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Spectrophotometric Study of Luminol in Dimethyl Sulfoxide–Potassium Hydroxide

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The spectrophotometric study of luminol (LH_2) in dimethyl sulfoxide (DMSO), DMSO-water solutions, and alkaline DMSO and DMSO-water solutions has been done, focusing on the effect of the KOH additon on LH_2 absorption and fluorescence properties. The absorption spectra indicate an acid-base equilibrium, and the luminol dianion (L^{2-}) formation at $3 \times 10^{-4} - 2.4 \times 10^{-3}$ *M* KOH. The decrease of the fluorescence intensity and the variation of the excitation spectra of LH_2-DMSO-KOH solutions with KOH concentration have been similarly explained. The acid-base process is reversible. The addition of HCl to the solution with 3.0×10^{-3} M KOH leads to an increase of the fluorescence intensity to its highest value, observed in pure DMSO. The addition of HCl to the LH_2-DMSO solution leads to the decrease of the fluorescence intensity as a result of the LH_3^+ cation formation. In LH_2-DMSO-water, the fluorescence band is shifted from 405 nm to 424 nm and increased in the intensity. In the presence of KOH (in LH_2-DMSO-water-KOH solution) a new band appears, with the maximum at 485 nm and the band at 405 nm decreased. The changes in fluorescence lifetimes also evidence the different chemical species formed.

KEY WORDS: Luminol fluorescence; alkaline dimethyl sulfoxide; luminol fluorescence lifetime.

INTRODUCTION

Luminol (LH_2) (5-amino-2,3-dihydro-1,4-phthalazinedione) chemiluminescence in water is mostly applied for analytical purposes, in special forensic medicine (to detect trace amounts of blood); this is why luminol reactions in water have been intensely investigated. Luminol is a fluorescent probe utilized in analytical and biochemical applications [1–9].

The chemiluminescence of the system luminol-DMSO has several practical applications [10], so it is useful to know more about the spectroscopic behavior of this system. The luminol fluorescence in different solvents and solvent mixtures or its photoprocesses in the presence of quenchers are much less studied, although some papers in this area have been published in the last years [11–19]. Thus, solvatochromic spectroscopy studies on LH_2 in polar and nonpolar solvents or their mixtures have been conducted [13,14].

LH_2 behavior in aqueous NaOH and H_2SO_4 solutions has been studied by steady-state fluorescence and time-resolved single-photon counting techniques [11]. It was found that both fluorescence quantum yields and rate constants are pH dependent, the maximum fluorescence intensity being observed at pH 5–6. The fluorescence quenching by very small NaOH concentrations (10^{-5} M) has been analyzed by Stern-Volmer equation.

Both the chemiluminescence and the fluorescence of LH_2 in water render one band with maximum at the same wavelength (425–430 nm). Therefore the emission

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appears from the same singlet excited state of aminophtalate dianion [7].

The situation is more complex in aprotic solvents, for instance in dimethyl sulfoxide (DMSO), an important solvent that is well suited for investigation of proton and electron transfer of organic molecules [20]. Solid KOH in dimethyl sulfoxide is a very strong base with pKa-value higher than 27 [21–24]. The strongly basic nature of the potassium salt of the conjugate base of DMSO (the dimsyl anion) enables evaluation of proton-transfer equilibrium [20] and facilitate the nucleophilic processes [24], the obtained nucleophile species being stable in this no aqueous medium. The fluorescence of LH_2 in DMSO has not been investigated in great detail. In DMSO with KOH one can expect the conversion of LH_2 in its anion from an acid-base reaction.

White and Bursey [1964] observed a chemiluminescence band with the maximum at 485 nm for LH_2 in DMSO and the same position for the fluorescence emission [25]. In our previous paper [8] we presented the LH_2 reaction with potassium superoxide (KO_2) in DMSO, in the presence of 18-Crown-6-ether (18C6), which ensures the superoxide solubilization through the $[18C6. . . K]^+O_2^-$ supramolecular hydrophobic complex formation. The chemiluminescence results from an acid-base reaction, followed by a redox reaction, and the emission band assigned to the aminophthalate anion has a maximum at 490–500 nm. For LH_2 in DMSO fluorescence band at 405 nm and one at 425–430 nm in DMSO-KO_2-18C6 have been observed [8]. These findings are evidences in another emissive species.

