The Red-Edge Effects in Laurdan Solutions

V. I. Tomin, M. Brozis, and J. Heldt

Institute of Physics, Pomeranian Pedagogical Academy,
76-200 Slupsk, Arciszewskiego str. 22B, Poland

Reprint requests to Prof. V. I. T; Fax (1033)-59-8405-339, E-mail: tomin@wsp.slupsk.pl

Z. Naturforsch. **58a,** 109 – 117 (2003); received January 14, 2003

The luminescence spectrum of laurdan in glycerol consists of two bands with maxima at 425 and 500 nm. The strong dependence of this spectrum on the energy of the excitation quanta is demonstrated. When, excited on the red-edge slope of the absorption band, it is investigated at various temperatures. The biggest red shift (about 10 nm) of λ_{max} is found for the long wavelength band at low temperature, where the solvent forms a rigid matrix. A small shift, about 3 – 4 nm, and a change of the luminescence band shape occurs on increasing the temperature to 370 K. Simultaneously, intensity changes occur in the excitation spectra when the maximum intensity of the two emission bands is recorded. From the received data, the differential excitation spectrum has been determined. The additional absorption contributes to the luminescence of the short wavelength band.

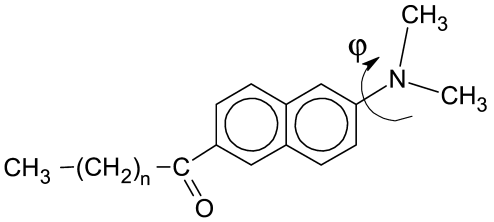
The deconvolution of the luminescence decay data obtained separately for the luminescence bands results in common components in the sub- and nanosecond time range with different participation. The experimental data testify the presence of an additional mechanism, responsible for the heterogeneity of the solution, which differs from the well-known solvent molecule reorientation.

Torsion oscillation of the $N(CH_3)_2$ functional group in the laurdan molecule is necessary to take into account as well as the statistical properties of the laurdan solvate shells. The observed luminescence properties are explained taking into account the simultaneous existence of local-excited and charge transfer states in the solute molecules.

Key words: Laurdan; Luminescence; Excitation Spectra; LE (local-excited) and CT (Charge Transfer) States; Inhomogeneous Broadening of Spectra; Intermolecular Relaxation.

1. Introduction

The fluorescence of organic molecules is widely used in natural sciences as probe in studies of the physico-chemical properties of solvents, surfaces, large biological molecules, membranes, cells, *etc.* [1 – 3]. Luminescent probes with complex multifunctional properties will become interesting and useful in the coming years. Therefore it is necessary to study special molecules for which the luminescence properties vary with excitation and which possess the ability of simultaneous creation of local-excited (LE), and charge transfer (CT) states [4 – 10]. In this connection, laurdan (6–dodecanoyl–2–dimethylaminonaphtalene) and prodan (6–propinyl–2–dimethylaminonaphtalene) molecules [11] are perspective objects.



Scheme 1: Chemical structure of laurdan.

The chemical structure of laurdan is given by Scheme 1. If $n = 1$ we have prodan, and if $n = 10$ we have laurdan. Both molecules are derivatives of naphthalene. A remarkable property of laurdan in comparison to prodan is its ability to precipitate selectively in various inhomogenous solvents, *i. e.*, membranes and cell structures. It plays an important role in the determination of its localisation and orientation as luminescence probe. Further, the laurdan shows a significant dipole moment change. The moments are 3,7 D and 10,2 D for the ground and excited state, respectively [12]. Their difference reaches 6,5 D, and such large dipole moment changes at the rather small size of the basic fragment, the naphthalene moiety, points to a strong sensitivity of these probe molecules to solvatochromic effects and to the dynamics of the microstructure in their nearest environment. As is known,

0932–0784 / 03 / 0200–0109 $ 06.00

the spectral heterogeneity of such complex molecules in solvents, found in [13 – 18], is directly connected to the variable polarity [2, 16].

This heterogeneity depends on the physical properties of the environment and has static and dynamic character [17,18]. The dynamic model [16,18] gives a possibility to study various molecular movements (both orientational and translational) in nano – and subnanosecond time ranges.

Another major peculiarity of laurdan for probing various objects, including biological, is the presence of conformational twisted states, so called CT states, connected with intramolecular charge transfer. The excitation of this state is connected with an originally excited LE state, from which the excitation energy is transmitted by configuration changes of the molecule to the state with the minimum internal energy.

For free molecules, such transitions are purely intramolecular. However in solvents they are connected with the opportunity to change the orientation of surrounding solvent molecules. Such a process is necessarily connected with structure changes as well as changes of the geometry and mobility of solvent molecules. These radiationless transitions, as it is usually considered, occur faster than the reorientations of all molecules in the solvate luminescence centre. It is characteristic that molecules which undergo transitions from LE to CT configurations show distinct changes of the luminescence spectra [4 – 9]. The emission from the CT state appears at longer wavelength than the emission from the LE band. In polar solvents, the difference between these two bands of laurdan amounts to 75 nm in some cases. At the same time, the LE band is very weak and practically not observable [6, 7].

