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CONCENTRATION DEPENDENCE OF SPECTRAL AND LUMINESCENCE FEATURES FOR SOLUTIONS OF PYRAZOLINE DYES

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A study on the concentration dependence of spectral and luminescence properties of Luminophor 59 solutions in toluene, dioxane, dimethylformamide, and ethanol has shown that this dye forms associates at concentrations greater than 10^{-5} mole/liter. This conclusion has been supported by the data on the luminescence parameters. The quantum yields and lifetimes of fluorescence have been determined for the dye studied.

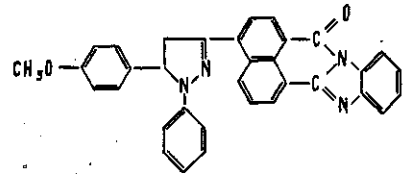
Pyrazoline dyes are promising compounds to be used in various fields of science and technology, in particular, in medical and biological research due to their specific physicochemical and spectral and luminescence properties. Their synthesis and the study of their spectral and luminescence characteristics have been initiated more than two decades ago. Previous studies [1–4] on the spectral features of these compounds were devoted to elucidating the nature and oscillator strength of electronic transitions, as well as their dipole momenta. However, data on the influence of the solvent identity on the physicochemical and optical parameters of these compounds are still lacking in the literature; the effect of dye concentration on the solution properties, in particular, the possibility of associate formation, have not yet been studied. Yet the elucidation of the correlation between the structure and spectral parameters of pyrazoline dyes would allow a purpose-oriented synthesis of molecules with desired properties, and the practical application of these dyes is related to variation of solution concentration over a wide range.

We have started a systematic study of the spectroscopic behavior of some pyrazoline dyes as a function of their molecular structure, the identity and acidity of the solvent, concentration and temperature of solutions. We have obtained some quantitative energy spectrum characteristics that were absent in the literature. In this paper, we present data on the spectral and luminescence properties of Luminophor 59 solutions in solvents of varied nature (toluene, dioxane, ethanol, and dimethylformamide) over a wide range of dye concentration. The knowledge of these parameters will facilitate the use of these compounds as fluorescent labels and probes, reporter groups, in the studies of proteins and nucleic acids.

Pyrazoline dyes show a number of properties required for medical and biological studies: they have various reactive sites, they show absorption and intensive fluorescence in the visible spectral region, and have a large extinction coefficient.

Absorption spectra were measured in the 250–750 nm spectral range with a UV-VIS Specord M40 spectrophotometer and the luminescence parameters, with a Jobin Ivon spectrofluorometer admitting a correction of emission spectra. Fluorescence lifetimes τ were measured with a pulsed fluorometer of picosecond resolution time. In measuring the luminescence quantum yields η a diluted solution of Rhodamine 6G in alcohol was used as a reference ($\eta = 0.94$ [5]).

The compound studied (1-phenyl-5-(*n*-methoxyphenyl)-3-[1,8-naphthoylene-1′,2′-benzimidazolyl-4] pyrazoline-Δ^2, or Luminophor Red 2G 600 RT) has the following molecular structure:



The shape of electron absorption spectra of Luminophor 59 is almost completely independent of the solvent used, while the positions of the absorption band maxima depend substantially on the identity of

solvents (Table 1), and this was noted by other workers [2]. As an example, the absorption spectrum of this compound in toluene at room temperature is shown in Fig. 1 (curve 1). As the Luminophor 59 concentration increases from 10^{-7} to 10^{-3} mole/liter, the maximum of the long-wavelength absorption band undergoes a bathochromic shift by 14 nm; the position of this band in dimethylformamide (DMF) and ethanol is almost independent of dye concentration.

Table 1

Positions of band maxima in the absorption and fluorescence spectra of Luminophor 59 in various solvents

Concentration, mole/liter	Dioxane		DMF		Toluene		Ethanol	
	λ_{max}^{abs}, nm	λ_{max}^{fl}, nm	λ_{max}^{abs}, nm	λ_{max}^{fl}, nm	λ_{max}^{abs}, nm	λ_{max}^{fl}, nm	λ_{max}^{abs}, nm	λ_{max}^{fl}, nm
6.5×10^{-8}	493	608	—	—	500	591	519	550
7×10^{-7}	497	608	—	—	500	592	519	550
7.9×10^{-6}	501	609	515	671	507	592	521	544 582 676
8.7×10^{-5}	502	612	515	680	514	595	521	542 582 676
1×10^{-4}	503	614	515	683	514	596	522	541 582 676
1×10^{-3}	506	618	515	692	514	598	522	—

