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The solvent effect on the excited-state proton transfer of lumichrome

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Abstract

The acetic acid (AA)-catalysed tautomerism of lumichrome (Lc) was investigated in 1,2-dichloroethane, acetonitrile and pure AA. The interactions between Lc and AA were studied by means of UV-Vis and fluorescence spectroscopy. The results suggest the formation of 1:1 Lc–AA hydrogen-bonded complexes in the ground state of Lc. The apparent equilibrium constants were one order of magnitude higher in 1,2-dichloroethane than in acetonitrile, indicating a solvent effect on the ground-state interactions. The dynamics of exited-state processes were studied using time-resolved methods. The results show that the mechanism of tautomerism depends on the solvent.

Keywords: Lumichrome; Acetic acid (AA); DMSO; Excited-state proton transfer

1. Introduction

Lumichrome (Lc; 7,8-dimethylalloxazine, see Fig. 1) and other compounds with alloxazinic structure represent a class of nitrogen heterocycles related to lumazine. Lumichrome, a decomposition product of biologically important flavins, may be associated with them in biological systems and may be involved in some biological processes [1]. The photochemistry of lumichrome is also of special interest [2–17]. Lumichrome is a multifunctional molecule with proton-donor and proton-acceptor sites and proton transfer reactions have been found to occur in the excited state of this compound. In this process, the proton from N(1) nitrogen atom of lumichrome molecule is transferred to the N(10) nitrogen atom, and the excited isoalloxazinic form is created [3,7,8,10,14,18–24]. It was shown that the excited-state isomerisation might take place in lumichrome and other N(1) unsubstituted alloxazines, in the presence of compounds having proton-donor and proton-acceptor functions and being able to form hydrogen bonds of appropriate strength and conformation with the alloxazinic molecules, i.e. carboxylic acids and water.

Excited-state proton transfer reactions in hydrogen-bonded systems constitute a wide class of processes which have been extensively studied from experimental and theoretical points of view due to their importance in chemistry and biochemistry [25–28]. A well-known agent used to promote excited-state alloxazine–isoalloxazine tautomerism is acetic acid (AA). The very first mechanism of an excited-state proton transfer of lumichrome in the presence of acetic acid has been proposed by Koziołowa and co-workers [3,13,19]. This mechanism assumes the formation of 1:1 eight-membered cyclic complexes between lumichrome and acetic acid with hydrogen bonds at N(1) and N(10) nitrogen atoms in the lumichrome molecule. The increase in the basicity of N(10) nitrogen atom and an increase in the acidity of N(1)–H group after excitation provide the driving force for proton shift between these two nitrogen atoms. Kasha proposed an analogous mechanism, with six-membered complex between lumichrome and acetic acid [10]. A remarkable case of an excited-state proton transfer occurs in solution of lumichrome in the presence of pyridine [3,19,29] as an active transporting medium of a proton from N(1) to N(10) position of lumichrome.

Considerable work has been done to study the mechanism of excited-state proton transfer reaction in lumichrome–acetic acid and other complexes [3,7,8,10,14,23,29–32], however, there are still several discrepancies in the results concerning the mechanism and kinetics of the alloxazine–isoalloxazine tautomerism reported in literature. The main controversies concern the rate of the excited-state process. Some results based on time-resolved studies have revealed a relatively high rate constant of the process studied (of order of $10^{12}\ s^{-1}$) [33], on the other hand, Choi et al. reported considerably lower rate constants of the excited-state

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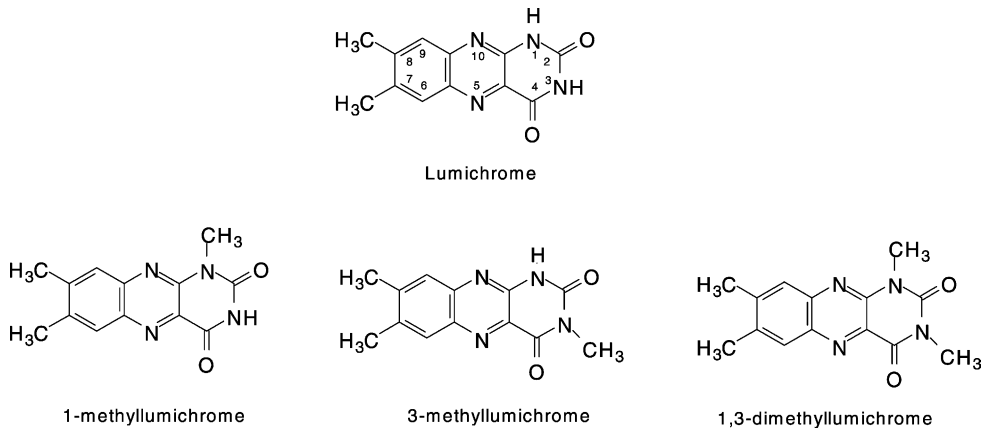


Fig. 1. Structure of the lumichrome, 1-methyllumichrome, 3-methyllumichrome and 1,3-dimethyllumichrome studied.

proton transfer [8]. However, Choi et al. estimated the rates of proton transfer by the steady-state ratios of the normal and tautomer emission, assuming a diffusional mechanism.

Recently, we have studied the photo-induced proton transfer of a set of differently substituted methyl- and cyano-alloxazines in 1,2-dichloroethane [14]. It has been found that in the presence of 0.8 mol dm^{-3} acetic acid the rise times of tautomeric forms are of order of hundreds picoseconds. The model proposed on the basis of the time resolved and steady-state results assumes a two-step excited-state reaction. The first step involves a formation of acetic acid–alloxazine complex with an appropriate structure permitting proton transfer, whereas the proton transfer itself is realised in the second step.

In this paper, we present the investigation on the mechanism and dynamics of the photo-induced proton transfer of lumichrome in the presence of acetic acid. The study was performed in a non-polar solvent (1,2-dichloroethane), a polar non-protic solvent (acetonitrile) and a polar protic solvent (acetic acid). The excited-state process was investigated using steady-state and time-resolved methods. The study reveals that solvent plays important role in hydrogen bonding interactions and an overall dynamics of phototautomerisation of lumichrome.