Thus, from this aspect laurdan molecules in organic solutions are unique luminescent probes which are widely used for the study of various biological systems [19 – 22]. The laurdan molecules can be located at definite places of complex organic structures and than give spectra and other characteristics of the luminescence, which are rich in information concerning the dynamics of the environment from picoseconds up to tens of nanoseconds.

Despite these studies on laurdan and its practical use [19 – 24], some of its basic properties have not been explained completely. For example, the model of the inhomogeneous broadening of the two luminescence bands of laurdan was not applied to explain the influence of the environment. Also, the mechanism of some red-edge excitation effects observed for laurdan in solutions has not been studied.

The purpose of the present work is to show the complex character of the inhomogeneous broadening of laurdan spectra in solutions, where we assume that three major mechanisms for this phenomenon exist. The fluorescence spectrum shift and broadening is considered in this paper. An important factor for the spectral displays is the distribution of molecules over various orientations of the dimethyl group relative to the naphthalene plane. This distribution changes with time and depends on the excitation wavelength.

Data on laurdan in glycerol are practically absent in the literature. The spectroscopic characteristics of this solvent are close to the properties of various liposomes and vesicles. To study them, laurdan has been used frequently in the past years [19 – 25].

2. Experimental Section

The studies on laurdan were carried out in glycerol at a concentration of $1.5 \cdot 10^{-5}$ M. The luminescence spectra were registered on a HITACHI F- 2005 spectrofluorimeter. The kinetic luminescence characteristics were measured by exciting the sample with short laser pulses (16 ps halftime duration), using the second harmonic $\lambda_{\mathrm{ex}} = 386$ nm of the Ti: sapphire laser (Spectra Physics Laser Company). The registration was carried out with the photon counting mode (TAC model TC 864 Tenneler) using a photon detector MCP-PMT R3809U-05, which is thermoelectrically cooled and equipped with a preamplifier (HAMAMATSU). A detailed description of the set-up is given in [26].

The processing of the received luminescence pulses was carried out with the modified method of generalised crossvalidation (MMGC), developed by the authors [27, 28]. In this method, the analysis of the data is made with minimal *a priori* assumptions of the decay model. Presently, it is one of the most productive methods to investigate emitting systems with complex multiexponential luminescence kinetic.

Distributions of the luminescence decay constants were obtained from the experimental emission kinetics by the MMGC. The results are discussed on the basis of the received distributions.

3. Experimental Results

The absorption, 1 – 3, and luminescence, 4 – 7, spectra of laurdan in glycerol are presented in Figure 1.

As can be seen the absorption spectra depend on temperature. In all cases the long wavelength band has a very long wing towards low energies. An appreciable absorption is still seen at a wavelength of 600 nm. The maximum of the band lies in the region of 390 – 400 nm from 258 up to 350 K. The absorption band is significantly broadened. It has a more symmetric structure at higher temperatures, *e.g.* above 350 K. At these temperatures the absorption maximum is slightly shifted to short wavelengths, *e.g.* to 380 nm, and doubles almost, whereas at the long wavelengths it decreases appreciably. Simultaneously, as can be seen in Fig. 1, a bigger absorption occurs at 200 – 280 nm.

Laurdan, in polar organic solvents, shows a relatively strong luminescence in the blue and green regions of the visible spectrum. At $T = 258$ K, the short wave component with maximum intensity at 420 – 425 nm is very strong, the other component is insignificant and noted as a shoulder in the band at the long-wave wing. The growth of temperature causes an increase of intensity of the long wavelength components at $\lambda = 500$ nm, whereas the emission of the other component decreases gradually. At 323 K the long wavelength component already dominates, and at 350 K the luminescence band is characterised by a smooth, almost symmetric spectrum consisting of the long wavelength component only (see Figure 1). This emission component $\mathbf{I_{CT}}$, appears from transitions, starting from the S_1(CT) state created in excited laurdan as a result of charge transfer between the twisted dimethyl function group and the naphthalene moiety. Figure 2 represents in semilogarithmic form the dependence of the luminescence intensity ratio of the intensity maximum of the $\mathbf{I_{LE}}$ to the $\mathbf{I_{CT}}$ band *versus* the temperature of the solution (curve 1). This dependence is graphically presented by a falling curve. The basic changes of the emission structure of laurdan appear in glycerol in the range from 258 up to 348 K. In this range of temperatures the logarithm of the intensity ratio is nonlinear and falls from 1 to -4.

On Fig. 3 the excitation wavelength dependencies of the luminescence spectra are given. All luminescence bands, obtained by varying λ_{ex}, show an appreciable change. If exciting at the maximum of the absorption band $\lambda_{\text{ex}} = 370$ nm, the luminescence spectrum is characterised by a blue short wavelength component of the emission spectrum. It results basically in the S_1(LE) transitions. Increasing the excitation wavelength up to the excitation on the red-edge of absorption band allows to excite and observe the emission spectrum of the second component, for which the centres of luminescence possess smaller electronic transition energies. It is obvious from Fig. 3 that, varying the λ_{ex} to larger values, the intensities $\mathbf{I_{LE}}$ and $\mathbf{I_{CT}}$ of the luminescence bands and their $\lambda_{\max}$ values change. This excitation wavelength dependence of $\lambda_{\max}$ of the CT band is graphically presented in Fig. 4, whereas ln [$\mathbf{I_{LE}}/\mathbf{I_{CT}}$ *versus* λ_{ex} depends on the temperature of the solution as pictured on Fig. 2 (curve 2) for 260 K. As it can be seen on Fig. 4, for $T = 260$ K the shift of the CT band is maximal, $\Delta\lambda_{\text{lum}}(\lambda_{\text{ex}}) \cong 10$ nm. This value decreases for higher temperatures and does not disappear even when heating the solution up to 350 K. At 325 K and 350 K the red shift equals 3 and 4 nm, respectively.

