

4-Amino-benzo[f]isoindole-1,3-dione Derivatives as Turn-on Fluorescent Indicators for Water Determination in Acetonitrile

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4-Amino-9-(2,5-dioxo-pyrrolidin-1-phenyl-3-yl)-1-phenyl-benzo[f]isoindole-1,3-dione (**1**) and 4-amino-9-(2,5-dioxo-1-(4-methylphenyl)-pyrrolidin-3-yl)-1-(4-methylphenyl)-benzo[f]isoindole-1,3-dione (**2**) were studied as fluorescent indicators for water determination in acetonitrile. The intensity of fluorescence of these compounds in acetonitrile solutions increases in presence of water along with red shift of the emission peak. Intensity-based and wavelength-based procedures of water determination in acetonitrile are suggested with detection limit of 0.2% (v/v) H₂O in both cases. The linear response covers a concentration range of 0.2–20% (v/v). The relative standard deviation at 1% (v/v) water content is 0.20. The interferences of acetic acid, ammonia and metal ions have been studied. Ammonia and acetic acid don't interfere with the determination of water by wavelength-based fluorescence technique. In intensity-based mode the determination of water is possible at concentration of these impurities up to 0.25 and 0.02 mol·L⁻¹, respectively. Cu(II) ions have almost no effect on fluorescence of indicators up to 1·10⁻⁵ mol·L⁻¹; Zn(II) ions enhance fluorescence of **1** at concentration $\geq 5 \cdot 10^{-6}$ mol·L⁻¹. The 4-amino-benzo[f]isoindole-1,3-dione-based fluorescent indicators are pH-insensitive.

Keywords: 4-amino-benzo[f]isoindole-1,3-dione derivatives, turn-on fluorescence detection, water content, acetonitrile, solvatochromic shift

Похідні 4-аміно-бензо[f]-ізоіндол-1,3-діону як флуоресцентні "turn-on" індикатори для визначення вмісту води в ацетонітрилі

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Досліджено флуоресцентні індикатори – 4-аміно-9-(2,5-діоксо-піролідін-1-феніл-3-іл)-1-феніл-бензо[f]-ізоіндол-1,3-діон (**1**) і 4-аміно-9-(2,5-діоксо-1-(4-метилфеніл)-піролідін-3-іл)-1-(4-метилфеніл)-бензо[f]-ізоіндол-1,3-діон (**2**), для визначення вмісту води в ацетонітрилі. В присутності води значно зростає інтенсивність флуоресценції цих сполук у ацетонітрильних розчинах, спостерігається bathochromic зсув максимуму смуги у спектрі емісії. Запропоновано два способи визначення вмісту води у ацетонітрилі – за зростанням інтенсивності флуоресценції і за зміною величини сольватохромного зсуву, кожний з межею виявлення 0.2 об. % H₂O. Лінійність градувального графіка зберігається у діапазоні 0.2–20 об. %. Відносне стандартне відхилення при вмісті води 1.0 об. % становить 0.20. Вивчено вплив оцтової кислоти, амонію і іонів металів, здатних до утворення стійких комплексів з аміногрупою. Встановлено, що амоній і оцтова кислота не впливають на визначення води при використанні як аналітичного сигналу величини сольватохромного зсуву. При детектуванні води за зміною інтенсивності флуоресценції їхній вміст не повинен перевищувати відповідно 0.25 і 0.02 моль/л. Іони Cu(II) при концентрації, що не перевищує 1·10⁻⁵ моль/л, не впливають на флуоресцентні властивості індикаторів. Іони Zn(II) підвищують інтенсивність флуоресценції при їхньому вмісті $\geq 5 \cdot 10^{-6}$ моль/л. Досліджувані флуоресцентні індикатори не чутливі до зміни pH середовища.

Ключові слова: похідні 4-аміно-бензо[f]-ізоіндол-1,3-діону, "turn-on" флуоресцентне детектування, вміст води, ацетонітрил, сольватохромний зсув

The water content in medicines, chemical products, foods, and synthetic fibers is one of the important characteristics. Water is the most common impurity in organic solvents; its concentration is an

important indicator of their quality. Therefore, the water determination in organic solvents is a routine analytical procedure which sometimes becomes a complex problem.

