{p2}) in equation (1).

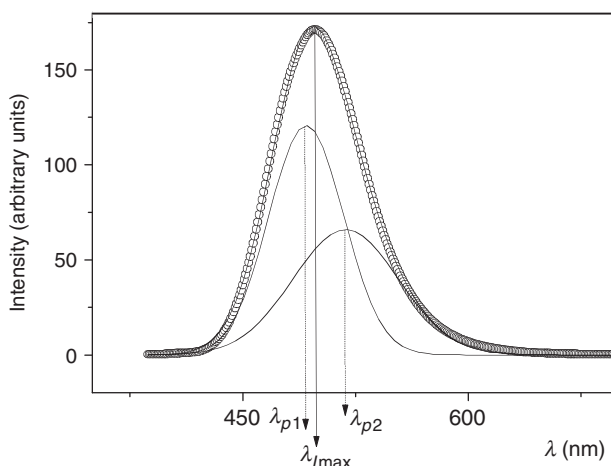


Figure 1. Emission spectra of PRODAN in a 60% W/W solution of ethanol in water. Symbols: Experimental data. Lines: Best fit by the sum of two Gaussian functions according to equation (1).

4. Experimental results

The same procedure was carried out for the aqueous solutions of all the three alcohols studied in this work. The fitting parameters for all the alcohol–water solutions are given in tables 1–3. Parameters are given together with the half-width of the 95% confidence interval, for instance, the 95% confidence interval for the lower wavelength maximum (λ_{p1}) is $[\lambda_{p1} - \Delta\lambda_{p1}, \lambda_{p1} + \Delta\lambda_{p1}]$.

For all the solutions studied, the fit of equation (1) is quite good, as indicated by the small relative width of the confidence intervals and the high value of the coefficient of determination (R^2), since in all cases, $R^2 > 0.993$.

From the results shown in tables 1–3 the dependence of λ_{p1} and λ_{p2} as a function of the alcohol mass concentration, C , was analyzed. For aqueous solutions of methanol, ethanol and 1-propanol, the position of the lower wavelength maximum (λ_{p1}) can be accurately fitted as a linear function of the mass concentration, C , of the solution, with ($R^2 > 0.995$). On the contrary, the dependence on C , of the higher wavelength maximum (λ_{p2}) clearly departs from linearity. In fact, the shift of λ_{p2} as a function of mass concentration may be roughly described as having three distinct regions: alcohol-rich, intermediate and water-rich. Results are plotted in figure 2(a), (b) and (c), respectively, including the best linear fit and R^2 coefficient.

The index of refraction (n) and static dielectric constant (ϵ) of the different solutions were measured. From the experimental values, the polarizability (Δf) was calculated according to the following equation:

$$\Delta f = \frac{2^*(\epsilon - 1)}{2^*\epsilon + 1} - \frac{2^*(n^2 - 1)}{2^*n^2 + 1}. \quad (2)$$

The results for all the solutions are given in tables 4–6. From the uncertainties in the experimental values, the relative uncertainty of the polarizability is estimated to be less than 2%.

Table 1. Fitting parameters for the fluorescence spectra of PRODAN in methanol–water solutions.

	C [%W/W]					
	0	20	40	60	80	100
λ_{p1} [nm]	517.1	512.3	506.6	501.4	493.9	488.6
$\Delta\lambda_{p1}$ [nm]	0.3	0.1	0.2	0.3	0.3	0.3
ω_1 [nm]	49.4	50.5	52.4	53.5	50.2	48.8
$\Delta\omega_1$ [nm]	0.3	0.4	0.4	0.7	1	1
A_1 [arbitrary units]	9.9	8.9	50.8	117.7	139.7	129.1
ΔA_1	0.2	0.2	0.4	0.8	0.8	0.8
λ_{p2} [nm]	543.4	540.7	542.2	541.0	520.2	515.1
$\Delta\lambda_{p2}$ [nm]	1.3	1.6	4.0	7.6	2.8	3.0
ω_2 [nm]	83.5	82.6	76.6	73.0	70.8	68.5
$\Delta\omega_2$ [nm]	0.7	0.9	2.5	5.0	1.2	1.4
A_2 [arbitrary units]	4.2	3.1	13.1	26.2	77.2	80.1
ΔA_2	0.1	0.1	0.2	0.3	0.6	0.6

Table 2. Fitting parameters for the fluorescence spectra of PRODAN in ethanol–water solutions.

