Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 150 (2015) 151–161

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Ionization and tautomerism of methyl fluorescein and related dyes

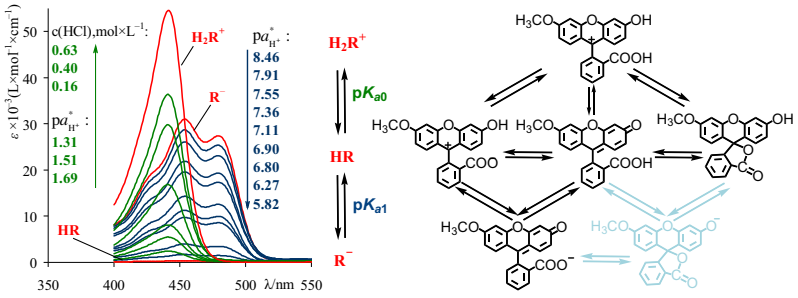
Nikolay O. Mchedlov-Petrossyan [a,*], Tatyana A. Cheipesh [a], Sergey V. Shekhovtsov [a], Andrey N. Redko [b], Vladimir I. Rybachenko [b], Irina V. Omelchenko [c], Oleg V. Shishkin [c,†]

[a] Department of Physical Chemistry, Kharkov V. Karazin National University, Kharkov 61022, Ukraine
[b] Institute of Physico-Organic Chemistry and Coal Chemistry, National Academy of Science of Ukraine, Donetsk 83114, Ukraine
[c] Institute for Single Crystals, National Academy of Science of Ukraine, Kharkov 61072, Ukraine

HIGHLIGHTS

- The molecular form of fluorescein methyl ether in water is a mixture of tautomers.
- The fractions of the zwitter-ion, quinonoid, and lactone are 11%, 6%, and 83%.
- In aqueous ethanol, the colorless molecular lactone predominates.
- The lactone structure of the monoanion in solution is unlikely.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 December 2014
Received in revised form 27 March 2015
Accepted 7 May 2015
Available online 20 May 2015

Keywords:
Fluorescein methyl ether
Molecular and nuclear magnetic resonance spectroscopy
Dissociation constants
Tautomerism
X-ray structure analysis

ABSTRACT

The protolytic equilibrium of methyl ether of fluorescein is studied in water, aqueous ethanol, and in other solvents. The constants of the two-step dissociation are determined by spectrophotometry. In water, the fractions of the zwitterionic, quinonoid, and lactonic tautomes are correspondingly 11%, 6%, and 83%, as deduced from the UV–visible spectra. Corresponding study of the ionization of the methyl ether ester of fluorescein, fluorescein ethyl ester, and sulfonefluorescein allows testing the correction of the attribution of the microscopic dissociation constants of methoxy fluorescein. The results of nuclear magnetic resonance and infrared spectroscopy, as well as the X-ray analysis confirm the predomination of the lactonic structure of the molecular species in solid state and in DMSO. Contrary to it, the spectroscopic studies in both hydrogen-donor bond (HDB) and non-HBD solvents confirm that the presence of lactonic monoanion is atypical for the dye under study and, with high probability, also for the mother compound fluorescein.

Introduction

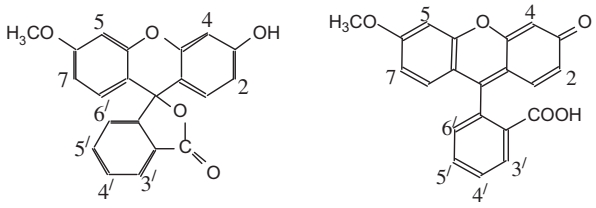
Nowadays, much attention has been given to the structure, absorption, and fluorescence of fluorescein dyes in solution [1–9]. The recent studies of the structure and spectra in the gas phase are of special interest [10–14]. The evident reason of such activities is the expanding applications of the abovementioned compounds in versatile fields, including biochemistry [15–18].

It should be mentioned, however, that the detailed ionization scheme of the mother compound, fluorescein, in water [4,6,19–23] and non-aqueous solvents was already studied in full and reported in a set of publications [24–29].

In order to further this research we studied the protolytic equilibrium of the monomethyl derivative of fluorescein, which may be represented as a colorless lactone, 3′-hydroxy-6′-methoxyspiro[isobenzofuran-1(3*H*),9′-(9*H*)-xanthene]-3-one, or as a quinonoid, 2-(6-methoxy-3-oxo-3*H*-xanthene-9-yl) benzoic acid:

* Corresponding author.
E-mail address: mchedlov@univer.kharkov.ua (N.O. Mchedlov-Petrossyan).
† Deceased.

http://dx.doi.org/10.1016/j.saa.2015.05.037
1386-1425/



At first glance, this compound is of less interest for the physico-chemistry of xanthenes, because within the reasonable pH range it cannot generate a double charged brightly emitting anion. However, the examining of the ionic equilibrium of methyl ether of fluorescein is necessary for better understanding the (partly hidden) properties of the parent dye.

The detailed ionization equilibrium of fluorescein in solutions is given in Scheme 1.

The structures possessing identical or similar chromophore systems are designed by the same letters, namely, $\mathbf{H_3Z^+}$ and $\mathbf{H_2Z^\pm}$, $\mathbf{H_2Q}$ and $\mathbf{HQ^-}$, $\mathbf{HX^-}$ and $\mathbf{X^{2-}}$. Whereas the neutral form exists in solution as a mixture of three tautomers, zwitterion $\mathbf{H_2Z^\pm}$, quinonoid $\mathbf{H_2Q}$, and colorless lactone $\mathbf{H_2L}$, only the carboxylate tautomer, $\mathbf{HQ^-}$, is typical for the monoanion. The phenolate tautomer $\mathbf{HX^-}$ appears in small quantities only in pure non-hydrogen bond donor solvents, such as DMSO, acetonitrile, and acetone [29], but it predominates in the gas phase [10–14]. For the derivatives bearing halogen atoms in the xanthene moiety, such as eosin, the zwitterionic tautomer is less typical, whereas the monoanion exists predominantly as $\mathbf{HX^-}$ [25,28].

The values of the tautomerization constants K_T, $K_T^{/}$, and $K_T^{//}$, and consequently the indices of the microscopic dissociation constants, p*k* (Scheme 1), were estimated for fluorescein in water and in several non-aqueous systems [6,7,20–22,28]. But for all that, some structures were excluded from consideration. For example, the anions–lactones $\mathbf{HL^-}$ and $\mathbf{L^{2-}}$ (not shown in Scheme 1) were regarded as less probable because in different solvents the variations of the maximal molar absorptivity of mono- and di-anions were rather of solvatochromic nature and occurred simultaneously with the shifts of the wavelength of the absorption band maximum. Such lactonic anions were registered only in the case of nitro derivatives of fluorescein [30].

The above-mentioned regularities of tautomerism of fluorescein dyes have been recently corroborated by quantum-chemical calculations [31].

The quantitative study of ionization and tautomerism of the methyl ether of fluorescein and several related dyes allows one to verify the assumptions used earlier and compare the p*k* values with those of fluorescein. The probable detailed protolytic equilibrium of methyl fluorescein is given in Scheme 2.

The probability of existence of the lactonic monoanion should be verified more directly than in the case of fluorescein, because here the R^- species predominates within a broad pH range. On the other hand, such a study allows elucidating how replacing of OH by OCH_3 influences the acidity of the COOH and the remaining OH group.

Some data on ethers and esters of fluorescein dyes are available in the literature [25–37]; Amat-Guerri et al. studied the ethers of eosin and rose bengal B and thus estimated the fractions of the lactonic monoanions of these dyes [37].

In this paper, we report the characterization of methyl ether of fluorescein and its methyl ester in solid (X-ray, IR spectra) and liquid (IR, ^{1}H NMR, and ^{13}C NMR spectroscopy) states, the dissociation constants of the dyes in 50 mass% aqueous ethanol by means of vis-spectroscopy, and the results of examination of the tautomeric equilibrium of methyl fluorescein. Two related dyes, sulfonefluorescein and ethyl ester of fluorescein, were also studied in order to compare all the p*k* values with those of fluorescein in the same mixed solvent.

Also, the protolytic equilibrium of methyl fluorescein was studied in water, despite the limited solubility of the neutral molecular species. This was necessary in order to compare the K_T, $K_T^{/}$, $K_T^{//}$, and p*k* values with those for fluorescein, taking into account some colliding information concerning the tautomerism of fluorescein just in water.

Indeed, Nagase et al. [38] proposed all the three tautomers, whereas Scharf [39] and Zanker and Peter [40] considered the equilibrium between $\mathbf{H_2L}$ and $\mathbf{H_2Q}$. Hioka and colleagues trend to such point of view in their recent paper [4].

Using the visible spectra of neutral species, Lindqvist [19,41] estimated the fractions of the tautomers: the ratio of $\mathbf{H_2Z^\pm}$, $\mathbf{H_2Q}$, and $\mathbf{H_2L}$ equals 2:1:5. We somewhat refined these approach; the percentages of the zwitterionic, quinonoidal, and lactoid tautomers was found to be 22%, 11%, and 67%, respectively [20]. Simultaneously a paper by Chen, Nakamura, and Tamura appeared where only the lactoid tautomer, $\mathbf{H_2L}$, was presumed [32]. Later on, Tamura and co-workers found the fractions of all the three tautomers of fluorescein in water close to ours: 20%, 13%, and 67%, respectively [21]. The corresponding values of 15, 15, and 70% were then published by Klonis and Sawyer [22].

Alternatively, Fompeydie and Levillain [25,26] considered the zwitterion rather as a kind of a transient form, whereas Diehl et al. [42] assumed that the molecular species of fluorescein exist in water just as this tautomer. However, Sjöback et al. [23] considered the quinonoid tautomer as 'generally believed to be prevalent' in aqueous solutions.

Hence, the verification of the problem using the methyl ether of fluorescein as a model compound seems to be pertinent.