Figure 5 shows general changes of the laurdan luminescence spectra noted on changing the excitation wavelength in the range 380 – 430 nm. The luminescence spectrum changes comprise the red shift band as a whole and a pronounced transformation of its contour, which is noted in the reduction of the band half width (FWHM). The changes are more pronounced if the sample is excited in the long wavelength tail of the absorption band. Figure 5 shows the excitation and the emission spectra of laurdan in glycerol obtained at 350 K. The excitation spectra are obtained recording them at $\lambda_{\text{ob}} = 425$ nm (curve 1) and 500 nm (curve 2), respectively. The fluorescence excitation spectrum obtained detecting at $\lambda\,\frac{\text{LE}}{\text{em}} = 425$ nm, is always broader than that registered at the long wavelength band maximum $\lambda\,\frac{\text{CT}}{\text{em}} = 500$ nm. Besides, it possesses a higher “intensity absorption coefficient” in the wavelength range at 330 – 340 nm.

The depopulation kinetics of the luminescence states, *e.g.*, S_1(LE) and S_1(CT), have been measured detecting the fluorescence decay curve exciting the fluorescent sample at the long wavelength absorption band $\lambda_{\text{ex}} = 386$ nm. The emitted photons were detected at the long, $\lambda\,\frac{\text{CT}}{\text{em}} = 500$ nm (curve 2), and at the short, $\lambda\,\frac{\text{LE}}{\text{em}} = 428$ nm (curve 1), wavelength maxima. Respective decay are shown on Figure 6. Comparing the two curves, it follows that the CT band characterises a process with longer decay time. On the bottom of Fig. 6 the experimental decay data, analysed using the modified method of generalised crossvalidation (MMGC) [27,28], are given as well. Analysing them it results that the “time – amplitude” pictures of both decay curves differ essentially. The decay of the LE band possesses luminescence components, the first about 0,1 ns, the second about 1 ns (curve 3, Fig. 6), and the third weak at about 6 ns. The CT emission

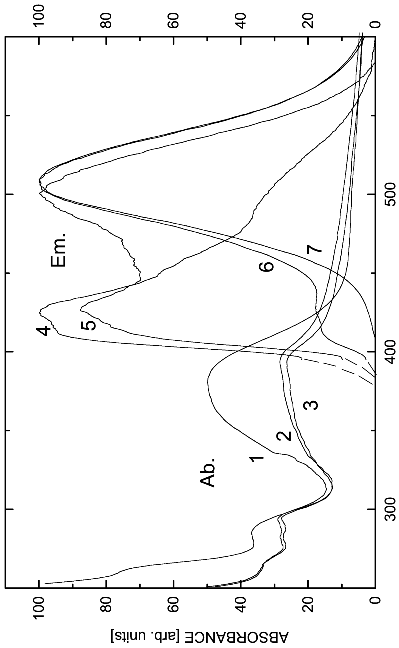


Fig. 1. Absorption (1 – 3) and luminescence (4 – 7) spectra of laurdan in glycerol at $T = 260$ K (3, 4), 295 K (2, 5), 320 K (6) and 350 K (1, 7). The excitation wavelength was $\lambda_{ex} = 380$ nm.

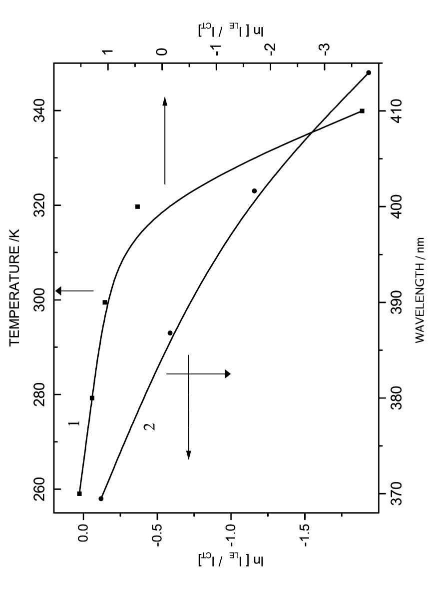


Fig. 2. The relative intensity, $\ln[\mathbf{I_{LE}}/\mathbf{I_{CT}}]$, of the laurdan emission bands *versus* the excitation wavelength λ_{ex}, 2 and temperature, 1. The $\mathbf{I_{LE}}$ and $\mathbf{I_{CT}}$ values are taken at the LE and CT band maxima. The appropriate axes are indicated by the arrows.