The aim of this study is to investigate in detail the fluorescence of LH_2 in DMSO and DMSO-water, focusing on the effect of KOH addition on its absorption and fluorescence properties. For a better evidence of the LH_2 emissive species in DMSO, DMSO-water, and alkaline DMSO, the time-resolved fluorescence measurements have been utilized.

EXPERIMENTAL

Materials and Solutions

Luminol, DMSO (spectroscopic grade), and KOH from Merck were used. The LH_2 concentration was 2.3×10^{-5} *M*. Solid KOH was solubilized in DMSO by heating and stirring to obtain a saturated solution of about 3×10^{-3} *M* KOH. This solution was diluted with DMSO.

Methods

The pH of the solutions was measured with Consort P500 (Sentek Limited) pH-meter. The pH-meter was calibrated with aqueous buffers, so the pH values of the LH_2-alkaline DMSO solutions are arbitrary values; that is why the concentrations of KOH are also indicated. The fluorescence spectra (emission and excitation) were recorded with Spex spectrofluorimeter, at 23°C. The emission and excitation spectra were corrected. The fluorescence quantum yield was determinate by comparison to diluted quinine bisulfate solution in 0.1 N H_2SO_4, with 0.55 absolute quantum yield [26]. The fluorescence lifetime of LH_2 was measured, at 21°C, using a single-photon counting technique. The excitation setup uses a mode-locked Nd-YAG laser (Spectra Physics Model 379.344S) and a dye laser. The excitation wavelength was 300 nm. The experimental method is described in [27]. Absorption spectra were recorded with a Shimadzu UV-VIS 2501 PC spectrophotometer, at 23°C.

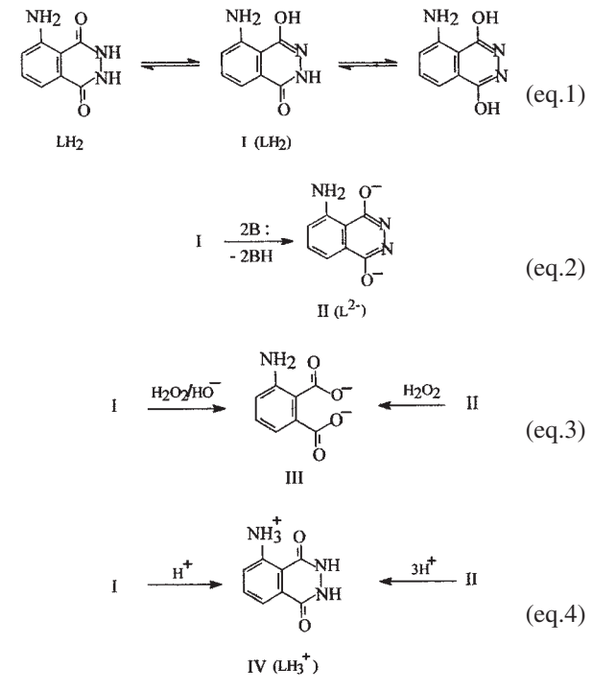
RESULTS AND DISCUSSION

The structure of LH_2 [Eq. (1)] confers acidic properties upon aqueous solution, so that in a base presence there results the dianionic species L^{2-} [Eq. (2)], which by oxidation with hydrogen peroxide yields the dianion III [Eq. (3)] with chemiluminescence emission [5,28]. On the other hand, at acidic pH the cation IV, LH_3^+, is produced by the protonation of the amino group of LH_2 (Eq. 4).

The Effect of KOH Concentration on the Absorption Spectra of LH_2-DMSO-KOH Solutions

Potassium hydroxide reacts with both DMSO and luminol; with luminol to form corresponding anion and water, and with DMSO to produce the dimsyl anion (the conjugate base of DMSO) and water (the dimsyl anon being less stable). Thus, in the LH_2-DMSO-KOH system, the trace of water is present.

