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Molecular Stark-effect spectroscopy of Prodan and Laurdan in different solvents and electric dipole moments in their equilibrated ground and Franck–Condon excited state

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Abstract

The results from electrooptical absorption measurements (EOAM) on the ground and excited Franck–Condon state dipole moments of Prodan and Laurdan in 1,4-dioxane and cyclohexane are presented. The ground and excited Franck–Condon state electric dipole moments as well as the respective transition moment of both probes are parallel. The electric dipole moments of Prodan and Laurdan in the ground state in cyclohexane and 1,4-dioxane have values within the range $(15.7–16.5) \times 10^{-30}$ C m. On optical excitation the dipole moments increase by $(42.1–49.5) \times 10^{-30}$ C m. The obtained results are compared with the values of the dipole moments of Prodan and Laurdan determined by other methods.

Keywords: Prodan; Laurdan; Dipole moments; Electrooptical method

1. Introduction

The fluorescent probe Prodan (6-propionyl-2-(dimethylamino)-naphthalene), introduced by Weber and Farris [1], has both an electron-donating amino group and an electron-acceptor carbonyl one, resulting in a large change of the electric dipole moment after excitation and extensive red shift of the fluorescence spectrum with increasing solvent polarity. Later several variants of Prodan have been synthesized, including the lipophilic derivative Laurdan [2]. When Prodan and Laurdan are incorporated into membranes or proteins they interact noncovalently with the environment and their fluorescence characteristics are sensitive to the physico-chemical microparameters as well as to the structure and molecular dynamics in biological systems. In membranes, Prodan localizes at the surface and Laurdan situates more deeply near the glycerol backbone [3]. Much experimental works using these probes have been done for detecting lipid phases, studying the influence of cholesterol and alcohols on membranes or assessing the polarity of lipid/buffer interfaces, etc. [4–16].

As was mentioned above, Prodan and Laurdan are polarity-sensitive fluorescent probes. Hence, if the electric dipole moments in the relevant electronic states are known, the local dielectric constant of an environment may be determined from the spectral position of the electronic spectra using appropriate equations [17]. The dipole moments of Prodan and Laurdan had already been studied experimentally by several groups [1,18–22], but determined values of the dipole moments have very different magnitudes. This is not surprising, because all variants of the solvatochromic and thermochromic shift methods, used in refs. [1,18–21], require an estimate of the probe cavity radius, which enters into the final equations for recalculation of the dipole moments in cubic power. Estimation of the Prodan cavity radius is not trivial and this problem is even more complicated for Laurdan, which possesses a long lauric acid tail.

The electrooptical method [23–24] (molecular Stark-effect spectroscopy in terminology of refs. [25–27]) provides valuable information about the values and directions of the dipole moments in the ground and excited states of solute molecules in individual solvents without an estimation of the probe cavity radius. In our previous works using electrooptical method we have studied the dipole moments of aminophthalimides

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Abbreviations: EOAM, electrooptical absorption measurements; Laurdan, 6-decanoyl-2-dimethylamine-naphthalene; Prodan, 6-propionyl-2-dimethylamino-naphthalene

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[28], laser coumarins [29–30], aminonaphthalenes [17], 4′-aminoflavonols [31–32] and cetocyanine dyes [33] in individual organic solvents. For a number of compounds our results are in good agreement with the data obtained by other methods.

In this paper, we present the results from electrooptical absorption measurements (EOAM) on the ground and excited Franck–Condon state dipole moments of Prodan and Laurdan in 1,4-dioxane and cyclohexane.