	C [%W/W]					
	0	20	40	60	80	100
λ_{p1} [nm]	517.1	508.9	500.9	492.3	484.5	474.5
$\Delta\lambda_{p1}$ [nm]	0.3	0.1	0.2	0.3	0.5	0.4
ω_1 [nm]	49.4	51.5	53.8	49.8	48.3	44.2
$\Delta\omega_1$ [nm]	0.3	0.4	0.4	0.6	1.0	1.3
A_1 [arbitrary units]	9.9	10.3	74.5	120.7	176.8	188.3
ΔA_1	0.2	0.2	0.7	0.9	1.0	1.0
λ_{p2} [nm]	543.4	539.7	541.6	518.5	512.9	502.5
$\Delta\lambda_{p2}$ [nm]	1.3	2.0	1.7	1.6	2.0	1.6
ω_2 [nm]	83.5	82.3	72.7	70.2	65.5	61.9
$\Delta\omega_2$ [nm]	0.7	1.1	0.7	1.1	0.8	1.8
A_2 [arbitrary units]	4.2	3.1	15.4	65.9	116.5	173.2
ΔA_2	0.1	0.1	0.2	0.6	0.9	0.8

Table 3. Fitting parameters for the fluorescence spectra of PRODAN in 1-propanol–water solutions.

	C [%W/W]				
	0	40	60	80	100
λ_{p1} [nm]	517.1	497.9	487.9	476.9	468.4
$\Delta\lambda_{p1}$ [nm]	0.3	0.2	0.2	0.3	0.3
ω_1 [nm]	49.4	53.5	50.3	44.2	41.0
$\Delta\omega_1$ [nm]	0.3	0.7	0.5	0.9	0.8
A_1 [arbitrary units]	9.9	33.1	47.2	50.0	69.9
ΔA_1	0.2	0.4	0.6	0.5	0.6
λ_{p2} [nm]	543.4	524.0	508.0	499.9	492.9
$\Delta\lambda_{p2}$ [nm]	1.3	1.4	2.0	1.7	1.3
ω_2 [nm]	83.5	83.2	77.5	67.0	63.4
$\Delta\omega_2$ [nm]	0.7	1.3	1.1	0.6	0.7
A_2 [arbitrary units]	4.2	10.2	26.0	43.0	57.7
ΔA_2	0.1	0.2	0.4	0.6	0.6