Experimental

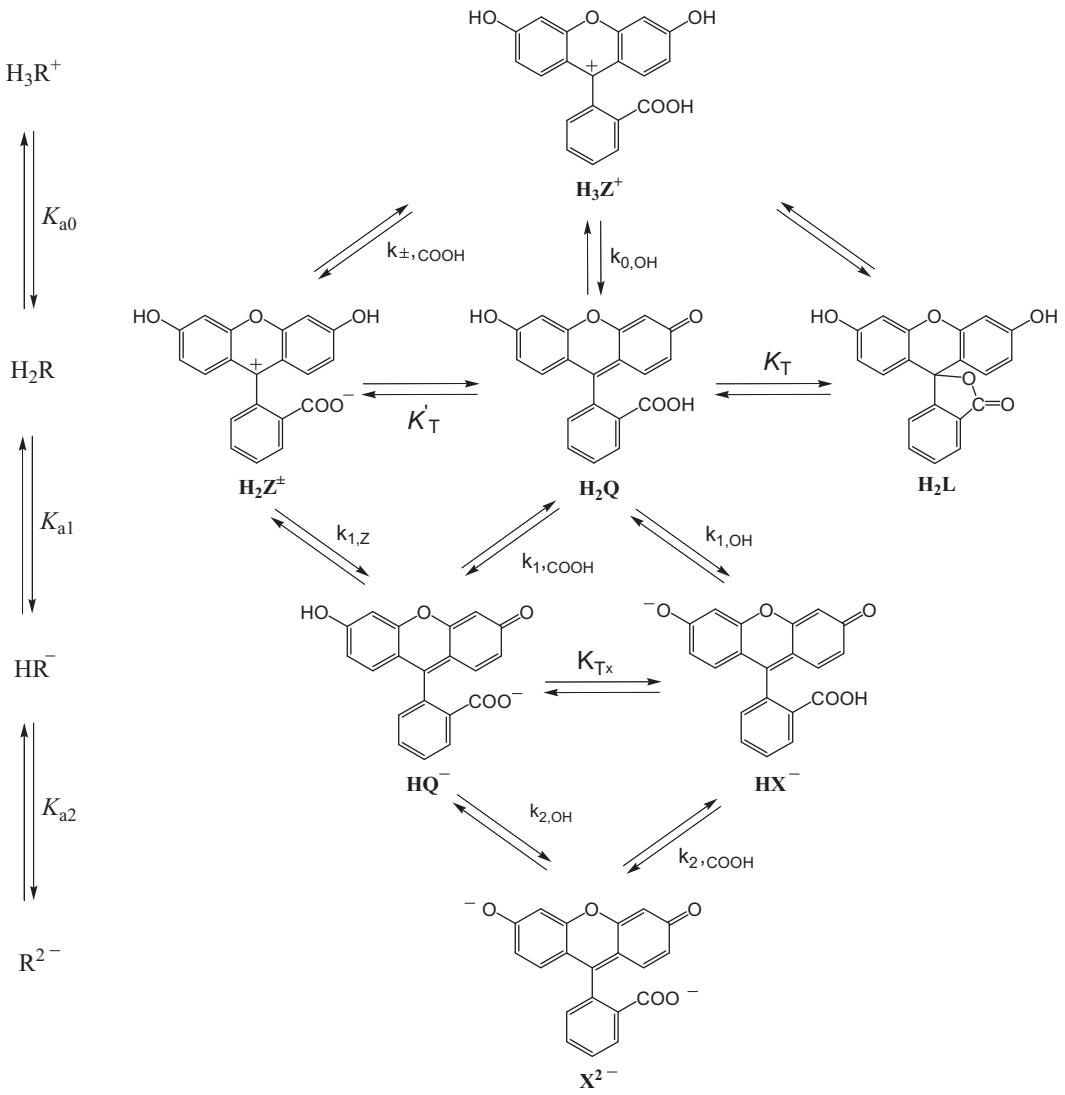
Materials

Solvents for synthesis were purified according to standard methods. Solvents for visible spectroscopic measurements and pK_a determination were of analytical and spectroscopic grade. Buffer solutions components, i.e., phosphoric, acetic, hydrochloric acids, as well as sodium chloride were of analytical grade. Sodium hydroxide solution was prepared using CO_2-free water and kept protected from the CO_2-containing air. 1,8-Diazabicycl o[5.4.0]undec-7-ene, or DBU (Merck), was used as commercially obtained.

Synthesis of the dyes

Methyl ether ester of fluorescein

This compound was synthesized following Fischer and Hepp [43]. The product was recrystallized from the $CCl_4/CHCl_3$ (3:1 by volume) mixture: 2.75 g was dissolved in 40 mL of the mixed solvent under heating. Then the solution was filtered and cooled to give the orange precipitate (1.9 g). ^{1}H NMR ($(CD_3)_2S{=}O$) δ/ppm: 8.19 (1H, dd, *J* = 7.1, *J* = 2.1, 3′-H), 7.94–7.68 (2H, m, *J* = 7.1, *J* = 2.1, 4′,5′-H), 7.47 (1H, dd, *J* = 7.1, *J* = 2.1, 6′-H), 7.21 (1H, d, *J* = 2.1, 4-H), 6.95–6.68 (3H, m, 1,2,8-H), 6.36 (1H, dd, *J* = 9.5, *J* = 2.1, 7-H), 6.21 (1H, d, *J* = 2.1, 5-H), 3.88 (3H, s, Ph-O-CH_3), 3.55 (3H, s, Ph-CO-O-CH_3). ^{13}C NMR ($(CD_3)_2S{=}O$) δ/ppm: = 183.89, 165.21, 163.92, 158.39, 153.60, 150.14, 133.92, 133.24, 130.73, 130.40, 130.08, 129.39, 129.51, 128.88, 116.65, 113.60, 114.31, 104.60, 100.60. IR/cm^{-1}, selected bands: 1726, 1642, 1587, 1509, 1453, 1256, 1211, 1105.



Scheme 1. Protolytic equilibrium of fluorescein: $K_T = [H_2L]/[H_2Q]$; $K_T' = [H_2Z^{\pm}]/[H_2Q]$; $K_T'' = K_T/K_T' = [H_2L]/[H_2Z^{\pm}]$; $K_{T_x} = [HX^-]/[HQ^-]$; $k_{\pm,COOH} = a_{H^+}a_{H_2Z^{\pm}}/a_{H_3Z^+}$; $k_{0,OH} = a_{H^+}a_{H_2Q}/a_{H_3Z^+}$; $k_{1,Z} = a_{H^+}a_{HQ^-}/a_{H_2Z^{\pm}}$; $k_{1,COOH} = a_{H^+}a_{HQ^-}/a_{H_2Q}$; $k_{1,OH} = a_{H^+}a_{HX^-}/a_{H_2Q}$; $k_{2,OH}a_{H^+}a_{X^{2-}}/a_{HQ^-}$; $k_{2,COOH} = a_{H^+}a_{X^{2-}}/a_{HX^-}$.

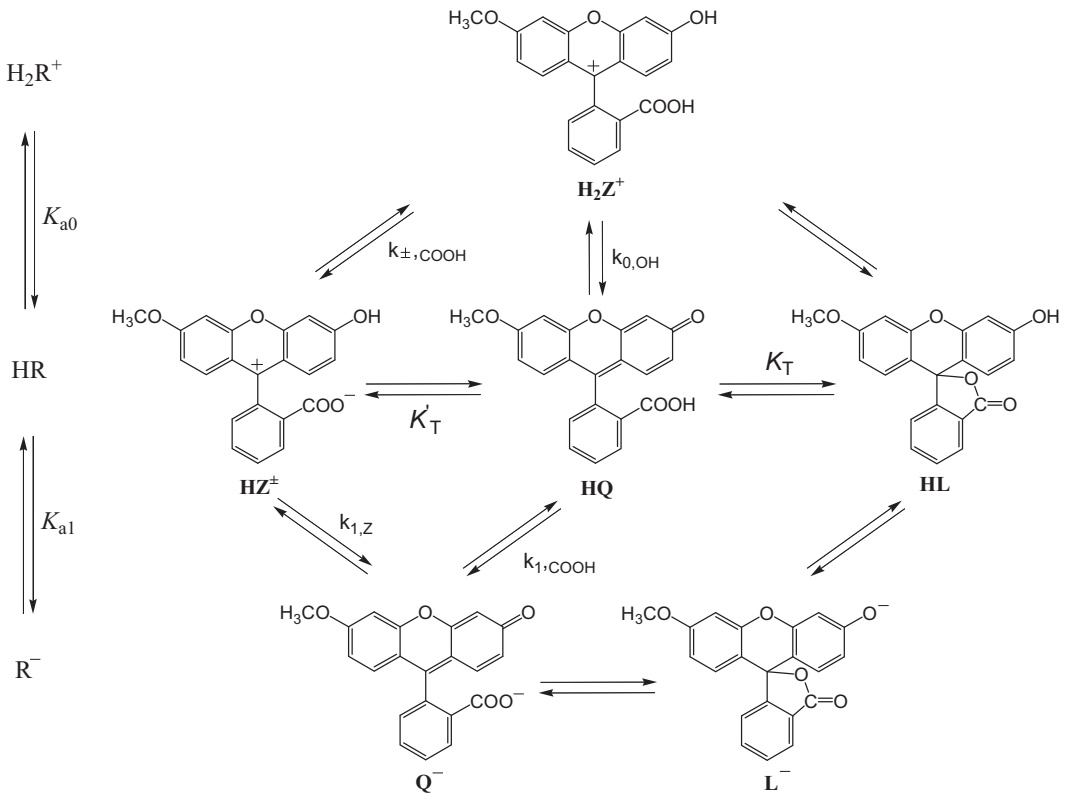
Methyl ether of fluorescein

According to the procedure described by Fischer and Hepp [43], 1.54 g of the above methyl ether ester in 15 mL of methanol and 0.8 g potassium hydroxide (1 mol L^{-1}) were heated under reflux for 2 h. After cooling of reaction mixture, 100 mL of distilled water was added. The transparent solution thus obtained was acidified by adding HCl. The resulting yellow flocky precipitate was filtered off, washed with distilled water and dried. This unpurified product (1.2 g) was recrystallized from ethanol–water mixture (50 vol%). The crystals thus obtained were used for X-ray analysis. ^{1}H NMR ($(CD_3)_2S{=}O$) δ/ppm: 10.14 (1H, s, Ph-OH), 7.98 (1H, d, J = 7.2, 3′-H), 7.83–7.64 (2H, m, J = 7.2, 4′,5′-H), 7.25 (1H, d, J = 7.2, 6′-H), 6.91 (1H, d, J = 2.1, 4-H), 6.73–6.51 (5H, m, 1,2,5,7,8-H), 3.79 (3H, s, Ph-O-CH_3). ^{13}C NMR ($(CD_3)_2S{=}O$) δ/ppm = 168.68, 161.03, 159.55, 152.50, 151.88, 151.77, 135.67, 130.16, 129.11, 128.96, 126.03, 124.67, 124.01, 112.79, 111.91, 110.99, 109.44, 102.2, 100.78, 82.71, 55.66. IR/cm^{-1}, selected bands: 1718, 1609, 1508, 1430, 1286, 1257, 1171, 1120.