2. Experimental

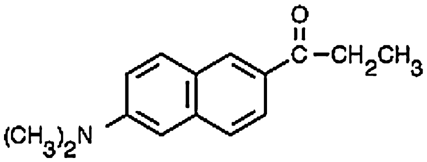
2.1. Materials and methods

Prodan was purchased from LAMBDA Fluoreszenztechnologie (Austria), Laurdan from FLUKA (assay ≥ 98%) (Fig. 1). The extra dry 1,4-dioxane and cyclohexane (water < 50 ppm) were bought from Acros Organics (NJ). The steady state absorption spectra were recorded by a spectrophotometer Perkin-Elmer (LS 50B). Electrooptical absorption measurements were performed by a set-up, which is a modern modification of the laboratory's apparatus described in ref. [24]. The high voltage was 18 kV for dc and 18 kV for variable current in 1,4-dioxane, as well 16 kV for dc and 16 kV for variable current in cyclohexane with the distance between the electrodes 3 mm.

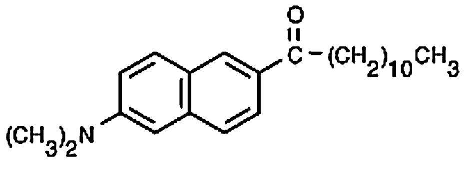
2.2. Electrooptical absorption measurements

To determine the ground and excited state dipole moments, we used electrooptical absorption measurements. Using Liptay's formalism [34] the effect of an external electric field E_f on the molar absorption coefficient $\kappa(\tilde{\nu})$ can be described by a quantity L, which is defined by

$$L = L(\tilde{\nu}, \chi) = \left[\frac{\kappa^{\mathrm{E}}(\tilde{\nu}, \chi) - \kappa(\tilde{\nu})}{\kappa(\tilde{\nu}) E_\mathrm{f}^2}\right] \tag{1}$$



Prodan



Laurdan

Fig. 1. Fluorescence probes studied in this work.

where κ^{E} is the molar absorption coefficient in the presence of an applied electric field E_f, κ the same without applied electric field, χ the angle between the direction of E_f and the electric field vector of the incident light and $\tilde{\nu}$ is the wave number. For a homogeneously broadened absorption band, L is given by the following equation

$$L = Dr + [1/6]Es + Frt + Gst + Hru + Isu \tag{2}$$

where the parameters r and s are determined by the angle χ and the quantities t and u depend on the first and second derivatives of the absorption spectrum

$$r = \frac{2 - \cos^2\chi}{5} \tag{3}$$

$$s = \frac{3\cos^2\chi - 1}{5} \tag{4}$$

$$t = \left(\frac{1}{hc}\right)\left(\frac{\kappa}{\tilde{\nu}}\right)^{-1}\frac{\mathrm{d}(\kappa/\tilde{\nu})}{\mathrm{d}\tilde{\nu}} \tag{5}$$

$$u = \left(\frac{1}{2}h^2c^2\right)\left(\frac{k}{\tilde{\nu}}\right)^{-1}\frac{\mathrm{d}^2(k/\tilde{\nu})}{\mathrm{d}\tilde{\nu}^2} \tag{6}$$

The coefficients D, E, F, G, H and I are connected with intrinsic properties of the solute molecules. Neglecting some explicit polarizability terms, D to I can be written as

$$D = \left(\frac{f_\mathrm{e}^2}{kT}\right)\mathbf{R}^{(1)}\boldsymbol{\mu}_\mathrm{g} \tag{7}$$

$$E = \left(\frac{f_\mathrm{e}}{kT}\right)^2[3(\mathbf{m}_\mathrm{a}\boldsymbol{\mu}_\mathrm{g})^2 - \boldsymbol{\mu}_\mathrm{g}^2] + \left(\frac{f_\mathrm{e}^2}{kT}\right)(3\mathbf{R}^{(2)} - \mathbf{R}^{(1)}\boldsymbol{\mu}_\mathrm{g} \tag{8}$$

$$F = \left(\frac{f_\mathrm{e}^2}{kT}\right)(\boldsymbol{\mu}_\mathrm{g}\,\Delta^\mathrm{a}\boldsymbol{\mu}) + f_\mathrm{e}^2\mathbf{R}^{(1)}\,\Delta^\mathrm{a}\boldsymbol{\mu} \tag{9}$$

