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Complex formation and photophysical properties of luminol: solvent effects

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Abstract

The photophysical properties of the lowest excited singlet state of luminol were studied in various protic and aprotic solvents with different dielectric constants using steady state and nanosecond emission spectroscopy at room temperature and 77 K. A red shift is observed in the emission spectra on going from aprotic to protic solvents. We show that this red shift is due to the formation of a relatively long- lived complex in the excited state between luminol and protic solvents. The decay rates correlate well with the solvent shifts. The dependence of the quantum yields of fluorescence on the excitation energy and solvent characteristics was studied.

Keywords: Luminol; Solvent effects; Complex formation

1. Introduction

Photoinduced hydrogen bonding interactions in proton donating and accepting solvents have been shown to be the primary events triggering many photoreactions which depend on the hydrogen bonding ability of the surrounding solvent medium. The fluorescence of aromatic compounds containing amino and imino groups has been studied extensively [1–10]. The spectroscopic properties of biological molecules containing indole groups, such as indoles, tryptophans, luminol, etc., in particular their fluorescence behaviour, have stimulated many photophysical studies [7,11–13]. These compounds have been the subject of many investigations because of their intense solvent-dependent fluorescence properties and Stokes shift. Both the emission spectra and quantum yields show a remarkable variation with the nature of the surrounding environment. In other words, the solvatochromic shifts originate from solute–solvent interactions. Polar solvents can interact in three different ways with amino and imino groups depending on the nature of the solvent [14]: (i) the type of interaction depends on the dielectric properties of the solvent; (ii) excited state complexes of varying stability may form depending on the solvent; (iii) in hydrogen bonding solvents, hydrogen bonding interactions with amino or imino groups may occur. A combination of these effects can explain the fluorescence properties of the molecules in polar protic and aprotic solvents.

It has been reported in Ref. [7] that the fluorescence spectrum of luminol shows a red shift on going from non-polar to polar solvents; this has been explained by the stabilization of the excited states in the more polar medium. This shift cannot be attributed to hydrogen bonding between the solute and the solvent, because a normal solvatochromic shift is observed; it has been suggested that it is due to a non-specific type of solute–solvent association known as "dielectric enrichment".

In this work, we report the fluorescence spectra and decay behaviour of luminol in different protic and aprotic solvents at room temperature and 77 K. We have shown that, in the excited state, there are at least two conformers present, and the spectral shift is due to a hydrogen bonding interaction. It is also shown that the measurable absorption spectral shift observed in the presence of water is due to hydrogen bonding between the solute molecules and water.

2. Experimental details

2.1. Materials

The solvents dimethylsulphoxide (DMSO), *N,N*-dimethylformamide (DMF), 1,4-dioxan (DIO), tetrahy-

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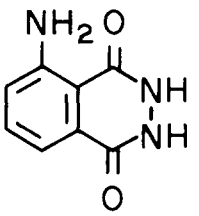
drofuran (THF) and 2,2,2-trifluoroethanol (TFE) (all from Fluka), normal alcohols such as methanol (MeOH), ethanol (EtOH), *n*-propanol (PrOH), butanol (BuOH), pentanol (PeOH) and hexanol (HeOH) (all obtained from Sigma or Aldrich Chemicals Co.) and acetone and acetonitrile (ACN) (E. Merck) were of spectroscopic grade and were further distilled before use. Analytical grade acetic acid and triply distilled water were used throughout. 3-Amino-phthalhydrazide (luminol, **I**) was obtained from Fluka AG and was used as received. The concentration of luminol was maintained at approximately $(2–3)\times10^{-5}$ mol dm^{-3} during the experiment.



I (luminol)

2.2. Instruments and technique

The electronic absorption spectra were obtained with a Shimadzu UV–visible spectrophotometer (UV 2100). Fluorescence spectra were taken with a Perkin–Elmer 44B fluorimeter. The decay time was determined using a time-correlated single-photon counting (SPC) system, the details of which have been described elsewhere [15]. The results were analysed with the software prepared by Photon Technology International (PTI, Global Fluorescence Analysis, version VI.1) or with standard DECON software.