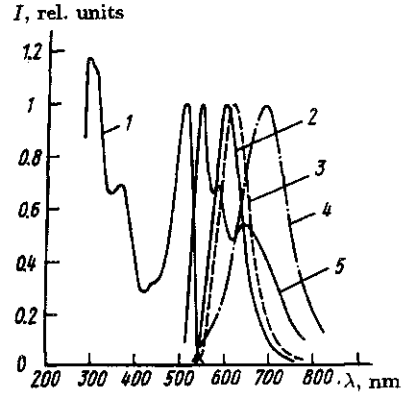


Fig. 1

Absorption and fluorescence spectra of Luminophor 59 in various solvents: absorption in toluene at $C = 10^{-4}$ mole/liter (1); fluorescence in toluene (2), dioxane (3), dimethylformamide (4), and ethanol (5) at $C = 10^{-4}$ mole/liter for all solvents.

The position of the Luminophor 59 luminescence spectra is strongly dependent on the solvent. For all the solvents studied (except ethanol), one maximum was observed upon excitation within the long-wavelength absorption band (Fig. 1, curves 2–5). An increase in the dye concentration by a factor of 10^4 results in a shift of the spectra from 5 to 20 nm toward longer wavelengths depending on the identity of solvent (Table 1). Following excitation at short wavelengths, a short-wavelength maximum was found to appear in the luminescence spectra of solutions of the dye in dioxane, toluene, and DMF; its relative intensity depends on the dye concentration and wavelength of the exciting radiation. These bands have not been observed in previous studies, and we shall consider their origin in our further discussion.

Quite a different behavior was shown by the concentration dependence of the Luminophor 59 luminescence in ethanol solutions (Fig. 2). At low concentrations (10^{-7} mole/liter), one maximum at $\lambda_{max}^{fl} = 550$ nm is observed in the fluorescence spectrum. When the dye concentration reaches 10^{-5} mole/liter, two new bands appear. At maximum concentrations of Luminophor 59 one can see three maxima at 540, 580, and 647 nm. The short-wavelength band maximum of fluorescence undergoes a hypochromic shift by 10 nm. Our experiments show that the absorption and fluorescence spectra are independent of pH; therefore, no ionic forms of the dye are formed in ethanol solutions. The spectral changes observed are probably due to the occurrence of intermolecular association of the dye molecules in ethanol solutions.

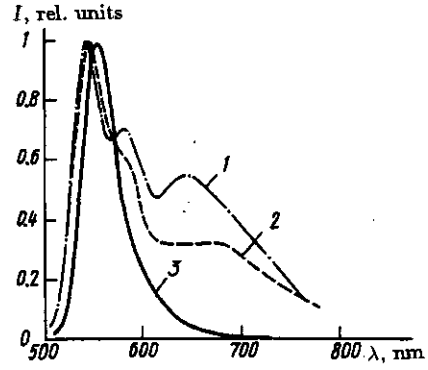


Fig. 2

Fluorescence spectra of Luminophor 59 in ethanol solutions at various concentrations: $C = 10^{-4}$ (1), 7.9×10^{-6} (2), and 7×10^{-7} mole/liter (3).

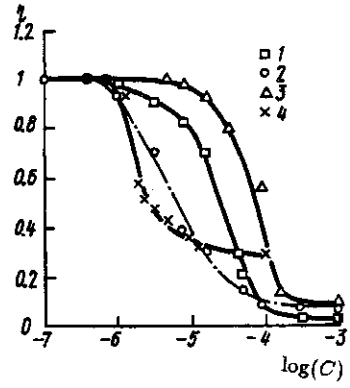


Fig. 3

Relative quantum yield η of fluorescence as a function of Luminophor 59 concentration in various solvents at room temperature: toluene (1), dioxane (2), dimethylformamide (3), and ethanol (4).

Figure 3 shows the relative quantum yields of luminescence η versus the concentration of the pyrazoline dye studied in various solvents at room temperature. The luminescence quantum yield is seen to substantially decrease for all the solutions beginning with the dye concentration $C = 10^{-5}$ mole/liter, but the $\eta(\log C)$ dependence is different for different solutions. For diluted solutions ($C = 10^{-7}$ mole/liter) when the $\eta(\log C)$ curve attains a plateau and the excitation occurs at the long-wavelength absorption band maximum, the η values become strongly dependent on the solvent: $\eta = 0.73 \pm 0.04$ for toluene, 0.28 ± 0.01 for dioxane, 0.08 ± 0.01 for DMF, and 0.06 ± 0.01 for ethanol. The lifetime of the Luminophor 59 excited state was measured for toluene and ethanol solutions: 4 ± 0.2 ns and 170 ± 10 ps, respectively.