The pH variation, at KOH concentrations greater than 3×10^{-4} *M*, leads to important modifications of absorption spectra. Figure 1 presents the absorption spectra of 3.2×10^{-5} *M* LH_2 in alkaline DMSO. One can observe two bands with maxima at about 300 and 360 nm, and the isosbestic points at 314, 344, and 373.5 nm, pointing to the appearance of a new species, that is luminol dianion [Eq. (2)].



Reaction scheme 1

For KOH concentrations lower than 3×10^{-4} *M* the absorption spectra are not modified and fluorescence is linearly quenched. Using a Stern-Volmer equation: $Q_0/Q = 1 + k_q\tau_0[KOH]$, where Q_0 and τ_0 are the quantum yield and the lifetime in absence of quencher, a bimolecular quenching rate constant k_q of 5.8×10^{10} M^{-1}/s^{-1} is obtained. When water was used as solvent, Bhattacharjee *et al.* [11] reported a value of 1.2×10^{12} M^{-1}/s^{-1}; therefore the quenching by KOH is stronger in water, as expected.

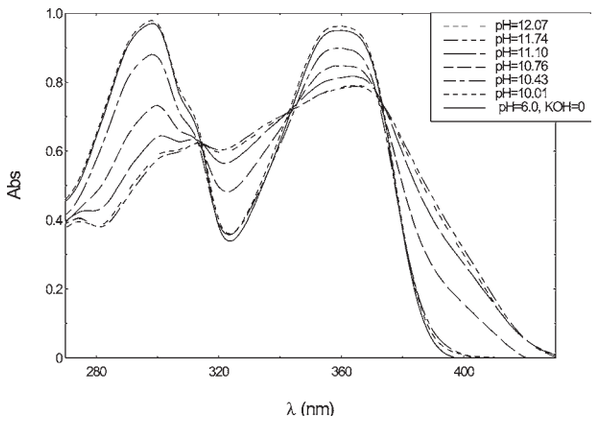


Fig. 1. Absorption spectra of 3.2×10^{-5} *M* luminol solution in alkaline DMSO. In Table I one can find the KOH concentrations corresponding to the pH values.

The Effect of KOH Concentration on the Fluorescence of LH_2-DMSO-KOH Solutions

Figure 2 shows that the fluorescence band intensity of LH_2 (maximum at 405 nm) decreases with increasing of the pH. For the solution with 2.1×10^{-3} *M* (pH = 12.07), the fluorescence spectrum has been multiplied by a factor of 100, so that one can observe a new band (very weak) with a maximum at 475–480 nm, and the band at 405 nm is almost completely quenched. These modifications have been assigned to the dianion L^{2-} [Eq. (2)] formation. Because of the water trace, one can also observe (on the multiplied spectrum) the emission band, characteristic of hydrated luminol. Table I presents the fluorescence quantum yield of LH_2-DMSO and LH_2-DMSO-KOH solutions. One can observe the quenching effect of KOH on the LH_2 fluorescence as a result of dianion formation.

The process is of acid-base type [Eqs. (1) and (2)]. Figure 2a shows that at gradual addition of HCl to the solution with pH 13.1 (certainly keeping constant LH_2 concentration), the fluorescenzce intensity increases, approaching the value in DMSO.

On the other hand, when HCl is added to the LH_2-DMSO solution (Fig. 2b), the fluorescence intensity decreases, probably as a result of the cation formation (Eq. 4). One can thus estimate that the LH_2 fluorescence is maximal for neutral solution.

Figure 3 shows the fluorescence excitation spectra obtained at $\lambda_{em} = 405$ nm. For a better comparison of these spectra, they have been normalized at 344 nm, so that the isosbestic points, at the same wavelengths as in absorption spectra (Fig. 1), could be observed. Both bands (300 and 360 nm) contribute to the fluorescence emission, with the second band being more efficient.

