Laurdan solvatochromism: influence of solvent polarity and hydrogen bonds

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Laurdan solvatochromism is generally exploited in biological studies, but some of its properties are not completely elucidated. Two of them are related to the dipole moment of Laurdan and its possibility to form hydrogen bonds. Using solvatochromic methods we determined the Laurdan dipole moments both in the ground and excited states. Using different solvent polarity scales we observed that in the excited state aside from the effect of solvent polarity, a specific solvent effect appears. We showed that this specific effect is caused by hydrogen bonds formed between Laurdan and polar protic solvents.

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1. Introduction

Laurdan (2-dimethylamino-6-lauroylnaphthalene), a derivative of Prodan (6-Propionyl-2-(dimethy-1-amino)-naphthalene), is a polarity sensitive probe, designed by Weber and Faris in 1979 [1] with the purpose to be used to study various chemical and biological environments, particularly the lipid bilayer. The fluorescent naphthalene moiety is linked to a 12-carbon aliphatic tail (Fig. 1), which assures to the probe a good anchorage in the hydrophobic core of the lipid bilayer. An important characteristic of Laurdan is the large bathochromic shift of its emission spectrum in solvents with high polarities (from ~ 400 nm in cyclohexane to ~ 500 nm in methanol). Similarly, when it is found into a lipid bilayer, where the fluorescence moiety is located at the level of the triglyceride backbone of the phospholipids [2], a red (Stokes) shift of the emission spectrum is detected with increasing polarity of the environment. This makes Laurdan one of the fluorescent dyes most used to characterize the lipid bilayer: lipid packing order and fluidity [3], changes in lipid order induced by cholesterol [4,5], phase coexistence in lipid mixture systems [6-8], and lipid phase transitions [9,10]. An important characteristic assessed by Laurdan is the presence of water penetration into lipid bilayer (the hydration level of the lipid bilayer) during phase transition or due to different physical and chemical factors [11,12].

Although the solvatochromic effect on Laurdan emission spectrum is widely accepted, the mechanism by which this occurs is not entirely elucidated. One of the mechanisms considers that this effect is caused by the solvent dipolar relaxation around the large dipole of Laurdan in its excited state [8]. The dipole – dipole interactions between the fluorescent naphthalene moiety of Laurdan in its excited state and the surrounding solvent molecules produce a rearrangement of the solvent dipoles, the energy consumed in this process causing the emitted light to have a longer wavelength.

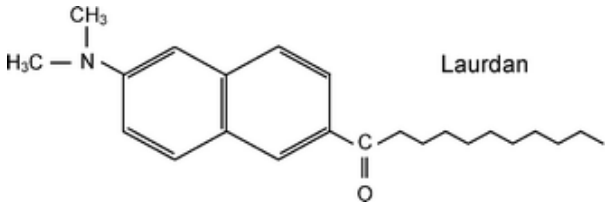

Fig. 1. Laurdan structure.

But the Laurdan dipole moment both in ground and excited states is still not well characterized. Most of the data have been obtained on Prodan, being very often extrapolated for Laurdan as having similar values. But even for Prodan there is a large range of values for dipole moment reported in the literature: 2.46 – 4.70 D, in ground state and 7.6 – 17.4 D in excited state. This large range of values is due to different techniques used to evaluate the dipole moment: fluorescence spectroscopy [13-16], dielectric spectroscopy [17] and electro-optical absorption [18]. For Laurdan only two reports for dipole moment value had been found in the literature and some differences with respect to Prodan have been noticed especially in the excited state [16,18].

Another aspect still not clear concerning the solvent – Laurdan interaction is its ability to form hydrogen bonds (H-bonds). The presence of carbonyl moiety confers, theoretically, the possibility of accepting protons for H-bonds even in lipid bilayers, mostly in the liquid-crystalline phase, when the water molecules are penetrating deeper into bilayer.

