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MONONITROFLUORESCEINS IN AQUEOUS MEDIA: ACID-BASE EQUILIBRIA, TAUTOMERISM, AND HYDROLYSIS OF DIACETATES

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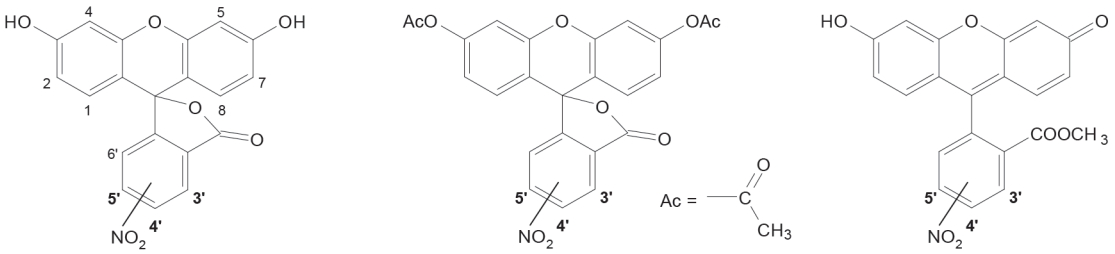
This article reports equilibrium and kinetic data for three fluorescein dyes containing the nitro group in the residue of the phthalic acid, 3′-, 4′-, and 5′-nitrofluorescein, in water and 50 mass % aqueous ethanol. These compounds, particularly the recently synthesized 3′-nitrofluorescein, were less considered polyprotic acids than many other fluorescein dyes. The dyes behave as diprotic acids, which can also be protonated: $H_3R^+ \rightleftharpoons H_2R \rightleftharpoons HR^- \rightleftharpoons R^{2-}$. The dissociation constants in water and 50 mass % aqueous ethanol were obtained in buffer solutions of diluted HCl using the spectrophotometric method accompanied by a potentiometric determination of pH at an ionic strength of 0.05 M and 25.0 ± 1 °C. The dye concentrations in the working solutions were, as a rule, within the range of $(5–10)\times10^{-6}$ M. The thermodynamic values of the dissociation constants were calculated using the Debye–Hückel equation, the second approach, for ionic activity coefficients. The absorption spectra in the visible region of the individual molecular and ionic forms were determined for the above-mentioned compounds and the ester of 3′-nitrofluorescein. For the last, the dissociation constants were determined in aqueous ethanol. Whereas in water the fraction of the zwitterionic tautomer of nitrofluoresceins increases as compared with the parent compound, in aqueous ethanol, the colorless lactone predominates. The microscopic dissociation constants were determined and their values are in line with the influence of the electrophilic nitro group. The alkaline hydrolysis of the nitrofluorescein diacetates was investigated in the two aforementioned solvents. The obtained results allowed us to single out the rate constants of the two-step kinetics of the hydrolysis of the diacetyl derivatives of nitrofluoresceins.

Keywords: Nitrofluorescein; acid-base dissociation; water-ethanol binary solvent; absorption spectra; dissociation constants; tautomerism; diacetylfluorescein; two-step alkaline hydrolysis; rate constants.

INTRODUCTION. This paper continues the research of alkaline hydrolysis of diacetylfluorescein [1]. Here, we present the study of diacetates of three nitrofluoresceins with the NO_2 group in the phthalic acid residue in water and 50 mass % aqueous ethanol. To better understand the kinetic data, we examined the acid-base and tautomeric equilibria of these nitrofluoresceins and an ester as model compound in the same solvent.



Scheme 1. The molecular structures of nitrofluoresceins, their diacetates, and esters.

The dissociation constants of 3′-, 4′-, and 5′-nitrofluorescein were determined via the spectrophotometric method. The hydrolysis of fluorescein diacetates and similar esters has been numerously studied in the literature [2–4]. Usually, a rate constant of the total hydrolysis was determined without dividing it into two separate constants of stepwise hydrolysis. We have developed an approach based on the knowledge of the spectrum of the single-charged fluorescein anion, which allowed calculating the first and second rate constant [1, 5]. Now, we decided to further this procedure by involving nitrofluoresceins. To the best of our knowledge, the acid-base and tautomeric equilibria of these dyes in aqueous media have not been studied so far. Therefore, a study of their protolytic equilibrium in itself is essential for understanding the behavior of fluorescein dyes in solutions.

The dyes behave as diprotic acids, which can also be protonated, Equations (1–3).

$$H_3R^+ \rightleftharpoons H_2R + H^+, \quad K_{a0} \qquad (1)$$

$$H_2R \rightleftharpoons HR^- + H^+, \quad K_{a1} \qquad (2)$$

$$HR^- \rightleftharpoons R^{2-} + H^+, \quad K_{a2} \qquad (3)$$

EXPERIMENT AND DISCUSSION OF THE RESULTS. The synthesis of the dyes and their diacetates was described in our previous publications [6, 7]. Hydrochloric, acetic, phosphoric, diethylbarbituric acids and analytical grade borax were used to create different pH values. The standard aqueous solution of NaOH was prepared from a saturated solution using CO_2-free water and kept protected from the atmosphere. Besides 96% aqueous ethanol, acetonitrile (Sigma-Aldrich, for HPLC, gradient grade, ⩾ 99.9%) was used to prepare the stock solutions of the dyes. The equilibrium and kinetic experiments were run on a Hitachi U-2000 spectrophotometer at 25.0±1 °C. Because of the limited solubility in water, the stock solutions of the dyes were prepared in acetonitrile, adding 0.013 M diazabicyclo[5.4.0]-undec-7-ene, DBU. The dye concentrations in the working solutions were within the range of $(5–10)\times10^{-6}$ M unless otherwise specified; hereafter, 1 M = 1 mole dm^{-3}. The pH values were determined using a glass electrode in a cell with a liquid junction. The glass electrode was calibrated with standard buffer solutions (pH 1.68, 4.01, 6.86, and 9.18 at 25 °C). When working in 50 mass % ethanol, the instrumental pH values, pH_{instr}, were corrected according to the literature data: $pH = pH_{instr} - 0.20$ [8]. The limiting alkaline forms of the dyes were obtained in diluted NaOH and, in some cases, with DBU (Merck, for synthesis). For each system, the number of solutions with different pH was 25 to 32. The CLINP chemometric program and wavelengths from 430 to 510 nm

with a step of 1 nm were used for calculations [7, 9]. The ionic strength I = 0.05 M was maintained by sodium chloride of analytical grade. In some acidic solutions, e.g., with pH in water below 1.3, the ionic strength in HCl solutions was higher. The hydrolysis kinetics of fluorescein dyes was studied as described previously [1]. The visible spectra were recorded from 400 to 600 nm during either 29 or 40 s for faster and slower reactions, respectively. The time of adding the buffer was considered as the moment of beginning the reaction.

