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SPECTROSCOPIC STUDIES OF DUAL FLUORESCENCE EFFECTS IN
A SELECTED 1,3,4-THIADIAZOLE DERIVATIVE IN ORGANIC SOLVENTS
AND AQUEOUS SOLUTIONS

BADANIA SPEKTROSKOPOWE EFEKTÓW PODWÓJNEJ
FLUORESCENCJI W WYBRANYM ZWIĄZKU Z GRUPY 1,3,4-TIADIAZOLI
W ROZPUSZCZALNIKACH ORGANICZNYCH ORAZ ROZTWORACH WODNYCH

Abstract

Spectroscopic studies of the fluorescence emission of selected 1,3,4-thiadiazoles in organic solvents and an aqueous solution were carried out. An interesting effect of pH-induced dual fluorescence was observed in the aqueous solution. The use of organic solvents resulted either in a single fluorescence maximum, double fluorescence (two well-resolved emission bands), or the dual fluorescence effect. The results obtained suggest that the fluorescence emission effects in 1,3,4-thiadiazoles are associated with both the conformational isomerism and the chromophore aggregation phenomena.

Keywords: double fluorescence, dual fluorescence effect, molecular spectroscopy, chromophore aggregation, 1,3,4-thiadiazole, charge transfer (CT)

Streszczenie

W niniejszym artykule przedstawiono wyniki badań emisji fluorescencji wybranych związków z grupy 1,3,4-tiadiazoli w rozpuszczalnikach organicznych oraz roztworach wodnych. W środowisku wodnym dla badanych związków zaobserwowano interesujący efekt podwójnej fluorescencji indukowany między innymi zmianami pH. W rozpuszczalnikach innych niż woda obserwowano efekt albo pojedynczej fluorescencji lub dwóch rozdzielonych pasm emisji fluorescencji oraz efektu podwójnej fluorescencji. Na podstawie prezentowanych wyników badań zaproponowano, że na obserwowane efekty fluorescencyjne wpływ mogą mieć zarówno zmiany konformacyjne jak również procesy związane z agregacją chromoforową.

Słowa kluczowe: efekt podwójnej fluorescencji, spektroskopia molekularna, agregacja chromoforowa, 1,3,4-tiadiazole, transfer ładunku (CT)

1. Introduction

The development of successful therapeutic agents against cancer and neurodegenerative disorders such as Alzheimer's or Parkinson's diseases is one of the most challenging tasks in contemporary medicinal science. Furthermore, the increased resistance of microbes to conventional antibiotics has become a major obstacle in the successful treatment of various infections. Therefore, there is a constant need for the development of new biologically active substances with potency against cancers, neurodegenerative disorders, and various microbes.

In this context, thiadiazole derivatives are of particular interest. These heterocyclic compounds were first obtained in 1882 by Fisher, but their detailed structural characterisation was not made until 1890 [1]. Although various isomers are known, 1,3,4-thiadiazoles are the most extensively studied. According to the numerous reports, a broad array of biological activity exhibited by 1,3,4-thiadiazoles results from the presence of thioimine moiety, which constitutes the 5-membered core ring [2, 3]. Due to this unique structural feature, 1,3,4-thiadiazoles can act as potential antiproliferative, antifungal, antibacterial, anti-inflammatory, or antiviral agents, just to name a few [4–13].

The biological activity of 1,3,4-thiadiazoles is often accompanied by unusual physicochemical properties which makes them an interesting subject for in-depth spectroscopic studies. With this in mind, a series 1,3,4-thiadiazoles substituted with resorcinyly moiety is particularly worth emphasizing. For instance, these compounds exhibit various solvent polarizability-induced effects such as the keto-enol tautomerism [40, 43], polymorphism, and the solvatomorphism of crystals [14–17]. Furthermore, through interaction with lipids, they influence the molecular organisation and other structural properties of biomembranes [18]. Various excited state-related effects, including the nature of the fluorescence emission properties of 1,3,4-thiadiazoles, were extensively studied, pointing at the differences between the dual fluorescence and double fluorescence phenomena [19, 41–42]. The most recent reports refer to 1,3,4-thiadiazoles as ligands in the synthesis of transition metal complexes with an implication for the antineurodegenerative activity [20, 21].