In this paper we bring under review these two features (the dipole moment of Laurdan and its ability to form H-bonds) using fluorescence spectroscopy techniques. Laurdan solvatochromism was investigated in solvents covering a broad polarity range (from non-polar to polar protic ones) and the data were analyzed by several models accepted in the literature (Lippert- Mataga model of solvent polarity [16], the empiric E_T^N polarity scale [19] and multiparametric Catalan scale [20]).

2. Experimental

2.1. Materials

Laurdan was purchased from Invitrogen/Molecular Probes (Eugene, OR, USA). All solvents used for the spectral study were of spectroscopic grade.

2.2. Measurements

All measurements were performed at room temperature on freshly prepared samples. UV/Vis absorption spectra were recorded within the 300-450 nm range on a Varian Cary-100 spectrophotometer (Santa Clara, CA, USA) using 1 cm length quartz cuvette in the path of radiation. Fluorescence spectra were recorded using a FluoroMax 3 spectrofluorimeter (Horiba Jobin Yvon, New Jersey, NJ, USA) in the appropriate conditions for each solvent. Laurdan concentration used was 1 μM for absorption measurements and 0.1 μM for fluorescence measurements. Measurements were also performed on Laurdan added to solvent mixtures: acetonitrile and toluene, or methanol and toluene. Each spectrum represents an average over three repeated measurements performed on the same sample.

2.3. Data processing

The evaluation of ground and excited state dipole moment values (μ_g and μ_e, respectively) was performed according to the extended Lippert - Mataga formalism [16]. The formalism computation was as follows:

$$\mu_g = \frac{A-B}{2}\sqrt{\frac{hcr^3}{2A}} \quad (1a)$$

$$\mu_e = \frac{A+B}{2}\sqrt{\frac{hcr^3}{2A}}, \quad (1b)$$

where:

- h is the Planck constant, c is the velocity of light and r is the Onsager radius, which for Laurdan is 5.5 Å, according to [16];
- A and B are the absolute values of slopes of linear relationships:

$$\nu_a - \nu_f = Af(\varepsilon, n) + ct \quad (2a)$$

$$\nu_a + \nu_f = -B[f(\varepsilon, n) + 2g(n)] + ct \quad (2b)$$

where ν_a and ν_f are the absorption and emission frequencies of maxima (cm^{-1}) respectively, and

$$f(\varepsilon, n) = \frac{2n^2+1}{n^2+2}\left(\frac{\varepsilon-1}{\varepsilon+2} + \frac{n^2-1}{n^2+2}\right) \quad (3a)$$

$$g(n) = \frac{3}{2}\frac{n^4-1}{(n^2+2)^2} \quad (3b)$$

where n and ε are the refractive index and the relative electric permittivity of the solvent.

The data in Eqs. (1) have been inserted in cgs unit system and the dipole moment resulted in esu·cm (1D = 10^{-18} esu·cm).

Table 1. Data obtained from the UV/Vis absorption and fluorescence spectra of Laurdan in solvents of varying polarities.

Solvent	n	ε	$f(\varepsilon,n)$	$f(\varepsilon,n)+2g(n)$	λ_a (nm)	ν_a (cm^{-1})	λ_e (nm)	ν_e (cm^{-1})
n-Hexane	1.3749	1.88	- 0.00253	0.50757	342	29,240	404	24,752
n-Decane	1.4097	1.99	0.00065	0.55716	343	29,155	405	24,691
Toluene	1.4979	2.27	0.00563	0.67766	350	28,571	423	23,641
Benzene	1.4969	2.38	0.02908	0.69982	351	28,490	427	23,419
Chlorobenzene	1.5210	5.62	0.39370	1.09542	355	28,169	428	23,364
Ethyl acetate	1.3724	6.02	0.48908	0.99583	349	28,653	439	22,779
THF	1.4072	7.58	0.54908	1.10227	351	28,490	443	22,573
1-Butanol	1.3993	17.51	0.75043	1.29311	367	27,248	485	20,618
2-Propanol	1.3772	19.92	0.77869	1.29187	362	27,624	479	20,877
Acetone	1.3586	20.70	0.79041	1.27863	352	28,409	452	22,124
Ethanol	1.3614	24.55	0.81293	1.30492	361	27,701	491	20,367
DMF	1.4305	36.71	0.83559	1.41964	357	28,011	463	21,598
DMSO	1.4793	46.68	0.84039	1.48834	360	27,778	465	21,505
Methanol	1.3284	32.70	0.85473	1.30221	362	27,624	501	19,960
Acetonitrile	1.3410	35.94	0.86014	1.32465	353	28,329	460	21,739

