ISSN 1070-3632, Russian Journal of General Chemistry, 2009, Vol. 79, No. 7, pp. 1437–1445. © Pleiades Publishing, Ltd., 2009.
Original Russian Text © N.A. Vodolazkaya, P.V. Shakhova, N.O. Mchedlov-Petrosyan, 2009, published in Zhurnal Obshchei Khimii, 2009, Vol. 79, No. 7, pp. 1081–1089.

Acid–Base and Tautomeric Equilibria of Fluorescein Dyes in Water Micellar Solutions of Zwitterionic Sulfobetaine Surfactant

N. A. Vodolazkaya, P. V. Shakhova, and N. O. Mchedlov-Petrosyan

Karazin Khar'kov National University,
pl. Svobody 4, Khar'kov, 61077 Ukraine
e-mail: mchedlov@univer.kharkov.ua

Received April 28, 2008

Abstract—Ionization constants of fluorescein and six its derivatives in water micellar solutions of zwitterionic surfactant, *N*-cetyl-*N,N*-dimethyl-3-ammonium-1-propanesulfonate were evaluated spectrophotometrically. On the basis of absorption spectra of dyes conclusions concerning the tautomerism of molecular and ionic forms were made and the relationships between the pK_a values were explained.

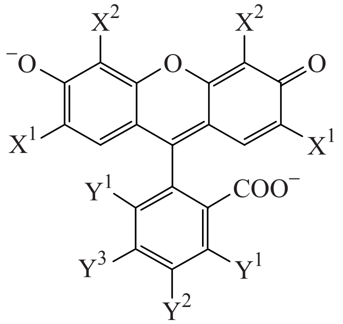
DOI: 10.1134/S1070363209070068

Hydroxyxantene dyes due to their unique photophysical properties are widely used in the investigations of true and colloid solutions as well as in biochemistry, biomedicine, photophysics, and neighboring sciences 1–4]. Absorption and fluorescence spectra of these dyes strongly depend on pH [1, 2, 5, 6]. That is why for the rational use of dyes it is necessary to have data on their ionization constants in corresponding solvents.

Nowadays fluorescein and some of its derivatives such as eosine, erytrosine, Bengal Rose and some others are among the most thoroughly studied [5, 7–13]. Meanwhile there exists a series of fluorescein dyes presenting significant theoretical and practical interest [1–3, 6], but significantly less studied or even never studied at all. From the other side, in spite of the great number of works dealing with the protolytic equilibria of various dyes and indicators, among them of hydoxyxantenes, in the micellar solutions of cationic, anionic, and nonionic colloid surfactants [5, 8, 10, 14–16], data on the ionization constants of organic reagents in the solutions of zwitterionic amphiphiles are practically absent. Meanwhile, just the micelles of such compounds resemble the phospholipid lyposomes.

That is why continuing the systematic studies of the protolytic properties of hydroxyxantene dyes [5, 7–10, 12] we have studied the acid–base and tautomeric transformations of seven fluorescein dyes in the micellar solutions of zwitterionic sulfobetaine surfactant, *N*-cetyl-*N,N*-dimethyl-3-ammonium-1-propanesulfonate, $n\text{-}C_{16}H_{33}N(CH_3)_2^+–(CH_2)_3–SO_3^-$. Crytical concentration of micelle formation of this amphiphile is about 5×10^{-5} mol l^{-1} [17], and the aggregation number $\geq10^2$ [18].

We have studied fluorescein **I**, fluorescein isothiocyanate *I* (**II**), 2,7-dichlorofluorescein **III**, 4,5-dibromofluorescein **IV**, 2,4,5,7-tetrabromofluorescein (eosine) **V**, 3',4',5',6'-tetrabromofluorescein **VI**, and 2,4,5,7,3',4',5',6'-octabromofluorescein **VII**. Their structural formulas are presented below.



I, $X^1 = X^2 = Y^1 = Y^2 = Y^3 = H$; **II**, $X^1 = X^2 = Y^1 = Y^3 = H$; $Y^2 = NCS$; **III**, $X^1 = Cl$, $X^2 = Y^1 = Y^2 = Y^3 = H$; **IV**, $X^1 = Y^1 = Y^2 = Y^3 = H$; $X^2 = Br$; **V**, $X^1 = X^2 = Br$, $Y^1 = Y^2 = Y^3 = H$; **VI**, $X^1 = X^2 = H$, $Y^1 = Y^2 = Y^3 = Br$; **VII**, $X^1 = X^2 = Y^1 = Y^2 = Y^3 = Br$.

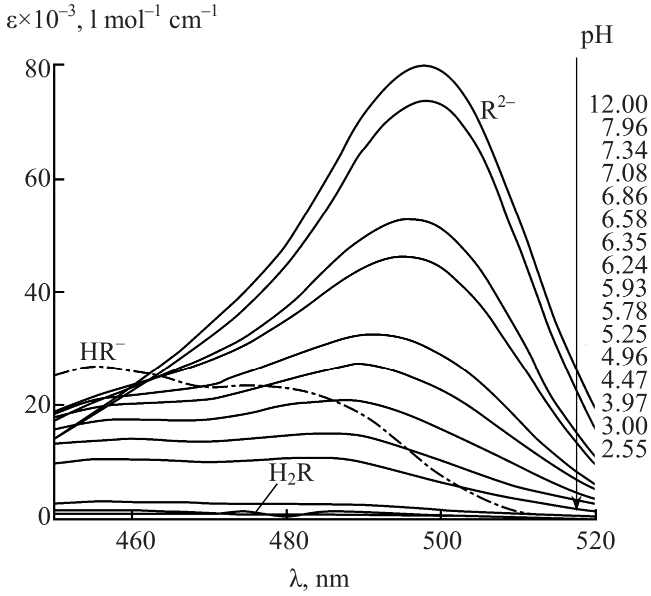


Fig. 1. Absorption spectra of fluorescein at varied pH in the micellar solution of *N*-cetyl-*N,N*-dimethylammonium-propanesulfonate. Spectrum of R^{2-} form was measured at pH 12, spectra of HR^- and H_2R forms were extracted from the spectra of equilibral mixtures by means of the established pK_a^a meanings.