The visible absorption spectra at different pH in water and the dependences of absorbance on pH are exemplified in Figures 1 and 2, respectively.

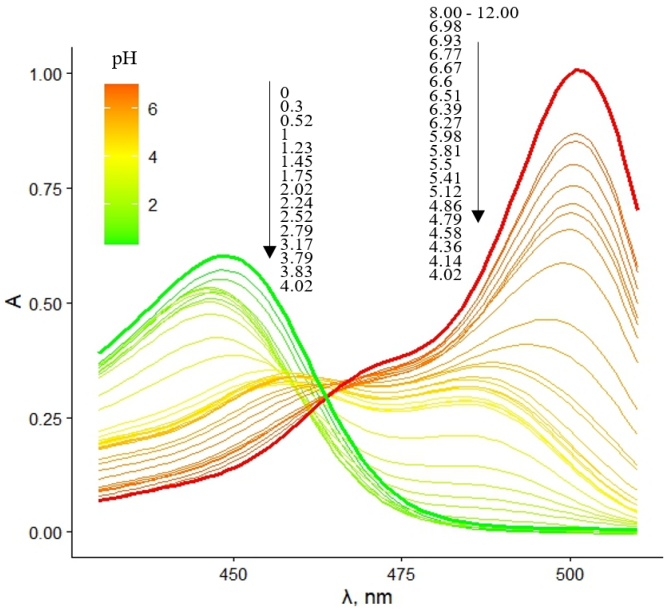


Fig. 1 – The absorption spectra of 3′-nitrofluorescein in aqueous solutions, 25 °C, at different pH values; The dye concentration 1.08×10^{-5} M; I = 0.05 M except forsolutions with pH < 1.3. The pH values are given in the activity scale down to pH 1.3; the concentration scale (–logarithm of HCl concentration) was used below this limit.

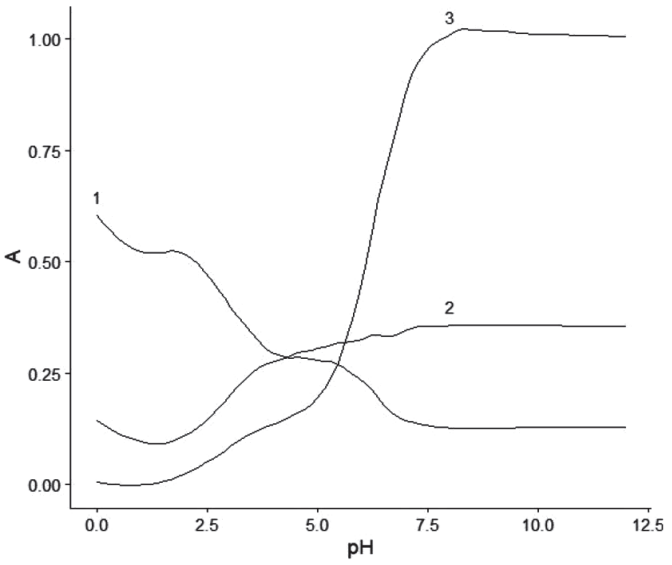


Fig. 2 – The dependence of absorbance of 3′-nitrofluorescein on pH at wavelengths 448 nm (1), 470 nm (2), and 501 nm (3); 25 °C, I = 0.05 M, except the solutions with pH below 1.3.

The results are presented in Table 1. The thermodynamic values of the dissociation constants were calculated using the Debye – Hückel equation, the second approach, for ionic activity coefficients. The values of activity coefficients in water are 0.822 and 0.457 for single and double-charged ions, respectively. The absorption spectra of the individual equilibrium forms of the dyes are presented in Figures 3–5. The spectra of the H_3R^+ and R^{2-} forms were measured directly in 1.0 M HCl and 0.042 M NaOH solutions, respectively, while those of H_2R and HR^- were singled out from the experimental data jointly with calculating the dissociation constants.

Table 1.

The parameters of ionic equilibria of nitrofluoresceins in water, 25 °C, I = 0.05 M[a,b]

Parameter [c]	3′-Nitrofluorescein [d]	4′-Nitrofluorescein	5′-Nitrofluorescein
pK_{a0}	1.06±0.01 (0.98)	1.86±0.01 (1.78)	1.87±0.02 (1.79)
pK_{a1}	2.82±0.01 (2.90)	3.66±0.01 (3.74)	3.68±0.02 (3.76)
pK_{a2}	6.28±0.01 (6.54)	6.36±0.01 (6.62)	6.54± 0.01 (6.80)
λ_{max}/ ε (H_3R^+)×10^{-3}	449 nm /55.72	443 nm /59.73	444 nm /60.29
λ_{max}/ε (H_2R)×10^{-3}	446 nm /43.89	439 nm /23.86	441 nm /26.56
λ_{max}/ε (HR^-)×10^{-3}	459 nm /31.44; 485 nm /29.96	457 nm /34.48 477 nm /33.44	458 nm /35.21 481 nm /33.84
λ_{max}/ε (R^{2-})×10^{-3}	501 nm /93.1	497 nm /85.65	499 nm /94.03
α ($H_2Z^{\pm}$)	≈ 0.8	0.36	0.43
α (H_2Q)	>0.01	0.11	0.07
α (H_2L)	≈ 0.2	0.53	0.50
$pK_{\pm,COOH}$	≈ 1.1	2.22	2.14
$pK_{0,OH}$	—[d]	2.74	2.93
$pK_{1,COOH}$	— [d]	2.78	2.60
$pK_{1,Z}$	≈ 2.7	3.30	3.39

Note. [a] The pK_a values in parenthesis are thermodynamic. [b]The molar absorptivities, ε, are expressed in $M^{-1}cm^{-1}$. [c]For fluorescein, the pK_a values are 2.22, 4.37, and 6.55; the thermodynamic values are 2.14, 4.45, and 6.80 [10]. [d]In this case, the data with pH below 1.3 were not used for calculations.

The spectra of the individual equilibrium forms of the dyes are presented in Figure 3. They were singled out from the total body of spectra by calculating the molar absorptivities of the forms H_2R and HR^- together with the dissociation constants, while those of the ions H_3R^+ and R^{2-} were determined directly at appropriate acidity.

To explain the obtained data, the detailed scheme of the acid-base and tautomeric equilibrium should be considered (Scheme 2) [10, 11].

