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PROTOLYTIC EQUILIBRIUM OF TETRA- AND PENTANITROFLUORESCEINS IN A BINARY SOLVENT ACETONITRILE – DIMETHYL SULFOXIDE (MASS RATIO 96 : 4)

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In this paper, the acid-base and tautomeric equilibria of four nitrofluorescein dyes, 2,4,5,7-tetranitrofluorescein, 2,4,5,7,4'-pentanitrofluorescein, 2,4,5,7,5'-pentanitrofluorescein, and 2,4,5,7-tetranitrofluorescein methyl ester, were studied. As reaction media, a binary solvent acetonitrile – dimethyl sulfoxide (96 : 4 by mass) was used. The acidity scale in this solvent was established previously. The indices of the dissociation constants of the dyes were determined using the spectrophotometric method.

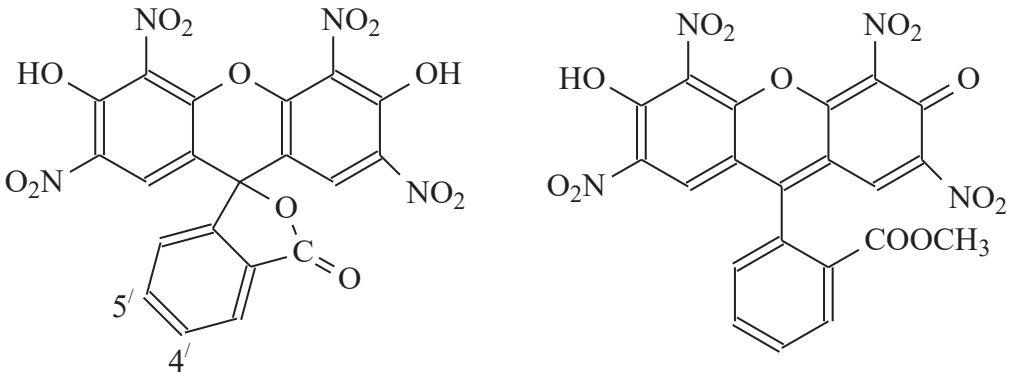
Interpreting the pK_a values requires an understanding of the state of tautomeric equilibria. The behavior of these compounds differs significantly from that of other fluorescein dyes, e.g., halogen derivatives. In the case of the first three compounds, i.e., for dyes with a free carboxylic group, the lactonic structure is predominant not only for the neutral form, but even for the double-charged anion. The single-charged anionic form exists as an equilibrium mixture of a colored (and fluorescent) tautomer and an almost colorless lactone. The fourth compound with esterified carboxylic group exhibits extreme stability in its anionic form.

Evaluation of the tautomerization constants made it possible to calculate the microscopic equilibrium constants of the stepwise dissociation of dye lactones, k_{1L} and k_{2L}. The consideration of the difference ($pk_{2L} - pk_{1L}$) allowed estimating the effective relative permittivity of the space between the ionizing groups basing on the Bjerrum – Kirkwood – Westheimer equation. Tautomerism of anions was discussed from the point of view of stabilization of symmetric structures.

Key words: nitrofluorescein dyes, acetonitrile-DMSO binary solvent, buffer solutions, absorption spectra, dissociation constants, tautomerism, anionic lactones.

INTRODUCTION. Fluorescein and its numerous halogen derivatives are widely used in chemistry and contiguous fields of science. Their behavior in solutions was a matter of numerous studies. Nitro derivatives of fluorescein, however, are much less explored. During past two decades, some publications devoted to acid ionization and tautomerism of nitrofluoresceins appeared from this laboratory [1–6]. In this paper, we continue our research in this direction.

The compounds studied are as follows: 2,4,5,7-tetranitrofluorescein, 2,4,5,7,4'-pentanitrofluorescein, 2,4,5,7,5'-pentanitrofluorescein, and 2,4,5,7-tetranitrofluorescein methyl ester. Except the first one [4, 6], they were synthesized and identified by us; the details will be published elsewhere.



Scheme 1 – Molecular structure of 2,4,5,7-tetranitrofluorescein and its methyl ester; the pentanitrofluoresceins bear the NO_2 group in 4' or 5' positions.

$$H_2R \rightleftharpoons HR^- + H^+, \ K_{a1} \qquad (1)$$

$$HR^- \rightleftharpoons R^{2-} + H^+, \ K_{a2} \qquad (2)$$

Fluorescein dyes bearing NO_2 groups in the xanthenes portion are relatively strong acids. Therefore, their investigation in terms of pK_a values should be conducted in a polar weakly basic solvent, e.g., in acetonitrile. However, even traces of water can distort the results obtained with this solvent. In order to avoid this interfering factor, a new binary solvent was proposed recently, namely, acetonitrile with 4 mass % dimethyl sulfoxide [7]. In this solvent, the acidity scale ($pa^*_{H^+}$) was established, and a set of buffer solutions was proposed. It was shown that the proton is associated with the molecules of the protophilic dimethyl sulfoxide, while other ionic and molecular species are solvated mainly by acetonitrile. Hence, this binary solvent can be regarded, as first approximation, as acetonitrile with lyonium ion $H(DMSO)_2^+$ [7].