3. Results and discussions

The UV/Vis absorption and fluorescence spectra were measured in solvents of different polarities. Their maxima positions are presented in Table 1 along with other properties of the solvents. The influence of three solvents (one from each class of solvents: non-polar, aprotic and protic) on Laurdan absorption and emission spectra are presented in Fig. 1.

As shown both in Table 1 and Fig. 1, the solvent polarity affects more the emission spectra as compared with the absorption ones. The solvatochromic effect of Laurdan is well depicted by the red shift of at least 80 nm between the spectra recorded in toluene, an apolar solvent, and the one recorded in methanol, a polar protic solvent.

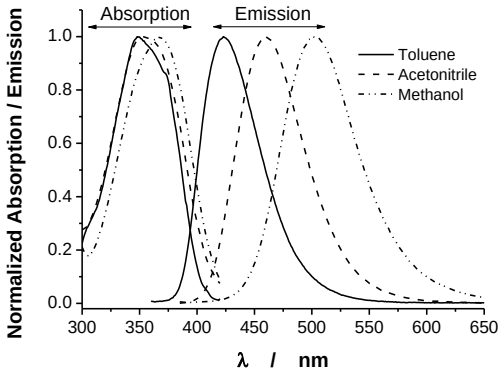

Fig. 1. Normalized absorption and emission spectra of Laurdan in homogeneous solutions. One solvent for each category (non-polar, aprotic, and protic) is presented in this graph.

In the following part we will first estimate, from the spectroscopic data, the changes of Laurdan dipole moments in ground and excited states. Thus, if the differences between absorption and emission maxima become larger with the increase of solvent polarity, the dipole moment difference (μ_e - μ_g) can be determined from the Lippert- Mataga equation.

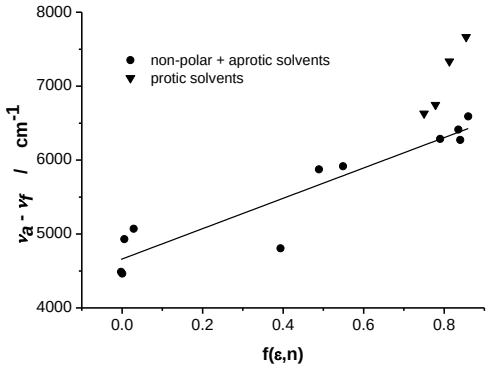

Fig. 2. Plot of ($\nu_a - \nu_f$) versus solvent polarity parameter, f(ε,n), in different solvents.

In Figs. 2 and 3 are presented the plots of ($\nu_a - \nu_f$) and ($\nu_a + \nu_f$) against the solvent polarity parameters $f(\varepsilon,n)$ and $f(\varepsilon,n)+2g(n)$, respectively.

A linear correlation between the ($\nu_a - \nu_f$) or ($\nu_a + \nu_f$) and the solvent polarity parameters reveals the effect of solvent polarity. As observed in Figs. 2 and 3, this is true with the exception of the polar protic solvents, which fall on a different line as compared with the rest of the solvents. This is indicative of a specific solvent effect, which in this case is due to hydrogen bonding.

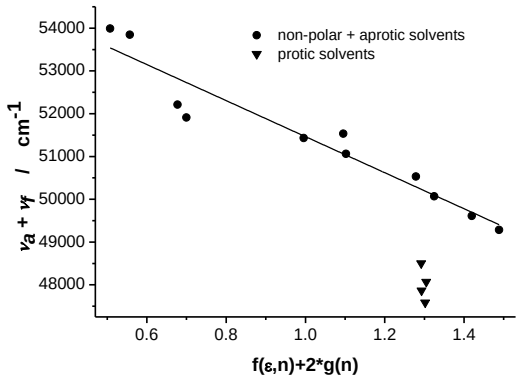

Fig. 3. Plot of ($\nu_a + \nu_f$) versus solvent polarity parameter, f(ε,n)+2g(n), in different solvents.