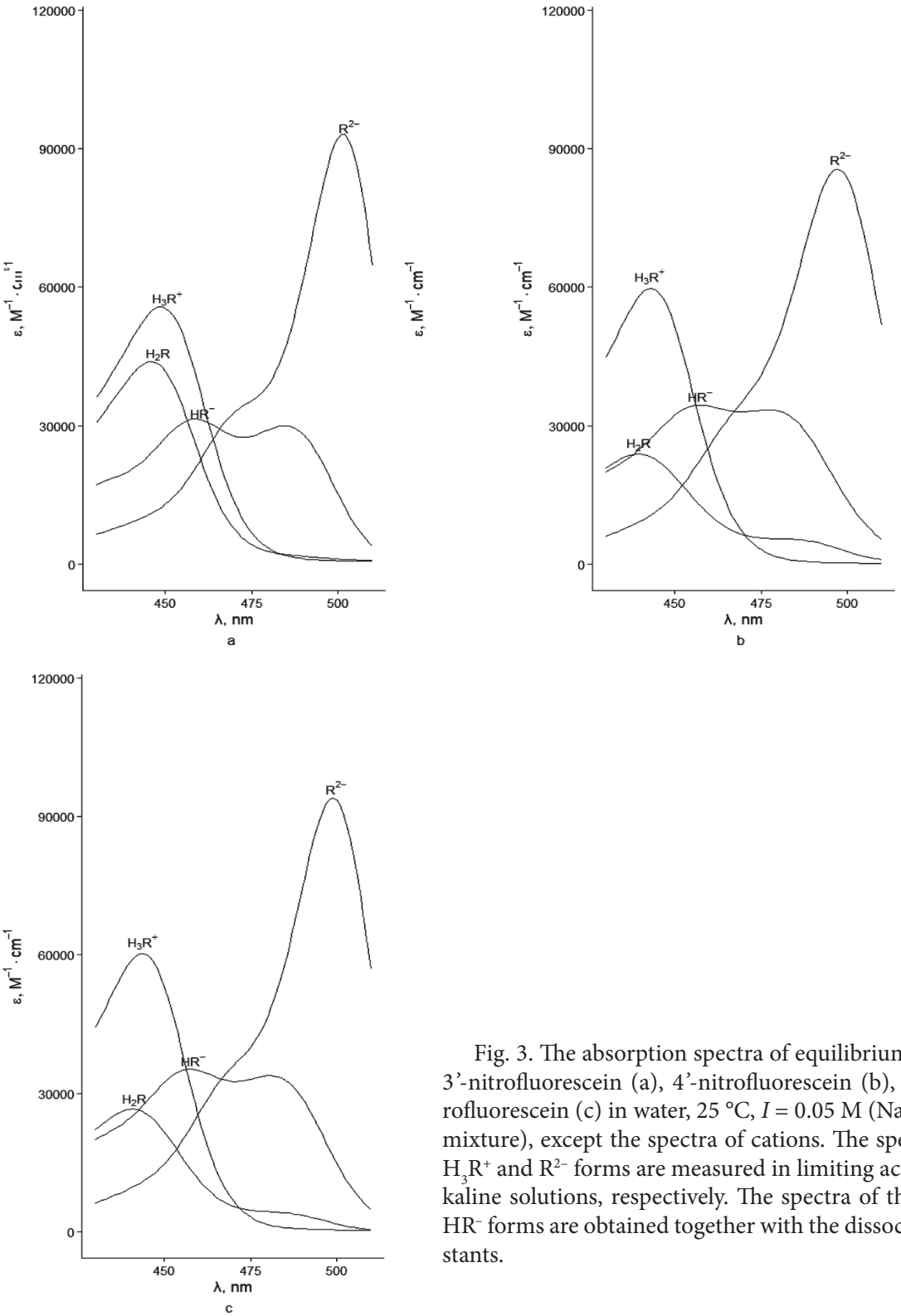
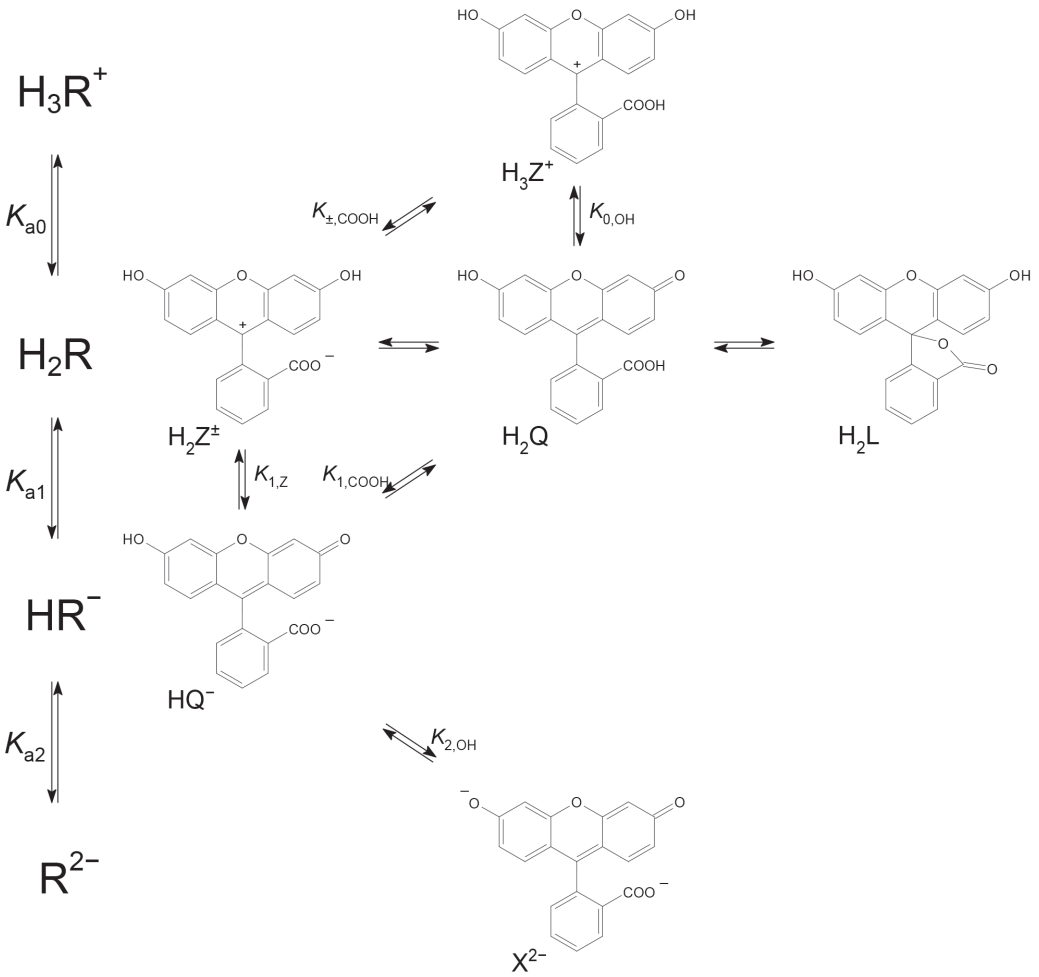


Fig. 3. The absorption spectra of equilibrium species of 3'-nitrofluorescein (a), 4'-nitrofluorescein (b), and 5'-nitrofluorescein (c) in water, 25 °C, I = 0.05 M (NaCl + buffer mixture), except the spectra of cations. The spectra of the H_3R^+ and R^{2-} forms are measured in limiting acidic and alkaline solutions, respectively. The spectra of the H_2R and HR^- forms are obtained together with the dissociation constants.