The dual fluorescence effect relies on the appearance of two (or more) bands of fluorescence emission as a result of an excitation with a specific wavelength [22, 23]. In 1,3,4-thiadiazoles, such effects may be induced either by pH, solvent polarity, temperature or pressure. The difference between the dual fluorescence effect and the effect of two distinctly separated fluorescence emission bands should also be emphasised. In the former, two distinct joined bands can be observed after electron excitation, whereas the latter is characterised by two clearly separate fluorescence emission bands resulting from two separate excitations. *N,N*-dimethylaminobenzonitrile (DMABN) is a frequently given example of a molecule exhibiting this effect [24]. The dual fluorescence effect is commonly explained as being a result of intramolecular charge transfer (CT) accompanied with twisting deformation of the molecule, referred to as 'twisted intramolecular charge transfer' (TICT) [25–28]. The TICT model was introduced in the nineteen-seventies the 20th century by K. Rotkiewicz, K.H. Grellmann and Z.R. Grabowski and remains one of the most popular theoretical models used in a large number of molecular spectroscopy studies. It has been reported that a significant change in the dipole

moment of the excited molecule compared to that in the ground state is required for TICT to take place [29, 30]. Molecules exhibiting effects associated with TICT states are also highly sensitive to solvent effects. Moreover, the initial and final orbitals have to be spatially separated from each other so that its dipole moment can undergo changes resulting from electron transition (and absence of molecule symmetry). Importantly, the acceptor and donor fragments of the electron have to be located in close proximity to molecules exhibiting CT (or TICT). An excited-state intramolecular proton transfer (ESIPT) is another process that allows the explanation of dual fluorescence effects [31–33]. This model requires a close proximity between the potential proton acceptor and the proton donor group [34]. Furthermore, molecules in which (intra- or intermolecular) ESIPT is observed have similar fragments with the -OH group bound *via* a hydrogen linkage with an electron-negative atom, e.g. N. This molecule conformation facilitates proton transfer along the hydrogen bond and thus processes associated with ESIPT. Additionally, molecular aggregation, which results in the formation of excimers may contribute to the dual fluorescence effect.

The main aim of this work is the spectroscopic investigation of mechanisms of inter- and intramolecular interactions in chosen 1,3,4-thiadiazole derivatives in organic solvents as well as in aqueous solutions with varied pH values. A well known 2-(4-fluorophenylamino)-5-(2,4-dihydroxyphenyl)-1,3,4-thiadiazole (FABT) served as the model compound in these studies. Although this compound was previously studied [4], its more detailed spectroscopic characterisation is given herein. Moreover, an in-depth examination of the nature of interactions in this group of compounds may reflect on the design of a further series of derivatives with higher biological activity and their potential practical applications.

2. Materials and methods

2.1. Materials

The 2-(4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole (FABT) (see Scheme 1) was synthesized in the Department of Chemistry of the University of Life Sciences in Lublin using the previously reported protocol [2]. The structure of FABT is present in the Cambridge Crystallographic Data Centre with entries: CCDC 768785–768787.

All solvents were purchased from Sigma-Aldrich and were of highest purity available. The following solvent systems were used: 1 – 1-butanol (Bu-OH); 2 – methanol (Me-OH); 3 – Acetonitrile (ACN); 4 – ethanol (Et-OH); 5 – acetone; 6 – dimethyl sulfoxide (DMSO); 7 – propane-2-ol (Pr-OH).

2.2. Methods

The pH values of all aqueous solutions were measured with an Elmetron CP-502 pH meter at room temperature. For propan-2-ol solutions, FABT was first dissolved in selected solvents and then the pH was changed by the slow addition of 0.1M HCl to the glass flask. For



water-FABT and other compound solutions, 0.1M NaOH was first added to water to obtain pH 12. Afterwards, the powdered FABT or another compound was dissolved in water. To obtain a certain pH in the water-FABT solution, 0.1M HCl acid was slowly added. The pH was continuously controlled.