2. Materials and methods

Lumichrome (Sigma Chemical Co.), hexafluoroisopropanol (Merck) and dimethyl sulfoxide (DMSO, Merck) were used as obtained. 1-Methyllumichrome, 3-methyllumichrome and 1,3-dimethyllumichrome were synthesised and purified as described in reference [19]. The solvents, 1,2-dichloroethane (Sigma), acetonitrile (Sigma) and acetic acid (Merck), were spectral grade and were used without further purification. The purity of the solvent was confirmed by the absence of fluorescence at the maximum sensitivity of the spectrofluorometer.

Absorption spectra were recorded on a Cary 5E spectrophotometer (Varian). Steady-state corrected fluorescence emission and excitation spectra were measured using an MPF-44A/E spectrofluorometer (Perkin-Elmer).

Time-resolved fluorescence measurements were conducted with a model C-700 fluorometer from Photon Technology International (Canada) Inc. The system utilises a nanosecond flash lamp as an excitation source and a stroboscopic detection system [34]. The concentration of lumichrome solution was about 2×10^{-5} mol dm^{-3}.

3. Results and discussion

3.1. Absorption spectra

The spectroscopic properties of lumichrome and other alloxazines in different solvents have been a subject of a number of previous works [19,31]. The two strong long-wavelength absorption bands of these compounds can be assigned to electric dipole allowed $\pi \rightarrow \pi^*$ transitions [31,35,36]. Koziołowa demonstrated that the exact positions of these two bands maxima are solvent dependent and was able to show that both long-wavelength maxima positions exhibit a linear correlation with the polarity of the solvents expressed in *Z*-values [19]. With increasing solvent polarity, both long-wavelength maxima show a red shift accompanied by a hypochromic effect of the first maximum and a hyperchromic effect of the second maximum. The deviations from the linear correlation observed for acetic acid, pyridine, and water have been interpreted in terms of specific solute–solvent interaction. Typical absorption spectra of lumichrome in 1,2-dichloroethane and acetonitrile are presented in Fig. 2.

The effect of an addition of acetic acid on the ground-state absorption spectra of lumichrome in 1,2-dichloroethane and acetonitrile is presented in Fig. 2. In the presence of acetic acid in 1,2-dichloroethane, a shift to the longer wavelength and an increase of the absorbance of the band with a

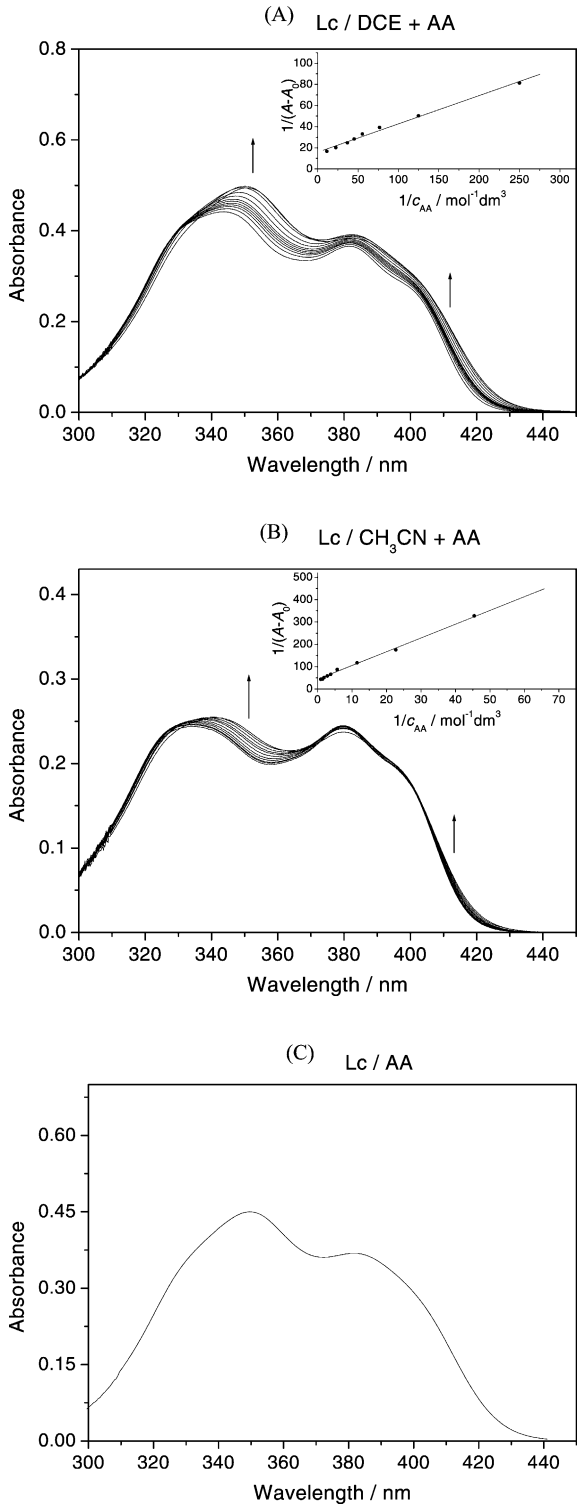


Fig. 2. Effect of varying acetic acid concentration on absorption spectra of lumichrome: (A) 1,2-dichloroethane (in the inset the Benesi–Hildebrand plot, $\lambda = 350$ nm, $K = 68\,\mathrm{mol^{-1}\,dm^3}$), concentration of acetic acid are: 0, 0.004, 008, 0.013, 0.018, 0.022, 0.03, 0.04, 0.09, 0.17, 0.35, 0.70 mol $\mathrm{dm^{-3}}$; (B) acetonitrile (in the inset the Benesi–Hildebrand plot, $\lambda = 345$ nm, $K = 6.1\,\mathrm{mol^{-1}\,dm^3}$), concentrations of acetic acid are: 0, 0.009, 0.02, 0.04, 0.09, 0.17, 0.26, 0.35, 0.52, 0.70, 1.05 mol $\mathrm{dm^{-3}}$. The arrows indicate increasing concentration of acetic acid. (C) Absorption spectrum of lumichrome in acetic acid.

maximum at 344 nm is observed. An increase of absorbance is observed also for the band with a maximum at about 382 nm. Similar, but relatively smaller changes in the absorption spectra of lumichrome are observed also in the presence of acetic acid in acetonitrile. These changes in the absorption spectra of lumichrome and other alloxazines have been studied previously and have been ascribed to the formation of hydrogen-bonded ground-state complexes between lumichrome and acetic acid [19]. The little red shift of the absorption bands indicates a larger stabilisation of the excited state due to the hydrogen-bonding interaction.