The sodium salt was prepared by addition of $NaHCO_3$ to the aqueous suspension of methyl ether of fluorescein. After evaporation of water from the solution obtained, the target product was extracted by 2-propanol and dried. ^{1}H NMR ($(CD_3)_2S{=}O$) δ/ppm: 8.08 (1H, d, J = 4, 3′-H), 7.58 (2H, m, J = 4, 4′,5′-H), 7.17 (1H, d, J = 4, 6′-H), 7.08 (1H, s, 5-H), 6.84 (1H, d, J = 8, 8-H), 6.80 (1H, d, J = 8, 7-H), 6.74 (1H, d, J = 8, 1-H), 6.32 (1H, d, J = 8, 2-H), 6.27 (1H, s, 4-H), 3.86 (3H, s, Ph-O-CH_3). ^{13}C NMR ($(CD_3)_2S{=}O$) δ/ppm = 178.55, 168.79, 162.82, 157.15, 153.15, 138.02, 137.15, 130.74, 130.48, 129.66, 129.07, 128.95, 127.66, 124.56, 114.51, 113.27, 112.70, 103.52, 100.31, 56.08.

Sulfonefluorescein

14 g of P_4O_{10} was carefully added to 10 mL of 85 mass% aqueous phosphoric acid. The stirred mixture was heated to 100 °C to give transparent solution. 5.0 g of ammonium salt of the 2-sulfobenzoic acid were slowly dissolved in the reaction mixture at 150 °C. Then 5.0 g resorcinol was gradually added, and the formed dark red product was heated to 170–190 °C for 3 h. Along with the progress of reaction, the target product precipitates in form of small glossy purple crystals, and the mass becomes more viscous. The reaction mixture was cooled to 80–90 °C and diluted with 150 mL ethanol–water mixture (50 vol%). After filtering off, washing with ethanol (95.6 mass%) and drying, 6.29 g of unpurified precipitate was obtained. As sulfonefluorescein is relatively poor soluble in the most of readily accessible solvents, the



Scheme 2. Protolytic equilibrium of methyl fluorescein: $K_T = [HL]/[HQ]$; $K'_T = [H_2Z^{\pm}]/[H_2Q]$; $K''_T = K_T/K'_T = [HL]/[HZ^{\pm}]$; $K_L = [L^-]/[Q^-]$; $k_{\pm,COOH} = a_{H^+}a_{HZ^{\pm}}/a_{H_2Z^+}$; $k_{0,OH} = a_{H^+}a_{HQ}/a_{H_2Z^+}$; $k_{1,Z} = a_{H^+}a_{HQ^-}/a_{H_2Z^{\pm}}$; $k_{1,COOH} = a_{H^+}a_{Q^-}/a_{HQ}$.

recrystallization was carried thought conversion of the compound into its soluble disodium salt and subsequent acidification of the solution by HCl [44]. The solution of the disodium salt (1 g) in 1000 mL of water was heated to boiling, then acidified by appropriate amount of HCl, and slowly cooled. This results in the precipitating of sulfonefluorescein in form of large purple crystals. The ethanol–water mixture (50 vol%) could also be used as a solvent instead of water (1 g salt per 50–100 mL). ^{1}H NMR ($(CD_3)_2S{=}O$) δ/ppm: 7.99 (1H, d, J = 7.4, 3^1-H), 7.68 (1H, t, J = 7.4, 5^1-H), 7.58 (1H, t, J = 7.4, 4^1-H), 7.37 (2H, d, J = 8.8, 1,8-H), 7.31–7.21 (3H, m, 6^1,4,5-H), 7.13 (2H, d, J = 8.8, 2,7-H). IR/cm^{-1}, selected bands: 1573, 1455, 1302, 1213, 1136, 1118, 1041.

The collection of the NMR and IR spectra is available in the Supplementary data.

Apparatus

The visible absorption spectra were run on a Hitachi U-2000 spectrophotometer. Fluorescence spectra were measured using Hitachi 850 apparatus. The pH determinations were performed using a potentiometer R 37–1 and a pH-meter pH-121, equipped with an ESL-63-07 glass electrode and an Ag|AgCl reference electrode, in a cell with liquid junction (1.00 mol L^{-1} KCl). The glass electrode calibration was performed with standard aqueous buffer solutions (pH 1.68, 4.01, 6.86, and 9.18 at 25.0 °C). In aqueous ethanol, the proton activity $a^*_{H^+}$ was standardized to infinite dilution in the given solvent, the experimental (instrumental) pH values were corrected for the diffusion potential and proton solvation by relation: $pa^*_{H^+} = pH_{instr} - 0.20$ [45,46]. The ^{1}H NMR spectra were recorded on Mercury Varian VX-200 spectrometer at 200 MHz and Bruker Avance II 400 at 400 MHz, the ^{13}C NMR spectra were recorded on Bruker Avance II 400 spectrometer at 100 MHz. The IR spectra were obtained by Dr. D. Yu. Filatov on Alpha FT-IR Spectrophotometer (Bruker) with Alpha-Platinum ATR module and either in KBr pellets or in DMSO solutions with application of FTIR spectrometer SPECTRUM ONE (PerkinElmer) in the range of 400–4000 cm^{-1} by Dr. D. S. Sofronov, Division of Functional Materials Chemistry, Institute for Single Crystals, National Academy of Science of Ukraine.

X-ray diffraction study: crystals of monomethyl fluorescein ($C_{21}H_{14}O_5$, Mr = 346.32) are triclinic, P$\bar{1}$, a = 7.956(2), b = 10.813(3), c = 10.831(2) Å, α = 94.84(2)°, β = 108.52(2)°, γ = 108.44(2)°, V = 820.3(3) Å^3, Z = 2, d_{calc} = 1.402 g sm^{-1}, μ = 0.101 mm^{-1}, $F(000)$ = 360. 7051 reflections (3750 independent, R_{int} = 0.106) were collected on an "Xcalibur-3" diffractometer (MoK$_\alpha$ radiation, CCD-detector, graphite monochromator, ω-scanning, $2\theta_{max}$ = 50°). Structure was solved by direct methods and refined against F^2 within anisotropic approximation for all non-hydrogen atoms by full-matrix least squares procedure using OLEX2 program package [47] with SHELXS and SHELXL modules [48]. All H atoms were placed in idealized positions (C–H = 0.93–0.96 Å, O–H = 0.82 Å) and constrained to ride on their parent atoms, with $U_{iso} = 1.2U_{eq}$ (except $U_{iso} = 1.5U_{eq}$ for methyl and hydroxyl groups). Final refinement was converged at wR_2 = 0.092 for all 2861 reflections (R_1 = 0.060 for 899 reflections with $F > 4\sigma(F)$, S = 0.78).

Atom coordinates and crystallographic parameters have been deposited to the Cambridge Crystallographic Data Centre (**CCDC 1026934**). These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Procedure

For pK_a determination, the stock solutions were prepared from weighted amount of dyes using 95.6 mass% ethanol as a solvent.

When preparing the initial solution of fluorescein methyl ether in aqueous solutions, small amount of NaOH solution was added for enhancing the dye solubility. In solutions of methyl ether ester of fluorescein and ethyl ester of fluorescein in 50 mass% C_2H_5OH, the $pa^*_{H^+}$ values did not exceed 11 in order to avoid the hydrolysis of the ester groups. The working solutions were prepared by mixing required volumes of the stock dye solution, aqueous buffer components and NaCl solutions with 95.6 mass % C_2H_5OH. Working dyes concentrations were ranged from 6×10^{-6} mol L^{-1} to 2.6×10^{-4} mol L^{-1}. Complete conversion of the dyes into the corresponding ultimate basic and acidic form was attained in the presence of 1×10^{-4} mol L^{-1} NaOH and 2 mol L^{-1} HCl respectively. The sets of 15–20 (for dyes dissociating in two steps) and 6 (for methyl ether ester of fluorescein) working solutions at different $pa^*_{H^+}$ (or pH) were utilized. The value of ionic strength was maintained constant (0.05 mol L^{-1}) by adding a proper amount of NaCl to the buffer components or to dilute NaOH and HCl solutions, except the experiments with high HCl concentrations.

The pK_a values were calculated jointly with the spectra of the neutral (for methyl ether and ethyl ester of fluorescein) or anionic (for sulfonefluorescein) species by the CLINP program [49]. The iterative procedure used was based on the minimizing the Huber's criterion function by the Gauss–Newton method. As a rule, the wavelengths around the absorbance maxima were considered as the most informative analytical positions.

The relative permittivity of the 50 mass% aqueous ethanol is equal to 49.0. The ionic activity coefficients of the ionic dye species were calculated using the Debye–Hückel equation (second approach, ionic parameter = 0.5 nm) to estimate the thermodynamic pK_a values. The corresponding corrections are +0.16, +0.47, and −0.16 for pK_{a1}, pK_{a2}, and pK_{a0} respectively.

The solutions for measuring the absorption spectra of the methyl ether anion and methyl ether ester neutral species in non-aqueous solvents were prepared by dilution of the stock solutions of the dyes either in acetone or in 95.6 mass% ethanol.

Results and discussion

Characterization of the samples

In the crystal, monomethyl fluorescein exists in the neutral lactone form (Fig. 1). The C(1)–O(4) bond length of 1.535(6) Å is rather long as compared to fluorescein and its structural analogues (mean value is 1.508 Å [50–53]). Also it is much longer than typical C–O bond length in common γ-lactones 1.464 Å [54]. Together with shortening of C(1)–C(2) and C(5)–O(2) bonds (1.497(6) Å and 1.344(6) Å, mean values are 1.527 Å and 1.370 Å, respectively [54]) that indicates presence of intramolecular π-conjugation in the methylresorcin moiety. This conjugation interaction does not affect the C(1) atom geometry, it retains tetrahedral configuration (valence angles are 101.0(3)–115.2(4)°).

Particularly interesting is that hydroxyl group, unlike methoxy group, does not take part in such conjugation: the C(1)–C(9) bond lengths is close to the mean value, and C(12)–O(3) bond of 1.388(6) Å is even longer than C_{ar}–OH bonds in fluorescein (1.352–1.364 Å according to [50–52]). Values of C(3)–O(1) and C(10)–O(1) bond lengths (1.393(6) Å and 1.378(7) Å) that are close to mean value for diaryl ethers (1.384 Å [54]) indicate that O(1) atom also does not take part in conjugation interaction in the molecule, as it was supposed previously for fluorescein analogues in solid state [55].