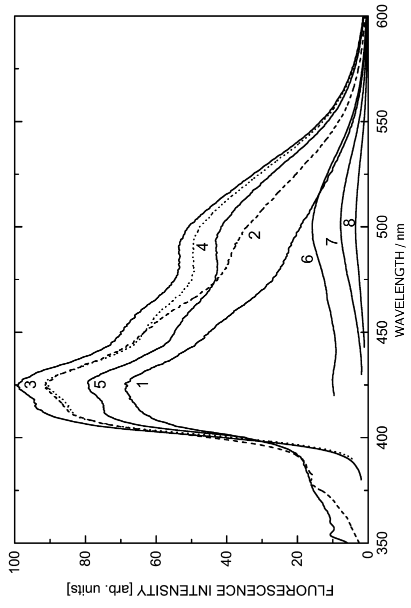


Fig. 3. Luminescence spectra of laurdan ($T = 260$ K) obtained by exciting the luminophor at $\lambda_{ex} = 320$ nm; 2, 340 nm; 3, 370 nm; 4, 380 nm; 5, 390 nm; 6, 410 nm; 7, 420 nm; 8, 430 nm.

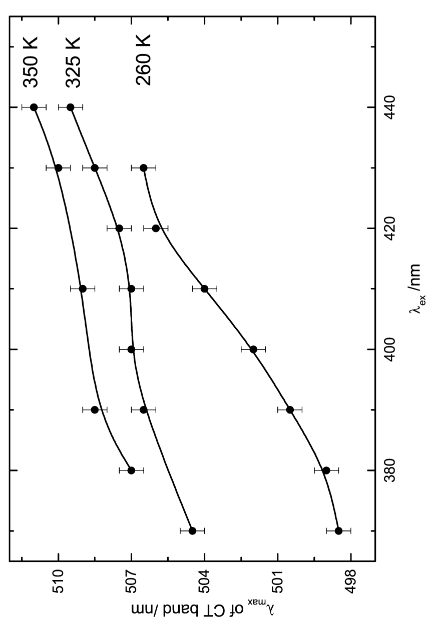


Fig. 4. Excitation wavelength dependencies of the luminescence maxima for three temperatures.

band (see curve 4, Fig. 6) is composed of the same time amplitude components. The first appears in the area of 0.1 ns, however with negative values, contrary to curve 3. The component appearing at about 1 ns possesses a smaller amplitude in comparison to that in the LE decay. The most intensive components, appearing at about 3 ns, show the very high amplitude of curve 4.

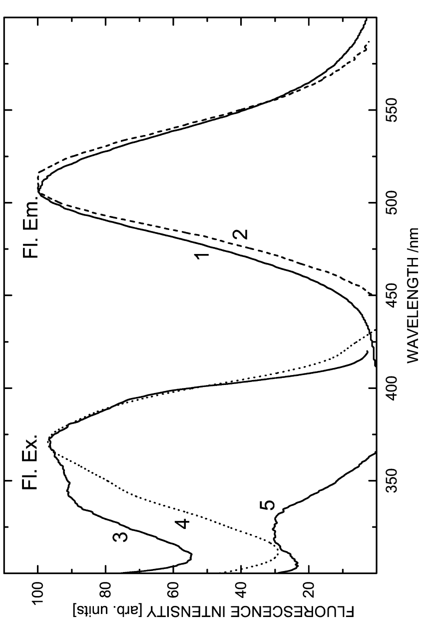


Fig. 5. Luminescence spectra of laurdan in glycerol, obtained at 350 K for the excitation wavelengths $\lambda = 380$ nm, 1 and 430 nm, 2. Luminescence excitation spectra obtained detecting the luminescence at $\lambda_{obs} = 425$ nm, 3 and 500 nm, 4, and their differential spectrum, 5.