Scheme 2 – Ionic and molecular structures of fluorescein in solution.

It is well known that the absorption spectra of the zwitterionic, $H_2Z^{\pm}$, and quinonoidal, H_2Q, tautomers in the visible are similar to those of the cation, H_3R^+ (structure H_3Z^+ in Scheme 1) and anion HR^- (structure HQ^- in Scheme 1), respectively [10, 11]. The dissociation of the carboxylic group is known to lead only to a slight hypsochromic shift of the band. The lactonic tautomer, H_2L, is colorless due to the sp^3-hybridization of the nodal carbon atom C_9. Using the spectra of the cationic and anionic forms instead of the corresponding tautomers of the H_2R form, respectively, and taking into account the above-mentioned slight shift of the absorption maximum resulting from the dissociation of the COOH group, the fractions α of the latter can be estimated (Table 1). In the case of 3′-nitrofluorescein, the fraction of the tautomer H_2Q is too small to be determined accurately.

Whereas for the unsubstituted fluorescein, α ($H_2Z^{\pm}$) = 0.22; α (H_2Q) = 0.11, and α (H_2L) = 0.67, the stability of the zwitterionic tautomer increases in the case of the nitro derivatives.

The apparent reason is the increase in the acidity strength of the NO_2 group. The 3′, 4′, and 5′ positions correspond to the *ortho*, *meta*, and *para* positions for the COOH group. The *ortho* effect is so pronounced, that the fractions of the quinonoid and lactone tautomers of the 3′-nitrofluorescein cannot be estimated accurately enough. For the other two dyes the fractions of the zwitterionic and quinonoidal tautomers can be assessed more reliably (Table 1). From Scheme 2, Equations (4) and (5) can be derived.

$$pK_{a0} = pK_{\pm,COOH} + \log \alpha(H_2Z^{\pm}) = \\ = pK_{0,OH} + \log \alpha(H_2Q) \quad (4)$$

$$pK_{a1} = pK_{1,COOH} - \log \alpha(H_2Q) = \\ = pK_{1,Z} - \log \alpha(H_2Z^{\pm}) \quad (5)$$

The thermodynamic values of the indices of the so-called microscopic dissociation constants for the unsubstituted fluorescein are as follows: $pK_{\pm,COOH}$ = 2.80; $pK_{0,OH}$ = 3.10; $pK_{1,COOH}$ = 3.49; and $pK_{1,Z}$ = 3.79 [10]. The decrease in the $pK_{1,COOH}$ values for the 4′-, and 5′-nitrofluoresceins as compared to the parent dye is 0.71 and 0.89, respectively, while for the $pK_{\pm,COOH,}$ the values are 0.58 and 0.66 for the same dyes. This corresponds to the *meta* and *para* effects of the nitro group in the benzoic acid and semi-quantitatively agrees with the standard values σ_m = 0.71 and σ_p = 0.78.

The corresponding effects for the $pK_{0,OH}$ values are 0.36 and 0.17, respectively, and for $pK_{1,Z}$, they are 0.49 and 0.40. Thus, despite the absence of conjugation between the benzoic and xanthene parts of the fluorescein molecule, the strong electrophilic properties of the NO_2 group, to some extent, affect the acidity of the OH group. Note that in this case, the influence of the 4′-substitution displays a more substantial effect, obviously because it is in the *para* position for the nodal carbon atom. It aligns with the electron transmittancein the fluorescein molecule, which was earlier studied in DMSO as a solvent [12].

Interestingly, the decrease in the $pK_{1,Z}$ value for the 3′-nitrofluorescein, 1.1, is even more pronounced. Here, the reason may be the steric factor that changes the angle between the two parts of the molecule. Note that the decrease in the pK_{a2} value, which corresponds to $pK_{2,OH}$ in Scheme 2, is 0.26; 0.18; and 0 for the 3′-; 4′-; and 5′-nitrofluorescein, respectively.

The visible absorption spectra at different pH in 50 mass % aqueous ethanol and the dependences of molar absorptivity on pH are shown in Figures 4 and 5, respectively.

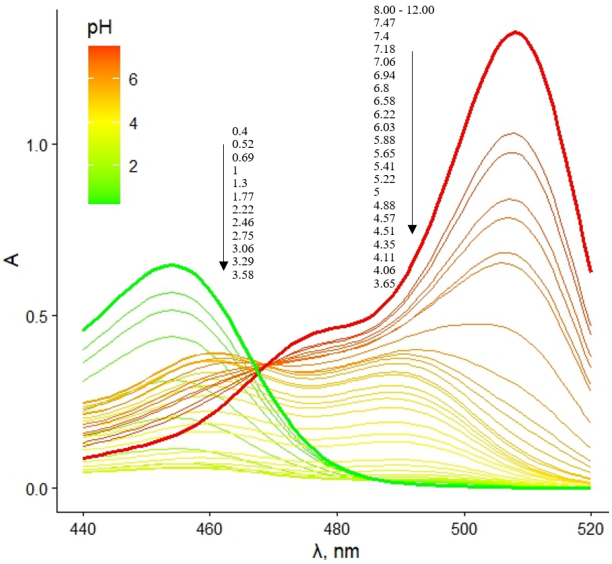


Fig. 4 – The absorption spectra of 3′-nitrofluorescein in 50 mass % aqueous ethanol solutions at different pH, 25°C; The dye concentration 1.31×10^{-5} M; I = 0.05 M except solutions with pH < 1.3. The pH values are given in the activity scale down to pH 1.3; the concentration scale (–logarithm of HCl concentration) was used below this limit.

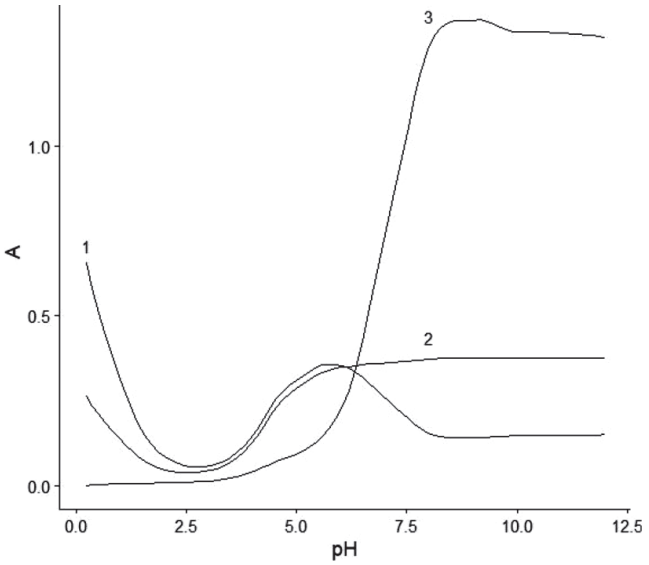


Fig. 5 – The dependence of absorbance of 3′-nitrofluorescein on pH in 50 mass % aqueous ethanol at wavelengths 454 nm (1), 470 (2), and 508 nm (3); 25 °C, I =0.05 M, except the solutions with pH below 1.3.