$$G = \left(\frac{f_\mathrm{e}^2}{kT}\right)(\mathbf{m}_\mathrm{a}\boldsymbol{\mu}_\mathrm{g})(\mathbf{m}_\mathrm{a}\,\Delta^\mathrm{a}\boldsymbol{\mu}) + \left(\frac{f_\mathrm{e}^2}{2}\right)\mathbf{R}^{(2)}\,\Delta^\mathrm{a}\boldsymbol{\mu} \tag{10}$$

$$H = f_\mathrm{e}^2(\Delta^\mathrm{a}\boldsymbol{\mu})^2 \tag{11}$$

$$I = f_\mathrm{e}^2(\mathbf{m}_\mathrm{a}\,\Delta^\mathrm{a}\boldsymbol{\mu})^2 \tag{12}$$

where k is the Boltzman constant, T the temperature, $\mathbf{m}_\mathrm{a}$ the unit vector in the direction of the transition moment for absorption, $\boldsymbol{\mu}_\mathrm{g}$ the equilibrated ground state dipole moment vector and $\Delta^\mathrm{a}\boldsymbol{\mu}$ is the change of the dipole moment vector upon excitation to the considered Franck–Condon excited state. The vectors $\mathbf{R}^{(1)}$ and $\mathbf{R}^{(2)}$ are related to the transition polarizability of the considered transition and describe the effects due to the electric field dependence of the transition moment. The field correction is done by the cavity field factor f_e [22].

The quantity $L(\tilde{\nu}, \chi)$ in the present work was determined for two values of the angle χ ($\chi = 0$ and $\chi = \pi/2$) and for a set of wave numbers within the first absorption band. Then the coefficients (8)–(12) and their standard deviations were obtained from fitting the experimental L values using the program SYSTAT Version 7.0, according to Eq. (2).

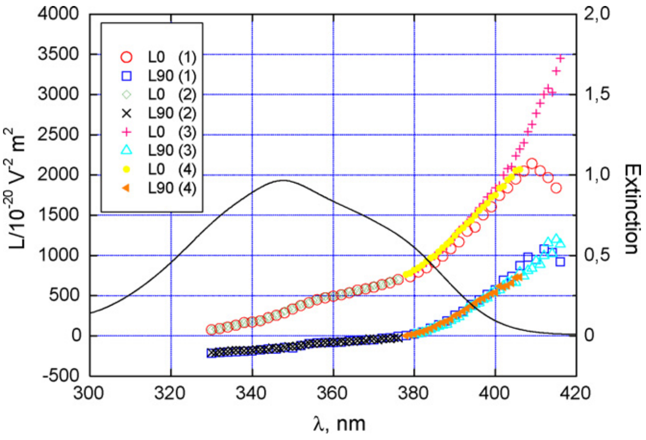


Fig. 2. Absorption (solid curve) and electrooptical absorption spectra (points) of Prodan in 1,4-dioxane at $T = 298$ K measured in different spectral intervals. The data scattering in the spectral region shorter than 400 nm is caused by low absorption and higher noise. In the left upper corner the measurement identification numbers are presented.

3. Results and discussion

The electrooptical absorption spectra of Prodan and Laurdan are accurately reproducible, especially in 1,4-dioxane in the spectral region shorter than 410 nm, where the absorption coefficient is sufficiently large for both probes. As an example, the experimental data points of the electrooptical absorption spectra of Prodan and Laurdan in 1,4-dioxane and cyclohexane are shown in Figs. 2–5.

From our measurements follow that for both probes in 1,4-dioxane and in cyclohexane as well the coefficient F equals G and H equals I, within the experimental error. The mostly closest coincidence of the coefficients for both probes take place in the spectral regions shorter 380 nm in 1,4-dioxane and 372 nm in cyclohexane. As an example, the electrooptical coefficients of Prodan in 1,4-dioxane are listed in Table 1. The above mentioned results means that $\mathbf{m}_a || \boldsymbol{\mu}_g || \Delta^a \boldsymbol{\mu}$. The same result follows from the slope of the function $L(\nu, \chi = 0) = f[L(\nu, \chi = \pi/2)]$ in mentioned above spectral regions, which for solute molecules with C_n symmetry is given in good approximation by the simple lin-