EXPERIMENT AND DISCUSSION OF THE RESULTS. Acetonitrile was stored several days over P_4O_{10} and distilled over dehydrated K_2CO_3. Water content was 0.015 % as determined by coulometric Karl Fischer method. Dimethyl sulfoxide was purified by freezing and distilled under vacuum; water content 0.023 %. Benzene was purified from water by P_4O_{10} and distilled; water content $\leq$0.01 %. Benzoic and salicylic acids were purified by

sublimation. Potassium benzoate and sodium hydrosalicylate were purified by re-crystallization. Picric acid was twice re-crystallized from ethanol and dried as described previously [7]. Tetra-*n*-butylammonium picrate was prepared by the reaction between picric acid and tetra-*n*-butylammonium iodide and purified by double re-crystallization from methanol. *p*-Toluenesulfonic acid monohydrate was re-crystallized from acetonitrile. 98% aqueous sulfuric acid was of analytical grade. Diazabicyclo[5.4.0]undec-7-ene (DBU) was used as purchased from Merck. 2,4-dinitrophenol was re-crystallized from benzene–cyclohexane mixture (1 : 1 by volume). The synthesis and purification of tetramethylammonium 2,4-dinitrophenolate was described previously [8]. The purity of 2,6-dinitrophenol, analytical grade, melting point 64 °C. Sodium 2,6-dinitrophenolate was prepared through the following procedure. 4.00 g of 2,6-dinitrophenol was solved under heating in 50 % aqueous ethanol, which contained 2.00 g sodium hydroxide. The hot solution was filtrated through a syringe membrane filter and cooled to +6 °C. On the next day, the deposit of the sodium salt of the 2,6-dinitrophenol was filtered, washed with 10 mL of 50 % aqueous ethanol and dried. The mass of the dried salt was 4.28 g. After re-crystallization from 50 mL of 50 % aqueous ethanol the mass of the dry salt was 3.74 g (yield 83.5%).

Absorption spectra of the dyes were run on a Hitachi U-2000 instrument, against the solvent blanks at 25 °C. The optical path length was 1.00 cm. Normally, the dye concentrations were within the range of $(0.3 – 4.7)\times10^{-5}$ M.

Following reagents and buffer systems were used for acidity variation: picric acid, benzoic acid – potassium benzoate, salicylic acid – sodium salicylate, 2,4-dinitrophenol – sodium 2,4-dinitrophenolate, 2,6-dinitrophenol – sodium 2,6-dinitrophenolate, picric acid, tetra-*n*-butylammonium picrate, *p*-toluenesulfonic acid, sulphuric acid (98 %), and DBU. For calculations of the $pa^*_{H^+}$ values, the following thermodynamic pK_{HA} values of acids were used as determined previously [7]: 3.30 (picric), 8.68 (2,4-dinitrophenol), 8.73 (2,6-dinitrophenol), 9.61 (salicylic), 13.98 (benzoic). The $K^f_{HA_2^-}$ values of homoassociation for the last two acids are 2.5×10^2 and 2.2×10^3 M^{-1}, respectively, the logarithms of the association constants of ions, $\log K_{ass}$, for sodium salts are 2.85, 3.46, and 3.46 for 2,4-, 2,6-dinitrophenolate and salicylate, respectively [7]. The relative permittivity of the binary solvent is 36.95 at 25 °C. The ionic activity coefficients of the buffer components and dyes were calculated using the Debye–Hückel equation, second approach; the *A* and *B* parameters are 1.58 and 0.48, respectively; the ionic parameter *a* was equated to 0.5 nm; *Ba* = 2.40. Molecular activity coefficients were taken as unity.

Determination of the pK_a *values of the dyes.* The dissociation constants were determined using the dependence of the absorption spectra on $pa^*_{H^+}$. These data are presented in Figures 1–5. The $pa^*_{H^+}$ values were calculated using the pK_{HA}, $\log K_{ass}$, and $K^f_{HA_2^-}$ values reported previously [7, 8]. For the picric acid and 2,6-dinitrophenol, the last value is close to zero owing to the well-known blocking effect, while for the 2,4-dinitrophenol it is unknown. Three schemes of $pa^*_{H^+}$ calculations were used. They are as follows:

(i) For salicylate, benzoate, 2,6-, and 2,4-dinitrophenolate buffer mixtures, a complete calculation of the equilibrium composition was made as described earlier [8]. For

the 2,6-dinitrophenol, the homoassociation is not typical. The $K^{f}_{HA_2^-}$ value for 2,4-dinitrophenolate was roughly estimated as 24 M^{-1}, because in pure acetonitrile it equals 2.5×10^2 M^{-1} [9], whereas in the binary solvent under study the $K^{f}_{HA_2^-}$ values are 1.8 to 8 times lower than in acetonitrile [8].

(ii) Diluted unbuffered solutions of the picric acid were used. In this case, the dye concentration was taken into account when calculating the equilibrium concentrations of all ionic and molecular species. The ratio of the molecular, H_2R, and ionic, HR^-, forms of the given dye was estimated from the absorption spectra. The sole exception was the case of the 2,4,5,7-tetranitrofluorescein methyl ester, because of the high concentration of the picric acid.

In the above cases, $pa^{*}_{H^+}$ was calculated as $-\log[H^+]-\log f_1$.

(iii) The limited acidity and basicity of the solvent system was created using the H_2SO_4 and DBU solutions, respectively. In some cases, *p*-toluenesulfonic acid tetra-*n*-butylammonium picrate were used.