There are numerous analytical approaches for the trace water determination in organic solvents such as the Karl Fischer titration [1,2], gas or liquid chromatography [3], microwave measuring techniques [4], voltammetry [5,6], and several spectroscopic techniques [3,7–10]. These methods require special equipment, toxic reagents, and complicated sample manipulations. They often are time-consuming; many of them are unsuitable for direct determination [3]. Fluorescent approach for water determination in organic solvents is one of the most sensitive and environmentally friendly methods with simple practical implementation. There are numerous fluorescent indicators developed for the water determination in acetonitrile, such as 8-hydroxyquinoline based probe [11], acridinyl derivatives [12], two-component coumarin phenylsemicarbazone/fluoride sensors [13], europium ion-based nanospheres [14], 2-(5-[4-dimethylaminophenyl]-2-oxazolyl)-benzoic acid [15] or series of N-heteroaryl-1,8-naphthalimides [16–18]. The use of 4-(2-dimethylaminoethoxy)-N-octadecyl-1,8-naphthalimide provides wide linear range (up to 79% v/v) and low detection limit of water (0.033% v/v) [19]. The main drawback of this method is the necessity to control the influence of pH value. The similar drawback has fluorescein and pyrene-appended fluorescein, which were found to provide high level of sensitivity [20,21]. However, the pH dependence of fluorescein fluorescence pose some limits for water determination in organic solvents containing traces of bases or acids [22]. Fluorescence PET sensors based on anthracene-aminoacid and anthracene-bisboronic acid ester have the detection limits for water in acetonitrile of 0.1 wt% and 0.013 wt%, respectively. Unfortunately, determination range is below 1 wt% of H₂O [23, 24].

The search for new indicators which demonstrate fluorescence enhancement in the presence of water with wide determination range and low pH-sensitivity is a challenging task. Isoindole and benzo[f]isoindole derivatives are perspective fluorescent probes in neurobiology, pharmacology, environmental analyses [25–27] because of their fluorescent properties in the visual spectral region. In this work we studied 4-amino-9-(2,5-dioxo-pyrrolidin-1-phenyl-3-yl)-1-phenyl-benzo[f]isoindole-1,3-dione (**1**) and 4-amino-9-(2,5-dioxo-1-(4-methylphenyl)-pyrrolidin-3-yl)-1-(4-methylphenyl)benzo[f]isoindole-1,3-dione (**2**) as fluorescent indicators for water determination in acetonitrile.

Experimental part

Reagents. Organic solvents used were of HPLC-grade (dimethyl sulfoxide and chloroform; LiChrosolv®, Merck, Germany; CHROMASOLV™, Sigma-Aldrich) or analytical reagent grade (toluene, acetone, ethanol, 2-propanol and butanol) and were used without further purification. Acetonitrile (Aldrich, 'anhydrous' grade) was additionally pre-dried. The actual water content ($0.008 \pm 0.001\%$) was verified using Karl Fisher method. All other chemicals were of analytical reagent grade. Double-distilled water was used throughout the study. The sample solutions of acetonitrile containing different amounts of water were prepared immediately prior to the measurement. Incremental amount of water was added to the solvent by micropipette.

Fluorescent indicators **1** and **2** (Figure 1) have been synthesized according to the previously described procedure [28]. The standard solutions of **1** and **2** were prepared by dissolving 1.5 mg of the corresponding substance in acetonitrile. The concentration of standard solutions were $1.3 \cdot 10^{-4}$ and $1.2 \cdot 10^{-4}$ mol·L⁻¹, respectively. Other solutions were prepared from the standard solutions by dilution with acetonitrile.

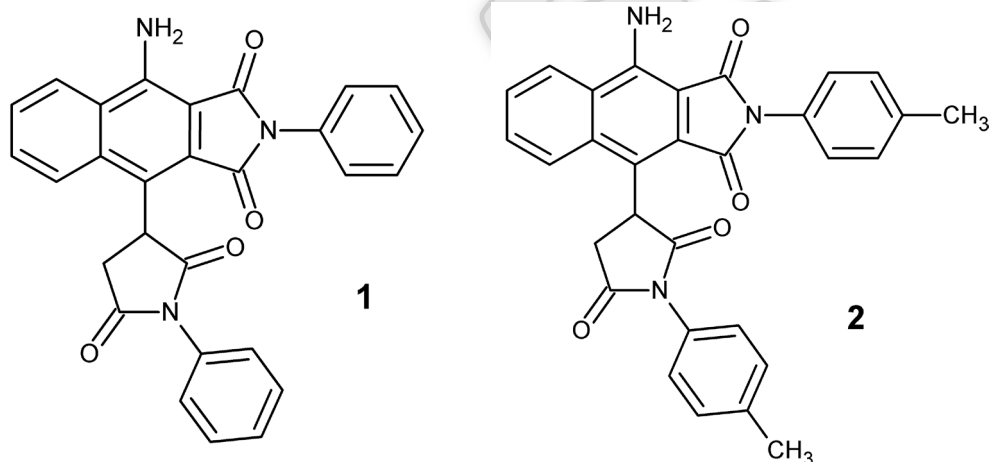


Fig. 1. Molecular structures of the 4-amino-9-(2,5-dioxo-pyrrolidin-1-phenyl-3-yl)-1-phenyl-benzo[f]isoindole-1,3-dione (**1**) and 4-amino-9-(2,5-dioxo-1-(4-methylphenyl)-pyrrolidin-3-yl)-1-(4-methylphenyl)benzo[f]isoindole-1,3-dione (**2**).