The logarithmic graphs of the SPC traces can be described by a double exponential function in all the solvents (except for the acidic solution of water, pH 5) of the form

$$F(t) = a_1 \exp(-t/\tau_1) + a_2 \exp(-t/\tau_2)$$

For an effective statistical test of the evolution of the compatibility of the experimental and simulated decay profiles, various statistical parameters, e.g. reduced χ^2 (χ_R^2) ($=1.0\pm0.2$), Durbin–Watson (DW) parameter (greater than 1.7) and random distribution of weighted residuals ($r(t_i)$), were checked [16].

3. Results and discussion

3.1. Spectral features at room temperature

The absorption spectrum of luminol exhibits two bands in the 360 and 300 nm regions (Fig. 1, Table 1) in all solvents used; the emission spectrum shows

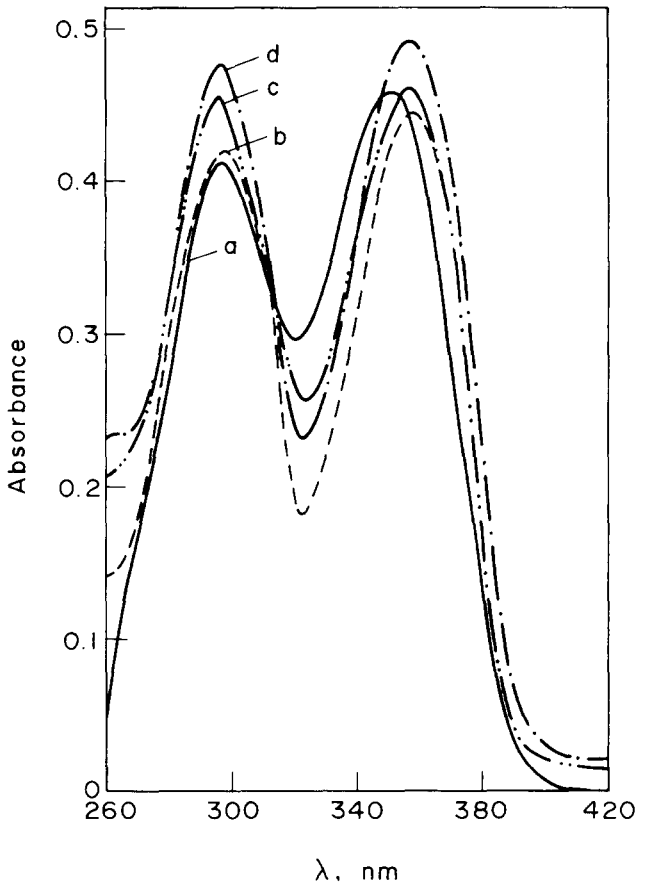


Fig. 1. Absorption spectra of luminol in: (a) water; (b) water–DMSO (2 : 3, v/v); (c) water–DMSO (3 : 2, v/v); (d) DMSO. Taken in a quartz cell (1 cm optical path length).

a single broad band at different positions (between 395 and 430 nm) depending on the nature of the solvent (Fig. 2, Table 1). The largest red shift is observed when the surrounding medium is a strong proton donor such as water (or TFE). From Figs. 1 and 2, it can be seen that there is no mirror image relationship between the emission and absorption spectra. Therefore the species present in the ground and excited states are different. It is also observed that, on addition of acid to luminol solutions of weaker proton donating solvents, such as normal alcohols, the 415 nm band (Table 1) is shifted to 430 nm. The red shift in the emission spectrum can be attributed to excited state complex formation, where the protic solvents act as proton donors. However, in the ground state, the band position does not show any measurable change before ($\lambda_{max}^{abs} = 356.2$ and 296.0 nm in ethanol, Table 1) and after ($\lambda_{max}^{abs} = 355.0$ and 295.2 nm) the addition of acid (approximately 10^{-2} mol dm^{-3}) in any of the solvents used except water. This proves conclusively that the red shift in the fluorescence emission is mainly due to an excited state complex.