The pK_a values of the dyes were re-calculated to the thermodynamic ones using the activity coefficients f_1 and f_2 for single and double charged anions. The value of the ionic strength was calculated for each working solution. It did not exceed 0.005 M in buffer solutions and diluted picric acid. The sole exception was the 2,4,5,7-tetranitrofluorescein methyl ester (Figure 1).

In the case of this compound, the dissociation of the HR form takes place at very low $pa^{*}_{H^+}$ values. The molecular spectrum was available only in benzene with small H_2SO_4 addition. Higher concentrations of the sulfuric acid and additions of the *p*-toluenesulfonic acid led to decolorization, obviously due to hydrolysis of the ester group and turning to the mother compound. The appearance of the R^- ion in pure benzene may be caused by small traces of water (see the Experimental section).

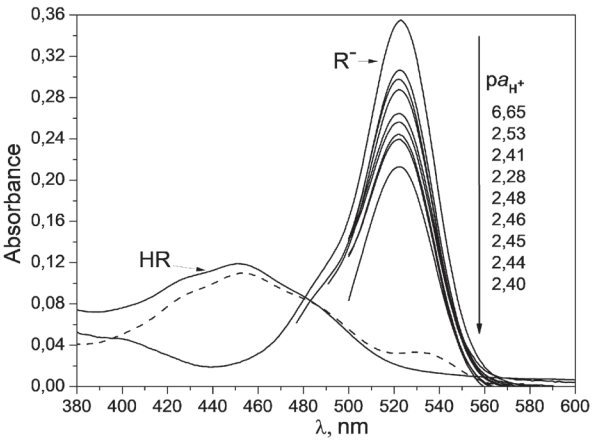


Figure 1 – Absorption spectra of 2,4,5,7-tetranitrofluorescein methyl ester (3.1×10^{-6} M) in the binary solvent at different picric acid solutions. The spectrum of the anionic form R^- was obtained in a salicylate buffer solution. The spectrum of the entire HR form was measured in benzene with 0.1 M sulfuric acid; without such acidifying, some amounts of the anion R^- are present in the solution (dotted curve).

Three wavelengths around the absorption maximum were used as analytical positions. Here, the absorption of the molecular form HR is small. The thermodynamic value pK_{a1} = 1.95±0.19 was obtained from measurements with 8 different $pa^{*}_{H^+}$ values, from 1.45 to 2.35. Here, the utilization of the Debye–Hückel equation is conventional. Note that in this binary solvent and in entire DMSO, the anion R^- exhibits bright fluorescence.

Much more diluted solutions of picric acid were used in the case of 2,4,5,7-tetranitrofluorescein (Figure 2a). Here, the neutral form H_2R is almost colorless due to predominance of the lactonic tautomer, as in other solvents [1,2,4,6].

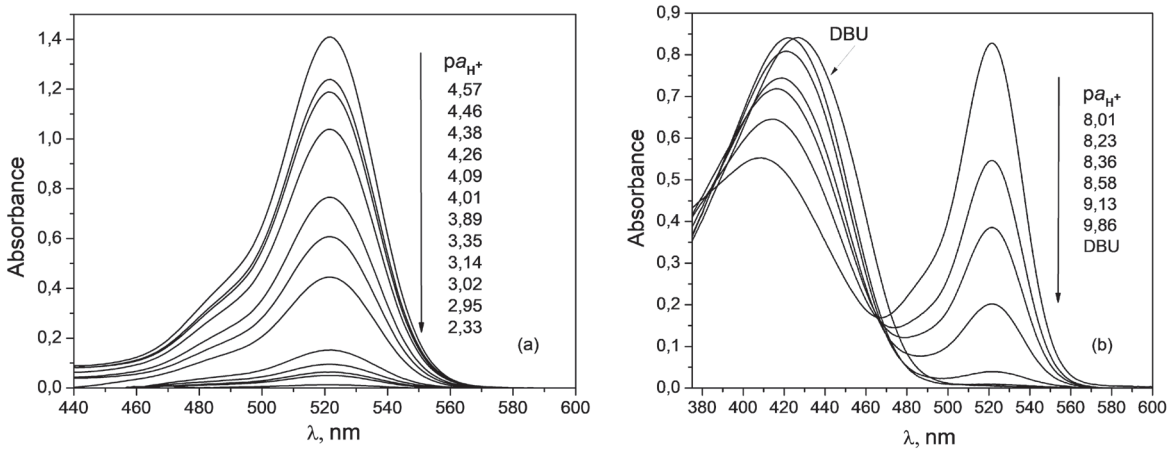


Figure 2 – Absorption spectra of 2,4,5,7-tetranitrofluorescein (4.2×10^{-5} M) in diluted picric acid (a) and in the salicylate buffer solutions (b). The R^{2-} spectrum was obtained in 0.02 M DBU solution.

The spectrum of the dianion R^{2-} of the dye is obtained in DBU solution (Figure 2). This absorption band is shifted to some extent as compared with the spectra in salicylate buffers. This can be explained by the specific interaction of this double-charged anion with either $DBUH^+$ cation or salicylic acid molecules. Such interactions may also somewhat contribute to the main equilibrium. The dependence of absorbance at 522 nm on $pa^*_{H^+}$ is given in Figure 3.