Therefore, a concentrational quenching was found to occur in all the dye solutions studied; as a rule, it is caused by association of dye molecules [6]. Small concentration-induced changes in the absorption spectra may point to the corresponding structure of associates [7] and their solvate shells [8].

To elucidate the origin of the observed spectral changes, we measured the temperature dependence of the spectral and luminescence characteristics of the solutions. Toluene 10^{-6} mole/liter solutions of Luminophor 59 were cooled down to a temperature of toluene crystallization (−95°C). The shift of luminescence spectra toward shorter wavelengths was found to reach 20 nm, while the fluorescence intensity of the band with a 590 nm maximum grew markedly (Fig. 4, curve 1). The intensity of luminescence from the dye solutions of high concentration (10^{-4} mole/liter) remains nearly unchanged as it cools to −50°C, and somewhat lowers with further temperature decrease (Fig. 4, curve 2).

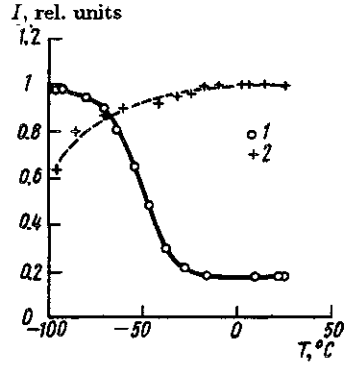


Fig. 4

Temperature dependence of fluorescence intensity for Luminophor 59 solutions in toluene: $C = 10^{-6}$ (1) and 10^{-4} mole/liter (2).

The efficiency of luminescence from the 10^{-5} mole/liter solutions of Luminophor 59 changed appreciably with decreasing temperature: the intensity of short-wavelength fluorescence band maximum (541 nm) decreases, and it vanishes almost completely at −80°C (Fig. 5, curve 1), whereas the intensity of the long-wavelength band (641 nm) increases by a factor of about 20 (Fig. 5, curve 2). The shift in the position of the long-wavelength band maximum toward longer wavelengths reaches 40 nm.

The results of the low-temperature experiments indicate conclusively that molecular association in the Luminophor 59 solutions studied occurs at dye concentrations greater than 10^{-5} mole/liter. In ethanol solutions, the associates are capable of luminescence. Their emission spectrum is shifted substantially (more than by 100 nm) to the red with respect to the fluorescence spectrum of monomers while the quantum yield of their luminescence is comparable to that of monomers. It should be noted that molecules of a pyrazoline dye show an associative ability at their low concentrations (about 10^{-5} mole/liter). Recently similar data were reported for aqueous solutions of pseudoisocyanine, which forms dimers at the same concentrations [9]. As follows from concentration dependences of the fluorescence quantum yields (Fig. 3), the efficiency of association is different for different solvents. This circumstance must be taken into account when encapsulating these dyes into polymer fluorescent latexes. The study of the concentration dependence of absorption and fluorescence spectra of dyes encapsulated into polyacrolein latexes has suggested a conclusion that changes in luminescence efficiency caused by increased concentration of dye molecules in latex particles

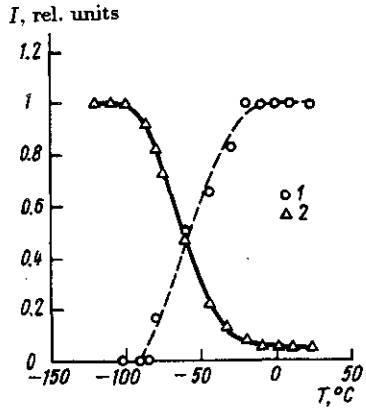


Fig. 5

Temperature dependence of fluorescence intensity for Luminophor 59 solutions in ethanol ($C = 10^{-5}$ mole/liter): short-wavelength band $\lambda^{fl}_{max} = 541$ nm (1) and long-wavelength band $\lambda^{fl}_{max} = 641$ nm (2).

are due to the formation of associates [10]. In view of this, the photoenergetics of the pyrazoline dyes studied in this work deserves a more detailed consideration.

Thus, the spectroscopic study showed that pyrazoline dyes, even at rather low concentrations, are capable of forming associates that can fluoresce. The data obtained help outline the directions of purpose-oriented synthesis of pyrazoline dyes with spectral and luminescence parameters required, which is important for their use for the diagnostic purposes.

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