3.2. Structure of hydrogen-bonded lumichrome–acetic acid complexes

In the lumichrome molecule there are several centres (oxygen atoms, nitrogen atoms, N–H groups), which may serve as hydrogen acceptors or hydrogen donors in the creation of hydrogen-bonded complexes. Additionally the acetic acid may act as a hydrogen-donor and acceptor agent. Thus, the lumichrome–acetic acid complexes may have various structures. The earlier investigation by Koziołowa [19] and Szafran et al. [20] suggests that the observed changes in the absorption spectra are a result of acetic acid binding at the N(10) nitrogen atom of the lumichrome molecule. This conclusion was mainly based on a comparison of the changes in absorption spectra for 9-methyl-substituted alloxazine and 1-methyl- and 3-methyl-substituted lumichromes in the presence of acetic acid. It was shown that the methyl groups at positions N(1) and C(9) restrict the possibility of hydrogen bond formation at N(10) but do not prevent it absolutely.

In this work, a further attempt was made to evaluate the structure of the hydrogen-bonded complexes. In order to determine the effect of binding position of the hydrogen donor or acceptor on the absorption spectra of lumichrome we have chosen to investigations compounds which may act as hydrogen donors or hydrogen acceptors: 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) and dimethyl sulfoxide, respectively. The corresponding absorption spectra of lumichrome in 1,2-dichloroethane are shown in Fig. 3.

The changes in the absorption spectra of lumichrome in 1,2-dichloroethane caused by increasing concentration of DMSO (hydrogen acceptor agent) are clearly different from those observed in the presence of acetic acid. The main difference is the shift of the absorption band with a maximum at 350 nm towards shorter wavelengths. Similar, but much smaller changes are observed for lumichrome in acetonitrile with increasing concentration of DMSO. To evaluate the possibility of hydrogen bonds formation at N(3) position, similar experiments with 3-methyllumichrome and 1,3-dimethyllumichrome have been curried out (spectra are not shown). The changes observed in the absorption spectra of the model compound 3-methyllumichrome as a result of an addition of DMSO, have been found very similar to those observed in the case of lumichrome. In contrast, for

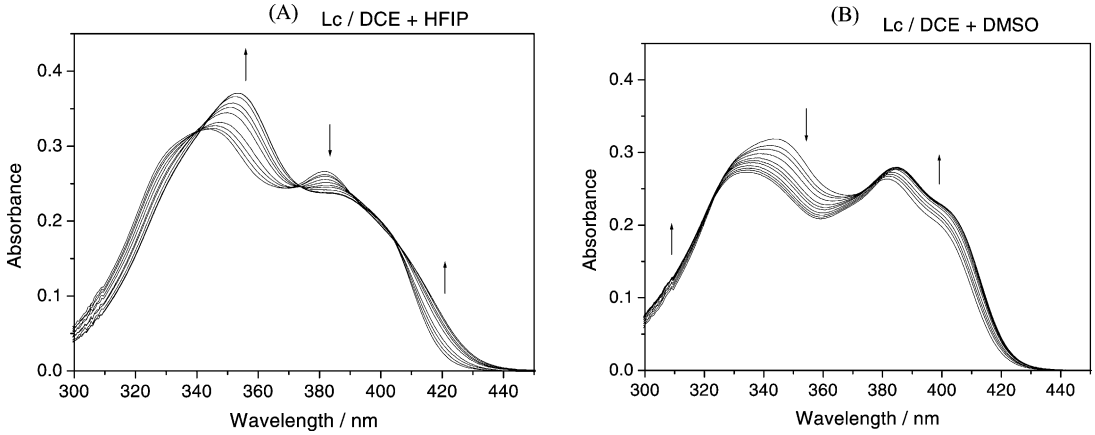


Fig. 3. Changes in absorption spectra of lumichrome in the presence of: hexafluoroisopropanol in 1,2-dichloroethane (A), dimethyl sulfoxide in 1,2-dichloroethane (B). The corresponding concentrations of HFIP are: 0, 0.01, 0.02, 0.04, 0.08, 0.12, 0.15, 0.23, 0.31, 0.39 mol dm^{-3}; the corresponding concentration of DMSO are: 0, 0.01, 0.03, 0.04, 0.06, 0.07, 0.08, 0.10, 0.11, 0.14, 0.21, 0.28, 0.42, 0.56, 0.85 mol^{-1} dm^3. The arrows indicate increasing concentration of HFIP or DMSO.

1,3-dimethyllumichrome no changes in the absorption spectra are observed in the presence of DMSO. These results suggest that the short-wavelength shift of the absorption band at 350 nm and long-wavelength shift of the maximum at 380 nm are caused by creation of the hydrogen bond at N(1)–H group of the lumichrome molecule.