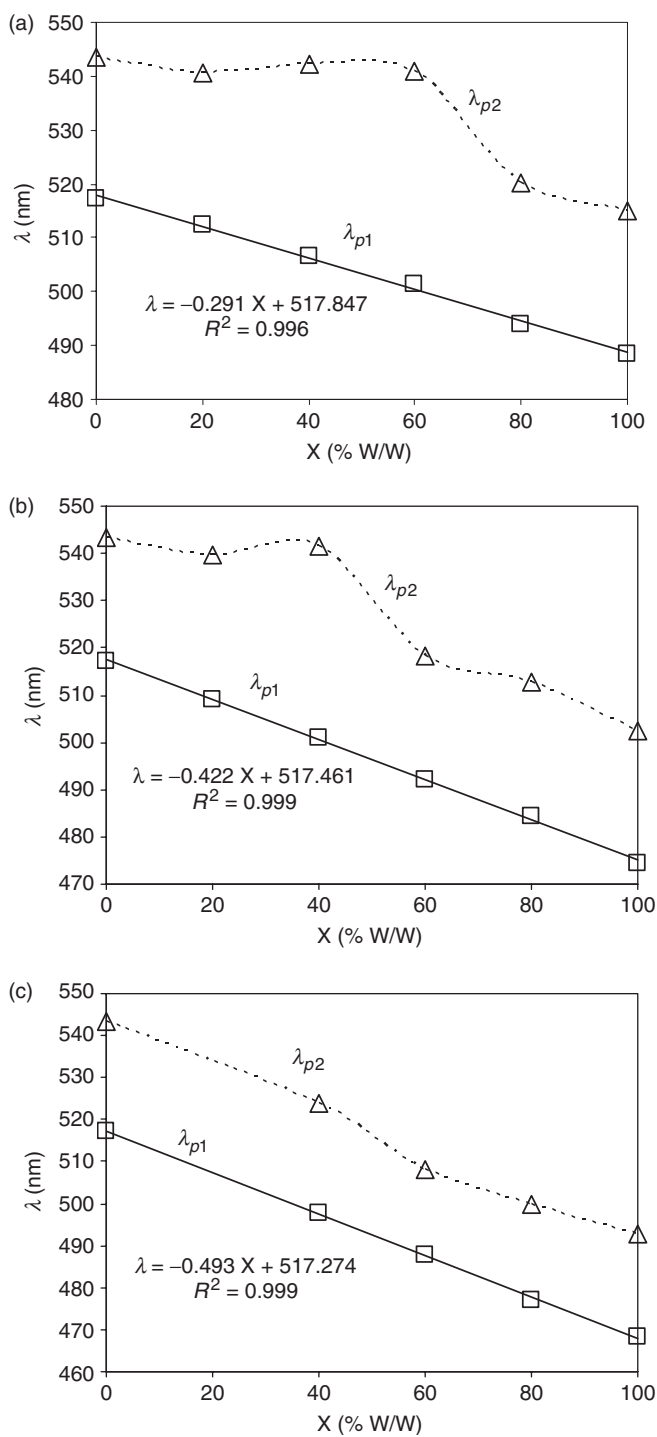


Figure 2. Dependence of λ_{p1} (\square) and λ_{p2} (Δ) on alcohol mass concentration, C [%W/W], for the (a) methanol-water solution, (b) ethanol-water solution, (c) 1-propanol-water solution. The dashed line is a guide for the eyes. The continuous line corresponds to the best fit of λ_{p1} as a linear function of C .

Table 4. Static permittivity (ϵ), index of refraction (n) and polarizability (Δf) in methanol–water solutions.

C [%W/W]	ϵ	n	Δf
0	78.5	1.3325	0.640
20	70.1	1.3326	0.638
40	61.5	1.3328	0.635
60	52.3	1.3329	0.630
80	40.1	1.3330	0.622
100	34.2	1.3332	0.615

Table 5. Static permittivity (ϵ), index of refraction (n) and polarizability (Δf) in ethanol–water solutions.

C [%W/W]	ϵ	n	Δf
0	78.5	1.3310	0.641
20	67.5	1.3415	0.630
40	56.7	1.3510	0.619
60	44.0	1.3560	0.608
80	29.9	1.3575	0.591
100	24.3	1.3560	0.581

Table 6. Static permittivity (ϵ), index of refraction (n) and polarizability (Δf) in 1-propanol–water solutions.

C [%W/W]	ϵ	n	Δf
0	78.5	1.3325	0.640
40	50.9	1.3570	0.611
60	36.9	1.3650	0.595
80	27.7	1.3740	0.575
100	20.1	1.3830	0.549

As shown in figure 3(a), (b) and (c), for the three alcohols, λ_{p1} is found to be a linear function of the solution polarisability, Δf , with $R^2 > 0.98$. In contrast, the dependence of λ_{p2} on Δf is not linear.

For aqueous solutions of DTAB, the analysis was made in a similar fashion to that used for alcoholic solutions. The experimental data were fitted with the sum of two Gaussian functions as indicated in equation (1). The values of the fitting parameters are shown in table 7, together with the half-widths of the 95% confidence interval. As in the case of alcohol–water solutions, it may be seen that equation (1) fits the data very well. For all the solutions $R^2 > 0.992$.