The electronic absorption spectra of FABT were recorded on a double-beam UV-Vis spectrophotometer Cary 300 Bio (Varian) equipped with a thermostat-equipped cuvette holder with a 6 × 6 multi-cell Peltier block. Temperature was controlled using a thermocouple probe (Cary Series II from Varian) placed directly inside the solutions of the samples. All UV-Vis absorption spectra were recorded at a temperature of 23°C.

Fluorescence excitation and emission spectra were recorded on a Cary Eclipse spectrofluorometer (Varian). Fluorescence spectra were recorded with a resolution of 0.5 nm and corrected for the lamp and photomultiplier spectral characteristics. The excitation and emission slits were set to 2 nm. Resonance light scattering (RLS) measurements were performed as in Pasternack and Collings [35, 36]. The excitation and emission monochromators of the spectrofluorimeter were synchronously scanned (0.0 nm interval between excitation and emission wavelengths); the slits were set to obtain a spectral resolution of 1.5 nm. All emission spectra, namely the fluorescence emission, fluorescence excitation and resonance light scattering excitation (RLS) were recorded at a temperature of $T = 23^{\circ}\text{C}$. The spectral analysis was performed with the use of Grams/AI 8.0 software (Thermo Electron Corporation).

3. Result and Discussion

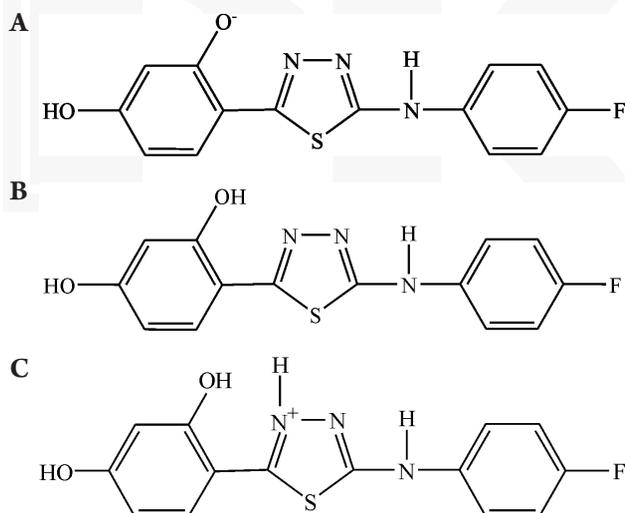


Fig. 1. Structures of FABT: A) – anionic form with deprotonated –OH group at the *ortho* position regarding the thiadiazole ring; B) – enolic form; C) – cationic form obtained by protonation of one of the thiadiazole nitrogen atoms

Fig. 1 illustrates the various forms of the FABT molecule which may occur depending on environment/solvent used. The structure 1A with deprotonated -OH group at the *ortho* position with respect to the thiadiazole ring is characteristic of basic pH (high pH values). The enolic form of FABT (Fig. 1B) is the most common form of this compound occurring within the pH range similar to that of physiological. Finally, Fig. 1C represents the cationic form of FABT, which according to crystallographic studies consists of the protonated thiadiazole ring, with the protonation occurring at the nitrogen atom (N3), which resides in close proximity to the resorciny] -OH group [17]. Although the rotation of resorciny] moiety is possible and may result in a conformation in which the -OH group resides near the thiadiazole sulphur atom, the crystallographic data suggests that the form 1C is more favourable [17, 19] and occurs in acidic solutions (low pH values). All forms of FABT presented in Scheme 1 are planar and do not occur in other conformations.