As a hydrogen-donor agent, 1,1,1,3,3,3-hexafluoroizopropanol was chosen. It was previously shown that this compound forms hydrogen bonds with lumichrome but does not promote the excited-state proton transfer [20,37]. Thus, it seems safe to assume that this compound acts mainly as a hydrogen-donor agent. The short-wavelength band in the absorption spectra of lumichrome in 1,2-dichloroethane in the presence of HFIP reveal a long-wavelength shift and an increase of the absorbance. The absorbance in the maximum of long-wavelength band is decreased and the absorbance in the short-wavelength region of this band is increased. Clear isosbestic points are observed at 340, 374, 404 nm. Similar, but relatively less pronounced changes are observed for lumichrome in acetonitrile with increasing concentration of HFIP. For HFIP the formation of hydrogen bonds with lumichrome molecule at N(10), N(5) nitrogen atoms and oxygen atoms have to be taken into account. However, due to a lower basicity of N(5) nitrogen atom, a hydrogen bonding formation at this position seems to be less probable. Moreover, hydrogen bonds at oxygen atoms should not affect the absorption spectra. These facts and observations lead to a conclusion that the shift of the two absorption bands of lumichrome to longer wavelengths is a result of the bonding of hydrogen donor at N(10) nitrogen atom.

Taking into regard the above results the long-wavelength shift of the absorption band at about 340 nm observed in the presence of acetic acid in both solvents used in this study, may be interpreted as a result of formation of hydrogen bond in which N(10) nitrogen atom is engaged. The long-wavelength shift of the absorption band at about 380 nm may be due to a formation of hydrogen bonds at the N(1) and N(10) nitrogen atoms as well. The results and their discussion allow us to ascribe the changes in the absorption spectra of lumichrome mainly to the creation of hydrogen bonds in which the N(1) and N(10) nitrogen atoms are involved. It should be stressed that the above results do not exclude a formation of hydrogen bonds with participation of the other lumichrome nitrogen and oxygen atoms. However, it seems that the changes in lumichrome absorption spectra are mainly connected with formation hydrogen bonds at N(10) and N(1) atoms.

Moreover, qualitative changes in the absorption spectra of lumichrome upon addition of acetic acid in 1,2-dichloroethane and acetonitrile may indicate different structure of hydrogen-bonded complexes in both solvents. Similar effect of HFIP and acetic acid on absorption spectra of lumichrome in acetonitrile may suggest that in this solvent occur single-hydrogen-bonded lumichrome–acetic acid complexes. In contrast, in 1,2-dichloroethane one can expect the existence of cyclic doubly-hydrogen-bonded complexes.

3.3. Equilibrium constants of the ground-state complexation

Analysis of changes in the absorption spectra of lumichrome in the presence of acetic acid and other hydrogen-donor or hydrogen-acceptor compounds allows determination of the equilibrium constants of complexation, *K*.

For the reaction of complexation:

$$\mathrm{Lc} + n\mathrm{D} \rightarrow \mathrm{Lc{-}D}_n$$

the equilibrium constant is given by the equation

$$K = \frac{[\mathrm{Lc{-}D}_n]}{[\mathrm{Lc}][\mathrm{D}]^n}$$

The number of hydrogen-donor and/or hydrogen-acceptor molecules engaged in complexation with a single lumichrome molecule, n, was on the basis of the following formula (cf. [38]).

$$\ln\left[\frac{A - A_0}{A_\infty - A}\right] = f(\ln[\mathrm{D}]) \quad (1)$$

The results indicate that the stoichiometry of lumichrome–acetic acid complexes in acetonitrile and 1,2-dichloroethane is 1:1. The values of n determined from Eq. (1) are: $n = 0.99$ in acetonitrile and $n = 0.90$ in 1,2-dichloroethane. Some deviation from 1:1 stoichiometry can appear at high acetic acid concentrations. The 1:1 stoichiometry has been also observed for HFIP and DMSO hydrogen-bonded complexes with lumichrome in acetonitrile and 1,2-dichloroethane.

Assuming the 1:1 stoichiometry of the complexes studied, the ground-state apparent equilibrium constants were determined using the following equation:

$$\frac{1}{A - A_0} = \frac{1}{(\varepsilon_K - \varepsilon_{\mathrm{Lc}})[\mathrm{Lc}]} + \frac{1}{K(\varepsilon_K - \varepsilon_{\mathrm{Lc}})[\mathrm{Lc}]}\frac{1}{[\mathrm{D}]} \quad (2)$$

where A_0 is the absorbance of the lumichrome solution without hydrogen-bonded agent, A the absorbance of the lumichrome solution in the presence of a hydrogen bonding agent, ε_K the molar absorption coefficient of Lc–D complex, $\varepsilon_{\mathrm{Lc}}$ is the molar absorption coefficient of lumichrome.

The Benesi–Hildebrand plot for lumichrome acetic acid system is shown in the inset of Fig. 2. These plots also suggest the existence of 1:1 complexes. However, the analysis of the plots shows a deviation from linearity for higher acetic acid concentrations. As a consequence, the determined K-values show systematic changes depending on the range of acetic acid concentration chosen for the calculation. The apparent equilibrium constants determined in 1,2-dichloroethane according to Eq. (2) vary from 73 to 51 $\mathrm{mol}^{-1}\,\mathrm{dm}^3$ when the acetic acid concentration increases from 0.009 to 0.70 $\mathrm{mol\,dm}^{-3}$, respectively.

The apparent equilibrium constants determined in acetonitrile according to Eq. (2) varies from 7.2 to 5.9 $\mathrm{mol}^{-1}\,\mathrm{dm}^3$ when the acetic acid concentration increases from 0.17 to 1.0 $\mathrm{mol\,dm}^{-3}$, respectively. The determined K-values for lumichrome–acetic acid complexes are about one order of magnitude lower in acetonitrile than that in 1,2-dichloroethane.