Instrumentation. Fluorescence excitation and emission spectra were measured at room temperature ($25 \pm 1^\circ\text{C}$) using Perkin Elmer LS 55 Spectrometer equipped with a xenon discharge lamp as a light source and gated photomultiplier with modified S5 response. All measurements were performed in a standard 1.0 cm quartz cell. The data were processed using the Perkin-Elmer FL WinLab 4.00.03. A scan speed of 400 nm/min was used with excitation slit width 5 nm and emission slit width 4 nm.

Intensity-based procedure for the determination of water in acetonitrile. An aliquot portion (0.2 mL) of the $5 \cdot 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ solution of **1** or **2** in a dry acetonitrile was added to the aliquot portion (10.0 mL) of sample solution of acetonitrile with 0 to 10% (v/v) water content in a 25.0 mL standard flask. The fluorescence intensity of corresponding solutions was measured in the Single Read mode at the emission wavelength of 500 nm with excitation at 255 nm. The concentration of water in acetonitrile was determined using the calibration curve plotted with the ΔI_{500} data as a function of the water content (% v/v), where ΔI_{500} equals the difference between fluorescence intensity of reagent in wet (I) and dry (I_0) acetonitrile at $\lambda_{\text{em}} = 500 \text{ nm}$ ($\Delta I_{500} = I - I_0$). Two series of acetonitrile solutions with a water content of 0; 1.3; 2.0; 2.6; 3.5; 4.0; 5.0; 6.0; 6.5; 10.0% (v/v) were prepared for this purpose. An aliquot portion (10.0 mL) of the each of the series solutions was mixed with 0.2 mL of $5 \cdot 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ solution of **1** or **2** in dry acetonitrile and their fluorescence intensity was measured according to the previously described procedure.

Wavelength-based procedure for the determination of water in acetonitrile. An aliquot portion (0.2 mL) of the $5 \cdot 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ solution of **1** or **2** in a dry acetonitrile was added to the aliquot portion (10.0 mL) of sample

solution of acetonitrile with 0 to 10% (v/v) water content in a 25.0 mL standard flask. Emission spectra of corresponding solutions were registered in a 420–570 nm range with excitation at 255 nm. Solvatochromic shift ($\Delta\lambda$) was calculated as a difference between maximum in the luminescence spectrum of **1** or **2** acetonitrile solution with (λ_{em}) and without (λ_{em}^0) water ($\Delta\lambda = \lambda_{\text{em}} - \lambda_{\text{em}}^0$).

The concentration of water in acetonitrile was determined using the calibration curve plotted with solvatochromic shift data (nm) as a function of the water content (% v/v). Two series of acetonitrile solutions with a water content of 0; 5.0; 10.0; 15.0; 20.0% (v/v) were prepared for this purpose. An aliquot portion (10.0 mL) of the each of the series solutions was mixed with 0.2 mL of $5 \cdot 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ solution of **1** or **2** in dry acetonitrile and their fluorescence spectra was recorded according to the previously described procedure.

Interferences caused by foreign species. To assess the selectivity of the procedure, the effect of foreign species commonly existed in the acetonitrile was investigated. For this purpose different amounts of these species were added to solutions containing 0.5 and 5.0% of water. The fluorescence intensities varying by 10% from the expected value were the criterion for the interference.

Results and discussion

Spectral characteristics in different solvents.

Fluorescence excitation, absorption and emission spectra of **1** and **2** were recorded in organic solvents of different polarity. Basic spectral characteristics of 4-amino-benzo[f]isoindole-1,3-dione derivatives in various solvents are summarized in Table 1.

Table 1. Spectral properties of studied 4-amino-benzo[f]isoindole-1,3-dione derivatives in various solvents.

Solvent	Solvent polarity E_T^N [29]	$\lambda_{\text{max}}^{\text{abs}}$, nm	$\lambda_{\text{max}}^{\text{em}}$, nm	Stokes shift $\Delta\nu$, cm^{-1}	Integral FL intensity I_Σ^*	$\lambda_{\text{max}}^{\text{abs}}$, nm	$\lambda_{\text{max}}^{\text{em}}$, nm	Stokes shift $\Delta\nu$, cm^{-1}	Integral FL intensity I_Σ^*
Compound 1						Compound 2			
Toluene	0.099	–	462	2925	0.37	–	462	3107	0.36
Chloroform	0.259	345 405	460	2952	0.67	332 397	459	3402	0.41
Acetone	0.355	348 407	460	2831	0.33	346 405	460	2952	0.83
DMSO	0.444	232; 284; 344; 406	470	3354	1.00	232; 284; 344; 406	472	3444	1.00
Acetonitrile	0.460	232; 284; 344; 406	462	2986	0.61	232; 284; 344; 406	462	2985	0.57

Table 1. (Contd.)