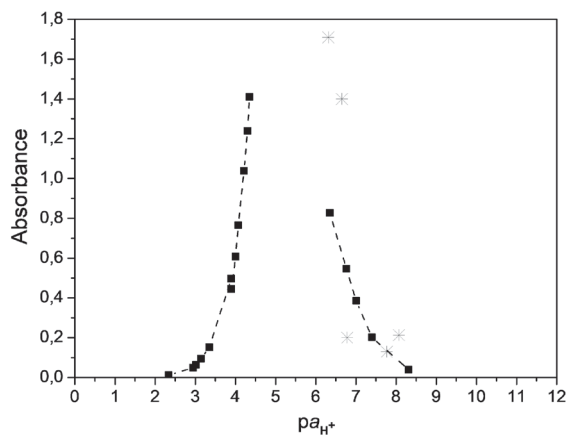


Figure 3 – Absorption of 2,4,5,7-tetranitrofluorescein (4.2×10^{-5} M) at 522 nm vs. $pa^*_{H^+}$. Picric acid solutions (left-hand side) and salicylate buffers (right-hand side); 2,6-dinitrophenolate buffer solutions shown with asterisks.

For a fixed wavelength, the following equation is valid:

$$A = \frac{A_{HR^-} a^*_{H^+} K_{a1} f_1^{-1} + A_{R^{2-}} K_{a1} K_{a2} f_2^{-1}}{(a^*_{H^+})^2 + a^*_{H^+} K_{a1} f_1^{-1} + K_{a1} K_{a2} f_2^{-1}} . \quad (3)$$

Here A stands for the absorbance at the current $a^*_{H^+}$ value; $A_{R^{2-}}$ and A_{HR^-} are absorbances under conditions of complete conversion of the dye into the corresponding form. Here, $A_{H_2R} = 0$. This equation can be represented in three forms for calculation of both dissociation constants and the A_{HR^-} value:

$$K_{a1}^{-1} = \frac{A_{HR^-} - A}{A} f_1^{-1} (a^*_{H^+})^{-1} + \\ + \frac{A_{R^{2-}} - A}{A} K_{a2} f_1 \, f_2^{-1} (a^*_{H^+})^{-2} \quad (4)$$

$$K_{a2} = \frac{A_{HR^-} - A}{A - A_{R^{2-}}} f_2 \, f_1^{-1} a^*_{H^+} - \\ - \frac{A}{A - A_{R^{2-}}} K_{a1}^{-1} f_2 \, (a^*_{H^+})^2 \quad (5)$$

$$A_{HR^-} = A\{a^*_{H^+} K_{a1}^{-1} f_1 \, + 1 + K_{a2} f_1 \, f_2^{-1} (a^*_{H^+})^{-1}\} - \\ - A_{R^{2-}} K_{a2} f_1 \, f_2^{-1} (a^*_{H^+})^{-1} \quad (6)$$

If the difference between the dissociation constants is big enough, the A_{HR^-} value can be determined directly and only the first items in the right-hand side of Equations 4 and 5 can be used for calculation of the constants. But if the yield of the single-charged anion, HR^-, does not reach 100 % at any $pa^*_{H^+}$ value, then an iterative procedure should be used. The method of calculations was as follows. As first approximation, A_{HR^-} was equated to the maximal absorption in Figure 3. The dissociation constants were calculated under the assumption that stepwise equilibria are isolated. Then the A_{HR^-} value was refined using Equation 6, and the K_{a2}^{-1} and K_{a2} values were successively improved via Equations 4 and 5.

In dilute picric acid solution, the $[HR^-]$ values were determined from the absorption spectra. The $a^*_{H^+}$ values were calculated using Equation 7, which is derived using the equations of mass action law for the picric acid, mass balance, and electroneutrality.

$$(a^*_{H^+})^2 + a^*_{H^+}\{K_{HPic}f_1^{-1} - [HR^-]f_1\} - \\ - K_{HPic}\{c_{HPic} - [HR^-]\} = 0 \quad (7)$$

First, the f_1 value was equated to unity. Then, the ionic strength was approximately equated to $a^*_{H^+}$, and after evaluation of the f_1 value the calculations were repeated. Finally, knowing $a^*_{H^+}$, $[HR^-]$, and f_1, the K_{a1} value was obtained. After five iterations, the results became stable. The $pa^*_{H^+}$ values indicated in Figures 2a and 3 are obtained as a result of the first iteration.

The calculations of the $pa^*_{H^+}$ values and ionic strengths of the buffer mixtures were performed by Dr. A. V. Lebed in this Department using the previously determined equilibrium constants and calculation procedure [7]. In Table 1, the data for the salicylate buffer solutions are presented. In Tables 2 and 3, analogous data for the dinitrophenols are given. These $pa^*_{H^+}$ s were used in further determinations of the dissociation constants. They can also be used for other purposes in this binary solvent.