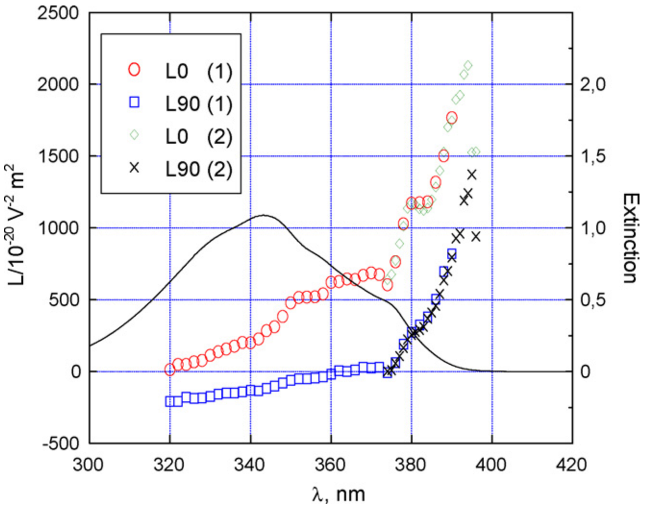


Fig. 3. Absorption (solid curve) and electrooptical absorption (points) spectra of Prodan in cyclohexane at $T = 298$ K measured in different spectral intervals. In the left upper corner the measurement identification numbers are presented.

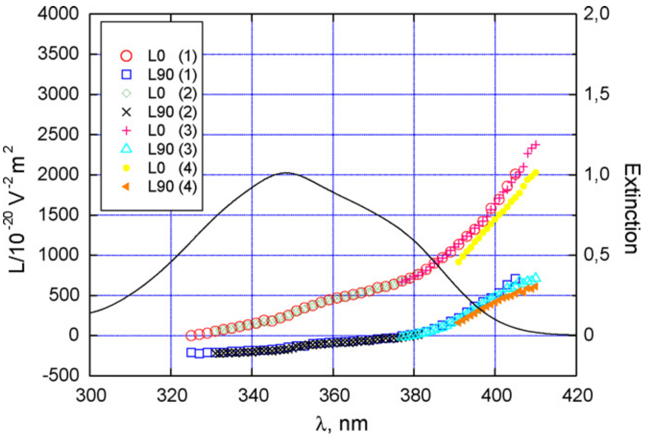


Fig. 4. Absorption (solid curve) and electrooptical absorption (points) spectra of Laurdan in 1,4-dioxane at $T = 298$ K measured in different spectral intervals. In the left upper corner the measurement identification numbers are presented.

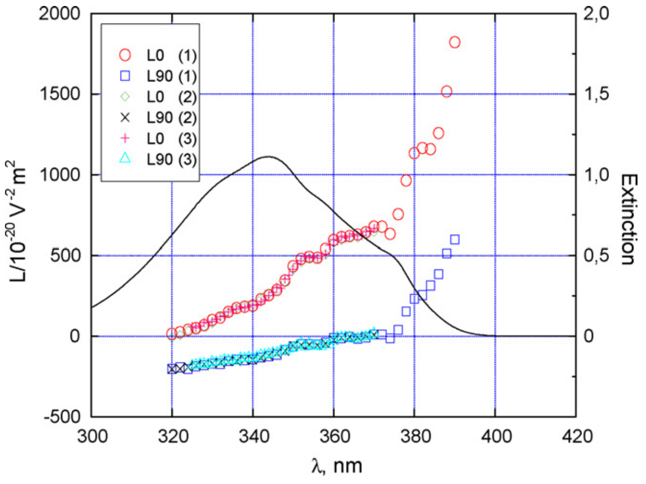


Fig. 5. Absorption (solid curve) and electrooptical absorption (points) spectra of Laurdan in cyclohexane at $T = 298$ K measured in different spectral intervals. In the left upper corner the measurements identification numbers are presented.