The Effect of H_2O and H_2O_2 Upon Absorption and Fluorescence of LH_2-DMSO-KOH

Figure 4 presents the normalized fluorescence spectra for LH_2 in DMSO ($\lambda_{em} = 405$ nm), in DMSO-H_2O, 1:3 molar ratio, ($\lambda_{em} = 424$ nm), and in DMSO-H_2O-KOH ($\lambda_{em} = 485$ nm). In the presence of water in DMSO, a shift of the emission maximum is observable ($\Delta\lambda_{em} = 19$ nm), and fluorescence intensity increases, the fluorescence intensity in water is about 30 times higher than the

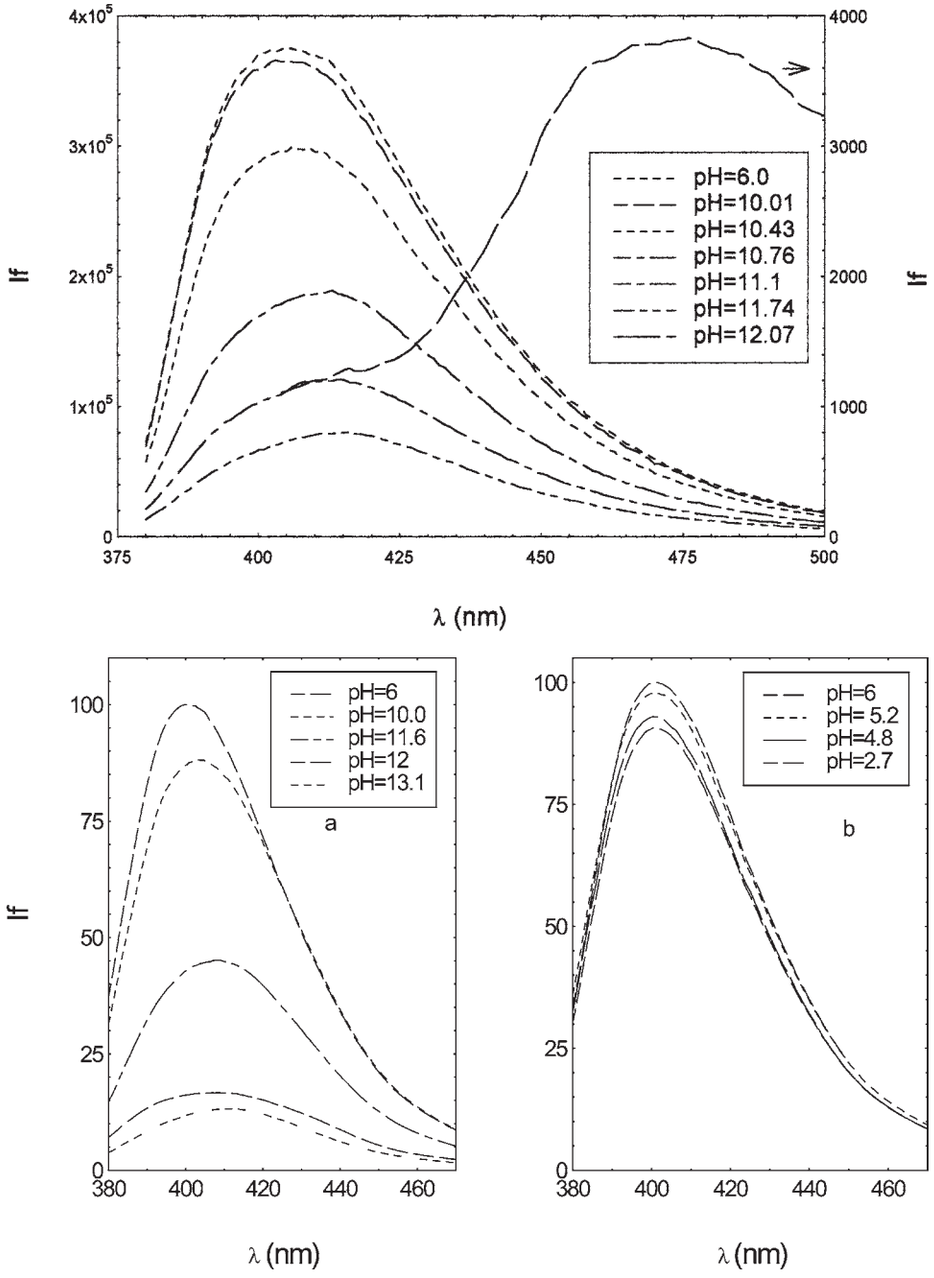


Fig. 2. The fluorescence spectra of 3.2×10^{-5} *M* luminol solution in alkaline DMSO. $\lambda_{ex} = 365$ nm. (For the curve at pH 12.07, the *y*-axis is in the right hand side). The effect on fluorescence intensity of HCl addition to the solution with pH 13.1 (Fig. 2a) and to the solution in DMSO (pH 6.0) (Fig. 2b).