Solvent	Solvent polarity E_T^N [29]	$\lambda_{\max}^{\text{abs}}$, nm	$\lambda_{\max}^{\text{em}}$, nm	Stokes shift $\Delta\nu$, cm^{-1}	Integral FL intensity I_Σ^*	$\lambda_{\max}^{\text{abs}}$, nm	$\lambda_{\max}^{\text{em}}$, nm	Stokes shift $\Delta\nu$, cm^{-1}	Integral FL intensity I_Σ^*
Compound 1					Compound 2				
2-Propanol	0.546	349; 414	462	2510	0.07	333; 410	466	2931	0.30
n-Butanol	0.586	348; 412	465	2766	0.08	335; 411	472	3144	0.12
Ethanol	0.654	345; 413	468	2846	0.08	333; 410	471	3181	0.26
Water	1.000	336; 409	497	4329	0.88	336; 409	499	4410	0.42
Water-acetonitrile (3:1) mixture	—	248; 280; 341; 407	479	3693	0.95	246; 280; 339; 409	478	3530	0.91

*— Relative to the most intensive emission measured in DMSO ($I_\Sigma = 1.00$).

The fluorescence intensity of solutions **1** and **2** depends on the nature of the solvent. In aprotic solvents, such as toluene, chloroform, acetone and DMSO, the fluorescence intensity increases with the increase in their E_T^N polarity parameter (Fig. 2). The exceptions are acetone and acetonitrile, for which the deviation from this correlation is observed for the first and for both substances, respectively. The fluorescence intensity of **1** and **2** in protic polar solvents (ethanol, 2-propanol, butanol) is lower in comparison with aprotic ones.

The dependence of the fluorescence intensity

on the polarity of the solvent in this case is less pronounced. The nature of the solvent also affects the spectral maxima positions. The maximum of the fluorescence band of **1** and **2** in toluene, chloroform, acetone, acetonitrile and 2-propanol is at 461–463 nm. In DMSO and protic solvents (ethanol and butanol), it is displaced by bathochromic at 6–10 nm (to 467–472 nm), which is accompanied by the appearance in the spectrum of a small shoulder in the region of 500 nm. The significant red shift in the band position of the fluorescence spectrum of aqueous solutions was observed (Fig. 3).

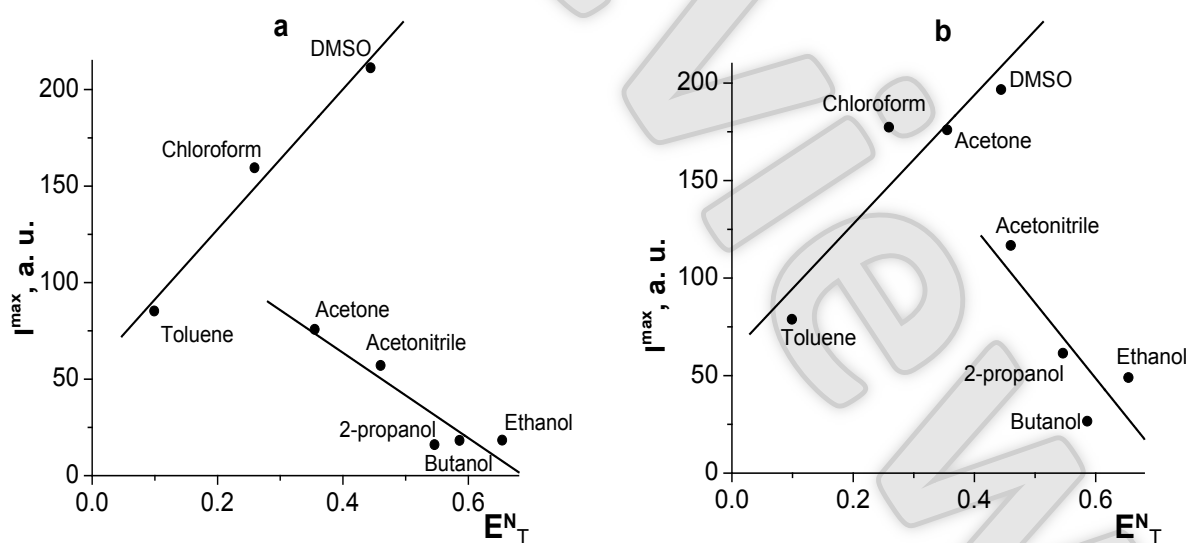


Fig. 2. Fluorescence intensity of **1** and **2** at band maximum in various solvents as a function of the E_T^N Reichardt parameter.