The experimental value, $\lambda_{I\max}$, and the fitted values λ_{p1} and λ_{p2} as a function of the molar concentration of DTAB, $C_{[\text{DTAB}]}$, are plotted in figure 4.

Experimental results show that λ_{p1} remains constant at practically the same value as in pure water (517.2 nm) up to DTAB concentrations approaching the CMC, and then decreases steeply to a constant value of 495.6 nm. However, the higher wavelength peak, λ_{p2} , decreases slightly from its value in pure water in an approximately linear way, without any abrupt changes at the CMC.

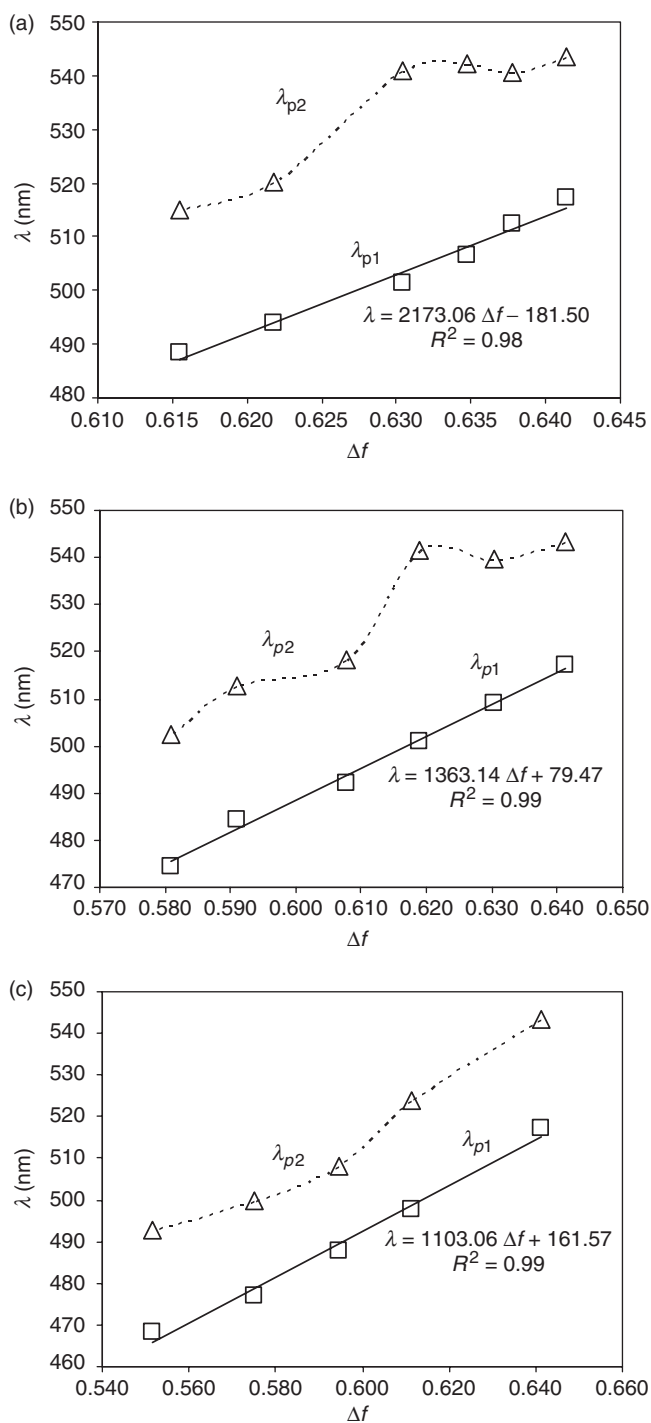


Figure 3. Dependence of λ_{p1} (\square) and λ_{p2} (\triangle) on polarizability, Δf , for the (a) methanol-water solution, (b) ethanol-water solution, (c) 1-propanol-water solution. The dashed line is a guide for the eyes. The continuous line corresponds to the best fit of X[P] as a linear function of Δf .