These results lead to a conclusion that the interactions between lumichrome and acetic acid are strongly modified by the solvent. In 1,2-dichloroethane, the solute–solvent interactions are expected to be weaker due to a lower polarity and aprotic character of the solvent. So one can expect stronger interaction between lumichrome and acetic acid. This suggestion is confirmed by a relatively high value of the apparent equilibrium constant of formation of lumichrome–acetic acid complexes in this solvent observed for the range of lower concentrations of acetic acid. With increasing acetic acid concentration the self-association of acetic acid may become an important process. The equilibrium constant for dimmerisation of acetic acid in 1,2-dichloroethane is equal 154 $\mathrm{mol}^{-1}\,\mathrm{dm}^3$ [39]. The poorer linear relationship in 1,2-dichloroethane for range of higher acid concentration relative to that in acetonitrile (see insets in Fig. 2) is likely due to the self-association of acetic acid and/or may be result of formation of lumichrome–acetic acid complexes with a stoichiometry different than 1:1. A 1:2 (lumichrome–acetic acid) complex, that would difficult proton transfer or ring closure, could be in the origin of the slow step, moreover that the non-linearity of the Benesi–Hildebrand plot (that applies strictly to 1:1 complexes) suggests precisely that. In more polar solvents, it is expected that the relatively strong lumichrome–solvent and acetic acid–solvent interactions restrict the reaction between lumichrome and acid. Thus, in acetonitrile the apparent equilibrium constant of complexation lumichrome–acetic acid is much lower than in 1,2-dichloroethane.

3.4. Emission spectra

To study the excited-state proton transfer properties, we have recorded the emission spectra of lumichrome varying the concentration of acetic acid. In contrast to the absorption spectra in these spectra the effects of the acetic acid on the lumichrome emission are dramatic. The emission spectra of lumichrome exhibit one broad band with a maximum at about 420–430 nm depending on the solvent. In the presence of acetic acid, a new band appears with a maximum at about 520 nm. The new emission is similar to the emission spectrum of lumiflavin, the compounds with isoalloxazinic structure, and has been identified as emission of the isoalloxazinic form appearing as a result of excited-state proton transfer from N(1) to N(10) [3,19]. The intensity of alloxazinic emission decreased and the intensity of isoalloxazinic emission increased with increasing acetic acid concentration. Clear isoemission points in the spectra are observed. For lumichrome in pure acetic acid, the alloxazinic emission is still observed (see Fig. 2). To help establish the role of N(1)–H and N(3)–H groups in proton transfer, model compounds 1-methyllumichrome (1,7,8-trimethylalloxazine) and 1,3-dimethyllumichrome (1,3,7,8-tetramethylalloxazine) were used. The methyl group at N(1) and/or N(3) allows the effect of lumichrome–acetic acid interaction to be selectively blocked. For N(1) substituted alloxazines, 1-methyllumichrome and 1,3-dimethyllumichrome, only alloxazinic emission is observed whose intensity decreases with increasing acetic acid concentration, see also [3,19].

From the changes in the emission spectra of lumichrome in the presence of acetic acid the apparent equilibrium constants for the complexation between lumichrome and acetic acid can be determined. According to the mechanism proposed by Koziołowa and co-workers [3,14,19,23] the proton transfer occurs in the excited cyclic complexes between lumichrome and acetic acid. The isoalloxazinic emission may originate only from the lumichrome molecules involved in such complexes. From the changes of the isoalloxazinic

emission of lumichrome in the presence of acetic acid the apparent equilibrium constant of the complexes formation can be determined from the equation

$$\frac{1}{I_F - I_F^0} = \frac{1}{a[\mathrm{Lc}]_0} + \frac{1}{a[\mathrm{Lc}]_0 K^*}\frac{1}{[\mathrm{AA}]} \quad (3)$$

where I_F^0, I_F is the fluorescence intensity monitored at the isoalloxazinic emission band without and in the presence of acetic acid, a the proportionality constant, $[\mathrm{Lc}]_0$ the concentration of lumichrome, [AA] is the acetic acid concentration.

The plots are linear in acetonitrile as shown in Fig. 4 (only a small deviation from linearity is observed for the higher acetic acid concentration). In 1,2-dichloroethane, the deviations from linearity are observed. The apparent equilibrium constant obtained using the above equation is $2.4 \pm 0.2\,\mathrm{mol}^{-1}\,\mathrm{dm}^3$ in acetonitrile. In 1,2-dichloroethane, the equilibrium constants are: $93 \pm 2\,\mathrm{mol}^{-1}\,\mathrm{dm}^3$ for the concentration range 0–0.022 mol dm^{-3} and $68 \pm 2\,\mathrm{mol}^{-1}\,\mathrm{dm}^{-3}$ for the concentration range 0–0.69 mol dm^{-3}. The K^*-values determined in different solvents are of the same order of magnitude as the respective K-values determined from absorption measurements. It can suggest that the number of molecules undergoing excited-state proton transfer is similar to that of molecules hydrogen-bonded to the acetic acid in the ground state, in a given solvent.

3.5. Excitation spectra

Excitation spectra of isoalloxazinic and alloxazinic forms of lumichrome in the presence of acetic acid are different (see Fig. 5).

In the excitation spectra of isoalloxazinic form, a shift to longer wavelengths is observed as compared to the excitation spectra of alloxazinic form. Also the effect of changing the excitation wavelength on the emission spectra is observed. In the presence of acetic acid the ratio of the emission intensity of isoalloxazinic to alloxazinic forms has a maximum for the excitation wavelength of about 350 nm and for the excitation in the long-wavelength region. The excitation spectra of both tautomeric forms of lumichrome and changes in emission spectra depending on the excitation wavelength correspond to the changes in absorption spectra of lumichrome in the presence of acetic acid. This observation suggests that the excited alloxazinic and isoalloxazinic forms have different precursors in the ground state. The precursors of excited isoalloxazinic form are the ground-state hydrogen-bonded lumichrome–acetic acid complexes.