Xanthene moiety is almost planar: two benzene rings are slightly rotated around each other, angle between planes is 6.2°. The C(1) atom deviates from the mean plane of the rest xanthene atoms on 0.22 Å (plane accuracy is 0.06 Å). That is typical for fluorescein structural analogues (mean deviation of the corresponding atom is 0.17 Å [53]). Xanthene and isobenzofuran moieties are almost orthogonal (angle between planes is 88.6(8)° that is equal to mean value for its structural analogues [53]). That causes presence of shortened intramolecular contacts C(15)···H(14) 2.68 Å and C(15)···H(7) 2.69 Å (sum of VdW radii is 2.87 Å [56]). In the crystal, molecules of monomethyl fluorescein form dimers due to intermolecular hydrogen bonds O(3)–H(3)···O(5)′ (1 − *x*, −*y*, 2 − *z*; H···O′ 2.18 Å, O–H···O 146°).

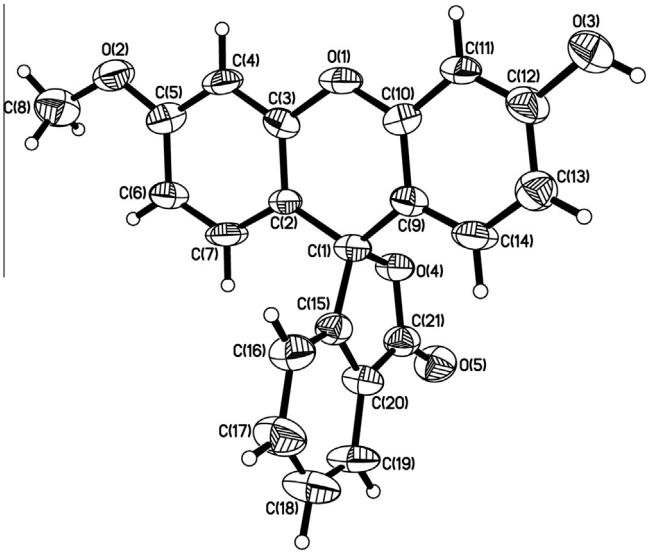


Fig. 1. Molecular structure of the monomethyl fluorescein according to the X-ray diffraction data. All non-hydrogen atoms are shown as 40% thermal ellipsoids.

The intensive band 1718 cm^{-1} in the IR spectrum of the solid methyl ether lactone of fluorescein should unambiguously be ascribed to the stretching vibrations of the C=O group of γ-lactone cycle. The X-ray data confirm that the sample under consideration exists without any solvent molecule. This is a remarkable observation, because the above cited authors state that the lactone of the parent compound can be prepared in solid state only in form of a solvate [51]. They report the value ν(C=O) = 1750 cm^{-1} for the complexes of fluorescein lactone with acetone or methanol.

On the other hand, Markuszewsky and Diehl determined the value 1730 cm^{-1} for the C=O group of the fluorescein lactone, with a shoulder at 1760–1770 cm^{-1} which they attributed to the lactone complex with 1,4-dioxane [48]. In earlier publications, the band at 1729–1730 cm^{-1} was reported [29,57].

The value 1750 cm^{-1} was registered by us for the molecular species of fluorescein in DMSO and $CHCl_3$ solutions [58,59]. It should be concluded that solvation of the lactone cycle results in 20–40 cm^{-1} increase in the ν(C=O) value.

In the IR spectrum of methyl ether of fluorescein in DMSO, the band ν(C=O) = 1756 cm^{-1} was registered, in line with the lactone structure, **HL**. The signal δ = 82.71 ppm registered by us in the ^{13}C NMR spectrum in DMSO-d_6 may be taken as a very strong support for the prevalence of the lactonic structure. It should be ascribed unequivocally to the central carbon atom of the last-named tautomer that is in the state of sp^3-hybridization; this value coincide with the published δ = 83.21 value for the 3′,6′-dimethoxyfluorane in $CDCl_3$ [60]. Meanwhile, the signal for the neutral species of the methyl ether ester **Q** is shifted towards the downfield, δ = 150.14 ppm, in accordance with the sp^2-hybridization.

On the other hand, very narrow shape of the 10.1 ppm signal in the ^1H NMR spectrum (see Supplementary data) gives evidence of the lack of any expressed specific interactions between DMSO and

the OH group of the **HL** species. This is in line with the weak acidity of the hydroxyl group of fluorescein dyes in DMSO [27,28,58].

The intensive bands 1726 cm^{-1} and 1642 cm^{-1} in the IR spectrum of the solid molecular form of methyl ether ester of fluorescein should be attributed to ν(C=O) of the $COOCH_3$ group and of the quinone in the xanthene moiety respectively. Fompeydie and Levillain reported the value of 1645 cm^{-1} for their 'non-lactone' sample, obtained from aqueous solution [26] (probably, quinonoid **H_2Q**). We registered the bands 1637, 1612, and 1642 cm^{-1} for the molecular forms of 6-hydroxy-9-phenyl fluorone, *n*-decyl ester of fluorescein, and ethyl ether ester of fluorescein, and 1720–1722 cm^{-1} for the last two dyes possessing the COOAlk groups.

Dissociation constants in 50 mass% aqueous ethanol

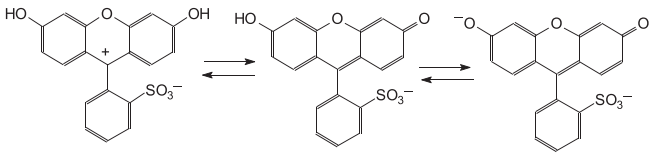
The numbering of the stepwise dissociation constants is as follows: the constant $K_{a(1-z)}$ corresponds to the step $H_jR^z \rightleftarrows H_{j-1}R^{z-1} + H^+$. Therefore, the constants determined for the fluorescein methyl ether describe the equilibria (1) and (2):

$$H_2R^+ \rightleftarrows HR + H^+; \quad K_{a0} \qquad (1)$$

$$HR \rightleftarrows R^- + H^+; \quad K_{a1} \qquad (2)$$

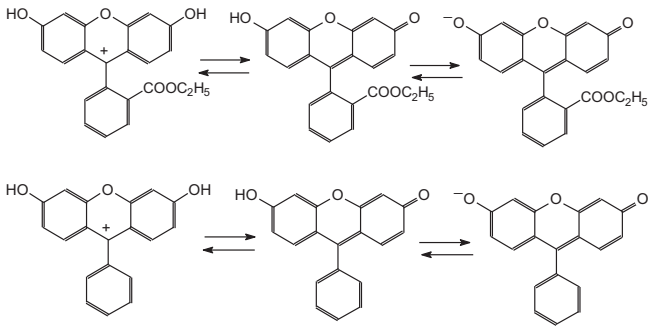
The absorption spectra at different pH values and the dependences of molar absorptivities vs. pH are exemplified in Figs. 2–6.

For fluorescein the cation H_3R^+ is a triprotic acid, and the third dissociation step is $HR^- \rightleftarrows R^{2-} + H^+$, with the equilibrium constant K_{a2} (Scheme 1). Sulfonefluorescein is also a triprotic acid, but the cation exists at rather low pH values and the K_{a0} was not determined here:



$H_2Z^{\pm}$ HQ$^-$ X^{2-}

The ethyl ester of fluorescein and the 6-hydroxy-9-phenylfluorone are dyes with "cut off" carboxyl group, and they are to be described via equilibria (1) and (2), but one should keep in mind that in this case it deals about the successively dissociation of OH groups: that of the cation (K_{a0}) and of the quinonoid molecule (K_{a1}):



H_2Z^+ HQ X$^-$

Finally, for methyl ether ester, only the first step ($HR^+ \rightleftarrows R + H^+$) is possible:

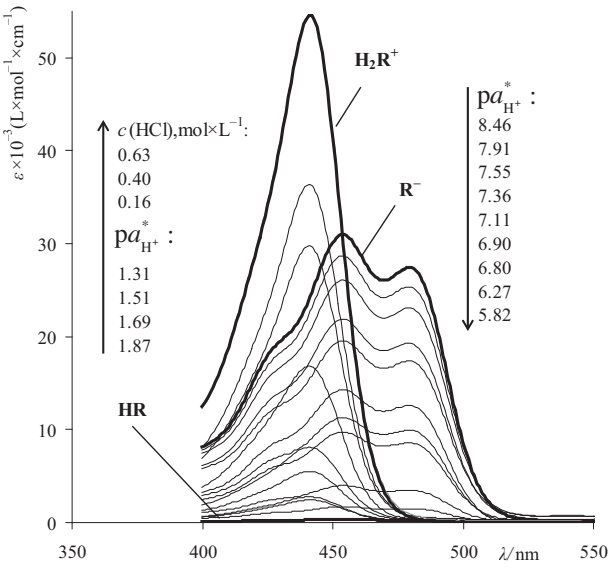


Fig. 2. Absorption spectra of fluorescein methyl ether in 50 mass% aqueous ethanol at different $pa^*_{H^+}$ and limiting dye forms.

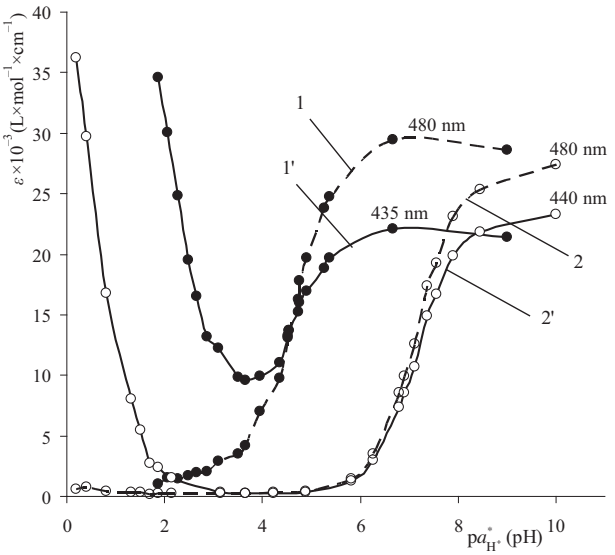
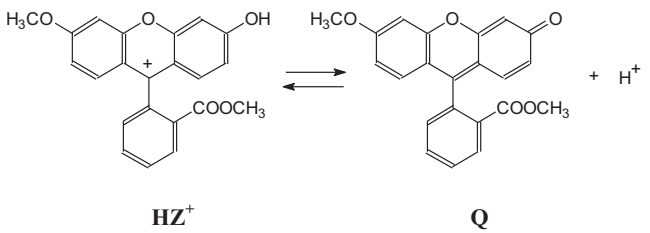


Fig. 3. Molar absorptivities of fluorescein methyl ether versus pH or $pa^*_{H^+}$ in aqueous (1, 1′) and 50 mass% aqueous ethanol (2, 2′) solutions, respectively.