Table 7. Fitting parameters for the fluorescence spectra of PRODAN in DTAB–water solutions.

	$C_{[\text{DTAB}]} [10^{-3} \text{ mole L}^{-1}]$									
	3	6	9	10	12	14	15	17	19	21
λ_{p1} [nm]	519.6	516.6	517.1	516	516.9	513.2	504.7	495.7	495.9	495.4
$\Delta\lambda_{p1}$ [nm]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2
ω_1 [nm]	49.7	50.0	48.1	49.6	49.5	52.0	59.0	53.6	55.5	56.1
$\Delta\omega_1$ [nm]	0.3	0.3	0.3	0.3	0.2	0.2	0.3	0.5	0.7	0.6
A_1 [arbitrary units]	9.1	14.0	15.4	16.9	15.6	17.5	21.4	48.5	61.4	57.8
ΔA_1	0.3	0.4	0.3	0.5	0.6	0.5	0.7	0.9	1.1	1.2
λ_{p2} [nm]	547.2	548.5	539.2	546.0	542.5	534.7	538.4	526.1	530.7	532.1
$\Delta\lambda_{p2}$ [nm]	0.9	1.5	0.6	1.1	0.7	0.5	1.9	2.6	6.3	2.3
ω_2 [nm]	83.4	79.8	86.1	80	83.9	89.8	89.4	80.3	76.7	78.1
$\Delta\omega_2$ [nm]	0.6	0.8	0.6	0.6	0.5	0.5	1.0	1.3	3.5	3.7
A_2 [arbitrary units]	3.4	4.7	7.1	6.2	6.1	7.0	4.7	15.7	15.7	12.5
ΔA_2	0.1	0.1	0.3	0.3	0.3	0.3	0.2	0.6	0.7	0.6

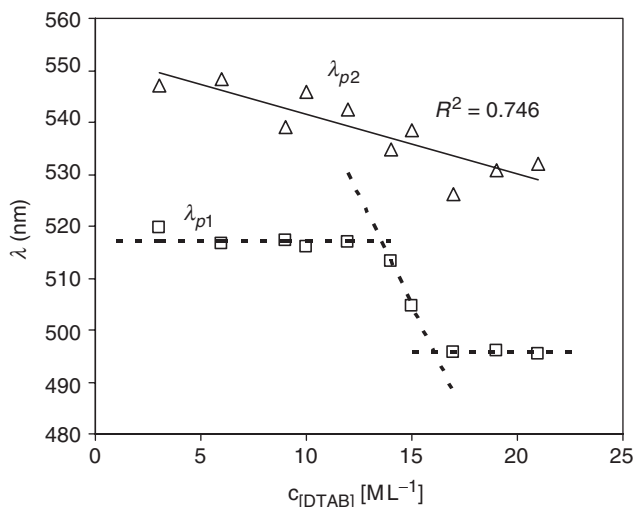


Figure 4. Dependence of λ_{p1} (\square) and λ_{p2} (Δ) on molar concentration of DTAB, $c_{[\text{DTAB}]}$, for the DTAB–water solution. The upper and lower horizontal dashed lines correspond to wavelength values of 517.2 nm and 495.6 nm respectively. The oblique dashed line is a guide for the eyes. The continuous line corresponds to the best fit of λ_{p2} as a linear function of Δf .