intensity in DMSO, for the same LH_2 concentration (10^{-5} *M*). In the presence of the water-KOH, two bands appear, one at 424 nm, due to the protonate LH_2, and the second at 485 nm, due to the KOH effect, in accordance with the Scheme 2. Bhattacharjee *et al.* have determined the quantum yield for LH_2 in water and obtained a value of 0.88 at pH 5.8 [11]. In 20% DMSO-80% H_2O the quantum yield decreases to 0.60, as is shown in Table I. In alkaline DMSO-H_2O (pH 11.01) the Q value decreases to 0.003, also due to the LH_2 dianion formation.

Table 1. Maximum Fluorescence Intensity (cps) of (3.2×10^{-5} *M*) Luminol and Quantum Yield (Q) in DMSO, Alkaline DMSO, DMSO-H_2O, and Alkaline DMSO-H_2O (λ_{ex} = 360 nm)

Solvent	[KOH] (*M*)	If (cps)	Q
DMSO	–	3.76×10^5	0.150
DMSO+KOH pH 10.01	3×10^{-4}	3.66×10^5	0.144
DMSO+KOH pH 10.20	4.5×10^{-4}	3.29×10^5	0.135
DMSO+KOH pH 10.43	6×10^{-4}	2.99×10^5	0.125
DMSO+KOH pH 10.76	9×10^{-4}	1.89×10^5	0.085
DMSO+KOH pH 11.10	1.2×10^{-3}	1.21×10^5	0.056
DMSO+KOH pH 11.74	1.8×10^{-3}	0.80×10^5	0.039
DMSO+KOH pH 12.07	2.1×10^{-3}	2.61×10^3	0.001
20% DMSO-80% H_2O	–	1.05×10^6	0.600

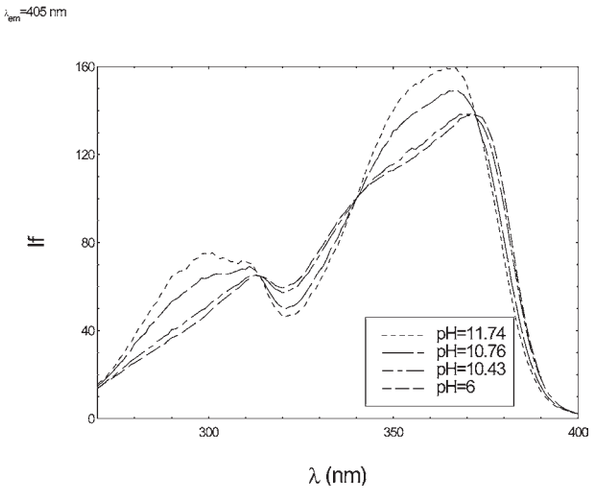
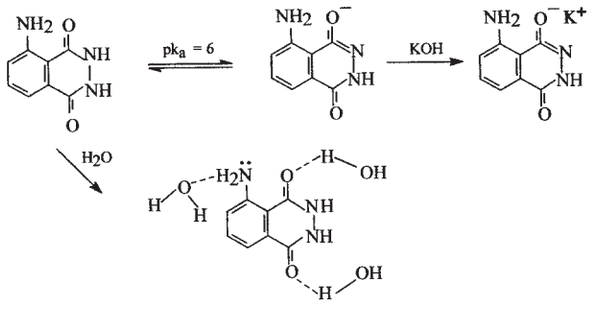


Fig. 3. The excitation spectra of 3.2×10^{-5} *M* luminol solution in alkaline DMSO. λ_{em} = 405 nm. The spectra are normalized at 340 nm.