HZ^+ Q

The pK_a data obtained are presented in Table 1; the main spectral data are compiled in Table 2. It should be emphasized that the spectra of the individual ionic and molecular forms (bold lines) have been singled out from the initial data during the calculation process.

For fluorescein methyl ether ester in 50 vol% CH_3OH, S. Niizuma et al. [62] reported pK_{a0} = 2.65. As the pK_{a0} values for similar

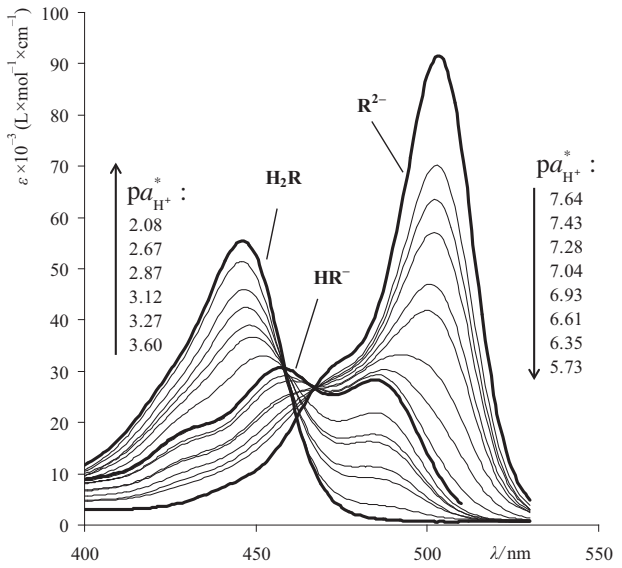


Fig. 4. Absorption spectra of sulfonefluorescein in 50 mass% aqueous ethanol at different $pa^*_{H^+}$ and limiting dye forms (heavy lines).

equilibria (dissociation of the cations of 6-hydroxy-9-phenyl fluorone and fluorescein ethyl ester) decrease on going from water (pK_{a0} around 3.0, see below) to water-organic mixtures, this result agrees with our one, because 50 vol% of methanol corresponds to ≈ 43 mass%.

Several data in aqueous alcohols are available in the literature for fluorescein. So, Frolov et al. [63] potentiometrically determined in aqueous 52.7 mass% 2-propanol the thermodynamic values $pK_{a1} = 6.90$ and $pK_{a2} = 7.78$ (at ionic strength 0.1 mol L^{-1}, the values are 6.62 and 7.44). Diehl et al. [42] obtained via the same method the values 6.38 and 7.16 in 50% aqueous ethanol at 0.1 mol L^{-1} (NaCl). Fompeydie and Levillain determined the values $pK_{a1} = 5.60$ and $pK_{a2} = 6.86$ spectrophotometrically in 50 vol% aqueous methanol (ionic strength not indicated); under the same conditions, the value for ethyl fluorescein: $pK_{a1} = 6.36$ [25].

For fluorescein methyl ether ester in 50 vol% CH_3OH, Niizuma et al. [62] reported λ_{max} = 444 nm and 458 nm for the cation and neutral form respectively.

Methyl fluorescein in water

During the study of fluorescein ether in water, we met difficulties caused by the rather limited solubility of the neutral (molecular) species. The initial solution was prepared in ethanol-free water, with addition of small amount of NaOH solution. In the working aqueous solutions, a slow decrease in absorbance, up to complete decoloration after 3 – 4 days, was observed within the pH range from 4.90 to 2.87. However, over 30 min no appreciable changes in absorbance of freshly prepared solutions occur. Thus, some of the results were obtained with oversaturated solutions. The pK_a values are presented in Table 3; the data for the related dyes obtained earlier are compiled ibidem (Fig. 7).

The λ_{max}/nm ($\varepsilon_{max} \times 10^{-3}$/L mol^{-1} cm^{-1}) for methyl fluorescein in water are as follows: R^-: 454 (31.9) and 474 (30.7); HR: 433 (7.6); H_2R^+: 437 (54.0). Naturally, the analogue of the $\mathbf{X^{2-}}$ species of fluorescein (Scheme 1, $R^{2-} = \mathbf{X^{2-}}$), possessing the band with $\lambda_{max} = 490.5$ nm (ε_{max} = 88.0 $\times10^3$) is absent among the species of its methyl ether (Scheme 2). But the band of the cationic form H_2R^+ (i.e., $\mathbf{H_2Z^+}$) coincides with that of the parent dye, $\mathbf{H_3Z^+}$: 437 nm (ε_{max} = 54.3 $\times10^3$). The similarity of the above R^- spectra of methyl fluorescein and that of HR^- of fluorescein [the $\mathbf{HQ^-}$ tautomer; $\lambda_{max} = 454–474$ nm; $\varepsilon_{max} = (32.7–33.8) \times 10^3$] give evidence for the structure of $\mathbf{Q^-}$ type. The fluorescence spectrum of this species in water was also measured; the quantum yield was estimated as $\varphi = 0.40$, referring to the $\varphi = 0.93$ value of the fluorescein dianion R^{2-} as standard. Note, that thus obtained value is in line with those reported for the HR^- monoanion of fluorescein in water: $\varphi = 0.36$ [22] and 0.37 [23]. The single-charged anion of fluorescein contains the OH group in the xanthene moiety (the $\mathbf{HQ^-}$ tautomer in Scheme 1), instead of the CH_3O group in the case of the $\mathbf{Q^-}$ anion of methyl ether. Finally, the relatively low ε_{max} values of the HR species of methyl ether permits to assume the substantial fraction of the colorless lactone **HL**; for the H_2R of the parent dye, the ε_{max} values 13.9 $\times10^3$ at 437 nm and (3–4) $\times10^3$ at λ within the range of 470–485 nm allowed to deduce the co-existence of the colored tautomers $\mathbf{H_2Z^{\pm}}$ and $\mathbf{H_2Q}$ with the colorless $\mathbf{H_2L}$ [20]. (The data for fluorescein in water are from Ref. [20].)

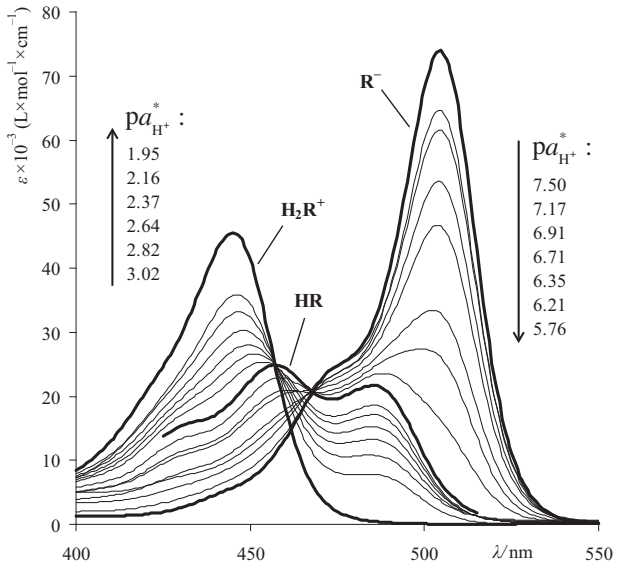


Fig. 5. Absorption spectra of fluorescein ethyl ester in 50 mass% aqueous ethanol at different $pa^*_{H^+}$ and limiting dye forms (heavy lines).

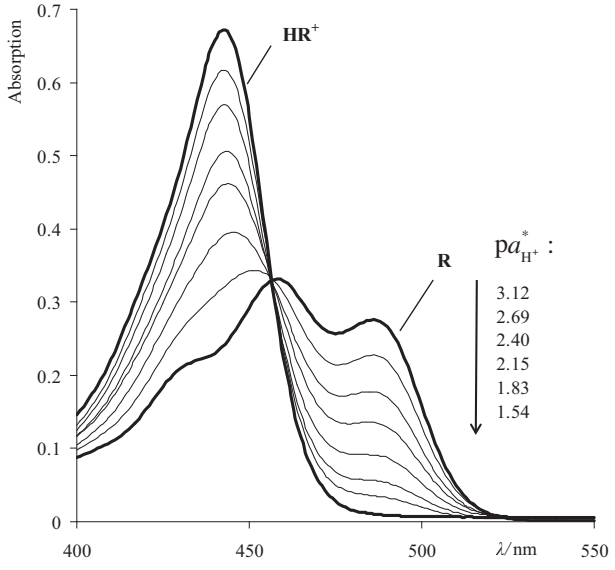


Fig. 6. Absorption spectra of fluorescein methyl ether ester in 50 mass% aqueous ethanol at different $pa^*_{H^+}$ and limiting dye forms (heavy lines).

Table 1
The thermodynamic values of the indices of dissociation constants in 50 mass% aqueous ethanol.

Compound	pK_{a0}	pK_{a1}	pK_{a2}
Methyl ether ester of fluorescein	2.28 ± 0.01	–	–
6-Hydroxy-9-phenyl fluorone[a]	2.34 ± 0.03	6.80 ± 0.08	–
Ethyl ester of fluorescein	2.05 ± 0.01	6.71 ± 0.01	–
Sulfonefluorescein	–	3.22 ± 0.01	7.63 ± 0.01
Methyl ether of fluorescein	0.39 ± 0.01	7.32 ± 0.01	–
Fluorescein[b]	0.94 ± 0.05	6.82 ± 0.05	7.66 ± 0.05

[a] Ref. [61].
[b] Ref. [64].

Tautomerism of the neutral form of the fluorescein methyl ether

The shape of the spectral curves of the methyl ether and methyl ether–ester obtained in the present paper agrees with those published by others [32–36]. It should be noted, that the presence of the charged COO^- group in the phthalic residue shifts the band of the xanthene moiety hypsochromically [7,22,25,27,28,37,58,64,65]. This finding has been supported via quantum-chemical calculations [66]. As it is seen from Table 2, the band of the dianion of fluorescein, structure $\mathbf{X^{2-}}$, is shifted by 8–10 nm towards the lower wavelengths against the bands of 6-hydroxy-9-phenylfluorone and ethyl ester of fluorescein (structures $\mathbf{X^-}$).