5. Discussion

The experimental results presented in this work may be explained by assuming that the fluorescence emission of PRODAN is originated in transitions from two excited states, which are close in energy but have dissimilar interactions with the probe environment. The excited state associated to λ_{p1} is perturbed only by non-specific, short-range hydrogen-bond interactions with alcohol or water molecules, as described by Kaatze *et al.* [16] in their study of dielectric relaxation of alcohol–water mixtures. Consequently, the spectral shift follows an ideal mixing law, in terms of the alcohol mass concentration, and there is also a linear dependence of λ_{p1} with solvent

polarizability. On the other hand, the perturbation of the excited state corresponding to λ_{p2} is also modified by the hydrogen-bond interactions within the solvent, between alcohol and water molecules. Therefore, the shift of λ_{p2} as a function of mass concentration is not linear, but it may be roughly described as having three distinct regions: alcohol-rich, intermediate and water-rich. It must be remarked that these three regions have also been described by Sato *et al.* [14, 17] in the study of microwave dielectric relaxation times in the aqueous solutions of methanol and ethanol. Their analysis explain the observed behaviour, by the effects at different alcohol concentrations of the long-range correlations introduced by the hydrogen-bond interactions between alcohol and water molecules.

In the case of aqueous solutions of DTAB, it is worth noticing that since PRODAN molecules are polar, they tend to migrate near the polar head of the surfactant molecules. Thus, it is reasonable to expect that the microscopic environment seen at short range by the probe is significantly altered at concentrations above the CMC. According to the previous discussion, this behaviour is reasonable since it may be assumed that the formation of micelles strongly alters the interactions at short range between water and PRODAN molecules. By contrast, the higher wavelength peak, λ_{p2} , is slightly shifted from its value in pure water in an approximately linear way, without any abrupt changes at the CMC. This is in agreement with the previous assumptions, since it is unlikely that, within the range of concentrations studied in this work, the presence of surfactant molecules could appreciably modify the correlations between water molecules originated by hydrogen bonds.

6. Conclusions

The steady-state fluorescent emission spectra of PRODAN in aqueous solutions of three straight-chain alcohols (methanol, ethanol and 1-propanol) were measured, for a wide range of concentrations. In all the solutions studied, experimental values of the intensity of the fluorescence emission, as a function of wavelength, were very accurately fitted by the sum of two Gaussian functions. In addition, the dependence of the wavelengths of the maxima of the Gaussian functions (λ_{p1} and λ_{p2}), on alcohol concentration, was determined. For the three alcohols studied in this work, the position of the lower wavelength maximum (λ_{p1}) can be very accurately fitted as a linear function of the mass concentration of alcohol in the solution, and also λ_{p1} is found to be a linear function of the solution polarizability (Δf). On the contrary, the dependence of the higher wavelength maximum (λ_{p2}) on mass concentration clearly departs from linearity.

These results may be explained by assuming that the fluorescence emission of PRODAN is originated in transitions from two excited states, which are close in energy but have dissimilar interactions with the probe environment. One of the states (associated to λ_{p1}) is perturbed only by non-specific, short-range hydrogen-bond interactions with alcohol or water molecules in the solution. On the other hand, the perturbation of the other excited state (corresponding to λ_{p2}) is also modified by the hydrogen-bond interactions between alcohol and water molecules.

The fluorescence of PRODAN was also studied in aqueous solutions of increasing concentration (from 3×10^{-3} to 21×10^{-3} mole L⁻¹) of DTAB, a well-known surfactant. In aqueous solutions, DTAB molecules form micelles at concentrations above the CMC. The results of the analysis of the fluorescence emission spectra show

that the position of the lower wavelength maximum (λ_{p1}) remains constant, at the same value as in pure water, up to DTAB concentrations approaching the CMC, and then decreases steeply to a different constant value. In contrast, the higher wavelength peak, λ_{p2} , slightly decreases from its value in pure water in an approximately linear way, without any abrupt changes at the CMC.

This behaviour is explained by assuming that the formation of micelles strongly alters the interactions at short range between water and PRODAN molecules (thus affecting λ_{p1}). However, within the range of concentrations studied in this work, it is unlikely that the surfactant could appreciably modify the correlations between water molecules originated by hydrogen bonds (and therefore λ_{p2} varies smoothly around the CMC).

In summary, the analysis of the PRODAN fluorescence spectra as the superposition of two Gaussian peaks provides a clearer understanding of its behaviour as a photo-physical probe, in aqueous solutions and complex fluids.

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