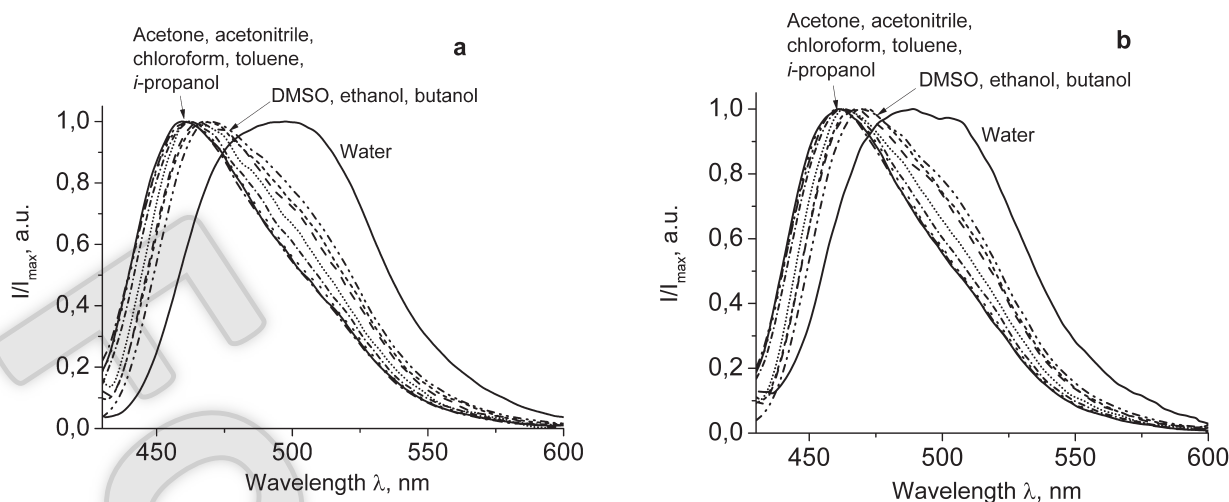


Fig. 3. Normalized fluorescence emission spectra of **1** (a) and **2** (b) in various solvents (Concentration, $\mu\text{mol}\cdot\text{L}^{-1}$: 1.0 (**1**, **2**). $\lambda_{\text{ex}} = 415 \text{ nm}$).

Spectral Changes Induced by the Presence of Water. Fluorescence emission spectra of **1** and **2** in acetonitrile solution contain single band with a maximum at 460 nm which corresponds to the blue fluorescence (Figure 4). As we have previously shown [28] fluorescence properties of **1** and **2** are influenced by the presence of water in the organic solvent. From a

Figure 4 we can see that increasing the water content in the acetonitrile enhances fluorescence intensity of **1** and **2** solutions. Water presence produces a red shift of the band in the fluorescence spectra of **1** and **2** acetonitrile solutions for 8.0–11.0 nm. This could be used for the water quantitation.

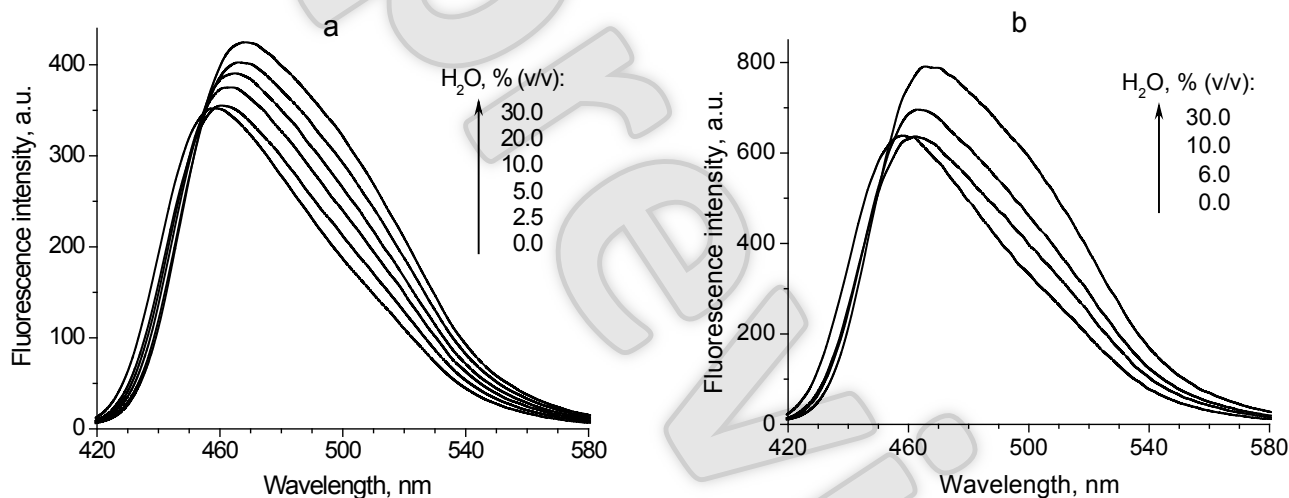


Fig. 4. Fluorescence emission spectra of **1** (a) and **2** (b) in acetonitrile solutions in the presence of increasing amounts of water (Concentration, $\mu\text{mol}\cdot\text{L}^{-1}$: 1.0 (**1**, **2**). $\lambda_{\text{ex}} = 255 \text{ nm}$).