Table 1

The $pa^*_{H^+}$ values and ionic strength of buffer solutions in acetonitrile with 4 mass % DMSO: salicylic acid + 0.00487 M sodium salicylate, 25 °C

Salicylic acid, M	$pa^*_{H^+}$	*I*, M
0.1733	6.27	0.0041
0.1722	6.28	0.0041
0.1570	6.35	0.0041
0.1037	6.69	0.0038
0.0948	6.76	0.0037
0.0689	7.01	0.0035
0.0584	7.14	0.0034
0.0555	7.18	0.0033
0.0420	7.40	0.0031
0.0118	8.31	0.0021
0.0050	8.86	0.0017
0.0022	9.32	0.0015

Table 2

The $pa^*_{H^+}$ values and ionic strength of 2,6-dinitrophenolate buffer solutions in acetonitrile with 4 mass % DMSO, 25 °C

2,6-Dinitrophenol	Sodium 2,6-dinitrophenolate	$pa^*_{H^+}$	*I*, M
0.3619	0.0510	6.76	0.0049
0.3547	0.0510	6.77	0.0049
0.3521	0.0510	6.78	0.0049
0.2997	0.0048	6.29	0.0012
0.2954	0.0048	6.23	0.0012
0.2881	0.0048	6.31	0.0012
0.1590	0.0048	6.57	0.0012
0.1362	0.0048	6.64	0.0012
0.1317	0.0048	6.65	0.0012
0.0090	0.0040	7.77	0.0011
0.0045	0.0040	8.07	0.0011

Table 3

The $pa^*_{H^+}$ values and ionic strength of 2,4-dinitrophenolate buffer solutions in acetonitrile with 4 mass % DMSO, 25 °C

2,4-Dinitrophenol	Sodium 2,4-dinitrophenolate	$pa^*_{H^+}$	*I*, M
0.1086	0.0040	6.30	0.0018
0.0698	0.0010	6.18	0.0006
0.0551	0.0008	6.25	0.0005
0.0549	0.0008	6.26	0.0005

table 3

2,4-Dinitrophenol	Sodium 2,4-dinitrophenolate	$pa^*_{H^+}$	*I*, M
0.0519	0.0005	6.14	0.0004
0.0455	0.0004	6.14	0.0003
0.0449	0.0004	6.15	0.0003
0.0270	0.0040	7.12	0.0014
0.0090	0.0040	7.68	0.0012
0.0045	0.0040	8.01	0.0012
0.0018	0.0040	8.42	0.0011

However, the data obtained with the two dinitrophenolate buffer systems were considered only as supporting information. The reasons are as follows. For the 2,4-dinitrophenol, the exact value of the homoassociation constant is unknown, whereas the concentrations of the 2,6-dinitrophenol, which allow receiving necessary acidities, are too high, and some additional interactions may take place. Only the results obtained with the salicylate buffer solutions were used for final calculations. Despite the lower pK_{HA} value of 2,6-dinitrophenol (8.73) as compared with that of salicylic acid (9.61), the latter is more suitable for creating lesser $pa^*_{H^+}$ values due to pronounced homoassociation effect.

As a rule, three wavelength around the absorption maximum of the HR^- anion were used. For instance, the final A_{HR^-} value at 522 nm (Figure 3) was 2.455. This value was used to calculate the molar absorptivity of the single-charged anion.

Spectrophotometric data for the two pentanitrofluoresceins (Figures 4 and 5) were processed in the same way.

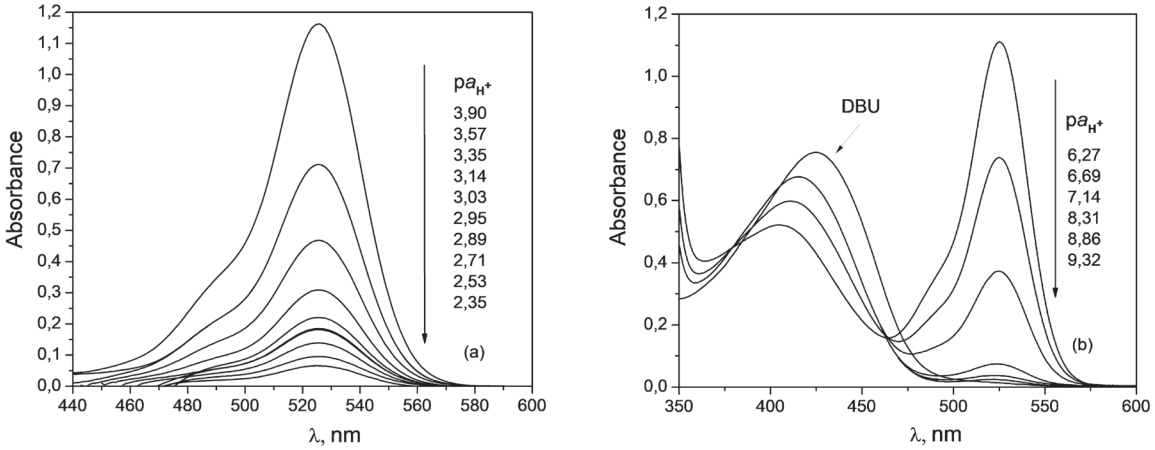


Figure 4 – Absorption spectra of 2,4,5,7,4'-pentanitrofluorescein (4.7×10^{-5} M) in diluted solutions of the picric acid (a) and in the salicylate buffer solutions (b). The R^{2-} spectrum was obtained in 0.02 M DBU solution.