In Table 2, the results for the equilibria in 50 mass % aqueous ethanol are presented. The relative permittivity of this solvent at 25 °C is 49.0 [11]. The thermodynamic values of the dissociation constants were calculated using the Debye – Hückel equation, the second approach, for ionic activity coefficients. The values of activity coefficients in water are 0.69 and 0.23 for single and double-charged ions, respectively. The spectra of the individual forms of the dyes are presented in Figure 6.

Table 2.

The parameters of ionic equilibria of nitrofluoresceins in 50 mass % ethanol, I = 0.05 M [a,b]

Parameter [c]	3′-Nitrofluorescein	4′-Nitrofluorescein	5′-Nitrofluorescein
pK_{a0}	0.94±0.005 (0.78)	1.12±0.004 (0.96)	0.92±0.008 (0.76)
pK_{a1}	4.33±0.01 (4.49)	5.26±0.01 (5.42)	5.30±0.01 (5.46)
pK_{a2}	6.97±0.01 (7.44)	7.03±0.01 (7.50)	7.12±0.01 (7.59)
$\lambda_{max}/\varepsilon$ (H_3R^+)×10^{-3}	454 nm / 49.39	448 nm / 57.474	449 nm / 58.175
$\lambda_{max}/\varepsilon$ (H_2R)×10^{-3}	444 nm / 2.631 479 nm / 1.645	459 nm / 1.183; 489 nm / 1.108	455 nm / 1.482 481 nm / 1.123
$\lambda_{max}/\varepsilon$ (HR^-)×10^{-3}	448 nm / 31.519 477 nm / 27.880	457 nm / 34.744; 482 nm / 32.195	459 nm / 38.132; 486 nm / 35.741
$\lambda_{max}/\varepsilon$ (R^{2-})×10^{-3}	508 nm / 101.03	503 nm / 90.206	506 nm / 111.796
α ($H_2Z^{\pm}$)	0.018	—	—
α (H_2Q)	0.058	0.034	0.035
α (H_2L)	0.929	0.966	0.965
$pK_{0,OH}$	2.02	2.43	2.22
$pK_{1,COOH}$	3.25	3.95	4.00

Note. [a]The pK_a values in parenthesis are thermodynamic. [b] The molar absorptivities, ε, are expressed in $M^{-1}cm^{-1}$. [c]For fluorescein, the thermodynamic pK_a values are 0.94, 6.82, and 7.66 [11].