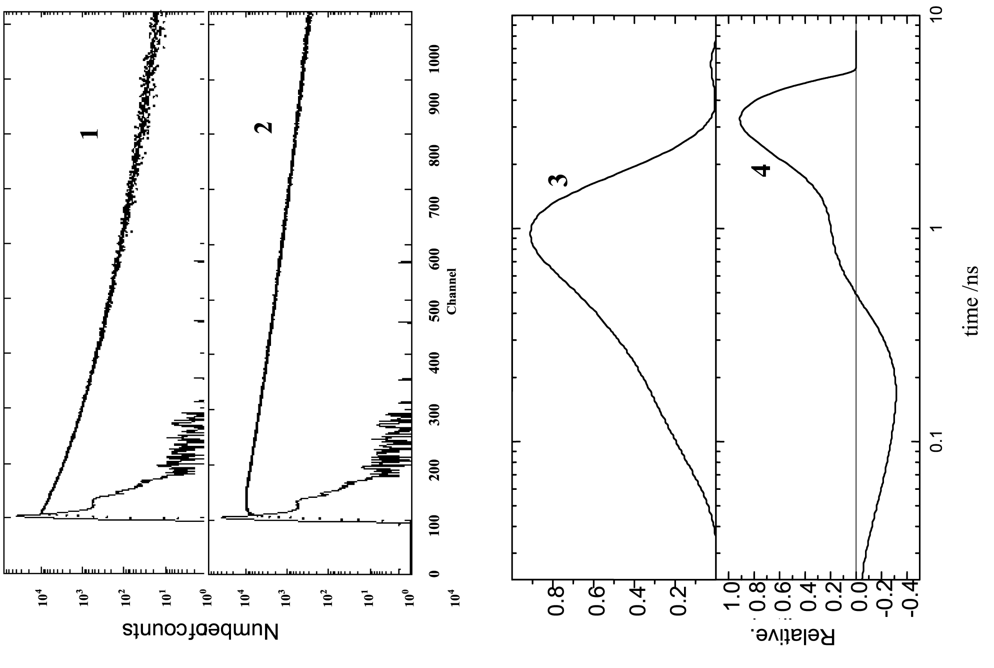


Fig. 6. Fluorescence decay curves of the LE (1 at $\lambda = 425$ nm) and CT (2 at $\lambda = 500$ nm) bands of the laurdan luminescence spectrum. The luminophor was excited at the maximum of the absorption band ($\lambda_{ex} = 386$ nm). Below is given the decomposition of the decay curve, found using the MMGC method (3 at $\lambda = 425$ nm and 4 at $\lambda = 500$ nm).

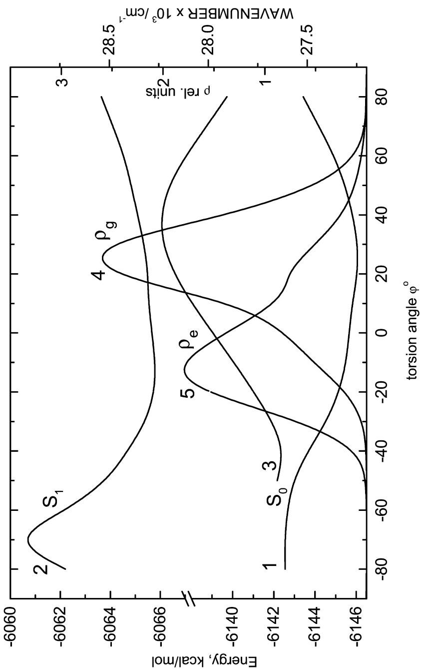


Fig. 7. The singlet state energy dependence: E_0, 1 and E_1, 2 *versus* the twisting angle φ and their difference, 3. Curves 4 and 5 represent the distribution function of the ground, $\rho_g(\varphi)$ and $\rho_e(\varphi)$, and excited states determined at room temperature.

4. Discussion

Let us consider the experimental results consistently. According to Grabowski and Rettig [4–6] the short and long wavelength bands appear as transitions from the local excited S_1(LE), and charge transfer S_1(CT) states, respectively. The two excited states correspond to two equilibrium distributions of the electronic energy density in the molecule. In the discussion we would like considered this concept and give further proves through a number of essential details, some of which, as we will show, requiring corrections.

In glycerol, at about 350 K, all reorientations of laurdan molecules, as well as the redistribution of the electron density inside the solute molecule, are carried out quickly in comparison with the lifetime of the exited states. Therefore, the laurdan fluorescence spectrum is formed mainly by the CT state emission (curve 7 on Fig. 1). The solvent viscosity increase, resulting from the temperature decrease, influences the transition speed of turning molecules from the S_1(LE) to the S_1(CT) state. As a consequence, at room temperatures the total fluorescence spectrum of laurdan consists of photons from both states in approximately equal amounts (curve 4, Fig. 1). Further cooling of the solution changes the intensity relation between the two bands in the direction of LE emission majority. At 260 K, in the total spectrum the luminescence emission from the CT state is not observed at all (see curve 4).

The presented data testify the strong influence of intramolecular interaction and molecular reorganisation inside laurdan solvates (luminescence shells) on the fluorescence spectrum. Similar results were observed by other authors for the same molecules dissolved in ethanol and other solvents [23], as well as for other molecules showing dual emission from the S_1(LE) and the S_1(CT) states [4–9].

To facilitate the interpretation of the dual luminescence characteristics it is useful to introduce and consider an additional parameter – the intensity ratio of both band maxima, *i.e.* $\mathbf{I_{LE}}/\mathbf{I_{CT}}$. The temperature dependence of it, *i.e.* $\ln[\mathbf{I_{LE}}/\mathbf{I_{CT}}]$ *vs* T (see Fig. 2, curve 1) may be explained using a four-level energy scheme in which the population densities of the S_1(LE) and S_1(CT) states are temperature dependent. As is known, the temperature affects the rate constants of the radiationless S_1(LE)→S_1(CT) transition [6], which is controlled by the viscosity and the association degree of the solvent. This statement is valid for the Stokes excitation at the maximum absorption band [6].

Figure 3 shows luminescence spectra of laurdan obtained for different excitation wavelength at 260 K. Comparing them with those pictured in Fig. 2 (determined for solutions at different temperatures), an essential difference is noted. It testifies the presence of certain molecular forms (conformations) of laurdan in the solution. Some of them should have the absorption spectra shifted to the red, and if the dye concentration is high enough they should be registered by the “red-edge” excited luminescence.