The UV-Vis spectroscopic studies carried out in the pH range of 1 to 12 reveal that the most notable shifts in the positioning of the absorption maxima occur in the pH range similar to that of physiological pH [19]. Also, a comparison of the absorption spectrum of FABT recorded at pH = 7, with those recorded at both acidic and basic conditions shows a respective hypsochromic and bathochromic shifts of the absorption maxima. Moreover, it has been reported that these changes are associated with the deprotonation of resorciny] *ortho* -OH (low pH) and protonation of the thiadiazole nitrogen (high pH) (Fig. 1A and 1C) [19]. These results are consistent with UV-Vis spectroscopic data obtained in this work.

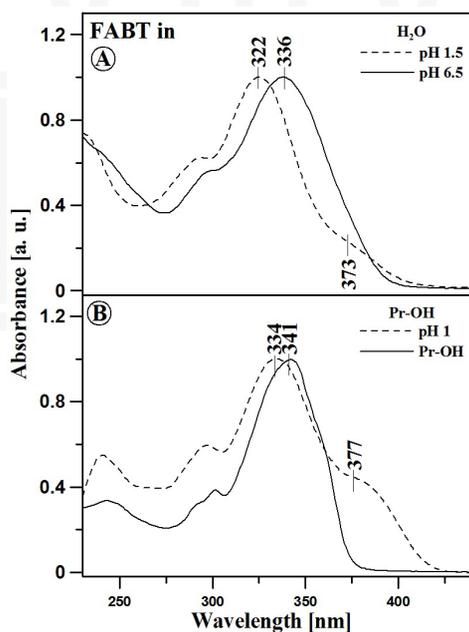


Fig. 2. UV-Vis absorption spectra of FABT: A) Spectra recorded in aqueous solutions at pH = 1.5 and 6.5; B) Spectra recorded in Pr-OH and acidified Pr-OH (so-called apparent pH = 1). The spectra are normalised to an a. u. value of 1 at their absorption maxima

The representative UV-Vis absorption spectra of FABT recorded in various conditions (pH and solvents) are given in Fig. 2. In aqueous solution at the pH of 6.5, FABT absorbs at 336 nm (29762 cm⁻¹), while at the pH of 1.5, its absorption maximum shifts to 322 nm (30960 cm⁻¹) and a new low intensity broad band appears at ~370 nm (26810 cm⁻¹). A similar trend is observed in Pr-OH solution, where the addition of 0.1M HCl (apparent pH= 1.5) results in shift of the main absorption maxima from 341 nm (29326 cm⁻¹) to 334 nm (29940 cm⁻¹), and appearance of a new absorption band at 377 nm (26525 cm⁻¹). The new, low energy absorption bands result most likely from aggregated FABT molecules [37]. Resonance light scattering (RLS) studies are in agreement with the UV-Vis absorption results, and confirm that the aggregation of FABT chromophores takes place (Fig. 6).

The formula (1) allows for the calculation of the distance between the neighbouring FABT chromophores based on the excitonic splitting theory and the UV-Vis studies [38].

$$R_{\beta} = 1.71 \sqrt[3]{\frac{\mu^2 \kappa}{n^2 \beta}} \quad (1)$$

Where μ represents the dipole moment of molecule transition (in the case of the analysed 1,3,4-thiadiazoles, its value is around 5D). The κ parameter is related to the orientation of molecules within the aggregate. The values of $\kappa = 1$ and $\kappa = -2$ correspond to 'card pack' and 'head-to-tail' orientations, respectively. N – is the refractive index, and β is the dipole-dipole interaction energy (in the classical approach). Formula (1) considers the aggregated structure formed as an effect of interactions between identical molecules. It assumes a model system, in which the transition dipole moments of neighbouring molecules are parallel. The transition dipole moment values calculated for the FABT monomer in selected solvents are given in Table 1. The calculated distance between FABT dimers in an aqueous solution equals 3.71 Å [19], in Pr-OH, it is 3.79 Å. The exciton splitting theory-based calculations are consistent with the crystallographic data. In particular, the distance between neighbouring molecules in crystals obtained from the aqueous solution equals 3.32 Å, while in crystal formed in Pr-OH, it is 3.39 Å [17].