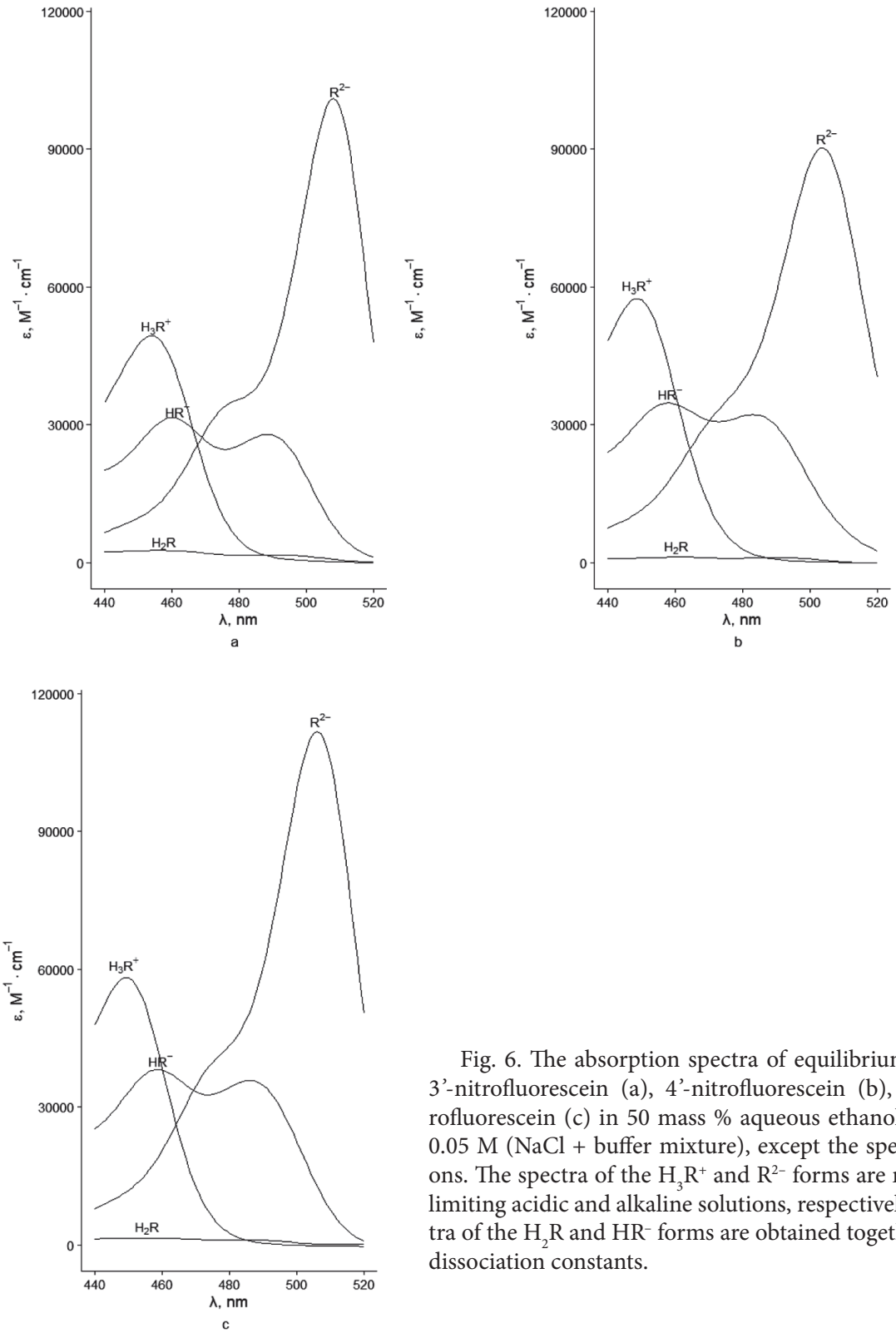


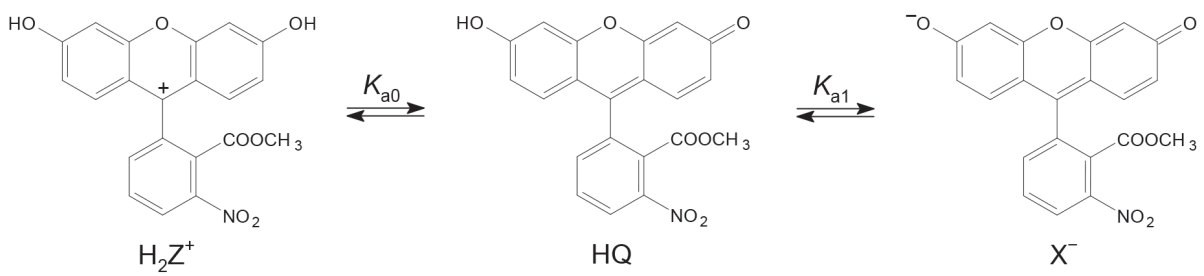
Fig. 6. The absorption spectra of equilibrium species of 3'-nitrofluorescein (a), 4'-nitrofluorescein (b), and 5'-nitrofluorescein (c) in 50 mass % aqueous ethanol; 25 °C, I = 0.05 M (NaCl + buffer mixture), except the spectra of cations. The spectra of the H_3R^+ and R^{2-} forms are measured in limiting acidic and alkaline solutions, respectively. The spectra of the H_2R and HR^- forms are obtained together with the dissociation constants.

The most striking difference when moving from water to this binary solvent is the drop in the molar absorptivity of the neutral form, H_2R. The apparent reason is the substantial shift of the state of the tautomeric equilibrium towards the colorless lactone. Following the above-described approach, the α (H_2Q) value was estimated, while no severe signs of the zwitterion were observed (Table 2). Only in the case of 3′-nitrofluorescein, some signs of the presence of $H_2Z^{\pm}$ were fixed. On the one hand, it reflects the instability of the highly polar tautomer in the organic environment. On the other hand, it proves the marked increase in the acidity of the COOH group in the presence of the NO_2 group in the *ortho* position, which leads to a small yield of $H_2Z^{\pm}$ in 50 mass % aqueous ethanol.

The $pK_{0,OH}$ values for 4′-nitro- and 5′-nitrofluorescein decrease by 0.31 and 0.71 units, respectively, when going from water to the aqueous ethanol, while the $pK_{1,COOH}$ values increase by 1.17 and 1.40 units, respectively. Such medium effects are typical for cationic and neutral acids, respectively. For fluorescein, these changes are –0.69 and +1.89, respectively [11]. Further, the difference between $pK_{1,COOH}$ of fluorescein, and the nitro derivative increases. For 4′-nitro- and 5′-nitrofluorescein,these values are 1.43 and 1.38, respectively. Comparing them with the values for an aqueous solution, 0.71 and 0.89, respectively, one should concludethat Hammett's ρ constant increases in nonaqueous media.

The $pK_{1,COOH}$ values of the nitro derivatives, 3.25, 3.95, and 4.00 (Table 2) are pronouncedly lower than that of fluorescein (5.38) [11]. This should be ascribed to the increase in the Hammett's constant ρ, which is typical for organic solvents [7].

To better understand the equilibrium in aqueous ethanol, we determined the dissociation constants of 3′-nitrofluorescein methyl ester (Scheme 3) synthesized previously [6]. The solubility of the methyl and ethyl esters in water is scarce; therefore the study was performed in aqueous ethanol.



Scheme 3 – Dissociation of methyl ester of 3′-nitrofluorescein in solution.

The absorption spectra at different pHs are shown in Figure 7. The equilibrium parameters are presented in Table 3. The pK_{a0}value of this dye corresponds to the $pK_{0,OH}$ of the parent compound (Table 2); considering the accumulation of errors in assessing the latter, the match can be regarded as satisfactory. This $pK_{0,OH}$ of fluorescein and its ethyl ester in 50 mass % aqueous ethanol are equal to 2.41 and 2.05, respectively [11].

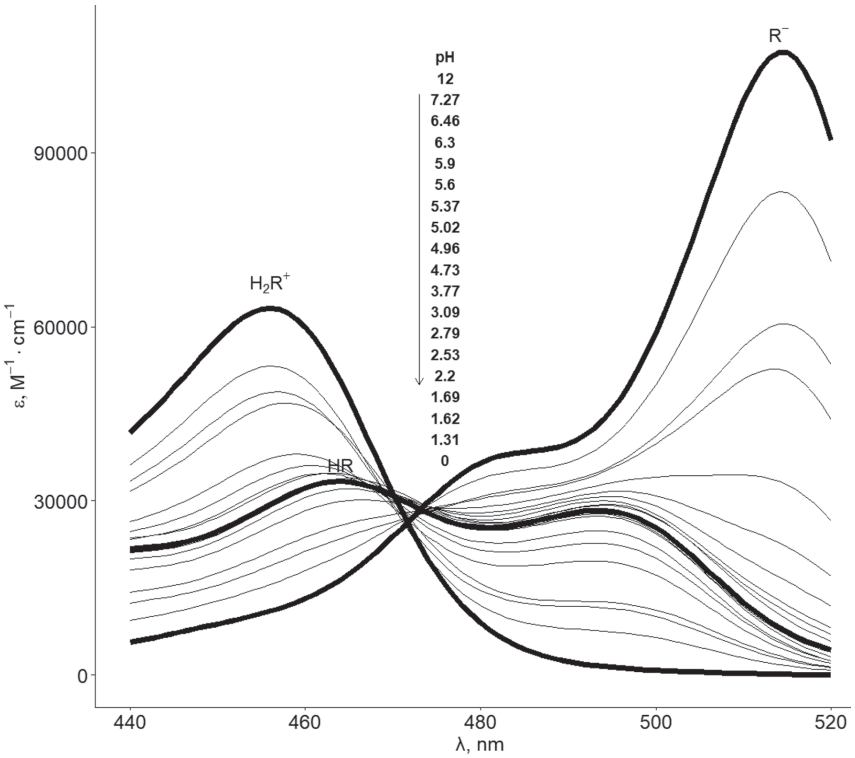


Fig. 7. The absorption spectra of 3'-nitrofluorescein methyl ester in 50 mass % ethanol, 25 °C, at different pH values; I = 0.05 M except for solutions with pH < 1.3. The pH values are given in the activity scale down to pH 1.3; the concentration scale (–logarithm of HCl concentration) was used below this limit. Bold curves represent absorption spectra of equilibrium species.