The effect of pH and metal ions on the fluorescence of 1 and 2. The effect of the pH on the fluorescence intensity of **1** and **2** was investigated in water-acetonitrile (1:4) mixtures at different pH. The pH of solution was created by addition corresponding buffer solutions. It was found that in the range of pH of 1.4–9.2 the variance of luminescence intensity does not exceed 4%.

Metal salts are quite common impurity type in organic solvents therefore their influence on fluorescent properties of **1** and **2** have been also studied. Zn(II) and Cu(II) were chosen as a model

metals as they form stable amino complexes. It has been found that Cu(II) ions have almost no effect on fluorescence of indicators up to $1.0 \cdot 10^{-5} \text{ mol}\cdot\text{L}^{-1}$. Zn (II) ions enhance fluorescence of **1** at $\geq 5.0 \cdot 10^{-6} \text{ mol}\cdot\text{L}^{-1}$.

The low reactivity of aminogroup towards metal ions and pH insensibility makes **1** and **2** suitable for practical allowing water determination in the acetonitrile contaminated with bases, acids and metal salts.

Intensity-based fluorescence method.

In the intensity-based fluorescent signaling systems, solvatochromic dyes were mostly used

as the water indicators. These methods usually employed fluorescence quenching effect of water [11–13,15,20]. Their main disadvantage is a low selectivity and sensitivity caused by interference of some quenchers. In contrast, methods based on the fluorescence enhancement response are considered much more sensible and selective.

Increasing the water content in the acetonitrile enhances the fluorescence intensity of **1** and **2** in

the range of 470–560 nm (see Figure 4). In order to choose the wavelength for the water quantification by fluorescence enhancement we have calculated the slope of calibration plots in this spectral range. The optimal wavelength lies within the 490–510 nm range (Figure 5). ΔI value at 500 nm was used as an analytical response for the water determination in acetonitrile. This provides the best sensitivity and satisfactory reproducibility.

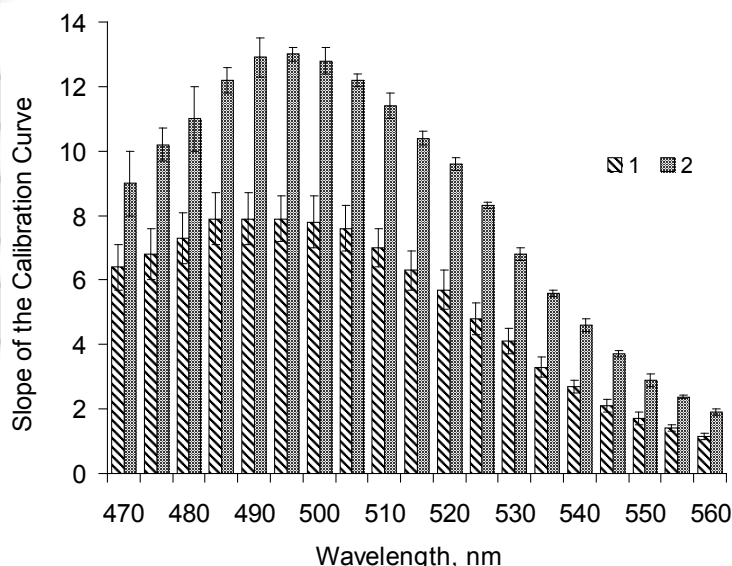


Fig. 5. Slope of the calibration plot ($\Delta I = (b \pm \Delta b) \cdot C_{H_2O}, \%$) for the water determination in acetonitrile by using **1** and **2** as a function of wavelength.

Fluorescence intensity of **1** and **2** at 500 nm correlates linearly with of water content in acetonitrile solutions in the range of 0.3–5.0 and 0.2–10% (v/v) respectively (Figure 6). Calibration curves for **1** and **2** could be represented by the following equations: $\Delta I_{500} = (10.7 \pm 0.3) \cdot C_{H_2O}, \%$ ($R = 0.998$) and $\Delta I_{500} = (12.8 \pm 0.2) \cdot C_{H_2O}, \%$ ($R = 0.998$). Detection limits were determined from 3 times of standard deviation of the blank noise and are 0.3% (v/v) and 0.2% (v/v) H_2O .

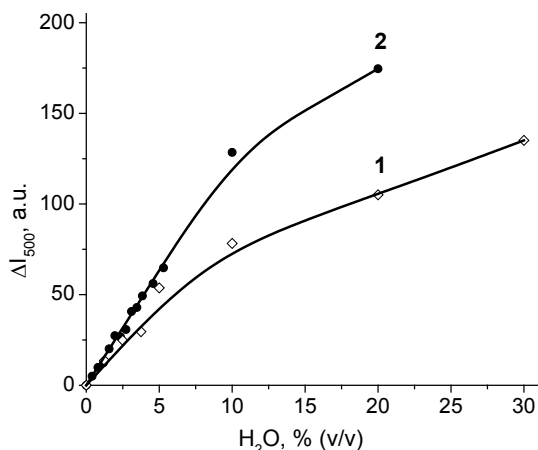


Fig. 6. The relative fluorescence intensity of **1** and **2** as a function of the water content in the acetonitrile solution (Concentration, $\mu\text{mol} \cdot \text{L}^{-1}$: 1.0 (**1**, **2**). $\lambda_{\text{ex}} = 255 \text{ nm}$).