Reaction scheme 2

The addition of hydrogen peroxide to the alkaline solution (Fig. 5) does not bring spectacular modifications; one can nevertheless observe the fluorescence intensity to increase, with the shift of the maximum (Fig. 5a)

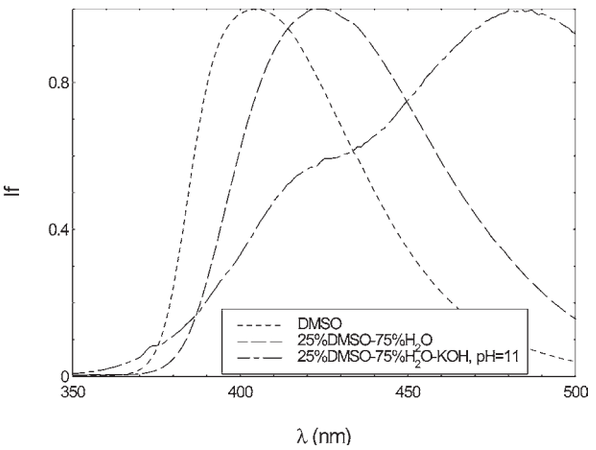
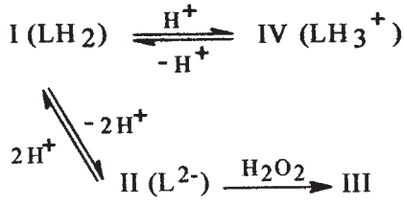


Fig. 4. The normalized emission spectra of luminol in DMSO, in DMSO-H_2O ($^1/_3$), and in DMSO-H_2O-KOH. λ_{ex} = 365 nm.

and the modification of absorption spectrum (Fig. 5b), due to the aminophthalate dianion formation, in accordance with Eq. (3). The modifications are not important because the hydrogen peroxide in the presence of KOH decomposes.

Thus, in solution, three species, I, II, and IV, can be in equilibrium, as a function of pH, with reversible effect on the fluorescence properties of the luminol (I). The oxidative process involve the species II, and the compound III is generated ireversibly, with the chemiluminescence emission, according to the Scheme 3.



Reaction scheme 3

The LH_2 Fluorescence Lifetime

Figure 6 shows the fluorescence decay curve for the LH_2-DMSO-H_2O-KOH system (λ_{em} = 424 nm). Table II presents the fluorescence lifetimes for different LH_2 solutions in DMSO and water. The lifetimes were obtained by fitting the experimental data with two or three exponentials. These values indicate the formation of the mentioned chemical species. Thus, in DMSO, the lifetime is 1.80 ns, value assigned to the LH_2 fluorescence (λ_{max} = 405 nm). In alkalinized DMSO solutions,