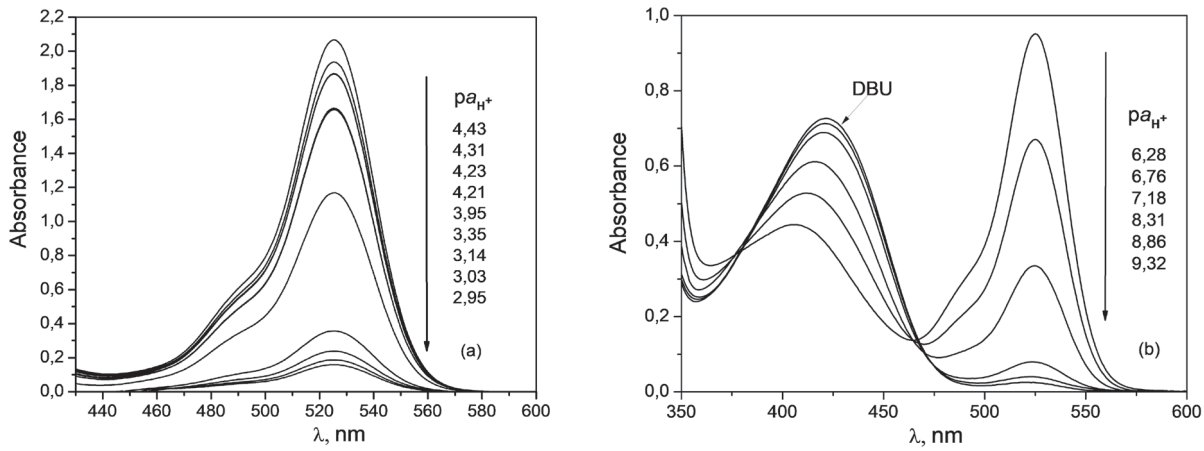


Figure 5 – Absorption spectra of 2,4,5,7,5'-pentanitrofluorescein (4.4×10^{-5} M) in diluted solutions of the picric acid (a) and in the salicylate buffer solutions (b). The R^{2-} spectrum was obtained in 0.02 M DBU solution.

The results are collected in Tables 4 and 5.

Table 4

Molar absorptivities of the dyes in acetonitrile – DMSO binary solvent (96 : 4 by mass).

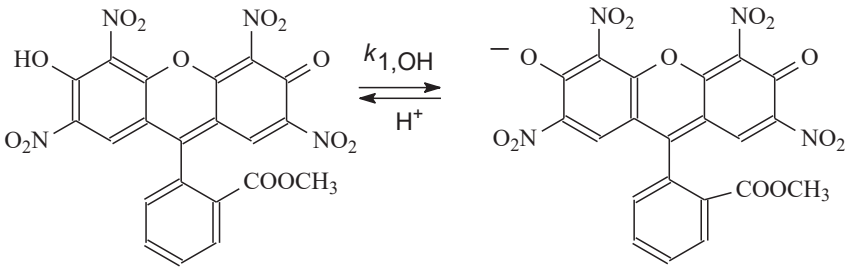
Dye	λ_{max}, nm $\left(E_{max}\times10^{-3}, M^{-1}cm^{-1}\right)$		
	Neutral	Monoanion	Dianion
2,4,5,7-Tetranitrofluorescein methyl ester	452 (37.9)[a]	523 (112.0)	—
2,4,5,7-Tetranitrofluorescein	—[b]	522 (58.4)[c]	422 (20.0)
2,4,5,7,4'-Pentanitrofluorescein	—[b]	526 (49.2)[c]	425 (16.1)
2,4,5,7,5'-Pentanitrofluorescein	—[b]	525 (64.0)[c]	421 (16.5)

Note. [a] In benzene with 0.1 M sulfuric acid. [b] Almost colorless. [c] Calculated from the equilibrium data jointly with the dissociation constants.

Table 5

Indices of the thermodynamic dissociation constants of the dyes in acetonitrile – DMSO binary solvent (96 : 4 by mass).

Dye	pK_{a1}	pK_{a2}	$pK_{a2} - pK_{a1}$
2,4,5,7-Tetranitrofluorescein methyl ester	1.95 ± 0.19	—	—
2,4,5,7-Tetranitrofluorescein	4.49 ± 0.08	6.48 ± 0.17	1.99
2,4,5,7,4'-Pentanitrofluorescein	4.02 ± 0.03	6.58 ± 0.10	2.56
2,4,5,7,5'-Pentanitrofluorescein	4.11 ± 0.08	6.50 ± 0.16	2.4