Wavelength-based fluorescence method. Along with increase of fluorescence intensity the presence of water in acetonitrile also shifts emission maximum for **1** and **2** (see Figure 4). Figure 7 depicts correlation plots of solvatochromic shift value versus water content in acetonitrile.

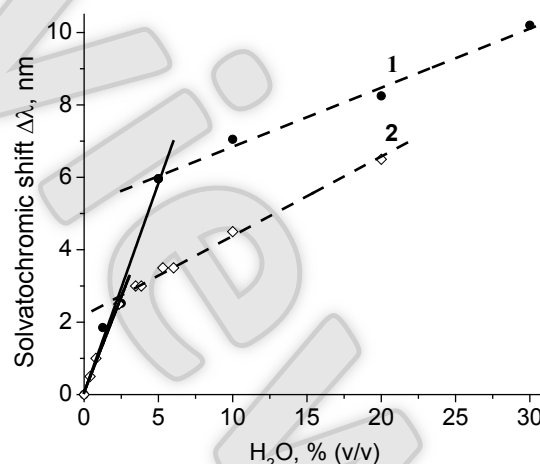


Fig. 7. The solvatochromic shift ($\Delta\lambda$) of **1** and **2** fluorescence band as a function of the water content in the acetonitrile solutions (Concentration, $\mu\text{mol} \cdot \text{L}^{-1}$: 1.0 (**1**, **2**)).

Two region of linear dependence between solvatochromic shift value and water concentration for each fluorescent indicator can be distinguished. Within the first region it was observed that increasing of water content up to 1% which causes emission band maximum shift on 1.2 and 1.1 nm for **1** and **2**, respectively (Table 2). The solvatochromic shift value at water content up to 5% (I) and 2.3% (II) is lower

than for the first concentration region. Thus the use of solvatochromic shift value as an analytical signal allows determining the micro quantities of water in acetonitrile. The wavelength-based fluorescent methods are free from the main drawbacks of intensity-based methods, such as external disturbance, photobleaching of the indicator dye, signal drift of the measuring equipment [30].

Table 2. Parameters of calibration plots and detection limits of water determination in acetonitrile by reading the solvatochromic shift value ($\Delta\lambda=(a\pm\Delta a)+(b\pm\Delta b)C$, %).

Indicator	Linear range, % (v/v)	$a\pm\Delta a$	$b\pm\Delta b$	R^2	Detection limit, % (v/v)
1	0.8–5.0	–	1.2 ± 0.1	0.991	0.8
	5.0–30.0	5.2 ± 0.2	0.16 ± 0.01	0.994	5.0
2	0.2–2.3	–	1.1 ± 0.1	0.998	0.2
	2.3–20.0	2.2 ± 0.1	0.22 ± 0.01	0.996	2.3

Determination of water in acetonitrile. The substance **2** was selected as fluorescence indicator for the water determination in acetonitrile because of it lower detection limit and wider range of calibration plot in comparison to **1**. The results of water determination in a commercially available dry acetonitrile (Aldrich

Chemical Co., ‘anhydrous’ grade) with precise water additives are presented in Table 3. It can be seen the methods are characterized satisfactory accuracy and reproducibility. The relative standard deviation doesn’t exceed 0.2.

Table 3. The results of water determination in acetonitrile (Aldrich Chemical Co., ‘anhydrous’ grade) using **2** as fluorescent indicator ($n=5$; $P=0.95$).

Sample	Analytical signal	H_2O , % (v/v)		S_r
		added	found, $x\pm\Delta x$	
1	ΔI_{500} , a.u.	1.0	1.4 ± 0.4	0.20
2	–“–	4.8	5.0 ± 0.2	0.02
3	–“–	6.2	5.8 ± 0.4	0.06
4	–“–	8.0	9 ± 1	0.09
5	$\Delta\lambda$, nm	1.0	0.9 ± 0.3	0.11
6	–“–	1.6	1.3 ± 0.4	0.14
7	–“–	4.8	6 ± 2	0.19
8	–“–	6.2	8 ± 3	0.16
9	–“–	12.0	13 ± 2	0.10
10	–“–	18.0	19 ± 2	0.10

The common impurities presented in acetonitrile are acetic acid and ammonia. These compounds can be formed by hydrolysis of this solvent [31]. The effect of acetic acid and ammonia on the determination of 0.5 and 5.0% H_2O in acetonitrile under optimal conditions was tested (Figure 8). In common these impurities caused the quenching of fluorescence of **2** in acetonitrile–water mixture. The maximum shift in the fluorescence spectra of **2** does not exceed ± 0.5 nm. So, these impurities in the tested amounts do not interfere in the determination of water with wavelength-based fluorescence technique.