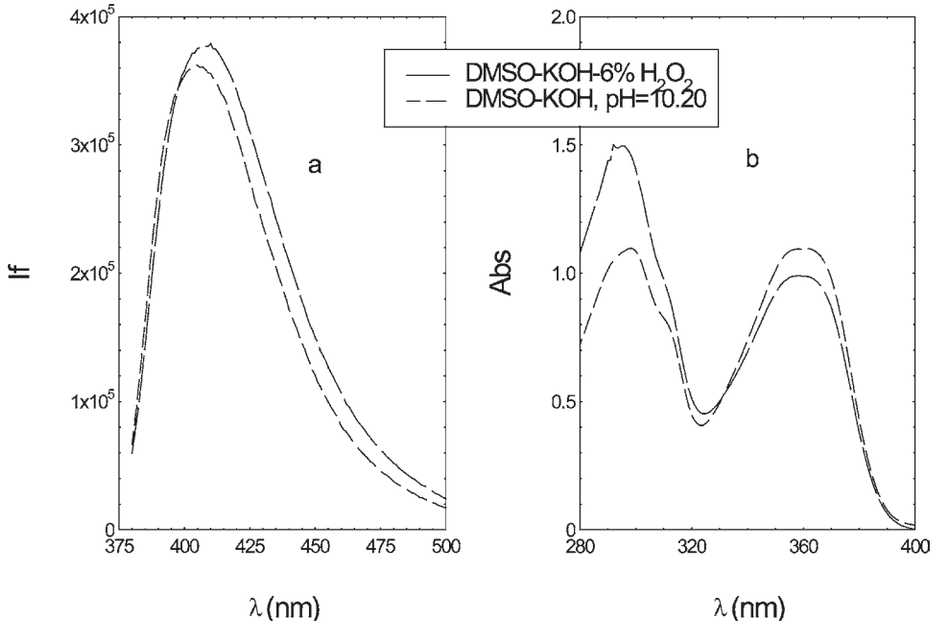


Fig. 5. The effect of hydrogen peroxide on the fluorescence (a) and absorption spectra (b) of 3.2×10^{-5} *M* luminol solution in alkalinized DMSO (pH 10.20). $\lambda_{ex} = 365$ nm.

the lifetimes are about 1.76–1.43 ns. The addition of water to the LH_2-DMSO solution leads to the formation of LH_3^+, with emission at 420–425 nm and with lifetime 10.0 ns (at neutral pH). In DMSO-H_2O-KOH with alkalinity increasing the lifetimes, measured at 425 nm, decrease in parallel with fluorescence intensity. The fluorescence band at 480 nm is very weak, more so at the excitation wavelength used in single-photon counting technique, $\lambda_{ex} = 300$ nm, while the emission bands of LH_2 and protonated LH_2 (due to the water trace) are much more intense, and so the correct determination of dianion emission is hindered. It is very probable that the lifetime is smaller than 1 ns, and at $\lambda_{em} = 480$ nm the emission of protonated LH_2 to interfere. In Table II one can observe at $\lambda_{em} = 480$ nm, in the case of LH_2-DMSO-KOH solutions, values of about 3 ns, which are assigned to the water trace in solutions.

CONCLUSIONS

The LH_2 behavior in DMSO, DMSO-water, DMSO-water-KOH, and DMSO-KOH has been studied, focusing on the effect of the KOH concentration on its absorption and fluorescence properties. The results are as follows:

1. For LH_2-DMSO-KOH solutions, at $3 \times 10^{-4} - 2.4 \times 10^{-3}$ *M* KOH (pH 10–12) the absorption spectra change, and the isosbestic points at 314, 344, and 375.5 nm indicate the acid-base equilibrium and the formation of luminol dianion L^{2-}. The decrease of the fluorescence intensity, in this KOH concentration range, and the variation of excitation spectra with KOH concentration have been similarly explained. For KOH concentrations smaller than 3×10^{-4} *M*, using a Stern-Volmer equation, a bimolecular quenching rate constant $k_q = 5.8 \times 10^{10}$ M^{-1}/s^{-1} has been obtained.
2. The acid-base process is reversible, the addition of HCl to the solution with pH 13 leads to the fluorescence intensity increase to its highest value, observed in DMSO, pH 6.
3. The addition of HCl to the LH_2-DMSO solution leads to the decrease of the fluorescence intensity as a result of the LH_3^+ cation formation.
4. In LH_2-DMSO-H_2O, one can observe the fluorescence band shift from 405 nm to 424 nm, and in presence of KOH (that is in LH_2-DMSO-H_2O-KOH solution) a new band appears, very weak, with maximum at 485 nm.
5. The fluorescence lifetime values have also evidenced the different chemical species formation and the quenching effect of KOH addition in the before mentioned solutions.