3.6. Time-resolved emission results

The information about the kinetics of the excited-state process is provided by the fluorescence lifetimes measurements. The decays of the emission of lumichrome in the presence and absence of acetic acid were measured in 1,2-dichloroethane and acetonitrile. The decays were monitored

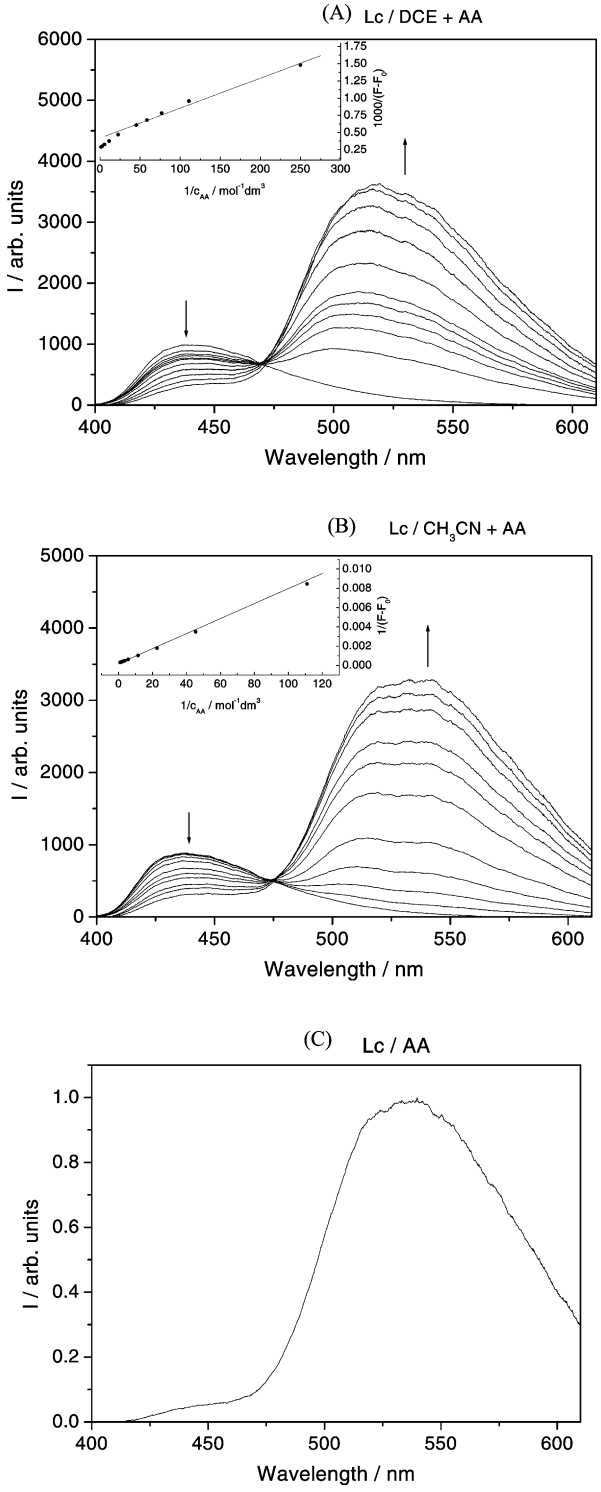


Fig. 4. Effect of increasing acetic acid concentration on fluorescence emission spectra of lumichrome: (A) in 1,2-dichloroethane (in the inset the Benesi–Hildebrand plot, $\lambda_{em} = 520$ nm, $K^* = 68\,\mathrm{mol}^{-1}\,\mathrm{dm}^3$), concentrations of acetic acid are: 0, 0.004, 0.009, 0.013, 0.017, 0.02, 0.04, 0.09, 0.17, 0.35, 0.70 mol dm^{-3}; (B) in acetonitrile (in the inset the Benesi–Hildebrand plot, $\lambda_{em} = 540$ nm, $K^* = 2.2\,\mathrm{mol}^{-1}\,\mathrm{dm}^3$), concentration of acetic acid are: 0, 0.004, 0.009, 0.013, 0.017, 0.02, 0.04, 0.9, 0.17, 0.35, 0.52, 0.70, $1.05\,\mathrm{mol}^{-1}\,\mathrm{dm}^3$. The arrows indicate the increasing concentration of acetic acid. (C) Fluorescence emission spectrum of lumichrome in acetic acid.

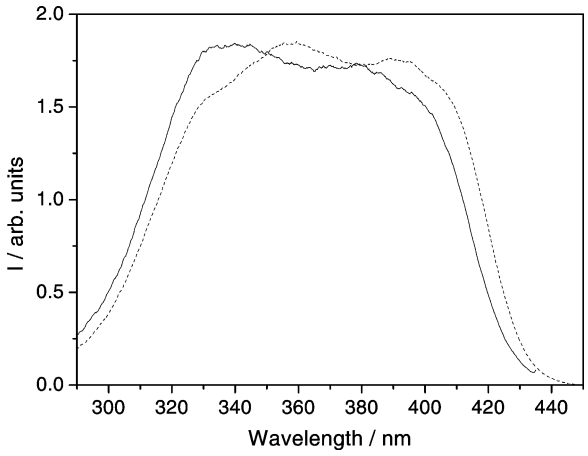


Fig. 5. Excitation spectra of alloxazinic ($\lambda_{em} = 430$ nm, solid line) and isoalloxazinic ($\lambda_{em} = 540$ nm, dashed line) forms of lumichrome in the presence of acetic acid ($c_{AA} = 0.09$ mol dm^{-3}) in 1,2-dichloroethane.

at the alloxazinic and isoalloxazinic emission bands. The fluorescence decay times are listed in Tables 1 and 2.

The decay of the emission of lumichrome in pure solvents, 1,2-dichloroethane and acetonitrile, is well described by single-exponential function. As shown in Tables 1 and 2, the kinetics of the excited-state proton transfer of lumichrome in the presence of acetic acid depends on the kind of solvent.

In acetonitrile, the decay of alloxazinic emission of lumichrome with and without acetic acid is single exponential in the whole range of acetic acid concentrations studied. The decay of the isoalloxazinic emission of lumichrome in the presence of acetic acid is described by a sum of single-exponential decay and a single-exponential rise. (It seems that the shorter decay time observed for isoalloxazinic emission for the concentration of acetic acid of 0.02 mol dm^{-3} and the single-exponential decay for concentration of 0.04 mol dm^{-3} can be due to overlapping of alloxazinic and isoalloxazinic emission. As a result, the rise time of isoalloxazinic emission is compensated by the decay of alloxazinic emission.) The decay times of the alloxazinic emission are close to the rise times of the isoalloxazinic emission for a given acetic acid concentration and the pre-exponential factors for these times are similar. These results allow a conclusion that in the excited state of lumichrome in the presence of acetic acid there is kinetic relationship between the excited alloxazinic and isoalloxazinic forms. The excited alloxazinic form is the precursor of the excited isoalloxazinic form. Single-exponential decay of the alloxazinic form in the presence of acetic acid suggests moreover, that in the excited state there is no equilibrium between both tautomeric forms. The relatively long rise times of the isoalloxazinic emission suggest that the excited-state process is relatively slow.