The spectroscopic data show that in glycerol a nonequilibrium conformational form of laurdan in the ground state exists. This means that laurdan in glycerol solution is a typical spectrally inhomogeneous system. As is well known, excitation wavelength dependence of the luminescence spectrum is an experimental criterion for inhomogeneous broadening [29]. The spectra presented in Fig. 3 confirm this criterion. The inhomogeneous broadening appears in both bands, *i.e.* it is connected with transitions from the S_1(LE) and the S_1(CT) states. The intensity relations plotted in Fig. 2 evidently show not only the inhomogeneous character of the luminescent solvates, but also demonstrate an opportunity to change simultaneously the ratio between the intensities of both emitting components by verifying the energy of the exciting quanta. Also, for different temperatures of the solutions the maximum of the CT band is red shifted on increasing the excitation wavelength (see Figure 4).

Now, let us discuss the luminescence maximum dependence on λ_{ex} at 260 K. At this temperature glycerol shows an almost frozen rigid matrix, at which the configuration relaxation times exceed essentially the laurdan lifetime in the excited S_1(LE) and S_1(CT) states. For this case it is quite natural to assume that we observe the LE fluorescence of laurdan being at various stages of the solution heterogeneity. For this temperature, the halfwidth of the inhomogeneous broadening function $\Delta\tilde{\nu}_{\text{in}}$, is calculated to be 700 cm^{-1} or $\Delta\lambda \approx 20$ nm. The calculations were performed using the formula 8-24 from [16] and values of the dipole moments 3,7 and 10,2 D for the ground and excited state of laurdan, respectively, and the Onsager sphere radius $a_0 = 5{,}5 \cdot 10^{-8}$ cm [12]. Obtained $\Delta\lambda$ value is rather large for inhomogeneous broadening and as such should cause a significant broadening of the luminescence bands.

In our case the wavelength shifts $\Delta\lambda_{\text{lum}}$ (λ_{ex}) are smaller than may be found in polar solutions (20–50 nm) for other molecules [3,16–18]. In the experiment, a more distinct display of the LE band is prevented by the weak selectivity of the non- monochromatic excitation light. A more intensive and monochromatic excitation light source (a frequency tunable laser) would allow to show all red-edge effects and get a more accurate $\Delta\tilde{\nu}_{\text{in}}$ value.

For higher temperatures (see Fig. 4) the excitation wavelength dependencies of the luminescence spectra testify the presence of heterogeneity of the luminescence solvates as well. At these temperatures a fast intermolecular relaxation takes place. The reorientation of the solution molecules (the reorientation time is smaller than 0.1 ns for the temperatures used [16 - 18]) is accomplished during the lifetime of the given luminescent states ($\tau_{\mathrm{Fl}} \approx 3$ ns). In this situation the spectral heterogeneity of the solution caused by fluctuations of solvate structures, including laurdan molecules, should not be registered in the stationary luminescence spectra. Thus we should not observe the bathochromic shift of the emission spectra decreasing the exciting light energy.

The nature of the spectral red shift (Fig. 4, curve 350 K) for the conditions of fast reorientation of the solvate molecules requires an additional explanation. Some details of it can been noted analysing the fluorescence excitation spectra obtained detecting the luminescence at $\lambda_{\mathrm{ob}} = 425$ nm and 500 nm, see Fig. 5 (curve 3 and curve 4). Each spectrum consists of two well revealed components with the maximum at $\lambda = 340$ and 370 nm, respectively. The excitation spectrum obtained detecting the luminescence at $\lambda_{\mathrm{ob}} = 425$ nm (curve 3), has a larger intensity in the region of 340 nm than those detecting the emission at $\lambda_{\mathrm{ob}} = 500$ nm. The differential excitation spectrum (see curve 5 on Fig. 5) explains these differences. It possesses an intensity maximum at $\lambda \cong 325$ nm. According to [20], the differences of the excitation spectra of laurdan and laurmen (where the dimethyl group is absent) show the influence of the dimethyl functional group on the absorption and emission process. It is obvious that the curve 5 on Fig. 5 reflects the LE states absorption, which leads to the emission of the LE band at 425 nm. Its distinction in the stationary absorption spectrum testifies, once again, the existence of the spectral heterogeneity of the laurdan solvates. The mechanism of the solvates heterogeneity is probably connected with a slow mechanism of intramolecular reorientation of the dimethyl group in comparison to the luminescent lifetime. In our opinion, the twisting motions of the dimethyl group around the C–N bond in laurdan are the main reason of the solvates heterogeneity, which must be considered.

Now we would like to make use of the important properties of laurdan in its free state obtained by quantum chemistry calculations (HyperChem v.5.0) in our previous paper [25]. Figure 7 shows the total energy of the first two singlet states (S_0 and S_1) dependence on the twisting angle φ of the mutual orientation of the dimethyl group with respect to the plane of the naphthalene moiety (similar results were obtained for prodan [30]). As can be seen on Fig. 7, the total energy of the S_0 and S_1 states shows minima at the φ values 27° and $-15°$, respectively. According to the calculations, the S_1(LE)$\rightarrow S_1$(CT) transitions are accompanied by an average change of the twisting angle of about 43°. The energy differences of the S_0 and the S_1 states (curve 3), which determine the frequency of the electronic transition, is not constant and depends on the angle φ [25]. Due to this dependence, an ensemble of laurdan molecules, which must by treated as a heterogenic system, has inhomogeneously broadened spectra. Their contours can be calculated if an appropriate distribution function is known [31].