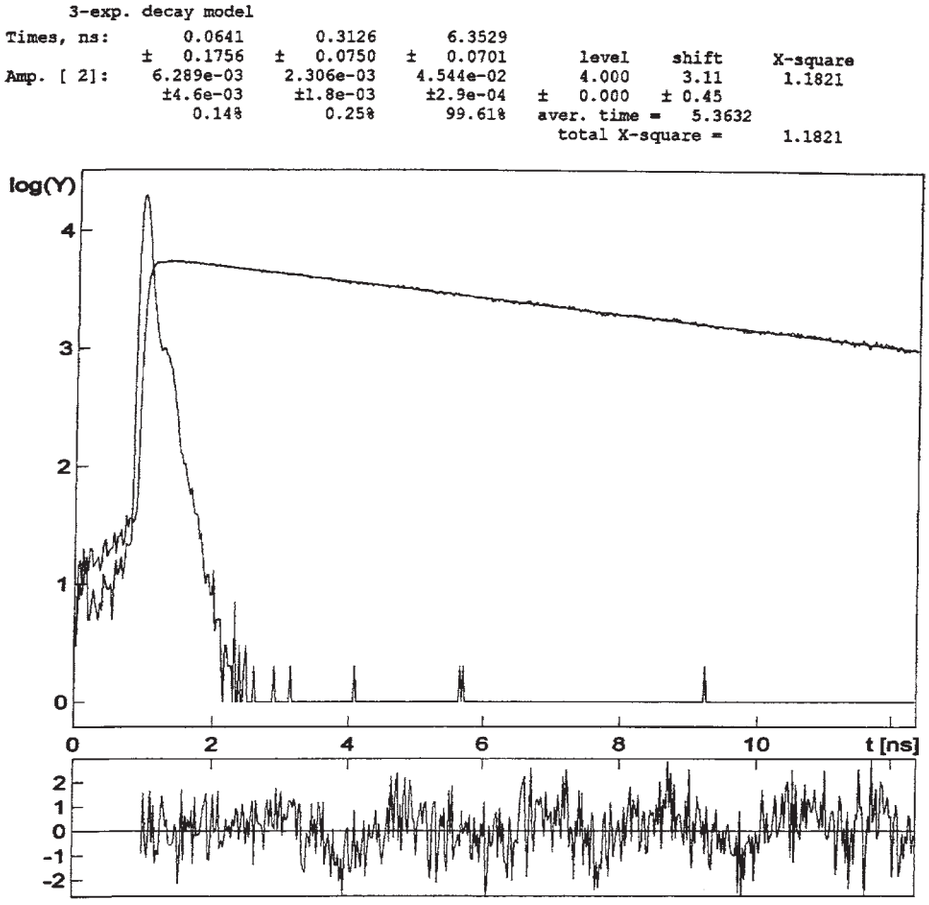


Fig. 6. The fluorescence decay curve of 5×10^{-5} *M* LH_2 /25%DMSO (alkaline)-75% H_2O solution, pH 9, $\lambda_{ex} = 300$ nm, $\lambda_{ex} = 424$ nm.

Table II. The Fluorescence Lifetime Values (t) for Luminol Solutions, Measured at Specified Wavelengths

Solution	λ_{em}(nm)	τ (ns)
2×10^{-4} *M* LH_2/DMSO	405	1.80 (99.4%)
5×10^{-5} *M* LH_2 /25%DMSO-75% H_2O, pH=9	424	6.56 (99.1%)
2×10^{-4} *M* LH_2/DMSO-KOH	480	2.67 (96%)
2×10^{-4} *M* LH_2/DMSO-KOH	420	1.57 (41%) 3.43 (53%)
LH_2/H_2O	425	10.1 (95%)
3.2×10^{-5} *M* LH_2/DMSO-NaOH-4% H_2O	480	3.00 (97%)
3.2×10^{-5} *M* LH_2/DMSO-NaOH-H_2O	405	1.77 (95%)
3.2×10^{-5} *M* LH_2/DMSO-NaOH-H_2O	425	4.33 (65%)
3.2×10^{-5} *M* LH_2/DMSO-KOH pH 10.73	480	1.76 (97%)
3.2×10^{-5} *M* LH_2/DMSO-KOH pH 11.10	425	1.73 (99%)
3.2×10^{-5} *M* LH_2/DMSO-KOH pH 10.43	405	1.55 (98%)
3.2×10^{-5} *M* LH_2/DMSO-KOH pH 10.43	425	1.71 (97%)
3.2×10^{-5} *M* LH_2/DMSO-KOH pH 10.43	480	1.43 (86%) 3.45 (14%)

Note: The exponential amplitudes are indicated between brackets. The table does not present the τ values smaller than 1 ns, because they are in the sensitivity limit of the apparatus, and therefore they are not significant.

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