The solutions of FABT were also examined by fluorescence emission spectroscopy and the representative set of spectra is given in Fig. 3, which illustrates the pH dependence of the fluorescence emission. It is noteworthy that the spectra recorded using an identical excitation wavelength (330 nm) differ significantly depending on the pH. At basic pH (10.5) only a single emission maximum is present at ~430 nm, while at acidic pH (starting from 6.5), this maximum shifts by approximately 15 nm to a higher energy region and an additional emission maximum of ~500 nm appears. These results are consistent with previously reported data [19] and correspond to the formation of FABT aggregates.

The comparison of emission spectra of FABT recorded in pure Pr-OH (panel A) with those in acidified Pr-OH solutions is given in Fig. 4. Regardless of the excitation wavelength used, only a single fluorescence maximum is present in spectra recorded in pure Pr-OH (Panel A). More notable changes are observed in acidified Pr-OH solutions (Panel B)

where the excitation wavelength-dependence of the emission maxima is clearly visible. The excitation wavelength of either 334 nm or 344 nm effects in only a single emission maximum. This is a characteristic feature of the FABT monomer and is only slightly broader than those recorded in pure Pr-OH. The excitation with use of the wavelength characteristic of the FABT aggregates (e.g. 377 nm) gives rise to an additional emission maximum at approximately 490 nm. It is worth emphasizing that this is not an effect of dual fluorescence regardless of the fact that two well resolved (only partly overlapping) emission bands are present. It is expected that the wavelength of 377 nm excites both the monomeric and aggregated form of FABT; therefore, two different absorption maxima are observed.

Excitation at 296 nm results in a similar pattern of two emission maxima; however, at short wavelengths, only the monomeric form absorbs; thus, the hypothesis that the observed emission maxima originate solely from the FABT monomer. This effect is called dual fluorescence. On the other hand, in both low pH aqueous solution and acidified Pr-OH, the emission maxima observed are of low intensity, which usually is attributed to aggregation. This in turn may suggest that the aggregation-related dual emission cannot be excluded.

In order to address that issue, a set of fluorescence excitation spectra was recorded (Fig. 5) at conditions similar to those, at which the fluorescence emission spectra were recorded. The excitation with 401 nm, and 410 nm (neutral and acidified Pr-OH solution, respectively) gave the spectra characteristic of the monomeric FABT and similar to those of the Uv-Vis absorption. The excitation at 484nm results in the appearance of a new band, with a maximum at 378 nm, with the characteristic broadening at the lower energy site, similar to that of the UV-Vis absorption spectra. Similar effects are observed in spectra recorded in water (not shown). In the case of the fluorescence excitation spectra recorded with use of long excitation wavelengths, additional bands (e.g. 357 nm) are present (see Fig. 5). This may serve as additional evidence for the formation of not only dimers but also more complex aggregates. Such structures may differ in size (see RLS results) and, depending on the excitation wavelength, can also give rise to variable fluorescence emission spectra.

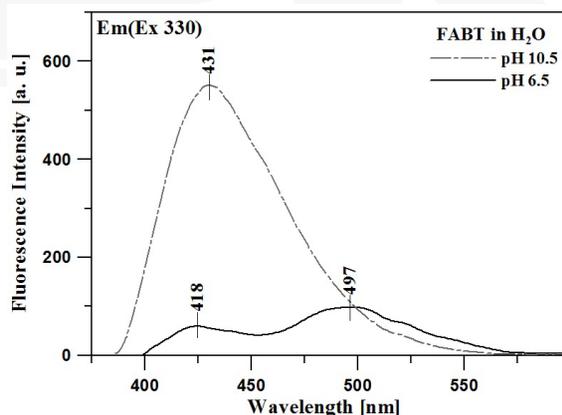


Fig. 3. Fluorescence emission spectra (Em) of FABT recorded in an aqueous solution at pH = 6.5 (black line) and 10.5 (dotted line) at an excitation wavelength of 330 nm