From the linear fit the parameters A and B were extracted, as defined in equations (2a) and (2b), respectively. These parameters were used to determine the μ_g and μ_e presented in Table 2.

Table 2. Dipole moment values in ground and excited states as derived from Eqs. (1a) and (1b). The values of A and B have been inferred by linear regression of data in Figs. 2 and 3.

A (cm^{-1})	B (cm^{-1})	r (Å)	μ_g (D)	μ_e (D)	μ_e - μ_g (D)	μ_e /μ_g
2,052	4,212	5.5	3.1	8.9	5.8	2.9

The values of μ_g and μ_e determined in our study are close to the ones reported by Kawaski, which used both the solvatochromic and thermochromic methods [16]. The values obtained by Nemkovich using electro-optical absorption measurements are much larger, and almost similar to those obtained for Prodan (see [17,18]). Because Laurdan has different properties compared to Prodan, one can expect different μ_g and μ_e values and the solvatochromic method can give an accurate result.

Solvatochromic studies can be further used to characterize the interactions between Laurdan and solvent molecules.

According to Catalan, a new term can be added to Eq. (2a) that includes the acidity (*SA*) of the solvent [20], the equation becoming:

$$\nu_a\text{-}\nu_f = Af(\varepsilon, n) + CSA + ct \quad (4)$$

where *C* is the slope for the linear dependence of SA.

SA values have been tabulated by Catalan and his colleagues [Catalan [21] web site]. Using this multi-parametric polarity scale, a better fit has been obtained (multiple linear regression algorithms): $A = 4{,}767\ cm^{-1}$; $C = -975\ cm^{-1}$; $R^2 = 0.924$ significantly higher than the fit with Lippert-Mataga equation (Fig. 4). Using this approach, a better quantitative description of the Stokes shift is obtained as the solvents polarity was extended into considering also the capacity of alcohols to provide protons.

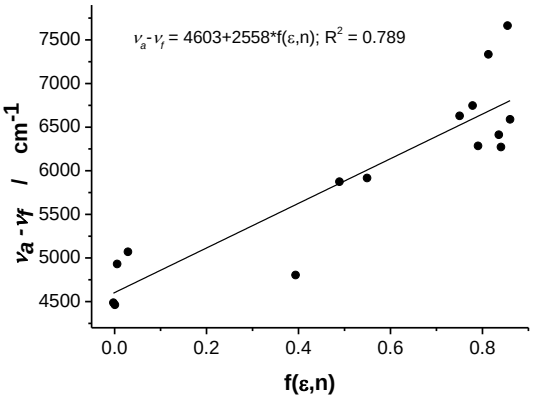

Fig. 4. Lippert-Mataga plot where all the solvents (including alcohols) have been considered in the linear fit.

The Stokes shift dependence on solvent polarity can be also investigated by another approach: the empirical scale E_T^N introduced by Reichardt [19]. Similar result was revealed when all the solvent used in our study were plotted against the E_T^N (Fig. 5). As in the Lippert-Mataga plots (see Fig. 2 and Fig. 3), the alcohols data are arranged on a different line unlike the rest of the solvents.

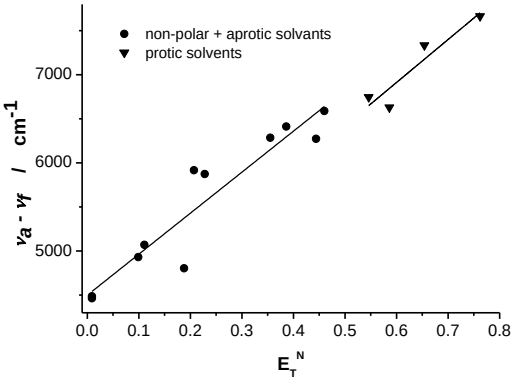

Fig. 5. Plot of Stokes shift in the E_T^N polarity scale.