However, with the gradual addition of water to a DIO solution of luminol, the 356 nm band shifts to 348 nm. This may be attributed to weak complex

Table 1
Spectroscopic properties of luminol in solution. Variation in the absorption (abs) and emission (em) maxima (λ_{max}), quantum yield (ϕ_f), decay time (τ) and pre-exponential factor (a) with solvent static dielectric constant D (λ_{exc} = 360 nm)

Solvent	D[c]	λ_{max}^{abs} (nm)	λ_{max}^{em} (nm)	Δ[d] (cm^{-1})	ϕ_f	τ_1 [a_1][e] (ns)	τ_2 [a_2][e] (ns)
Water[a]	80.2	347.8 296.2	430	5496	0.81 (0.35)	1.9 [0.43]	9.5 [0.57]
Acidic water[b]	–	–	430	–	0.86	–	6.8 [1.0]
TFE	39.5	–	430	–	0.75	2.9 [0.32]	8.4 [0.68]
EtOH	24.3	356.2 296.0	415	3978	0.50	1.7 [0.95]	4.5 [0.05]
MeOH	32.6	–	415	–	0.55 (0.26)	1.3 [0.69] (2.5) [0.54]	3.2 [0.31] (7.4) [0.45]
PrOH	20.3	–	415	–	0.52	1.6 [0.85]	3.8 [0.15]
PeOH	13.9	–	415	–	0.52	1.1 [0.74]	2.8 [0.26]
HeOH	13.3	358.4 292.0	415	3805	0.50	1.0 [0.82]	2.6 [0.18]
DMSO	48.0	357.2 297.4	410	3605	0.45 (0.22)	1.1 [0.83] (2.2) [0.69]	2.2 [0.17] (5.5) [0.31]
DMF	36.5	–	410	–	0.42	0.8 [0.79]	2.1 [0.21]
ACN	37.5	354.0 293.4	405	3557	0.31	0.5 [0.82]	1.3 [0.18]
Acetone	20.7	–	405	–	0.28	0.7 [0.05]	1.2 [0.95]
THF	7.6	353.0 291.6	395	3012	0.31	0.6 [0.4]	1.2 [0.60]
DIO	2.2	–	395	–	0.26 (0.12)	0.8 [0.37] (2.7) [0.32]	1.6 [0.63] (7.5) [0.68]

[a] pH 6.3.
[b] pH 5.
[c] Taken from Ref. [17].
[d] Stokes shift (Δ) calculated using the first absorption band.
[e] τ values are correct to within $\pm$0.2 ns; the statistical parameters in the deconvolution process are given in the text. The values given in parentheses are for 77 K. The decay functions were taken at the corresponding emission maxima.

formation between water and luminol in the ground state. The carbonyl groups in luminol (**I**) are weak electron acceptors because of the presence of two electron donating NH groups [7]. This provides a possible site of hydrogen bonding interaction between water/alcohol and luminol. The difference between the Stokes shift (Δ, Table 1) in normal alcohol and water is mainly due to the proton donating capabilities of these solvents and, consequently, the site of complexation. It should be noted that normal alcohols can act both as proton donors and acceptors. Due to the presence of alkyl groups in normal alcohols, they can release electrons towards the oxygen atom of the hydroxyl group due to the +I (inductive) effect [18]. Hence normal alcohols are weak electron donors. However, the proton donating property of these alcohols is much stronger than the electron donating capability. Therefore it is probable that the normal alcohols will act as proton donors, and the NH groups of luminol will accept protons from the alcohols to give a hydrogen bonding interaction. However, for stronger complexation, acid is necessary, which will enhance the hydrogen bonding interaction between normal alcohols and luminol. Stronger hydrogen bonding interactions may facilitate the stabilization of the emitting state of luminol causing a large red shift in H_2O or acidic alcohols. On the other hand, a small red shift is also observed in highly polar aprotic solvents, such as DMSO, DMF and ACN (Table 1). The small shift in this case indicates a different type of interaction between luminol and electron donating aprotic solvents. The large ϵ value (approximately 20 000 dm^3 mol^{-1} cm^{-1} in water) indicates that luminol absorption is due to a $\pi\pi^*$ transition. It is well known that OH and NH_2 groups become more acidic due to $\pi\pi^*$ excitation. Hence it is reasonable to propose that the amino (NH_2) group in luminol may be the possible site of interaction with electron donating polar aprotic solvents. Accordingly, the presence of at least two conformers or two different types of hydrogen bonded complex in the excited state is possible.