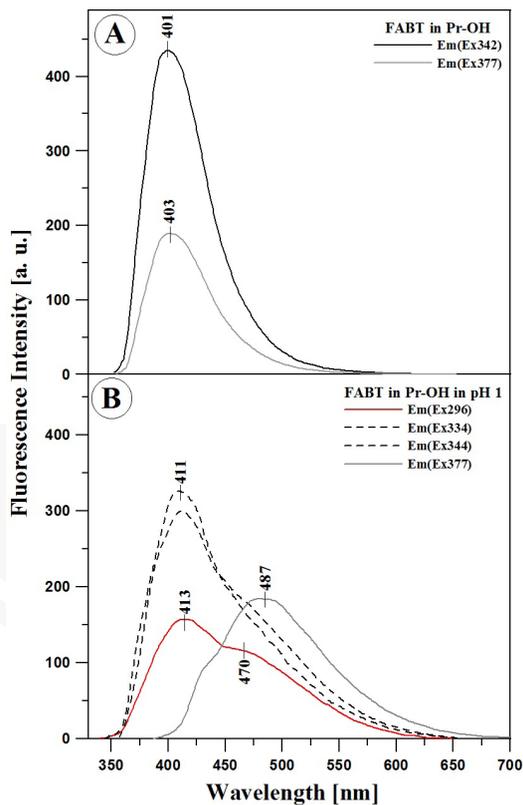


Fig. 4. Selected fluorescence emission spectra of FABT recorded in Pr-OH (Panel A) and in acidified Pr-OH (apparent pH = 1) (Panel B). Em(Ex), refers to the emission recorded for the respective excitation wavelength

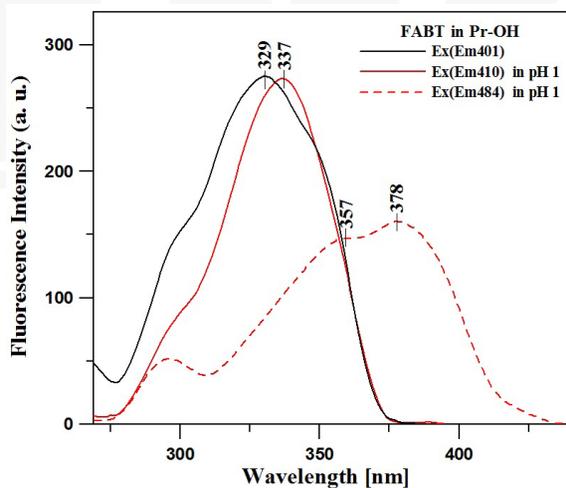


Fig. 5. Selected fluorescence excitation spectra (Ex) of FABT recorded in Pr-OH (black) and in acidified solution of Pr-OH (apparent pH = 1) (red, and red dotted). Em401, Em410, and Em484 refer to the respective excitation wavelengths

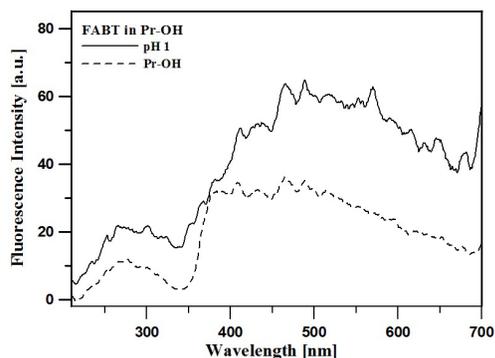


Fig. 6. RLS spectra of FABT recorded In Pr-OH (dotted line) and in an aqueous solution at pH 1 (black line)

The aggregation of FABT molecules is also supported by the resonance light scattering spectroscopy results (Fig. 6). The RLS spectra obtained are characteristic of highly intensive bands, which according to Parkash and Pasternack [35, 36], are associated with the presence of aggregates and are consistent with the results of Uv-Vis, fluorescence emission, and excitation spectroscopy. It is also worth emphasizing that the large number of RLS bands is most likely as a result of the formation of a large number of various aggregated structures. The highest intensity of RLS signals are observed in the spectra recorded at the pH range of 1–7, this is characteristic of the dual fluorescence of FABT. At the pH above 7 the intensity of RLS bands decreases significantly, and the disappearance of the long wavelength emission band in the fluorescence spectra is observed (the dual fluorescence emission phenomenon disappears).