Figure 7 shows the Boltzmann probability distribution function of the molecular energies $\rho_g(\varphi)$ and $\rho_e(\varphi)$ (the energy depends on the φ angle) of the S_0 and S_1 states of laurdan at room temperature. As can be seen, they show a wide and strong overlap. This means that there are laurdan molecule configurations which can be mutually considered as common for two states. Thus a direct excitation of the CT state configuration of laurdan is basically possible and the probability of such an absorption process is considerably high. The transition wavenumber dependence, $\tilde{\nu}_{\mathrm{el}}(\varphi)$, is the basis for the internal mechanism of electronic spectra broadening of the free molecules. This spectra broadening can be calculated taking the sum over all possible electronic transitions between the ground S_0 and excited S_1(LE) or S_1(CT) states. The appropriate formulas and the results of such calculations are presented in [31]. They show the relevance of the angular distributions, which account for the time and energy dependence of the emitted quanta. Also, this dependence is responsible for important luminescent properties of laurdan in solution.

Let us consider the results of the luminescence decay measurements of the LE and CT emission modes (see Figure 6). Analysing the decay constants and amplitude distribution in the common time region (about 0.1 and 1 ns), it follows that the contribution of the shortest component (0.1 ns) appears in the decomposition of the decay curves 3 and 4 with opposite signs. It shows that the excitation energy form the S_1(LE) state is transferred to the S_1(CT) state with an appropriate rate constant. As has been mention earlier, the transformation of the LE molecular form to the CT form is accompanied by a change of the angular energy distributions $\rho_{\mathrm{e}}(\varphi)$ (curve 5, Figure 7). It occurs with a characteristic decay time of one broad component in the luminescence decay, *e.g.*, from 0,3 ns up to 1 – 2 ns (see Fig. 6, curves 3,5). Thus, exciting the laurdan solution with high energy photons causes the LE emission band to be broadened. It corresponds to the excitation in the maximum of the distribution function $\rho_{\mathrm{g}}(\varphi)$ (see Figure 7). The excited molecules emit in part photons which constitute the LE component, and in part photons of relaxed components. This dual behaviour influences the shape of the stationary luminescence spectrum, whereas the shift of the excitation wavelength to the red presumably results in excitation of solvates possessing transitions with smaller electronic energies. This leads to an intensity decrease of the LE component (see Fig. 3) and its shift to the longer wavelength.

Further, we suppose that the absorption band ($\lambda \approx$ 325 nm) corresponding to the differential excitation spectrum (see curve 3, Figure 5) indicates the radiation of the component decaying with the shortest time (about 0,1 ns). This fast component, described by the negative part of curve 4 in Fig. 6, participates in the depopulating process of the S_1(LE) state. The fluorescence decay component in the time range of 1 ns, appearing with different participations at both wavelengths of registration expresses the emission of the LE band. The fluorescence components detected with a decay time of 2 ns and 6 ns are responsible for the CT emission.

On the basis of the decay constants we can discuss the kinetics of the radiation process in connection with the energy angular distributions caused by the - $N(CH_3)_2$ twisting phenomenon. Twisting of the -$N(CH_3)_2$ group will occur in molecules possessing longer decay times than the reorientation relaxation time of the solvent molecules in solvates (luminescence centres). Classically speaking, it means that the rotation of these functional groups does not occur in a free volume but in the solvation shell. In favour of such a heterogeneous mechanism argue also the results of Viard's *et al.* [23]. They demonstrated the luminescence heterogeneity of laurdan in nonpolar solutions (in oils), where the configurational broadening of the electronic spectrum is small in comparison to that of polar solvents. In the same paper [23] the characteristics of the inhomogeneous broadening of the LE band of laurdan in ethanol are found and measured at low temperature. The value of the LE band broadening is significant, $\Delta\lambda \sim 20$ nm, and convincing demonstrated its appearance in the normal LE fluorescence of laurdan.

5. Conclusion

The presented data as well as the results of other authors allow to draw the following more general conclusions. The laurdan solution is spectrally an inhomogeneous system, and for it various mechanisms are responsible. On the basis of the data available, we would like to present three major points.

1. In the ground and excited states, Laurdan dissolved in glycerol, presents simultaneously a set of various conformational forms. The clearly expressed conformational components are the LE and the CT states. The calculated value of the inhomogeneous broadening parameter equals $\Delta\lambda = 75$ nm. The presence of different conformational forms is a result of the physicochemical properties of laurdan molecules, *i.e.*, its ability to create conformational structures possessing different twisting angles. The distribution function of the twisting angle dependence is broad.

2. The LE and CT states of the laurdan are rather steady in time and have their own electronic absorption and emission spectra. In order to interpret the experimental data it is necessary to consider these conformations as laurdan molecules in various microstructure solvate shells. The broadening parameter of laurdan in glycerol is $\Delta\lambda = 20$ nm. The spectral broadening characteristics of the S_1(CT) state were observed in the present work.

3. It has been shown that the fluorescence band broadening mechanism is connected with the electronic transition wavenumber dependence $\tilde{\nu}_{el}(\varphi)$ on the twisting angle φ, *i. e.*, the angle between the mutual orientation planes of the dimethyl group and naphthalene moiety [29]. In solutions, this mechanism should be valid for all possible conformational states of laurdan. Relaxation over twisting coordinates influences (basically enlarges) the relaxation time to establish the full equilibrium $\tau_{\mathrm{rel}}(\varphi, R)$ in the luminophor solvates (R is the local electric field intensity in the solvate). The proposed model allows to explain all spectral properties of laurdan collected by us in pure solvents, including the ones received using the time resolved spectroscopy method in nano – and picosecond limits of times.