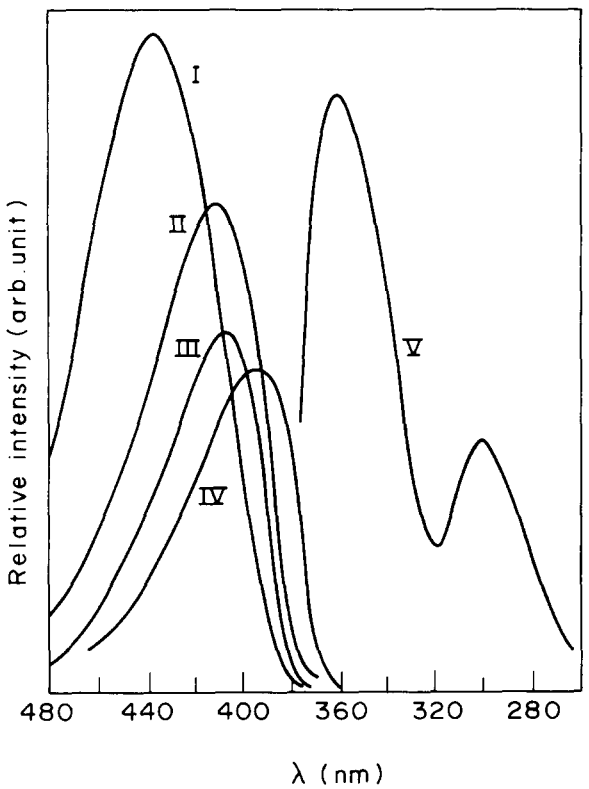


Fig. 2. Emission spectra of luminol (approximately 2.5×10^{-5} mol dm^{-3}) in TFE (I), EtOH (II), DMSO (III) and DIO (IV) and excitation spectrum in ACN (V).

The excitation spectrum of luminol fluorescence in all solvents exhibits two bands which are similar in position to those of the absorption spectrum (even in pure water and in the presence of acid). In the excitation spectrum, the band in the 360 nm region is always stronger than the 300 nm band. These observations show that all the species responsible for emission at different positions in different solvents originate from the same ground state conformer.

The absorption and excitation spectra of luminol indicate the presence of two excited electronic states (S_1 and S_2). It is pertinent to assume that, on interaction with the solvent molecules, excited luminol relaxes from the S_2 ($\pi\pi^*$) state to the S_1 ($\pi\pi^*$) state and, after complex formation (with the solvent), emits from the more stabilized S_1 ($\pi\pi^*$) state. Stabilization due to hydrogen bonding will lower the energy of the excited state, giving rise to a stronger red shift in protic solvents. The appearance of a single broad emission band indicates that the energy gap between the two states is very small or they are coupled by vibronic interaction in the excited state. The large Stokes shift in H_2O or TFE (approximately 5000 cm^{-1}) shows a larger stabilization of the excited state relative to the ground state as mentioned by Ghoneim [7]. This indicates a different type of ground state complexation compared with the other solvents. It can be explained by ground state complex formation between the amino electron lone pair (NH_2) and a water proton. This complex relaxes from the excited Franck–Condon state to another type of more stabilized hydrogen bonding complex. The loss of mirror image symmetry between the absorption and emission spectra can be interpreted in terms of conformational changes which occur during relaxation of the emitting states or during stabilization (by interaction with the solvent) from the S_2 ($\pi\pi^*$) state to the S_1 ($\pi\pi^*$) state. Since the absorption spectrum and the relaxed excited electronic spectrum are dissimilar and the fluorescence band width is always wider than the absorption band width, we can say that the mirror image relationship between the absorption and emission spectra is not respected. The relaxation due to solvent interaction in weak electron donating solvents, such as THF, is expected to be low, reflecting a relatively small Stokes shift in this solvent (Table 1). It is also observed that stabilization is highest in proton donating solvents, such as H_2O or TFE. The difference in Stokes shift (Δ) between DMSO and THF (Table 1) indicates that the interaction with the solvent plays an important role in the relaxation of the excited states [19].