The spectroscopic experiments carried out at various temperatures revealed a similar trend, specifically, that the highest intensity RLS signals were observed at low temperatures, while the temperature increase resulted in lowering the intensity of the RLS bands, as well as the disappearance of the dual fluorescence emission [19]. This in turn suggests a direct link between the aggregation and dual fluorescence emission, but it does not exclude other factors which may contribute to the spectroscopic effects observed in FABT.

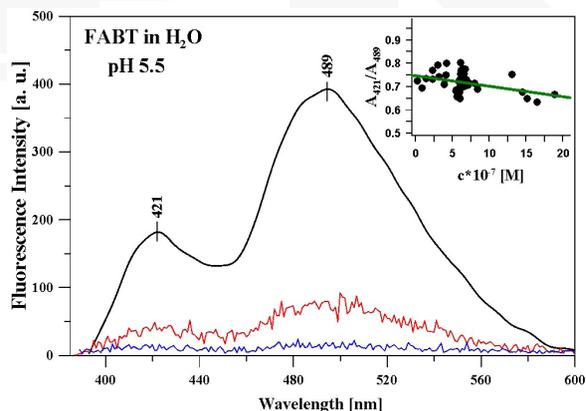


Fig. 7. Fluorescence emission spectra of FABT in aqueous solutions at pH = 5.5 recorded for three different concentrations. Insert: Ratio of the fluorescence maxima as a function of the FABT concentration

Another set of experiments carried out in this work was the examination of the concentration-dependence of dual fluorescence emission in FABT (Fig. 7). The spectra were recorded using the aqueous solution of FABT at pH = 5.5, which in these conditions, exists as a stable dual fluorescence emitting form. All spectra in this experiment set were recorded using the identical excitation wavelength (328 nm). The dual fluorescence effect was observed even at concentrations below 10^{-7} M and the intensities of emission bands were proportional to the FABT concentrations tested. The Figure 7 inset depicts the ratio of the fluorescence maxima as a function of the FABT concentration. It is clearly visible that the dual fluorescence emission effect depends on the concentration of the compound and is observed even at very low concentrations, which is consistent with previously reported data [20]. It is also clear that in aqueous solutions, the concentration increase effects in a variable ratio of fluorescence bands intensity. In particular, the higher the concentration, the more intensive the emission band at the lower energy side of the spectrum. This effect is clearly associated with the aggregation of the FABT chromophore, which occurs in an aqueous solution at increased levels of concentration [19].

It is not only the aggregation of the molecules that are known to contribute to the dual fluorescence effect but also conformational changes [19]. Usually, this effect is observed in conformation, where the resorcynyl -OH group resides in close proximity to one of the nitrogen atoms of the thiadiazole ring. The single fluorescence band or two resolved fluorescence maxima (but not originating from dual fluorescence effect) are characteristic of the opposite conformation, at which the resorcynyl -OH resides near the thiadiazole sulphur atom [19]. Secondly, the crystallographic studies on FABT report that the protonation site of thiadiazole ring which occurs at low pH also contributes to the fluorescence pattern [17, 39].

Comparison with other compounds which demonstrate dual fluorescence leads to the conclusion that this phenomenon in FABT is not related to TICT, PICT or ESPIT and cannot be fully explained based on these theories [25–34]. The results obtained suggest that the dual fluorescence effect in FABT and similar thiadiazole derivatives is more likely resultant of both chromophore aggregation and molecular conformation. The aggregation may induce changes in electron density, which may trigger a specific intramolecular charge transfer. This, in turn effects in the dual fluorescence emission. This explanation is further supported by calculations carried out based on the spectroscopic data, such as the dependence of the dipole moment (calculated from an integration of the absorption spectrum) on the Debay polarity (function dependent on the environment/solvent dielectrical constant) (Fig. 8).