Acknowledgements

The authors express their gratitude to Mgr K. A. Kozyra for the luminescence decay data, Mgr K. Hubisz for the performed measurements of the absorption spectra, PhD E. P. Petrov for the analysis of the luminescence kinetics, using the modified method of generalised crossvalidation, and Professor H. Diehl (University of Bremen) for the laurdan sample. This work was partially supported by the research grants of the Pomeranian Pedagogical Academy at Slupsk, Projekt NW/6/1239/02.

[1] G. E. Dobretsov, Fluorescence probes in investigations of cells, membranes and lipoproteins, Nauka, Moscow 1989, p.276.

[2] A. P. Demchenko, J. Lumin. **17**, 19 (2002).

[3] A. P. Demchenko, Luminescence and Dynamics of Protein Structure. Naukova Dumka: Kiev 1988.

[4] Z. R. Grabowski, K. Rotkiewicz, A. Siemiarczuk, D. J. Cowley, and W. Baumann, Nouv. J. Chim. **3**, 43 (1979).

[5] R. Grabowski, Acta Phys. Polon. **A71**, 743 (1987).

[6] E. Lippert, W. Rettig, V. Bonacic-Koutecky, F. Heisel, and J. A. Miehe, Photophysics of Internal Twisting, in Advances in Chemical Physics. Ed. I. Prigogine and Stuard, A. Rise. (1987), p.68.

[7] J. Heldt, D. Gormin, and M. Kasha, J. Amer. Chem. Soc. **110**, 8255 (1988).

[8] J. Heldt, D. Gormin, and M. Kasha, Chem. Phys. Lett. **150**, 433 (1989).

[9] J. Heldt, D. Gormin, and M. Kasha, Chem. Phys. **136**, 321 (1989).

[10] J. Heldt and M. Kasha, J. Mol. Liq. **41**, 308 (1989).

[11] G. Weber and F. J. Farris, Biochemistry **18**, 3073 (1979).

[12] A. Kawski, B. Kukliński, P. Bojarski, and H. Diehl. Z. Naturforsch. **55a**, 817 (2000).

[13] W. C. Galley and R. M. Purkey, Proc. Natl. Acad. Sci. USA **67**, 1167 (1970).

[14] A. N. Rubinov and V. I. Tomin, Opt. Spectr. **29**, 1082 (1970).

[15] G. Weber and M. Sinitsky, Proc. Natl. Acad. Sci. USA **65**, 823 (1970).

[16] N. A. Nemkovich, A. N. Rubinov, and V. I. Tomin, Inhomogeneous Broadening of Electronic Spectra of Dye Molecules in Solutions in Topics in Fluorescence Spectroscopy, Principles, ed. J. R. Lakowicz; Plenum Press New York **2**, 367 (1991).

[17] V. I. Tomin and A. N. Rubinov, Zh. Prikl. Spectr. **35**, 237 (1981).

[18] A. N. Rubinov, V. I. Tomin, and B. A Bushuk, J. Luminescense **26**, 377(1982).

[19] T. Parasassi, G. d. Stasio, G. Ravangan, R. M. Rusch, and E. Gratton, Biophys. J. **60**, 179 (1991).

[20] T. Parasassi, E. K. Krasnowska, L. Bagatolli, and E. Gratton, J. Fluorescence **8**, 365 (1998).

[21] M. Viard, J. Gallay, M. Vincent, and M. Paternostre, Biophys. J. **80**, 347 (2001).

[22] T. Parasassi and E. Gratton, J. Fluorescence **5**, 59 (1995).

[23] M. Viard, J. Gallay, M. Vincent, O. Meyer, B. Robert, and M. Paternostre, Biophys. J. **73**, 2221 (1997).

[24] M. Brozis, K. A. Kozyra, V. I. Tomin, and J. Heldt, Zh. Prikl. Spectr. **69**, 412 (2002).

[25] M. Brozis, V. I. Tomin, and J. Heldt. Zh. Prikl. Spectr. **69**, 589 (2002).

[26] J. Karolczuk, D. Komar, Kubicki, T. Wrozowa, K. Dobek, B. Ciesielski, and A. Maciejewski, Chem. Phys. Lett. **344**, 154 (2001).

[27] E. P. Petrov, Vth International Conference on Methods and Applications of Fluorescence Spectroscopy, Berlin, Germany, 21-24 September 1997, Book of Abstracts. – Köster, Berlin 1997, p.142.

[28] E. P. Petrov, J. V. Kruchenok, and A. N. Rubinov, J. Fluorescence **9**, 111 (1999); Erratum, J. Fluorescence **9**, 397 (1999).

[29] W. Demtröder, Laser Spectroscopy, Springer-Verlag, Berlin 1988.

[30] A. B. J. Parusel, F. W. Schneider, and G. Kohler, J. Mol. Structure (Theochem) (1997), p.341.

[31] M. Brozis and V. I. Tomin, Zh. Prikl. Spectr. (in print).