Concerning the determination of water with the intensity-based fluorescence technique the species was assumed not to interfere, when the effect of each foreign species on the fluorescence intensity is less than 10%. In the presence of acetic acid, the fluorescence intensity decreases by 10% at a concentration of 177–180 mmol·L⁻¹ in a solution containing 0.5% H_2O and at a concentration of 230–245 mmol·L⁻¹ in a solution with 5.0% H_2O . Similarly, in the presence of ammonia, a 10% decrease in the fluorescence intensity was observed at a concentration of 15 and 22 mmol·L⁻¹ in the determination of H_2O 0.5 and 5.0%,

respectively. Therefore, when determining the water content in acetonitrile, the concentration of acetic acid and ammonium should not exceed 180–245 mmol·L⁻¹

and 15–20 mmol·L⁻¹, respectively, according to the proposed procedure.

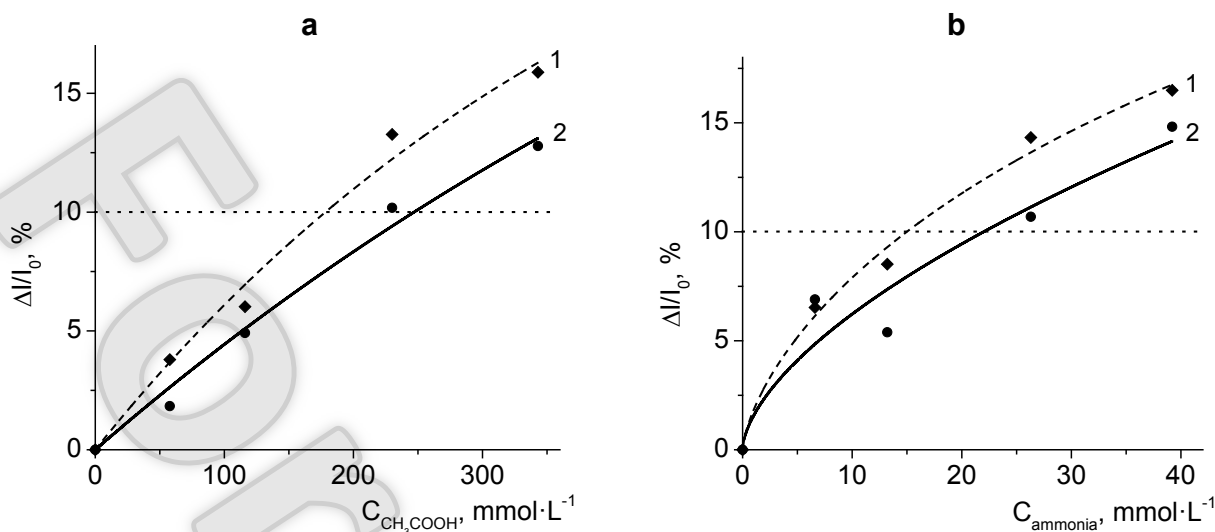


Fig. 8. Effect of acetic acid (a) and ammonia (b) on the determination of 0.5 (1) and 5.0% (2) of water in acetonitrile using the intensity-based fluorescence mode ($\lambda_{em} = 500$ nm).

Conclusions

4-Amino-benzof[f]isoindole-1,3-dione derivatives are sensitive to solvent polarity. The fluorescence intensity of acetonitrile solutions changes in the presence of trace amount of water. There is a red shift in the fluorescence spectrum. Two possible ways of detection of water content in acetonitrile are proposed: 1) intensity-based fluorescent quantitative mode and 2) wavelength-based fluorescent mode, in which the water concentration could be determined by reading the solvatochromic shift value of fluorescent indicator. In comparison with known fluorescent indicators, the advantage of using 4-amino-benzof[f]isoindole-1,3-dione derivatives is the increase in fluorescence intensity with increasing water content in acetonitrile. This allows to avoid the influence of the most

common fluorescence quenchers on results of the determination of traces of water. A second method for determining water in acetonitrile is less sensitive. But in this case the results of the determination of water are less affected by factors such as photobleaching of the indicator dye and stability of the measuring equipment. The 4-amino-benzof[f]isoindole-1,3-dione based fluorescent indicators have low sensitivity to the presence of bases, acids and metal cations and could be applied for the analysis of contaminated acetonitrile. Acetic acid and ammonia have no effect on determination of water using wavelength-based fluorescence technique. In intensity-based mode the determination of water is possible at concentration of these impurities up to 0.25 and 0.02 mol·L⁻¹, respectively.

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