Table 1
Electrooptical coefficients obtained by EOAM for Prodan in 1,4-dioxane at $T = 298$ K for two independent measurements

Coefficient	Measurement	
	First	Second
D ($\times 10^{-20}$ V^{-2} m^2)	51 ± 8	51 ± 5
E ($\times 10^{-20}$ V^{-2} m^2)	4983 ± 141	4947 ± 93
F ($\times 10^{-40}$ C V^{-1} m^2)	2857 ± 136	2837 ± 96
G ($\times 10^{-40}$ C V^{-1} m^2)	2888 ± 136	3035 ± 96
H ($\times 10^{-60}$ C^2 m^2)	1399 ± 836	1420 ± 568
I ($\times 10^{-60}$ C^2 m^2)	1335 ± 836	1132 ± 568

The coefficients and their standard deviations were obtained from fitting of the experimental L values by the program SYSTAT Version 7.0 according to Eq. (2). The spectral region of electrooptical measurements is 376–330 nm.

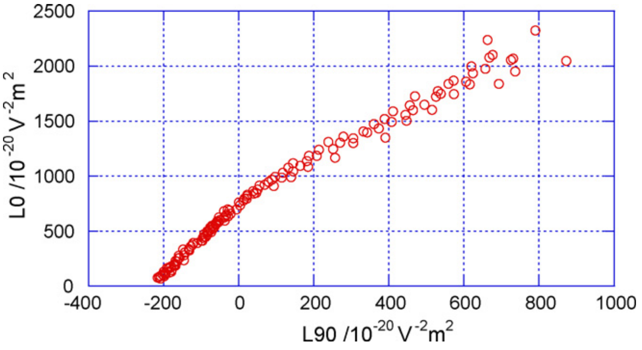


Fig. 6. Plot of $L(\nu, \chi = 0)$ vs. $L(\nu, \chi = \pi/2)$ of Prodan in 1,4-dioxane at $T = 298$ K. The points show the experimental data for all measurements presented in Fig. 2.

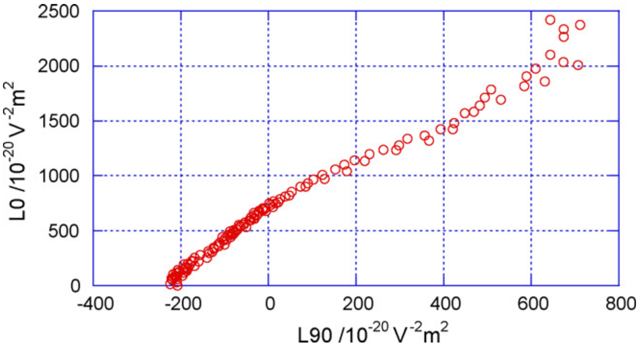


Fig. 7. Plot of $L(\nu, \chi = 0)$ vs. $L(\nu, \chi = \pi/2)$ of Laurdan in 1,4-dioxane at $T = 298$ K. The points show the experimental data for all measurements presented in Fig. 4.

ear relationship [27,28]:

$$L(\nu, \chi = 0) = AL\left(\nu, \chi = \frac{\pi}{2}\right) + \frac{Bf_e^2\mu_g^2}{6k^2T^2} \tag{13}$$

where $A = (1 + 2\cos^2\theta)/(2 - \cos^2\theta)$ and $B = (3\cos^2\theta - 1)/(2 - \cos^2\theta)$. Here, θ is the angle between $\mathbf{m}_a$ and $\boldsymbol{\mu}_g$.