The comparison of the λ_{max} values of the methyl ether anion, structure $\mathbf{Q^-}$ (Scheme 2), and fluorescein monoanion ($\mathbf{HQ^-}$), with those of the molecular species of ether–ester (structure **Q**) and 6-hydroxy-9-phenylfluorone and ethyl ester of fluorescein (**HQ**) also confirm this regularity (Table 1). The ε_{max} values are very close for $\mathbf{Q^-}$ and **Q** species of methyl ether and the ether-ester, respectively. However, on going from 50% to 96% ethanol or entire organic solvents, the molar absorptivities exhibit some differences.

The absorption spectrum of the methyl ether molecular form HR in 50% ethanol resembles by shape that of the anion of the same dye (Figs. 2 and 8). Here, the band of the **HQ** tautomer is also batochromically shifted against the band of the anion $\mathbf{Q^-}$, whereas the tremendous difference in intensity is certainly caused by the high fraction of the colorless tautomer **HL**.

The fractions of the tautomers of the neutral form HR may be estimated using the relation $\varepsilon = \varepsilon_{HZ^\pm} \times \alpha_{HZ^\pm} + \varepsilon_{HQ} \times \alpha_{HQ}$ at a fixed wavelength, with understanding that $\alpha_{HL} = 1 - \alpha_{HZ^\pm} - \alpha_{HQ}$. As it was earlier assumed for fluorescein [6,7,19,20,58], the absorptivities of the zwitterionic and quinonoidal tautomers, especially around the band maxima (Fig. 8), may be equated to those of the cation and monoanion, respectively. Therefore, before using the molar absorptivities $\varepsilon_{H_2Z^+}$ and ε_{Q^-} instead of $\varepsilon_{HZ^\pm}$ and ε_{HQ}, respectively, the spectra of the ionic forms were shifted to several nanometers in order to reach the coincidence of their maxima with those of the corresponding tautomers.

Table 3
The thermodynamic values of the indices of dissociation constants in water.

Compound	pK_{a0}	pK_{a1}	pK_{a2}
Methyl ether of fluorescein	1.94 ± 0.01	4.73 ± 0.01	–
Ethyl ester of fluorescein[a]	2.94 ± 0.07	6.31 ± 0.03	–
6-Hydroxy-9-phenyl fluorone[b]	3.10 ± 0.02	6.28 ± 0.06	–
Sulfonefluorescein[c]	–	3.22 ± 0.11	6.76 ± 0.03
Fluorescein[d]	2.14 ± 0.01	4.45 ± 0.02	6.80 ± 0.01

[a] Ref. [64].
[b] Refs. [20,61,64].
[c] Refs. [27,58].
[d] Refs. [6,20,27,28].

In water solution, the molecular spectrum (Fig. 8) gives evidence for the presence both of the zwitterion $\mathbf{HZ^\pm}$ and quinonoid **HQ**. Again, the band 433 nm is hypsochromically shifted as compared with the cationic band of this dye in water (437 nm), thus confirming the influence of the COO^- group on the xanthene portion.

The fractions of the tautomers $\mathbf{HZ^\pm}$ and **HQ** in water are 0.11 and 0.06 respectively, and the rest refer to the lactone **HL** (0.83). Consequently, $K_T = 13.8$; $K_T^{/} = 1.83$. In 50% ethanol, the zwitterionic tautomer does not manifest itself in the spectrum of the molecular species, whereas the fractions of the quinonoid and lactone are 0.01 and 0.99; $K_T = 99$. The corresponding fractions for the parent compound fluorescein are in water [6,20,27,28]: 0.22 ($\mathbf{H_2Z^\pm}$), 0.11 ($\mathbf{H_2Q}$), and 0.67 ($\mathbf{H_2L}$), while in 50 mass% ethanol [64]: 0.0325 and 0.967 ($K_T = 29.7$). One may note that the tendency to lactone formation is somewhat more expressed in the case of the fluorescein methyl ether.

At this place it should be noted that all these calculations, as well as the above cited for the parent compound fluorescein, are made with the connivance about the absence of the colorless tautomer of the monoanion (Scheme 2). Therefore, it was necessary to examine this statement.

Examining the possibility of lactone–monoanion formation

One of the aims of the present study was to verify the absence of the monoanion-lactone of the methyl ether and thus to conclude about the corresponding tautomerism of the fluorescein (Scheme 2, $\mathbf{Q^-} \rightleftarrows \mathbf{L^-}$). The intensity of the methyl ether anionic band in water and in 50% aqueous ethanol stays practically constant. For estimating the molar absorptivities of fluorescein methyl ether monoanion in methanol, 95.6% aqueous ethanol, 1-butanol, dichloromethane, and acetone (Fig. 9), the working solutions were prepared by diluting of initial dye solution in acetone with appropriate solvent. The volume fraction of acetone in target mixture equals 1%. In water and aqueous ethanol, the complete

Table 2
Spectral characteristics of dyes in 50 mass % aqueous ethanol.

Dye	λ_{max}/nm ($\varepsilon \cdot 10^{-3}$/L mol^{-1} cm^{-1})			
	Dianion	Monoanion	Neutral form	Cation
Fluorescein methyl ether (in water)	–	452–456 (30.9) 472–476 (29.8)	432–434 (7.61)	436–438 (54.0)
Fluorescein methyl ether	–	452–455 (30.9) 478–481 (27.4)	454–457 (0.33) 481–488 (0.26)	441 (54.5)
Fluorescein methyl ether ester	–	–	457–459 (29.1), 486 (24.5)	442–443 (56.9)
6-Hydroxy-9-phenylfluorone	–	503	457, 485	445
Fluorescein ethyl ester	–	504–505	456–459, 483–487	445
Sulfonefluorescein	503 (91.6)	455–458 (30.7) 483–487 (28.3)	445–447 (54.6)	–
Fluorescein[a]	495 (88.5)	455 (34.3)	455 (0.978)	445 (62.4)

[a] Ref. [64].

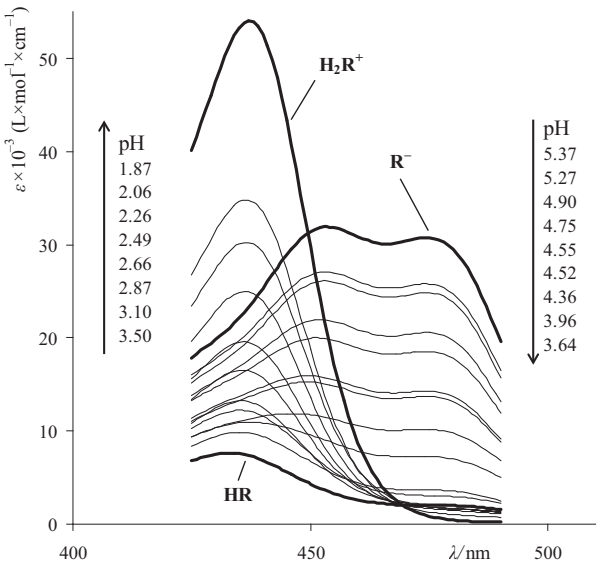


Fig. 7. Absorption spectra of fluorescein methyl ether aqueous solutions at different pH and limiting dye forms (heavy lines).

transformation of the dye into the anion was reached by introducing appropriate amounts of diluted NaOH. In non-aqueous solutions, DBU with concentration circa 0.02 mol L^{-1} was used as a deprotonating agent.

The spectra of the anion exhibit strong variation of the shape and still more of intensity. It should be noted that the effects are less marked in the hydrogen bond donor (HBD) solvents as compared with that in the non-HBD ones. This may be connected with the character of solvation of the COO^- group. On the other hand, the variations are much less expressed for the neutral quinonoid form of the ether-ester, which is unable to lactonization (Fig. 9). Therefore, we had to clarify if these alterations in molar absorptivity are caused either by entire solvatochromic effects of the colored species $\mathbf{Q}^-$ or by formation of the colorless lactone $\mathbf{L}^-$.

Therefore, the study was furthered with the help of IR and NMR spectroscopy.

In the IR spectra of sodium salt of fluorescein methyl ether in solid state as well as in DMSO solution the band corresponding

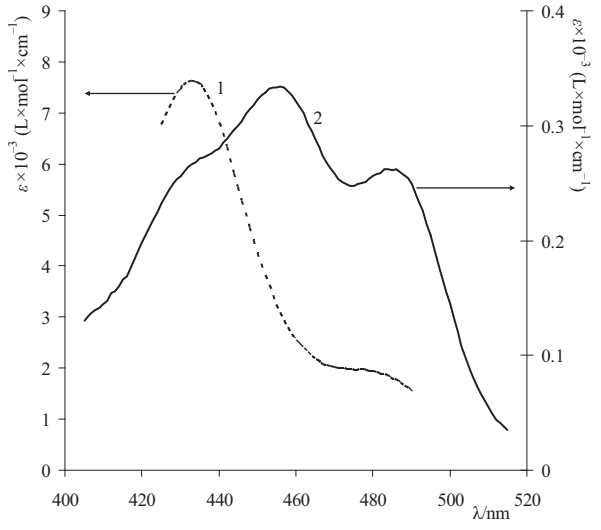


Fig. 8. Absorption spectra of neutral form of fluorescein methyl ether in aqueous (dashed line) and 50 mass% aqueous ethanol (solid line) solutions.

the stretching vibrations of the C=O is poorly expressed. The distinct decrease of this band intensity was observed while deprotonation of neutral form in solution occurs under the action of NaOH to the DMSO solution.

In the ^{13}C NMR spectrum of the sodium salt of fluorescein methyl ether, one carbon signal was not observed. This missing signal was assigned to the central carbon atom by HMBC and HSQC experiments and is due to the equilibrium quinonoid – lactone. The last-named structure may be either neutral, **HL**, which can be formed as a result of incomplete deprotonation of the dye or hydrolysis by residual water in DMSO-d_6, or anionic, $\mathbf{L}^-$.

After adding NaOH to the solution of neutral form in DMSO, the signal δ = 153.33 ppm was registered that indicates complete conversion to the anionic form. The value of chemical shift agrees well with the value for the central carbon signal of methyl ether ester (δ = 150.14 ppm). Thus, the NMR data do not give evidence of existence of the $\mathbf{L}^-$ species for fluorescein methyl ether.

The addition of trifluoroacetic acid to the salt solution leads to restoring of the neutral lactonic structure and appearing of signal 83.09 ppm of sp^3 hybridization of the central atom.

Note, that the presence of excess of NaOH results in appearance of signal 72.63 ppm that is due to the hydroxylation of central atom with possible destruction (rupture) of the xanthene moiety.