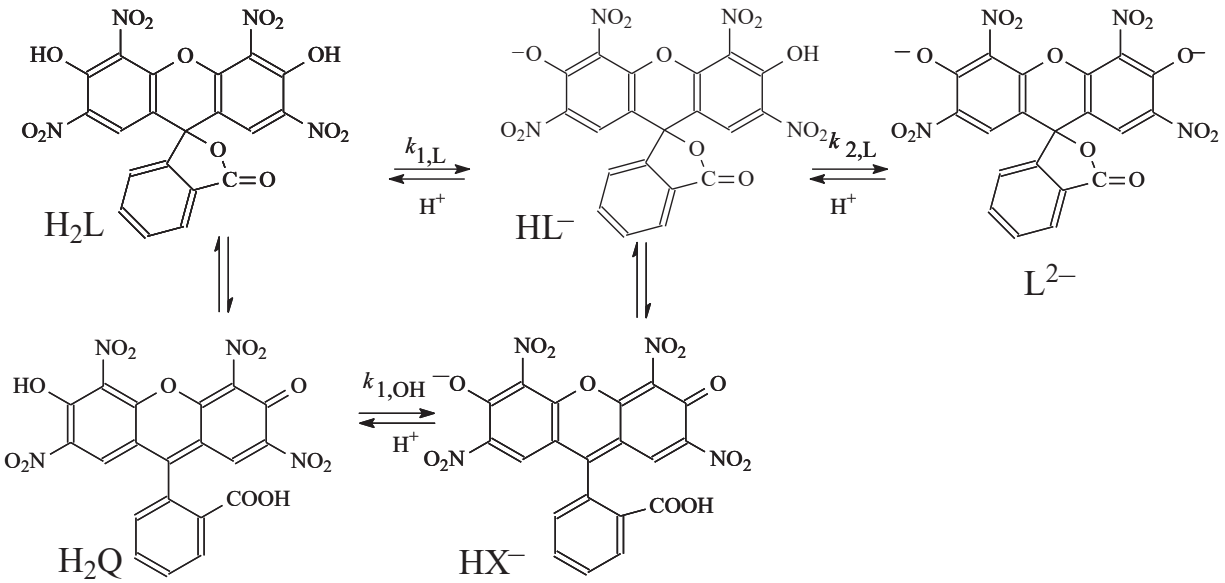
HQ X^-

Scheme 2 – Dissociation equilibrium of 2,4,5,7-tetranitrofluorescein methyl ester.

Tautomerism of the dye anions. The neutral HR and anionic R^- forms of the methyl ester of 2,4,5,7-tetranitrofluorescein exists as colored quinonoidal structures HQ and X^-, respectively (Scheme 2).

The esterification of the carboxylic group makes the formation of lactone impossible. The absorption spectra were presented above in Figure 1.

In contrast, the lactone tautomer H_2L (Scheme 3) predominates in the case of neutral species of the other three dyes. The H_2R forms are almost colorless, and the fraction of molecules existing as quinonoidal tautomer, α_{H_2Q}, are out of detection limits in spectra. Moreover, unlike most fluorescein dyes, 2,4,5,7-tetranitro derivatives, both with and without a nitro group in the phthalic acid residue, are prone to lactonization even in the dianionic form R^{2-}. The band maxima of the dianions of the tetra- and pentanitroderivatives are located close to the UV portion of the spectrum (Figures 2b, 4b, and 5b; Table 4) because of the "nitrophenolate" absorption of the lactones L^{2-} (Scheme 3). Long-wavelength absorption, as for X^- anions in Figure 1, for the dianions R^{2-} is not observed.



Scheme 3 – Detailed scheme of protolytic equilibrium of 2,4,5,7-tetranitro dyes with free COOH group.

Such deeper colored species appear only for HR^- anions of the dyes with four nitro groups in 2, 4, 5, and 7 positions. The bands resemble that of the monoanion of the ester (Figure 1) but the molar absorptivities are substantially lower, as is seen in Table 4. This allows to assume that the HR^- forms consist of an equilibrium mixture of intensively colored HX^- tautomer and an lactonic anion HL^-. Note that the single-charged anions are fluorescent owing to the presence of the HX^- tautomer.

If the E_{max} value of the HX^- tautomer of 2,4,5,7-tetranitrofluorescein is equated to that of the R^- (= X^-) anion of the methyl ester of the dye (Table 4), the α_{HX^-} value can be estimated as 58.4/112.0 = 0.52. Accordingly, $\alpha_{HL^-} = 1 - \alpha_{HX^-} = 0.48$. Analogous evaluations of the tautomer fractions were made for the two pentanitrofluoresceins (Table 6).

Knowing these values, the so-called microscopic dissociation constants, *k*, can be estimated. From the detailed dissociation scheme (Scheme 3), equations (8)–(10) can be derived:

$$\mathrm{p}k_{2,\mathrm{L}} = \mathrm{p}K_{\mathrm{a}2} + \log \alpha_{\mathrm{HL}^-} \qquad (8)$$

$$\mathrm{p}k_{1,\mathrm{L}} = \mathrm{p}K_{\mathrm{a}1} + \log \alpha_{\mathrm{H_2L}} - \log \alpha_{\mathrm{HL}^-} \qquad (9)$$

$$\mathrm{p}k_{1,\mathrm{OH}} = \mathrm{p}K_{\mathrm{a}1} + \log \alpha_{\mathrm{H_2Q}} - \log \alpha_{\mathrm{HX}^-} \qquad (10)$$

The $\mathrm{p}k_{1,\mathrm{L}}$ value can be calculated by taking $\alpha_{\mathrm{H_2L}}$ close to unity. The results are collected in Table 6. The tautomerization constants of the $HX^- \rightleftharpoons HL^-$ equilibrium are 0.92, 1.27, and 0.75, respectively. Also, the fractions of the tautomer H_2Q may be estimated. For example, if the $\mathrm{p}k_{1,\mathrm{OH}}$ value of 2,4,5,7-tetranitrofluorescein is equated to that of its methyl ester, a $\alpha_{\mathrm{H_2Q}}$ value of 1.5×10^{-3} follows from equation (10). Hence, the negligible content of this tautomer is confirmed in such an indirect way.

Table 6

Fractions of tautomers and the indices of the microscopic dissociation constants.