3.2. Quantum yield of fluorescence (ϕ_f)

We observed a moderately good correlation between the Stokes shift (Δ) and ϕ_f of the protonated species. From Table 1, it can be seen that ϕ_f increases on going from aprotic to protic solvents. A large ϕ_f value is observed in H_2O or TFE where the red shift reaches a maximum. This variation in the quantum yield is due to the variation in the excited state population of the hydrogen bonded complex as judged by the quantum yield of the protonated species. On the other hand, the increase in ϕ_f value with increasing red shift indicates an enhancement in the fluorescence lifetime (τ_f) and radiative rate (k_f^r). This is discussed in the next section. It is also observed that ϕ_f increases with an increase in the excitation wavelength (λ_{exc}) without any change in the position of the band. This indicates that a similar Franck–Condon envelope is involved in all cases irrespective of the excitation wavelength. As the experimental temperature is decreased to 77 K, a considerable decrease in ϕ_f is observed in all solvents. Therefore it is reasonable to assume that the population of the species responsible for the emission must decrease at 77 K. Some of these results are shown in Table 1.

3.3. Decay behaviour of luminol fluorescence

The fluorescence decay of luminol in all solvent media is measured on the nanosecond time scale. After deconvolution, a biexponential decay curve is obtained in most solvents, indicating that the measured fluorescence decay is adequately described by a double

exponential function. The weighted residuals appear to be better distributed when a double exponential decay is fitted. However, in water and TFE, the decay curves can also be fitted by a single exponential function at $\lambda_f > 430$ nm. At all wavelengths up to 430 nm, the fluorescence decay is best described by a double exponential function with two different lifetimes. In acidic water solution, a single exponential decay with one lifetime is obtained. All the measured lifetime values are displayed in Table 1. Two representative decay curves are shown in Fig. 3. The biexponential behaviour of the decay curves indicates that two different hydrogen bonded complexes are present in the excited state. The fluorescence decay in acidic water shows single exponential behaviour. It is probable that, as complex formation is complete (in acidic medium), the number of conformations will decrease and decay will show predominantly single exponential behaviour. It should be noted that the decay curves in normal alcohols can be described by single exponential behaviour after the addition of acid (approximately 10^{-2} mol dm^{-3}). The double exponential decay behaviour of luminol suggests that preferential populations of excited state species are present and their lifetimes depend on the nature of the interaction with solvent molecules.