Hence, the existence of lactoid monoanion of methyl fluorescein in solution seems to be unlikely. The same may be deduced for the parent compound fluorescein; such conclusion is helpful in understanding the equilibrium of the latter dye (Scheme 1), because the yield of the species HR^- of fluorescein in solution is relatively small, especially in organic solvents, and thus its UV–vis and in particular IR and ^{13}C NMR spectra are less available.

In the case of ethers of eosin and rose Bengal B, the fraction of the lactonic monoanion is very high [37]. Such difference between these dyes and the methyl ether of the unsubstituted fluorescein should be easily explained by the dramatic strengthening of the acidity of the hydroxy group due to introduction of the two Br or I atoms in the *ortho*-position.

Microscopic dissociation constants

From the Schemes 1 and 2, the following equations may be derived:

$$pK_{a0} = pk_{0,OH} - \log(1 + K_T + K_T^{/}) \quad (3)$$

$$pK_{a1} = pk_{1,COOH} + \log(1 + K_T + K_T^{/}) \quad (4)$$

The difference between fluorescein and its methyl ether consists in the possibility of the parent compound to dissociate in an additional step (the pK_{a2} value). Also, it possesses two hydroxy groups in the xanthene portion, contrary to the sole OH group of the ether.

Knowing the fractions of the tautomers **HQ** and $\mathbf{HZ}^{\pm}$ (see above), one can estimate the microscopic ionization constants. In Table 4, they are compared with those of fluorescein. The similarity of the corresponding p*k* values of fluorescein and its methyl ether is striking. In the case of $pk_{1,COOH}$ and even $pk_{\pm,COOH}$ it is natural because of the absence of conjugation between the xanthene moiety and the phthalic acid residue.

For the $pk_{1,Z}$ and $pk_{0,OH}$ values, however, it is an insubstantial coincidence. Indeed, the existence of two OH groups in fluorescein, fluorescein ethyl ester and 6-hydroxy-9-phenyl fluorone means that the last-mentioned p*k* must be log 2 = 0.301 higher if ascribed to a single OH group. But the $pk_{0,OH}$ values of sole OH group of the methyl ether ester (2.28) and methyl ether (2.31) are close to the

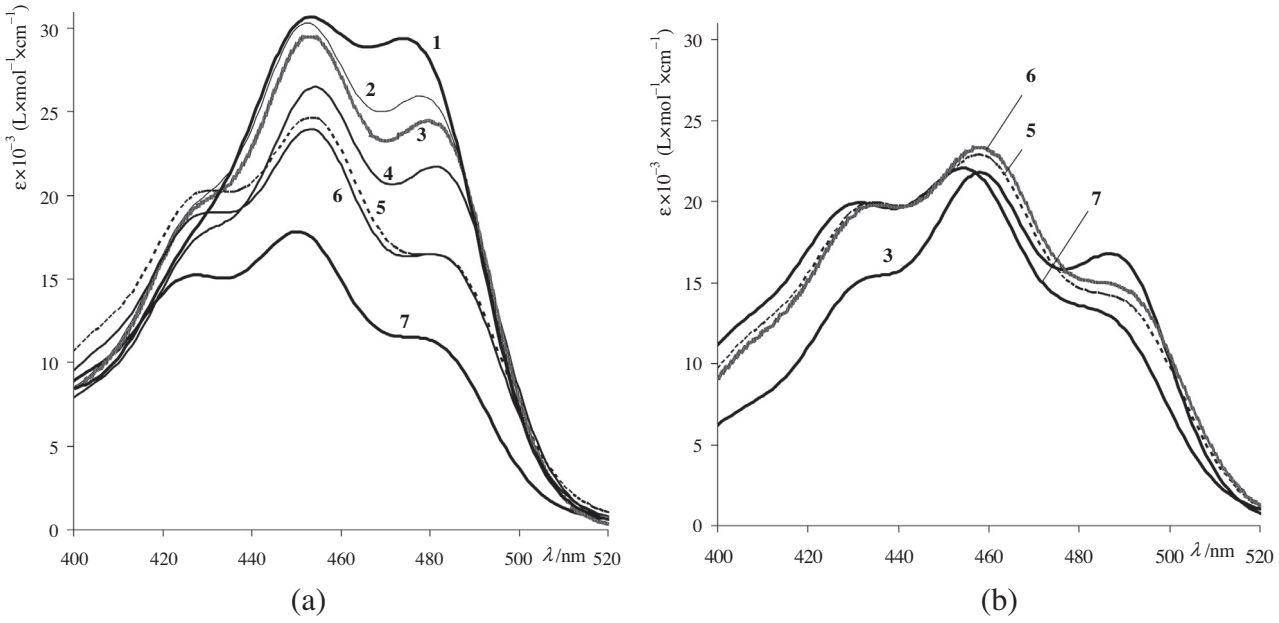


Fig. 9. Absorption spectra of fluorescein methyl ether monoanion (a) and fluorescein methyl ether ester (b) in water (1), methanol (2), 95.6% aqueous ethanol (3), 1-butanol (4), dichloromethane (5), DMSO (6) acetone (7). Solutions 1–6 contain 1 vol% acetone.

Table 4
The indices of the microscopic ionization constants of methyl ether of fluorescein and those of the parent compound.

pk	Water		50 mass% aqueous ethanol	
	Methyl ether of fluorescein	Fluorescein	Methyl ether of fluorescein	Fluorescein
p$k_{1,\mathrm{COOH}}$	3.48	3.49	5.40	5.38
p$k_{1,Z}$	3.78	3.79	–	–
p$k_{0,\mathrm{OH}}$	3.19	3.10	2.31	2.41
p$k_{\pm,\mathrm{COOH}}$	2.89	2.80	–	–

values of 6-hydroxy-9-phenyl fluorone (2.34) and fluorescein (2.41); only for the ethyl ester of fluorescein the p$k_{0,\mathrm{OH}}$ value 2.05 is really lower. Hence, it may be concluded that the substitution of the OH group in the xanthene moiety by the OCH_3 group somewhat increases the acidity of the remaining hydroxyl group. In methyl alcohol [56], this effect is even more pronounced: the p$k_{0,\mathrm{OH}}$ value of fluorescein ethyl ether ester was found to be 4.79 ± 0.03, whereas for fluorescein ethyl ester and 6-hydroxy-9-phenyl fluorone: p$k_{0,\mathrm{OH}} = 5.23 \pm 0.10$ and 5.14 ± 0.03, respectively. The low value of 4.8 for the unsubstituted fluorescein may be explained by the contributory uncertainty of the estimation of the small fraction of the quinonoid tautomer H_2Q absolute methanol [65].

In order to estimate the fraction of the **HZ**$^{\pm}$ tautomer in aqueous ethanol, the data for the dye sulfonefluorescein should be used. Indeed, its pK_{a1} value is in fact p$k_{1,Z}$. On the other hand, from Scheme 2 the following equation may be derived:

$$\log K_T^{/} = \mathrm{p}k_{0,\mathrm{OH}} - \mathrm{p}k_{\pm,\mathrm{COOH}} = \mathrm{p}k_{1,Z} - \mathrm{p}k_{1,\mathrm{COOH}} \quad (5)$$

Using the pK_{a1} value of sulfonefluorescein, which is in fact p$k_{1,Z}$, and the p$k_{1,\mathrm{COOH}}$ value of fluorescein methyl ether, one can obtain $\log K_T^{/} = -2.18$. It means that the fraction of the **HZ**$^{\pm}$ tautomer is 150 times lower than that of the quinonoid **HQ**, which is in turn only as low as 0.01. Such dramatic destabilization of the dipolar tautomer is in line with behavior of the parent compound fluorescein [6,7,27–29,58,59,64,65]. The zwitterionic tautomer of sulfonefluorescein, however, predominates in any solvent, owing to the weak basicity of the SO_3^- group; recently it was confirmed by quantum-chemical calculations [31].

Moreover, one may estimate the p$k_{\pm,\mathrm{COOH}} = 4.49$ value of fluorescein methyl ether on aqueous ethanol by using the above $\log K_T^{/}$ value and p$k_{0,\mathrm{OH}} = 2.31$.

The pK_{a2} values of fluorescein (7.66) and sulfonefluorescein (7.63) coincide. As the lactone-like (sultone) tautomer of the HR^- species of the latter dye is improbable, the same may be deduced for the fluorescein monoanion as well. The difference δpK_a between the pK_{a2} of these dyes and the average pK_{a1} value of ethyl ester of fluorescein and 6-hydroxy-9-phenyl fluorone (which are also rather close: 6.71 and 6.80) is 0.89, while in water δpK_a equals to 0.48. These differences should be explained in terms of the Bjerrum – Kirkwood – Westheimer equation [28], which describes the influence of the charged group on the pK_a of the ionizing one.

$$\delta \mathrm{p}K_a = \frac{e^2 N_A}{4\pi \times 8.854 \times 10^{-12} \times 2.303 RTrD_{eff}}. \quad (6)$$

Here e is the elemental electrical charge, N_A is the Avogadro number, R is the gas constant, T is the absolute temperature, r is the distance between the ionizing and charged groups, and D_{eff} is the effective permittivity of the space between the two above groups.

It should be noted that somewhat smaller difference following from the data by Fompeydie and Levillain in 50 vol% aqueous methanol (fluorescein: p$K_{a2} = 6.86$, ethyl fluorescein: p$K_{a1} = 6.36$) [25] may be explained by the higher relative permittivity value of the solvent as compared with our ethanol–water mixture. Also, there are no indications of the thermodynamic character of their pK_a s; if they are expressed in the concentration scale, the re-calculation to the thermodynamic values will increase the discussed difference.

The same equation explains the difference between the p$k_{1,Z} = 3.22$ value of sulfonefluorescein in 50% ethanol and the five p$k_{0,\mathrm{OH}}$ of fluorescein, its ethyl ester, methyl ether, methyl ether-ester, and 6-hydroxy-9-phenylfluorone (2.05–2.41) in the same solvent. Here, δpK_a is within the range of 0.81–1.17.

Conclusions

The spectroscopic study of the protolytic equilibria in solutions of methyl ether and methyl ether-ester of fluorescein, as well as related compounds sulfonefluorescein, ethyl ester of fluorescein, and 6-hydroxy-9-phenylfluorone enable better understanding of the acid-base dissociation and tautomerism of the widely used unique parent dye fluorescein. The neutral (molecular) form of fluorescein methyl ether in solution is an equilibrium mixture of three tautomers: zwitter-ionic, **HZ**$^{\pm}$, quinonoid, **HQ**, and colorless lactonic one, **HL**. In water, the fractions are correspondingly 11%, 6%, and 83%. On going from water to 50% aqueous ethanol, the fraction of the dipolar tautomer drops sharply to ca. 0.01%, that of the quinonoid decreases markedly (1%), whereas the colorless lactone predominates. The last-named tautomer was liberated and characterized by the X-ray analysis that revealed a substantially longer C–O bond for the central carbon atom as compared with fluorescein and common γ-lactones.