Dye	α_{HX^-}	α_{HL^-}	$pk_{1,L}$	$pk_{2,L}$	$pk_{2,L} - pk_{1,L}$	ε_{eff}
2,4,5,7-Tetranitrofluorescein	0.52	0.48	4.8	6.2	1.4	32
2,4,5,7,4'-Pentanitrofluorescein	0.44	0.56	4.3	6.3	2.0	18
2,4,5,7,5'-Pentanitrofluorescein	0.57	0.43	4.5	6.2	1.7	24

Thus obtained $pk_{1,L}$ and $pk_{2,L}$ values allow to evaluate the difference between the stepwise dissociation of the OH groups of the lactone H_2L, $pk_{2,L} - pk_{1,L}$ (Table 6). The effect can be explained by the Bjerrum – Kirkwood – Westheimer equation (11), where the term $\log 4$ reflects the statistical factor.

$$pk_{2,L} - pk_{1,L} = \log 4 + \frac{e^2 N_A}{2.303RT \times 4\pi\varepsilon_0 \varepsilon_{eff} r} = 0.602 + \frac{24.7}{\varepsilon_{eff} r} \quad (11)$$

Here, R, T, and ε_0 have their usual meanings, ε_{eff} is the effective relative permittivity of the space permeated by the electric field lines, r is the distance (in nm) between the dissociating group and the negatively charged substituent [10]. For more details, see the book by Vereshchagin [11]. The r = 0.95 nm value for these dyes was estimated by Dr. V. S. Farafonov in this Department. Thus estimated ε_{eff} values are presented in Table 6. The average value is 25, while the relative permittivity of the solvent equals 36.95. This allows concluding that the influence of charged group occurs both through the aromatic molecule and through the solvent.

Finally, the reasons of the strongly pronounced lactonization of molecules and dianions, on the one hand, and commensurable concentrations of HL^- and HX^- tautomers should be explained. Electro-deficient nitro groups reduce the electron density on the nodal carbon atom thus favoring the intramolecular acid-base reaction, i.e., lactone formation. This leads to lactonization even in the case of dianions. On the other hand, the structure HL^- is an asymmetrical one. In contrast, the negatively charged chromophore system of the quinonoidal structure HX^- is symmetrical, because the image is conventional and both oxygen atoms are equal [12]. Therefore, the equipotential charge distribution favors the stability of the structure. In addition, the presence of DMSO molecules, which are hydrogen bond acceptors, stabilizes the COOH groups.

CONCLUSIONS. The pK_a values of 2,4,5,7-tetranitrofluorescein, 2,4,5,7,4'-, and 2,4,5,7,5'-pentanitrofluoresceins in acetonitrile with 4 mass % DMSO are within the range of 4.0 – 6.6. Whereas the lactone tautomers predominate in the case of the neutral and dianionic forms, H_2R and R^{2-}, respectively, the equilibrium concentrations of the colored quinonoid and almost colorless lactone tautomers of the HR^- forms of the dyes are commensurable; the corresponding tautomerization constants are close to unity. The microscopic dissociation constants of lactones and the difference between their indices, $pk_{2L} - pk_{1L}$, are estimated.

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ПРОТОЛІТИЧНА РІВНОВАГА ТЕТРА- І ПЕНТАНІТРОФЛУОРЕСЦЕЇНУ У БІНАРНОМУ РОЗЧИННИКУ АЦЕТОНІТРИЛ — ДИМЕТИЛСУЛЬФОКСИД (МАСОВЕ СПІВІДНОШЕННЯ 96 : 4)

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У цій роботі досліджено кислотно-основні та тавтомерні рівноваги чотирьох нітрофлуоресцеїнових барвників: 2,4,5,7-тетранітрофлуоресцеїну, 2,4,5,7,4'-пентанітрофлуоресцеїну, 2,4,5,7,5'-пентанітрофлуоресцеїну та метилового естеру 2,4,5,7-тетранітрофлуоресцеїну. Як реакційне середовище було обрано бінарний розчинник асцетонітрил – диметилсульфоксид (96 : 4 за масою). Шкалу кислотності в цьому розчиннику було розроблено раніше. Константи дисоціації цих барвників було визначено за допомогою спектрометричного методу.

Інтерпретація значень pK_a вимагає розуміння положення тавтомерних рівноваг. Поведінка цих сполук суттєво відрізняється від такої для інших флуоресцеїнових барвників, наприклад, галоген-похідних. У разі перших трьох сполук, тобто для барвників з вільною карбоксильною групою, лактонний тавтомер є домінуючим не тільки для нейтральних форм, але навіть і для двічі заряджених аніонів. Однозарядні аніонні форми в розчині існують як рівноважна суміш забарвленого (та флуоресціюючого) тавтомера та практично безбарвного лактону. Четверта сполука з естерифікованою карбоксильною групою проявляє високу стабільність в аніонній формі.

Оцінка констант тавтомерних рівноваг дозволила розрахувати мікроскопічні константи дисоціації лактонів барвників, k_{1L} and k_{2L}. Аналіз різниці ($pk_{2L} - pk_{1L}$) дозволяє оцінити ефективну діелектричну проникність простору між групами, що іонізуються, базуючись на рівнянні Б'еррума – Кірквуда – Вестхаймера. Тавтомерію аніонів барвників обговорено з точки зору стабілізації симетричних структур.

Ключові слова: нітрофлуоресцеїнові барвники, бінарний розчинник ацетонітрил-ДМСО, буферні розчини, спектри поглинання, константи дисоціації, тавтомерія, аніонні лактони.

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