The difference between the pK_{a1} = 6.59 of the methyl ester and pK_{a2} = 7.44 should be ascribed to the influence of the negative charge of the carboxylate group on the dissociation of the OH group in the resorcinol part, according to the Bjerrum – Kirkwood – Westheimer equation [7, 11]. So, the difference between the $pK_{a2}$of fluorescein and pK_{a1} of its ethyl ester is 0.95 [11].

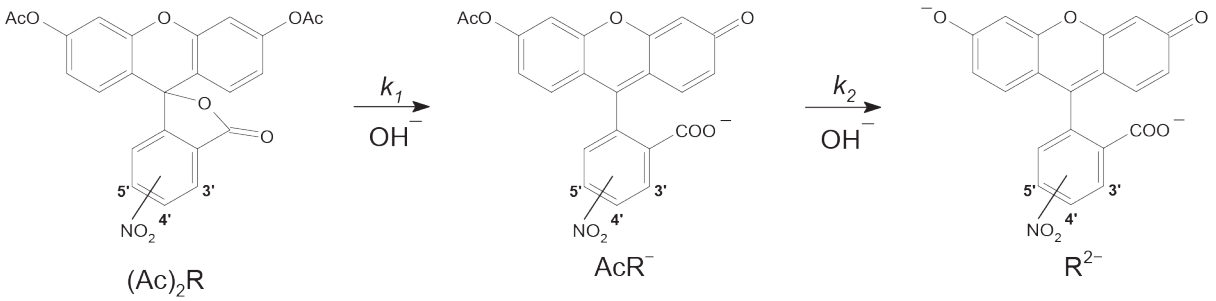
The final part of this communication is devoted to the kinetics of hydrolysis of the diacetyl derivatives of the three nitrofluoresceins. The two-step hydrolysis (Scheme 4) was described using the pseudo-first-order rate constants, k', at the fixed pH value: $k' = k\, c_{OH^-}$.

Table 3

The parameters of equilibria of 3′-nitrofluorescein methyl ester (Scheme 3) in 50 mass % ethanol [a,b]

pK_{a0}	1.79±0.01 (1.63)
pK_{a1}	6.43±0.02 (6.59)
λ_{max} / ε (H_2R^+)×10^{-3}	456 nm / 63.19
λ_{max} / ε (HR)×10^{-3}	462 nm / 33.34 492 nm / 28.34
λ_{max} / ε (R^-)×10^{-3}	514 nm / 107.29

Note. [a]I = 0.05 M . [b]The molar absorptivities, ε, are expressed in $M^{-1}cm^{-1}$.



Scheme 4 – Alkaline hydrolysis of the diacetyl derivative of nitrofluoresceins.

The spectral data are typified in Figure 8. The normalized spectra demonstrate two types of colored forms. The main idea is to equating to the absorption spectrum of the AcR^- ion to that of HR^- of the corresponding fluorescein dye [1, 5]. The spectrum of the final product of hydrolysis, R^{2-}, was already determined above. This made it possible to calculate the current concentrations of the AcR^- and R^{2-} forms at each moment. Then, the current concentrations of the colorless $(Ac)_2R$ form can be easily calculated.

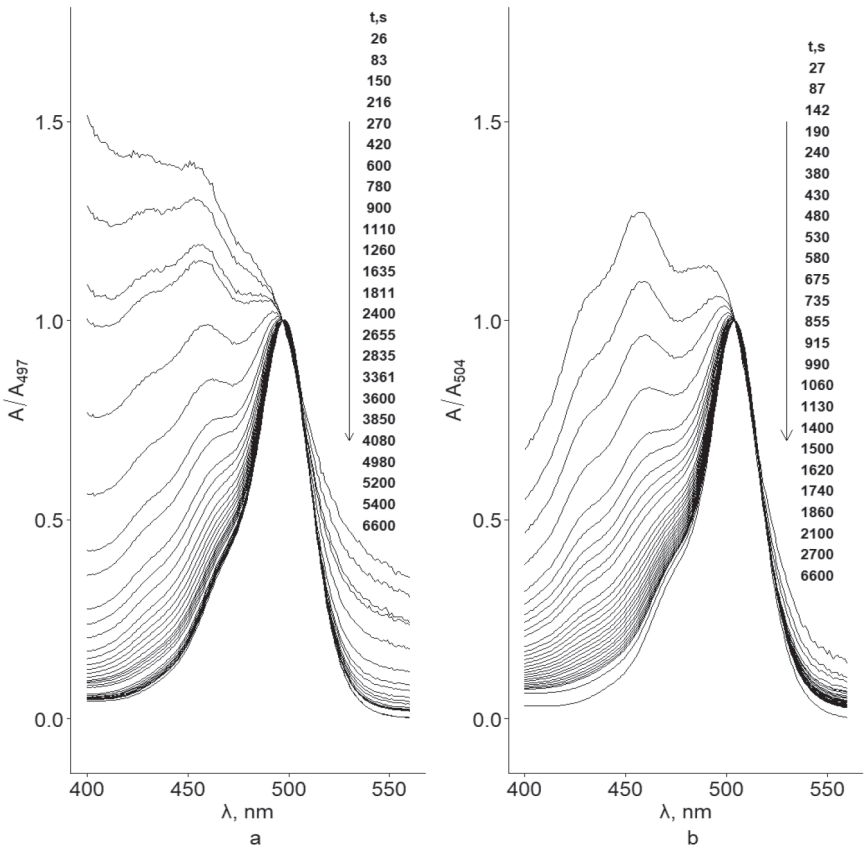


Fig. 8 – Normalized absorption spectra during the hydrolysis of 4′-nitrofluorescein and in water (a) and in 50 mass % aqueous ethanol (b), 25 °C, I = 0.05 M (NaCl + buffer mixture).

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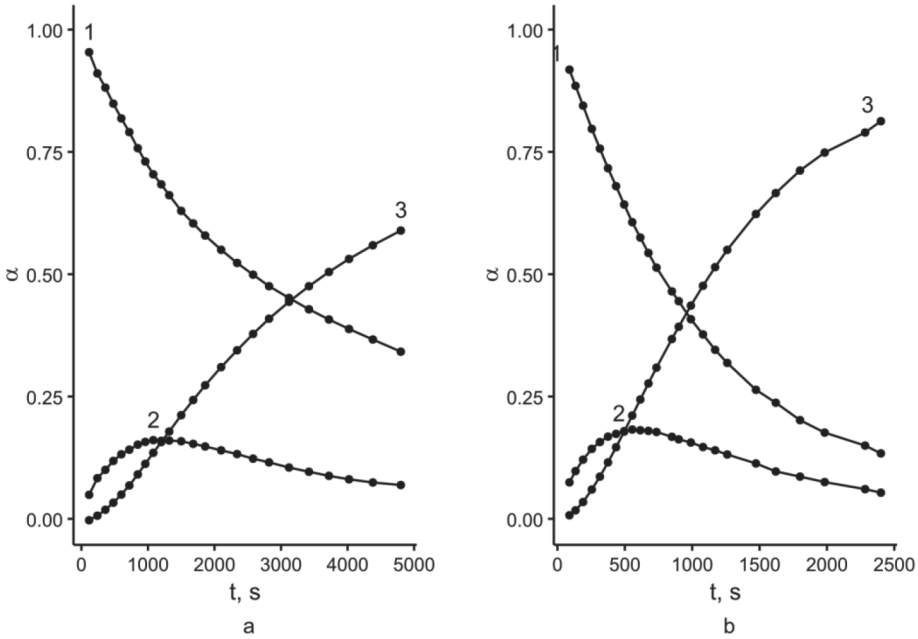

Fig. 9 – Dependences of the molar fractions of $(Ac)_2R$ (1), AcR^- (2), andR^{2-} (3) of 5′-nitrofluorescein in water (a) and in 50 mass % aqueous ethanol (b), 25 °C, I = 0.05 M (NaCl + borate buffer).