The kinetics of the excited-state proton transfer in 1,2-dichloroethane is more complicated. For the acetic acid concentrations within the range of 0–0.04 mol dm^{-3}, the decay of the alloxazinic and isoalloxazinic emission is single exponential. The lack of measurable rise time for the

Table 1
The fluorescence lifetimes for alloxazinic and isoalloxazinic forms of lumichrome for various acetic acid concentration in 1,2-dichloroethane

Acetic acid concentration (mol dm^{-3})	425 nm			580 nm		
	τ_F^1 (ns) (a^1)	τ_F^2 (ns) (a^2)	χ^2	τ_F^1 (ns) (a^1)	τ_F^2 (ns) (a^2)	χ^2
0	0.61	–	0.946	–	–	–
0.009	0.61	–	0.904	–	4.33	0.875
0.017	0.60	–	1.042	–	4.58	0.744
0.04	0.61	–	0.842	–	4.54	0.658
0.09	0.55 (0.99)	3.43 (0.01)	1.032	–	4.38	0.819
0.35	0.50 (0.98)	3.93 (0.02)	1.189	0.30 (−0.46)	4.06 (0.54)	0.705
0.7	0.45 (0.98)	3.00 (0.02)	0.864	0.45 (−0.38)	3.76 (0.62)	0.946
Acetic acid (17.4 mol dm^{-3})	0.23 (0.99)	2.52 (0.01)	0.735	–	2.44	1.333

τ_F^1, τ_F^2: fluorescence lifetimes; a^1, a^2: pre-exponential factors ($|a^1| + |a^2| = 1$).

Table 2
The fluorescence lifetimes for alloxazinic and isoalloxazinic forms of lumichrome for various acetic acid concentration in acetonitrile

Acetic acid concentration (mol dm^{-3})	425 nm		580 nm		
	τ_F (ns)	χ^2	τ_F^1 (ns) (a^1)	τ_F^2 (ns) (a^2)	χ^2
0	0.64	1.050	0.71	–	0.845
0.02	0.63	0.845	0.62 (0.44)	8.74 (0.56)	1.341
0.04	0.60	1.189	–	8.50	1.316
0.09	0.57	0.708	0.43 (−0.49)	7.86 (0.51)	1.718
0.17	0.56	0.823	0.50 (−0.42)	7.83 (0.58)	1.133
0.35	0.45	0.872	0.40 (−0.50)	7.35 (0.50)	1.106
0.7	0.37	0.998	0.35 (−0.48)	6.64 (0.52)	1.381

isoalloxazinic emission can suggest that the excited-state process can occur in a time shorter than the time resolution of our instrument. Moreover, for this range of acetic acid concentration the decay time of the alloxazinic emission is practically constant. On the other hand, in the emission spectra of lumichrome for this range of acetic acid concentration, a decrease of intensity of alloxazinic emission and an increase of intensity of isoalloxazinic emission have been observed. These results suggest that there is no kinetic connection between the excited alloxazinic and isoalloxazinic forms. The excited alloxazinic and isoalloxazinic forms have different precursors in the ground state.

For the range of higher concentration of acetic acid (0.09–0.7 mol dm^{-3}) the decay kinetics of tautomeric forms of lumichrome become similar as in acetonitrile, suggesting the kinetic connection between the two excited forms and a lower rate of the excited-state process. It is not clear whether the double-exponential decay of alloxazinic emission is a result of an equilibrium between both excited forms or the overlapping of isoalloxazinic and alloxazinic emission band.

In pure acetic acid, the fluorescence decay monitored at alloxazinic emission band is described as a sum of two one exponential functions with decay times 0.23 and 2.5 ns. The decay of isoalloxazinic emission is well fitted by a single-exponential decay function, with a time of 2.4 ns. This decay time is in good agreement with the fluorescence lifetime 2.5 ns reported for lumichrome in acetic acid by Fugate and Song [7].

3.7. The mechanism of the excited-state proton transfer

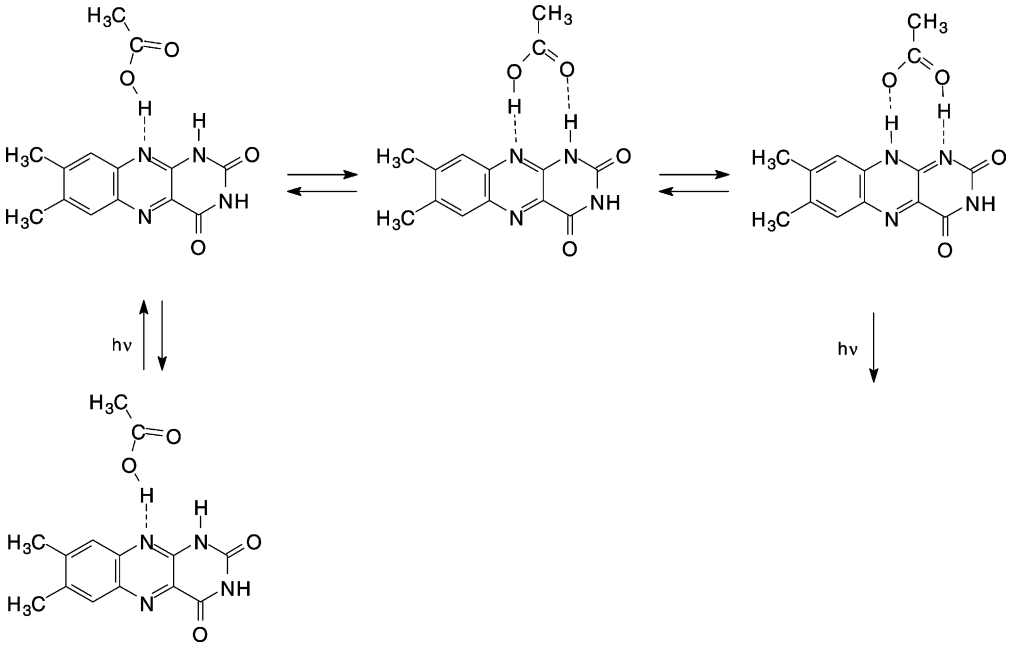
For a system undergoing the excited-state proton transfer the appearance of the dual emission can be due to (1) the excited-state reaction between two tautomeric forms, separated by an energy barrier or (2) the coexistence of different conformers in the ground state.