The results presented in Figs. 2, 3, and 5 strengthen the statement that Laurdan is involved in hydrogen bonds with protic solvents.

In order to further investigate this possibility we analyzed the emission maximum shift of Laurdan in mixtures of two solvents. The mixtures used were chosen based on the following criteria: (i) one solvent, which remains common in all mixtures, is an apolar one with the electric permittivity close to that of the hydrophobic core of the lipid bilayer, to mimic this environment where Laurdan is mostly used, and (ii) for the other solvent of the mixture, the first time is used a solvent able to form H-bonds (i.e., polar protic) and the second time, one solvent who is not able to form H-bonds (i.e., polar aprotic), but both of them having very close polarity values (according to Eq. (2a)). The mixtures used for this study are: acetonitrile (polar aprotic) in toluene (apolar) and methanol (polar protic) in toluene. Toluene has an electric permittivity of 2.27, and acetonitrile and methanol have very similar polarities (0.860 and 0.855). If this conditions are fulfilled and if there are differences in the way the emission maxima changes with the increase of solvent polarity, this can be caused only by H-bonds formed by Laurdan with methanol molecules. A similar approach with acetone instead of toluene was used by Rottenberg for studies on Prodan [22].

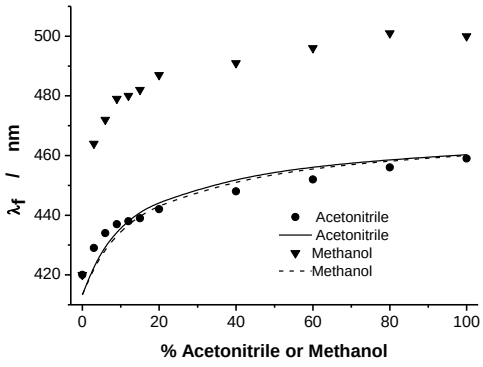

Fig. 6. Laurdan emission peak position as function of the acetonitrile or methanol concentration in toluene. The curves fitting the experimental data are theoretical predictions considering only the solvents polarity effect on Laurdan as described by Lippert-Mataga equation.

Our results are presented in Fig. 6. In both mixtures, the emission peak position is red shifted with the increase of the polar solvent concentration. However, the shift induced in the presence of methanol is more than two times higher comparing with the effect of acetonitrile. Using a simple model for computing the polarity of mixture (model that assume the equivalent electric permittivity and refractive index of the mixture as a linear combination of pure solvents values weighted by the concentration of each solvent in the mixture [23]) very similar curves have been predicted (continuous and dashed curves in Fig. 6) for both of the used mixtures. This simple

model reasonably describes the acetonitrile effect on Laurdan emission, but not of methanol. The difference between the shift induced by methanol and the predicted one is 42 ± 2 nm, that represents ~ 50 % of the total shift produced by methanol (80 nm). This result is in agreement with the measurements made on Prodan in mixtures of acetonitrile and methanol with acetone [22]. Molecular dynamics simulation of emission of Acdan (it has the same fluorescence naphthalene moiety as Prodan and Laurdan) in liquid-crystalline phase of a lipid bilayer suggests that only the polarity of the local environment is not enough to explain the red shift of Laurdan emission from 440 nm, in gel phase, to 490 nm, in liquid-crystalline phase [24]. Hypothesizing two H-bonds with water per Acdan molecule the predicted red shift almost covered the experimentally observed shift. Our results sustain indirectly the ability of Laurdan to accept protons when is involved in H-bonds with solvent molecules.

4. Conclusions

The presented data show that aside from the solvatochromic effect observed in solvents with different polarities, Laurdan can also form hydrogen bonds in protic solvents. The Laurdan dipole moments both in the ground and excited states can be easily determined with the solvatochromic method and are significantly different from the ones determined for Prodan.

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