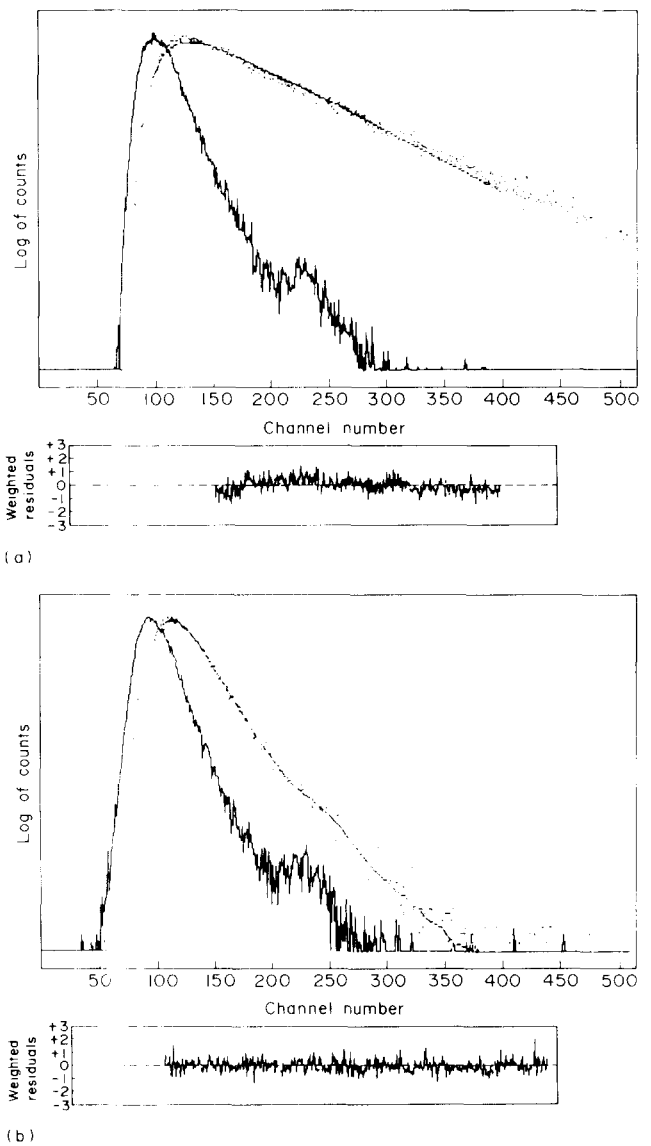


Fig. 3. Fluorescence decay curves of luminol: (a) in acidic water solution (pH 5); (b) in DMSO. Resolution, 0.083 ns per channel. The distribution of weighted residuals is also shown: (a) single exponential fit; (b) double exponential fit.

It can be seen from Table 2 that the decay time (τ_f) measured in aprotic solvents is very low compared with that measured in water and TFE. The origin of this behaviour can be related to the assignment of the emission to two different types of hydrogen bonded complex of luminol. This is due to the fact that aprotic solvents act as electron donors, whereas water and TFE are strong proton donors. From Table 2, it can be seen that the τ_f values are dependent on the nature of the solvent and also on the spectral position. The mean fluorescence lifetime (τ_f^m) can be calculated from the following equation: $\tau_f^m = \Sigma a_i \tau_i / \Sigma a_i$ [20] (Table 2). As ϕ_f (in Table 1) includes the total integrated fluorescence spectrum, it is reasonable to use these values with τ_f^m to calculate the radiative (k_f^r) and non-radiative (k_f^{nr}) decay rate constants from the following equation

$$1/\tau_f^m = k_f = k_f^r + k_f^{nr};\ \phi_f/\tau_f^m = k_f^r$$

The values are displayed in Table 2.

It can be seen from Table 2 that the τ_f^m values increase significantly on decreasing the temperature to 77 K. However, the radiative decay rate constant (k_f^r) decreases measurably in spite of the increase in τ_f^m.

Table 2
Mean fluorescence lifetime (τ_f^m) and radiative (k_f^r) and non-radiative (k_f^{nr}) decay rate constants of luminol in solution. The values in parentheses are for 77 K

Solvent	τ_f^m (ns) [a]	k_f^r (10^9 s^{-1})	k_f^{nr} (10^9 s^{-1})
Water	6.2	0.13	1.10
TFE	6.6	0.11	1.22
EtOH	1.8	0.27	1.73
MeOH	1.9	0.29	1.53
	(4.7)	(0.06)	(3.79)
PrOH	1.9	0.28	1.65
PeOH	1.5	0.34	1.59
HeOH	1.3	0.39	1.61
DMSO	1.3	0.35	1.87
	(3.7)	(0.06)	(4.49)
DMF	1.0	0.39	1.99
ACN	0.6	0.48	2.74
Acetone	1.1	0.24	3.33
THF	0.9	0.32	2.90
DIO	1.3	0.20	3.65
	(6.0)	(0.02)	(8.31)

[a] Values are correct to within ±0.1 ns.

Thus the increase in τ_f^m at low temperature can be related to the increase in k_f^{nr}. Table 2 shows that a measurable increase in k_f^{nr} over k_f^r is observed only in aprotic solvents. Again, k_f^{nr} does not control all the excited state dynamics of luminol, particularly in protic solvents. More importantly, the excited state dynamics of luminol are mainly dependent on the specific interactions with the solvent in the excited state.

4. Conclusions

From the experimental observations, it can be concluded that luminol emission depends largely on hydrogen bonding with the solvent molecules and originates from two possible hydrogen bonded conformers. The possible sites of interaction with electron donating aprotic solvents and proton donating protic solvents are different. Normal alcohols can interact on both sites. The solute–solvent interaction plays a major role in the spectral properties of luminol.

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