Figs. 6 and 7 show the respective plot of $L(\nu, \chi = 0)$ versus $L(\nu, \chi = \pi/2)$ for Prodan and Laurdan in 1,4-dioxane. The points represent the experimental data from various experiments. From the quite good linearity of the function $L(\nu, \chi = 0) = f[L(\nu, \chi = \pi/2)]$ in interval $L90 \leq 0$ ($\lambda \sim 380$ nm) follows that the first absorption band of the studied probes here is homogeneous and that the angle between the vectors $\mathbf{m}_a$ and $\boldsymbol{\mu}_g$ must be constant over the measured wave number interval ($\lambda \leq 380$ nm) in agreement with Eq. (13). From the slope of the function $L(\nu, \chi = 0) = f[L(\nu, \chi = \pi/2)]$ for Prodan and Laurdan in 1,4-dioxane ($\lambda \leq 380$ nm) and cyclohexane ($\lambda \leq 372$ nm) we found that the coefficient $A \cong 3$. From Eq. (13) follows that in this case $\mathbf{m}_a || \boldsymbol{\mu}_g || \Delta^a\boldsymbol{\mu}$.

Special measurements were performed at the red slope of the absorption spectrum of Prodan and Laurdan (see Figs. 2–5), where the function $L(\nu, \chi = 0) = f[L(\nu, \chi = \pi/2)]$ is non-linear (Figs. 6 and 7). Unfortunately, due to the large noise (see Figs. 6 and 7) it is not possible to determine the electrooptical coefficients here with high enough accuracy. Hence, the mechanism of such non-linearity the function $L(\nu, \chi = 0) = f[L(\nu, \chi = \pi/2)]$ at the moment is not clear and want of additional study.

The coefficients (7)–(12) and their standard deviations were obtained with the assumption $F = G$ and $H = I$ in the spectral regions $\lambda \leq 380$ nm in 1,4-dioxane and $\lambda \leq 372$ nm in cyclohexane. Using the symmetry condition $\mathbf{m}_a || \boldsymbol{\mu}_g || \Delta^a\boldsymbol{\mu}$ the values of the dipole moments $\boldsymbol{\mu}_g$ and $\Delta^a\boldsymbol{\mu}$ were calculated from

$$\mu_g = \left(\frac{kT}{f_e}\right)\sqrt{\frac{E - 6D}{2}} \tag{14}$$

$$\Delta^a\mu = \frac{(kT/f_e^2)F}{\mu_g} \tag{15}$$

The dipole moment in the excited Franck–Condon state ($\boldsymbol{\mu}_e^{FC}$) was determined by the formula

$$\Delta^a\boldsymbol{\mu} = \boldsymbol{\mu}_e^{FC} - \boldsymbol{\mu}_g \tag{16}$$

which is sufficiently valid for low polar solvents. The dipole moments of Prodan and Laurdan obtained as an average from several independent EOAM measurements in different solutions are shown in Table 2.

As follows from the table, the values of the dipole moment in the ground state μ_g and the change of the dipole moment vector after excitation to the Franck–Condon state $\Delta^a\mu$ are practically the same for Prodan and Laurdan, in a given solvent.

The values of $\Delta^a\mu$, at first, depends on the charge transfer from an amino group to carbonyl one. The diffusion motion of the long lauric acid tail in Laurdan, in principle, can change the orientation of the carbonyl group and may influence on the charge distribution in molecule and the efficiency of charge transfer. But from obtained results follow, that the lauric acid tail in the case of Laurdan does not influence on the distribution of the charges in the molecule and the efficiency of charge transfer. In addition, the polarity of the solvent does not essentially change the dipole moment values and therefore not the efficiency of intramolecular charge transfer, in Prodan and Laurdan (see Table 2).

Table 2
Average values of the dipole moments of Prodan and Laurdan in 1,4-dioxane and cyclohexane at $T = 298$ K

Molecule	$\boldsymbol{\mu}_g$ (10^{-30} C m)	$\Delta^a\boldsymbol{\mu}$ (10^{-30} C m)	$\boldsymbol{\mu}_e^{FC}$ (10^{-30} C m)
Prodan in cyclohexane (372–320 nm)	15.7 ± 0.1	42.4 ± 0.1	58.1 ± 0.1
Prodan in 1,4-dioxane (376–330 nm)	16.4 ± 0.1	46.9 ± 0.3	63.3 ± 0.3
Laurdan in cyclohexane (370–320 nm)	15.6 ± 0.1	42.1 ± 0.2	57.7 ± 0.2
Laurdan in 1,4-dioxane (380–330 nm)	16.5 ± 0.1	49.5 ± 0.2	66.0 ± 0.2

Mean values and errors are from several independent measurements. In the brackets the spectral regions of electrooptical measurements are listed. $\boldsymbol{\mu}_g$ is the dipole moment in the equilibrium ground state, $\Delta^a\boldsymbol{\mu}$ the change of the dipole moment vector after excitation to the Franck–Condon state and $\boldsymbol{\mu}_e^{FC}$ is the dipole moment in the excited Franck–Condon state.