The obtained results show that an increase in the Debay function is accompanied with a notable change in the dipole moment value of the examined molecules. According to the literature, such an effect is usually associated with inter- or intramolecular charge transfer, and supports the postulated intramolecular charge transfer origin of dual fluorescence emission in FABT.

The data from an integration of the absorption spectrum served as data for the calculation of the dielectric constant ϵ and the series of solvent polarity-related parameters (Tab. 1) – these results, together with charge transfer effects in FABT and other similar thiadiazole derivatives will be a subject of future studies.

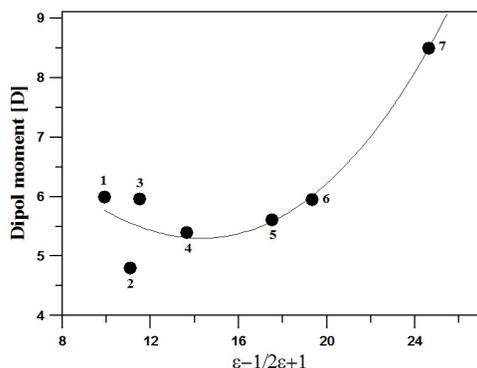


Fig. 8. Dependence of the dipole moment (calculated from an integration of the absorption spectrum) on the Debye polarity function (function dependent on environment/solvent dielectrical constant). A 2nd degree curve was fitted to data points 1–6 (details of assignment of the solvents to the respective numbers are provided in the Material and Methods section)

Table 1. Dielectric constant, dipole transition moment, and Debye polarity function values calculated for the selected solvents

FABT in	ϵ	$\epsilon - 1/2\epsilon + 1$	μ (D)
Pr-OH	20.18	11.09	4.81
DMSO	47.24	24.62	8.52
Me-OH	33.00	17.51	5.63
Et-OH	25.30	13.65	5.45
ACN	36.64	19.32	5.95
Acetone	21.00	11.51	5.96
Bu-OH	17.84	9.92	5.99

4. Conclusions

In conclusion, a series of spectroscopic experiments on 1,3,4-thiadiazole derivative FABT was carried out. Spectroscopic techniques such as UV-Vis absorption, steady state fluorescence, fluorescence excitation, and resonance light scattering revealed the highly complex nature of the dual fluorescence effect which is characteristic of this group of compounds. The results obtained confirmed that the dual fluorescence of FABT may occur in aqueous solutions at various pH levels – this is consistent with previously reported data. Additionally, a similar dual fluorescence effect is observed in organic solvents, both neutral and acidified with HCl. It is clear that both pH-dependent and solvent-dependent conformational changes in FABT notably influence its spectroscopic properties as well as the aggregation behaviour. In particular, the conformation in which the resorcynyl –OH group resides near the thiadiazole N atom is considered to be the main form responsible for the dual emission. Secondly, the

RLS and fluorescence excitation spectra together with a series of fluorescence emission measurements carried out on various concentrations suggest that the aggregation of FABT significantly contributes to the dual fluorescence effect. Moreover, the aggregation-related effects may take place both in organic and aqueous solutions.

An examination of the fluorescence excitation spectra allowed for the assignment of the fluorescence bands to the specific FABT conformations. These conformations, together with the aggregation are most likely responsible for the specific charge distribution around the FABT molecule, which in turn, introduces an intramolecular charge transfer (CT) and gives rise to the dual fluorescence effect. It is also worth emphasizing that the dual fluorescence effect can be observed upon excitation with high energy (short wavelength) at low pH values – this clearly indicates the involvement of CT processes. To sum up, it is worth stressing that the fluorescence effects observed in the analysed 1,3,4-thiadiazole compound are inherent traits of these compounds (as well as structurally similar molecules). This indicates that the several effects, which have not been analysed in other molecules in such systems, are involved in the observed fluorescence effects.

Acknowledgments

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