Table 4.

The rate constants of hydrolysis of nitrofluoresceins in solution, 25 °C, I = 0.05 M.

Compound	pH	k_1', s^{-1}	k_2', s^{-1}	k_2/k_1
		Water		
Diacetyl fluorescein [1]	9.72	3.5±0.2	4.5±0.3	1.29
Diacetyl 3′-nitrofluorescein	9.67	1.39±0.04	6.72±0.2	4.84
Diacetyl 4′-nitrofluorescein	9.74	4.29±0.13	12.2±0.4	2.85
Diacetyl 5′-nitrofluorescein	9.74	2.44±0.13	9.56±0.5	3.91
		50 mass % aqueous ethanol		
Diacetyl fluorescein [1]	10.50	13.10±0.4	26.00±0.8	1.98
Diacetyl 3′-nitrofluorescein	10.36	7.16±0.08	27.83±0.3	3.89
Diacetyl 4′-nitrofluorescein	10.38	11.28±0.33	24.69±0.25	2.19
Diacetyl 5′-nitrofluorescein	10.28	8.55±0.19	27.71±0.6	3.24

The dependence of the fractions of the three forms on time is demonstrated in Figure 9. The results of the kinetic study are presented in Table 4.

A comparison of the rate constants for a given compound in water and aqueous ethanol demonstrates either no significant changes or deceleration of the hydrolysis in the sec-

ond solvent. For all the nitro derivatives, the k_2/k_1 ratio is higher than that for the parent dye. However, the reasons are not so clear.

The influence of the nitro group on the k_2' value is similar to that of the K_{a2}. Although there is no conjugation between the two parts of the molecule, the electrophilic properties of this substituent result in a slight increase in both K_{a2} and k_2'. In water, the pK_{a2}s for fluorescein, 3′-, 4′-, and 5′-nitrofluorescein are 6.80, 6.54, 6.62, and 6.80, respectively, whereas the k_2' values after re-calculation to pH 9.74 are 4.93, 7.89, 12.2, and 9.56 s^{-1}, respectively. In 50 mass % ethanol, the correspondent pK_{a2} values are equal to 7.66, 7.44, 7.50, and 7.59, while k_2' = 18.8, 27.83, 25.57, and 33.31 s^{-1} (at pH 10.36).

The influence of the nitro group on the first rate constant is less clear, on the one hand, due to the sp^3-hybridization of the nodal carbon atom C_9, and on the other hand, because of the lack of the equilibrium constant that can be compared with the k_1' rate constant. In any case, after reduction to the same pH, the smallest first rate constant is observed for 3′-nitro fluorescein, while the largest one – is for the 4′- derivative. Note that 4′ is the *para* position to the nodal carbon atom.

CONCLUSIONS. The dissociation constants of 3′-, 4′-, and 5′-nitrofluorescein were determined in water and 50 mass % aqueous ethanol. The state of the tautomeric equilibrium of the molecular forms of the nitrofluoresceins substantially differs from that of the parent dye. A feature of the tautomerism of molecular forms of dyes is a sharp increase in the proportion of zwitterion and destabilization of the colorless lactone compared to un-substituted fluorescein. By contrast, the lactone tautomer predominates in aqueous ethanol. The influence of the nitro groups on the rate of hydrolysis is relatively small due to the lack of conjugation between the two parts of the molecule. Nevertheless, for all the nitro derivatives, the k_2/k_1 ratio is higher than that for the parent dye.

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МОНОНІТРОФЛУОРЕСЦЕЇНИ У ВОДНИХ СЕРЕДОВИЩАХ: КИСЛОТНО-ОСНОВНІ РІВНОВАГИ, ТАУТОМЕРИЗМ ТА ГІДРОЛІЗ ДІАЦЕТАТІВ

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У статті наведено рівноважні та кінетичні дані для трьох флуоресцеїнових барвників, що містять нітрогрупу в залишку фталевої кислоти, 3′-, 4′- і 5′-нітрофлуоресцеїну, у воді та 50 мас.% водному етанолі. Ці сполуки, зокрема нещодавно синтезований 3′-нітрофлуоресцеїн, вважали менш поліпротонними кислотами, ніж багато інших флуоресцеїнових барвників. Барвники поводяться як дипротонні кислоти, які також можуть бути протонованими: $H_3R^+ \rightleftharpoons H_2R \rightleftharpoons HR^- \rightleftharpoons R^{2-}$. Константи дисоціації у воді та 50 мас.% водному етанолі отримували в буферних розчинах розведеної HCl за до-

помогою спектрофотометричного методу з потенціометричним визначенням pH за іонної сили 0,05 M і 25,0 ±1 °C. Концентрації барвників у робочих розчинах, як правило, були в межах (5–10)×10^{-6} M. Термодинамічні значення констант дисоціації розраховували за допомогою рівняння Дебая – Хюккеля, другий підхід для коефіцієнтів іонної активності. Визначено спектри поглинання у видимій області окремих молекулярних та іонних форм для вищезазначених сполук та складного ефіру 3′-нітрофлюоресцеїну. Для останнього також визначали константи дисоціації у водному етанолі. Якщо у воді частка цвіттер-іонного таутомера нітрофлюоресцеїнів збільшується порівняно з вихідною сполукою, то у водному етанолі переважає безбарвний лактон. Визначено мікроскопічні константи дисоціації, значення яких відповідають впливу електрофільної нітрогрупи. Лужний гідроліз діацетатів нітрофлуоресцеїну досліджували у двох вищезгаданих розчинниках. Отримані результати дозволили виділити константи швидкості двостадійної кінетики гідролізу діацетил-похідних нітрофлуоресцеїнів.

Ключові слова: нітрофлуоресцеїн, кислотно-основна дисоціація, водно-етанольний бінарний розчинник, спектри поглинання, константи дисоціації, таутомерія, двоступінчатий лужний гідроліз, константи швидкості.

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