Table 3
Literature values of the dipole moments of Prodan and Laurdan determined by different methods

Molecule, method (authors)	μ_g (10^{-30}C m)	$\Delta\mu$ (10^{-30}C m)	μ_e (10^{-30}C m)
Prodan, solvatochromic shift; thermochromic shift (Weber and Farris)		66.71; 49.37	
Prodan, solvatochromic shift (Balter et al.)	9.67	26.69	36.36
Prodan, solvatochromic shift (Catalan et al.)	15.68	37.35	39.03
Prodan, CNDO/S calculations (Nowak et al.)	12.58	29.42	42.0
Prodan, solvatochromic shift (Kawski et al.)	8.21	16.37	24.58
Thermochromic shift (Kawski et al.)	9.34	16.01	25.35
Laurdan, solvatochromic shift; thermochromic shift (Kawski et al.)	11.54; 12.54	23.82; 21.62	35.36; 34.16

The conversion factor for the dipole moment is $\mu_{(SI)}$ (C m) = 3.33564 × $\mu_{(CGS)}$ (D), where 1D = 10^{-18} CGS units. μ_g is the dipole moment in the equilibrium ground state, $\Delta\mu$ the difference between the value of the dipole moments in equilibrated excited and ground state and μ_e is the dipole moment in the equilibrium excited state.

Table 3 compares the values of the dipole moments of Prodan and Laurdan as communicated in literature and determined by different methods. The value of $\boldsymbol{\mu}_g$ of Prodan obtained in our work (see Table 2) is very close to the value determined by Catalan et al. (ref. [19]) using a modified Lippert equation, which takes into account the acidity of the solvent. The change of the dipole moment vector of Prodan after excitation (Table 2) is close to $\Delta\mu$ (Table 3) calculated by Weber and Farris (ref. [1]) using the thermochromic shift method. On the other hand, one should be careful when comparing values of $\Delta^a\mu$ (Table 2) and $\Delta\mu$ (Table 3) since during the relaxation of the probe molecule from the Franck–Condon to equilibrium state some internal relaxation processes may change the value of the dipole moment.

Rollinson and Drickamer [35] and Viard et al. [36] proposed the presence of two emitting states in Prodan and Laurdan (locally excited and charge transfer), in analogy to *para*-(9-anthryl)-dimethylaniline, a compound with twisted intramolecular charge transfer [37]. The locally excited state should predominate in low polar aprotic solvents and in frozen polar solvents [36]. Hence, the values of the dipole moments determined in the present work may be attributed to the locally excited state. Although it is assumed in literature [38], that the locally excited state is not highly polar. The data presented in Table 1 clearly show that this state is quite polar.

4. Conclusions

As follows from our experiments in the case of Prodan and Laurdan the ground and excited Franck–Condon state electric dipole moments are parallel to each other and also parallel to the transition moment. The electric dipole moments of both compounds in the ground state in cyclohexane and 1,4-dioxane have values within the range (15.7–16.5) × 10^{-30} C m. By optical excitation the dipole moments increase by (42.1–49.5) × 10^{-30} C m. The values of the dipole moment in the ground state μ_g and the change of the dipole moment vector after excitation to the Franck–Condon state $\Delta^a\mu$ for Prodan and Laurdan are practically equal in a given solvent. This means, that the long lauric acid tail in the case of Laurdan does not influence on the distribution of the charges in the molecule. The polarity of the solvent does not change the values of the dipole moments of Prodan and Laurdan in the Franck–Condon state much.

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