In the first case, there should be a kinetic connection between two excited tautomeric forms. The excited alloxazinic form should be a precursor of the excited isoalloxazinic form. In acetonitrile, the following Scheme 1 may be proposed.

The results obtained in acetonitrile are in accordance with this model. In the ground state, complexes between lumichrome and acetic acid are formed with the hydrogen bond at N(10) nitrogen atom. After excitation, changes in the electron density distribution lead to an increased acidity of N(1)–H group and increased basicity of N(10) nitrogen atom. The increase of the acidity of N(1)–H group brings about the reorientation of molecules and formation of cyclic, doubly-hydrogen-bonded complexes. In such complexes, the proton is transferred from N(1) to N(10) nitrogen atom and the isoalloxazinic structure is formed.

On the basis of literature data, one could conclude that the excited-state proton transfer in complexes with “appropriate structure” is very fast—occurring in pico- or femtoseconds [40,41]. But the rate constant of the excited-state reaction determined by us in acetonitrile is about 10^9 s^{-1}, indicating that the process is relatively slow. Thus, it seems that the process in the excited state consists of two steps: the formation of complexes with “appropriate structure” and the proton transfer in the complexes formed. The first step should be slower and determine the observed rate of overall process.

The experimental data show however that the mechanism of excited-state proton transfer is different in 1,2-DCE than in acetonitrile. The kinetics of the excited-state process depends on the concentration of acetic acid.

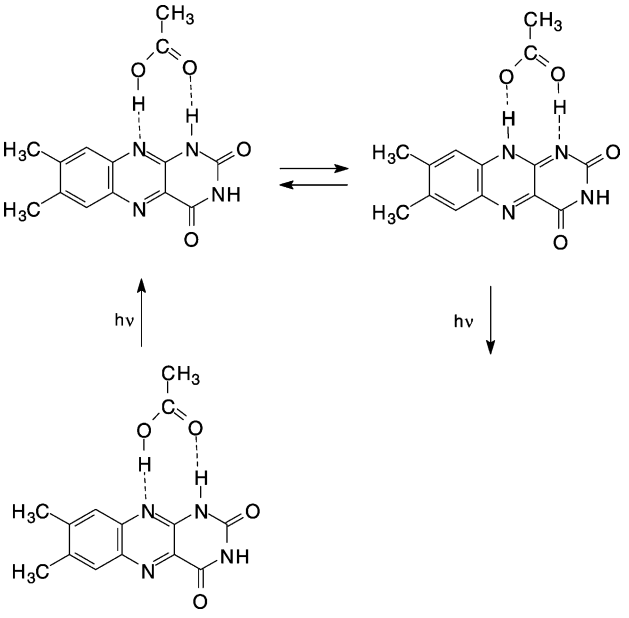


Scheme 1.

In the second case, the emission of both tautomeric forms may be a result of a coexistence of different conformers in the ground state. Some of the ground-state structures are ready to proton transfer, some are unable to undergo this process. In such a case, there is no kinetic relation between the excited tautomeric forms. The kinetic results in 1,2-dichloroethane for lower acetic acid concentrations indicate that such a situation is observed. In this case, a formation of cyclic, doubly-hydrogen-bonded complexes between lumichrome and acetic acid in the ground state is proposed. After absorption of light in the excited complexes the proton transfer occurs and the emission of isoalloxazinic form is observed. The process that leads to formation of isoalloxazinic structure is very fast, and no rise time for the tautomeric form is observed. So we propose that in this case the cyclic, doubly-hydrogen-bonded complexes are formed already in the ground sate. The following Scheme 2 may be proposed for lumichrome tautomerisation in 1,2-dichloroethane for lower acetic acid concentrations.

In this model, the excited-state process consists of only one step—a fast proton transfer in the complexes with "appropriate structure".

Kinetic data show that for the range of higher acetic acid concentrations the rate of the excited-state process has slowed down. For higher concentrations of acetic acid the creation of 1:1, open, single-hydrogen-bonded complexes may be preferable (Scheme 1) due to the increasing polarity of solution. On the other hand, complexes with stoichiometry different than 1:1 may be formed in the ground state. A stoichiometry of complex different than 1:1, would difficult proton transfer or ring closure. Reorientation of these complexes and creation of cyclic structures after excitation may be the slower process and might determine the observed rate of the excited-state reaction.



Scheme 2.

In pure acetic acid, a single-exponential decay and no rise time for isoalloxazinic emission is observed. These results suggest that the doubly-hydrogen-bonded, cyclic complexes between lumichrome and acetic acid exist in the ground state. Upon excitation a rapid, excited-state proton transfer occurs in these complexes.

4. Conclusion

In summary, the presented results suggest the important role of environment on the mechanism and dynamics of the excited-state tautomerisation of lumichrome. It seems that kinetics of the excited-state process is determined by the structure of the ground-state complexes between lumichrome and acetic acid molecules. Depending on the structures of these complexes, the process in the excited state may involve one or two steps. In non-polar solvent, the formation of complexes with the cyclic structure in the ground state seems to be preferable. In polar solvent, the single-hydrogen-bonded complexes are formed and a geometrical adjustment to the cyclic structure in the excited state is needed.

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