Another problem that has been considered is the possibility of formation of monoanion having lactonic structure **HL**. The utilization of UV–visible, IR, and ^{13}C NMR spectroscopy allows to consider the anion–lactone of fluorescein methyl ether as unlikely. The same may be deduced for the parent compound fluorescein; such conclusion is helpful in understanding the equilibrium of the latter dye (Scheme 1), because the yield of the species HR^{-} of fluorescein in solution is relatively small, especially in organic solvents, and thus its UV–vis and in particular IR and ^{13}C NMR spectra are less available.

The microscopic dissociation constants of fluorescein methyl ether in water and in 50% ethanol coincide with the corresponding quantities for fluorescein, thus confirming the adequacy of the general scheme of protolytic equilibria in solution.

Acknowledgements

The authors express their gratitude to Dr. D. Yu. Filatov and Dr. D. S. Sofronov for measuring the IR spectra and to Dr. A. D. Roshal for fluorescence measurements.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2015.05.037.

References

[1] X. Li, X. Gao, W. Shi, H. Ma, Chem. Rev. 114 (2014) 590.
[2] D.S. Pellosi, B.M. Estevao, C.F. Freitas, T.M. Tsbone, W. Caetano, N. Hioka, Dyes Pigm. 99 (2013) 705.
[3] A. Niazi, A. Yazdanipour, J. Ghasemi, M. Kubista, A. Sheyda, M. Alikhah, Croat. Chem. Acta 82 (2009) 753.
[4] V.R. Batistela, J. da Costa Cedran, H.P. Moisés de Oliveira, I.S. Scarminio, L. Ueno, A.E. da Hora Machado, N. Hioka, Dyes Pigm. 86 (2010) 15.
[5] X.-F. Zhang, Photochem. Photobiol. Sci. 9 (2010) 1261.
[6] L.N. Bogdanova, N.O. Mchedlov-Petrossyan, N.A. Vodolazkaya, A.V. Lebed, Carbohydr. Res. 345 (2010) 1882.
[7] N.O. Mchedlov-Petrossyan, N.A. Vodolazkaya, Y.A. Gurina, W.-C. Sun, K.R. Gee, J. Phys. Chem. B 114 (2010) 4551.
[8] V.R. Batistela, D.S. Pellosi, F.D. de Souza, W.F. da Costa, S.M. de Olivera Santin, V.R. de Souza, W. Caetano, H.P. Moisés de Oliveira, I.S. Scarminio, N. Hioka, Spectrochim. Acta A 79 (2011) 889.
[9] N.G. Greeneltch, A.S. Davis, N.A. Valley, F. Casadio, G.C. Schatz, R.P. Van Duyne, N.C. Shah, J. Phys. Chem. A 116 (2012) 11863.
[10] H. Yao, J.D. Steill, J. Oomens, R.A. Jockusch, J. Phys. Chem. A 115 (2011) 9739.
[11] P.D. McQueen, S. Sagoo, H. Yao, R.A. Jockusch, Angew. Chemie Int. Ed. 49 (2010) 9193.
[12] H. Yao, R.A. Jockusch, J. Phys. Chem. A 117 (2013) 1351.
[13] T. Tanabe, M. Saito, K. Noda, J. Phys. Conf. Ser. 388 (2012) 022106.
[14] T. Tanabe, M. Saito, K. Noda, E.B. Starikov, Eur. Phys. J. D 66 (2012) 163.
[15] T. Ueno, Y. Urano, K. Setsukinai, H. Takakusa, H. Kojima, K. Kikuchi, K. Ohkubo, S. Fukuzumi, T. Nagano, J. Am. Chem. Soc. 126 (2004) 14079.
[16] Y. Urano, M. Kamiya, K. Kanda, T. Ueno, K. Hirose, T. Nagano, J. Am. Chem. Soc. 127 (2005) 4888.
[17] L.D. Lavis, R.T. Raines, ACS Chem. Biol. 3 (2008) 142.
[18] A. Persat, M.E. Suss, J.G. Santiago, Lab Chip 9 (2009) 2454.
[19] L. Lindqvist, Ark. Kemi 16 (1960) 79.
[20] N.O. Mchedlov-Petrossyan, Zhurn. Anal. Khim. 34 (1979) 1055.
[21] Z. Tamura, T. Morioka, M. Maeda, A. Tsuji, Bunseki Kagaku 43 (1994) 339.
[22] N. Klonis, W.H. Sawyer, J. Fluoresc. 6 (1996) 147.
[23] R. Sjöback, J. Nygren, M. Kubista, Spectrochim. Acta A 51 (1995) L7.
[24] M. Shibata, M. Nakamizo, H. Kakiyama, Nippon Kagaku Kaishi 1972 (1972) 681.
[25] D. Fompeydie, F. Onur, P. Levillain, Bull. Soc. Chim. Fr. 9–10 (1979) 375.
[26] D. Fompeydie, P. Levillain, Bull. Soc. Chim. Fr. 11–12 (1980) 459.
[27] N.O. Mchedlov-Petrossyan, R.S. Mayorga, J. Chem. Soc. Faraday Trans. 88 (1992) 3025.
[28] N.O. Mchedlov-Petrossyan, V.I. Kukhtik, V.D. Bezugliy, J. Phys. Org. Chem. 16 (2003) 380.
[29] N.O. Mchedlov-Petrossyan, N.A. Vodolazkaya, N.V. Salamanova, A.D. Roshal, D.Y. Filatov, Chem. Lett. 39 (2010) 30.
[30] N.O. Mchedlov-Petrossyan, N.A. Vodolazkaya, Y.N. Surov, D.V. Samoylov, Spectrochim. Acta A 61 (2005) 2747.
[31] A.V. Lebed, A.V. Biryukov, N.O. Mchedlov-Petrossyan, Chem. Heterocycl. Compd. 50 (2014) 336.
[32] S.-C. Chen, H. Nakamura, Z. Tamura, Chem. Pharm. Bull. 27 (1979) 475.
[33] Z.-G. Zhao, T. Shen, H. Xu, Spectrochim. Acta A 45 (1989) 1113.
[34] H. Zhang, M. Zhang, T. Shen, J. Photochem. Photobiol. A 103 (1997) 63.
[35] A. Song, J. Zhang, M. Zhang, T. Shen, J. Tang, Colloids Surfaces A 167 (2000) 253.
[36] X.-F. Zhang, Q. Liu, H. Wang, Z. Fu, F. Zhang, J. Photochem. Photobiol. A 200 (2008) 307.
[37] F. Amat-Guerri, M.M.C. López-González, R. Sastre, R. Martínez-Utrilla, Dyes Pigm. 13 (1990) 219.
[38] Y. Nagase, T. Ohno, T.J. Goto, Pharm. Soc. Japan 75 (1953) 1039.
[39] J.-H. Scharf, Mikroskopie 11 (1956) 261.
[40] V. Zanker, W. Peter, Chem. Ber. 91 (1958) 572.
[41] M.M. Martin, L. Lindqvist, J. Lumin. 10 (1975) 381.
[42] H. Diehl, N. Horchak-Morris, A.J. Hefley, L.F. Munson, R. Markuszewski, Talanta 33 (1986) 901.
[43] O. Fischer, E. Hepp, Berichte der Dtsch. Chem. Gesellschaft 46 (1913) 1951.
[44] W.R. Orndorff, R.S. Vose, J. Am. Chem. Soc. 46 (1924) 1836.
[45] R.G. Bates, Determination of pH, Khimiya, Leningrad, 1972.
[46] E. Kozáková, B. Cséfalvayová, J. Zajacová, Chem. Zvesti 36 (1982) 345.
[47] O.V. Dolomanov, L.J. Bourhis, R.J. Gildea, J.A.K. Howard, H. Puschmann, J. Appl. Crystallogr. 42 (2009) 339.
[48] G.M. Sheldrick, Acta Crystallogr. A 64 (2008) 112.
[49] http://www-chemo.univer.kharkov.ua/kholin/clinp.html.
[50] R.S. Osborn, D. Rogers, Acta Crystallogr. B 31 (1975) 359.
[51] I.N. Polyakova, Z.A. Starikova, B.V. Parusnikov, I.A. Krasavin, G.M. Dobryakova, B.V. Zhadanov, J. Struct. Chem. 25 (1985) 752.
[52] D.-K. Bučar, S. Filip, M. Arhangelskis, G.O. Lloyd, W. Jones, CrystEngComm 15 (2013) 6289.
[53] F.H. Allen, Acta Crystallogr. A 58 (2002) 380.
[54] H.-B. Burgi, J.D. Dunitz, Structure Correlation, VCH, Weinheim, 1994.
[55] U. Anthoni, C. Christophersen, P.H. Nielsen, A. Püschl, K. Schaumburg, Struct. Chem. 6 (1995) 161.
[56] Y.V. Zefirov, Crystallogr. Rep. 42 (1997) 865.
[57] M. Davies, R.L. Jones, J. Chem. Soc. (1954) 120.
[58] N.O. Mchedlov-Petrossyan, R.S. Mayorga, Y.N. Surov, Zhurn. Obsh. Khim. 61 (1991) 225.
[59] N.O. Mchedlov-Petrossyan, Y.N. Surov, S.I. Egorova, R.S. Mayorga, E. Arias Kordova, Dokl. Akad. Nauk SSSR 317 (1991) 152.
[60] SDBSWeb: http://sdbs.db.aist.go.jp.
[61] N.O. Mchedlov-Petrossyan, Dokl. Akad. Nauk SSSR 293 (1987) 1178.
[62] S. Niizuma, K. Kikuchi, H. Kokubun, J. Photochem. 36 (1987) 51.
[63] B.Y. Frolov, M.A. Babashov, S.G. Kudryavtsev, Zhurn. Fiz. Khim. 66 (1992) 2970.
[64] N.O. Mchedlov-Petrossyan, Kharkov Univ. Bull. 626 (2004) 221.
[65] N.O. Mchedlov-Petrossyan, L.V. Vasetskaya, Zhurn. Obsh. Khim. 59 (1989) 691.
[66] N.O. Mchedlov-Petrossyan, V.V. Ivanov, Zhurn. Fiz. Khim. 81 (2007) 117.