pK_a Values corresponding to the step-by-step ionization of dyes [Eqs. (1)–(3)] were evaluated at 25.0ºC and the ion force 0.05 mol l^{-1} (NaCl and the components of buffer solution) by means of the spectrophotometric method.

$$H_3R^+ \rightleftarrows H_2R + H^+,\ K_{a,0}, \quad (1)$$

$$H_2R \rightleftarrows HR^- + H^+,\ K_{a,1}, \quad (2)$$

$$HR^- \rightleftarrows R^{2-} + H^+,\ K_{a,2}. \quad (3)$$

Concentrations of dyes were as a rule about 10^{-6}–10^{-5} mol l^{-1}, and the concentration of $n\text{-}C_{16}H_{33}N(CH_2)_2^+$–$(CH_2)_3$–$SO_3^-$ was 1.5×10^{-3} mol l^{-1}. Hence, one micelle of surfactant contained not more than one molecule of dye.

While studying the acid–base equilibria of the indicator dyes the key characteristics are the so-called apparent pK_a^a values (upper index means apparent) [8, 10, 14, 16]. The concentration ratio of the equilibral forms of indicator ($HB^z \rightleftarrows B^{z-1} + H^+$) in the colloid system is determined spectrophotometrically, and the pH_w value characterizing the continuous or voluminous (water) phase is as a rule determined potentiometrically [Eq. (4)]:

$$pK_a^a = pH_w + \log [HB^z]/[B^{z-1}]. \quad (4)$$

In fact, K_a^a value is the constant of the two-phase acid–base equilibrium under the condition of the complete or partial binding of both or one of the equilibrium forms of indicator with the micellar pseudophase. Last term emphasizes the conventionality of using the term “phase” to such nano-dimentional objects as the micelles of surfactants [16].

Examples of typical dependences of the absorption spectrum and molar absorption coefficient of the dye solution on pH are presented in the Figs. 1, 2. Spectra of R^{2-} ions were measured at pH 9–12 in the borate buffers and in the diluted NaOH solutions. These and all other spectra were obtained at the constant ion force 0.05 mol l^{-1} maintained by NaCl addition to the phosphate and acetate buffer mixtures and to the HCl solutions. Exclusion was made for the experiments in the acidic media especially while obtaining of spectra of H_3R^+ cations of compounds **I, II** in the concentrated hydrogen chloride solutions when the ion force was not maintained constant.

In all the cases at the exclusion of eosine **V** Eqs. (2) and (3) strongly overlap. Due to that the previously

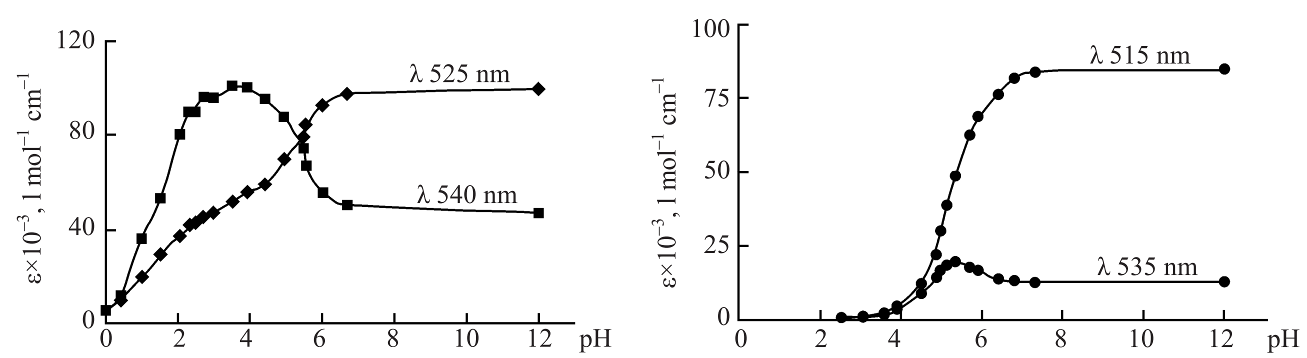


Fig. 2. Dependence of molar absorption coefficient of 2,4,5,7-tetrabromofluorescein (a) and 2,7-dichlorofluorescein (b) on pH in the micellar solution of cetyldimethylammoniumpropanesulfonate (1.5×10^{-3} mol l^{-1}), ion force 0.05 mol l^{-1} (NaCl).

Table 1. $\mathrm{p}K_\mathrm{a}^a$ values for dyes in 1.5×10^{-3} mol l^{-1} water solution of $n\text{-}C_{16}H_{33}N(CH_3)_2^+\text{–}(CH_2)_3\text{–}SO_3^-$, ion force 0.05 mol l^{-1} (buffer + NaCl), 25ºC

Compound	$\mathrm{p}K_{\mathrm{a},0}^{a}$ [a]	$\mathrm{p}K_{\mathrm{a},1}^{a}$	$\mathrm{p}K_{\mathrm{a},2}^{a}$	K_T
Fluorescein **I**	≈0.1	5.95±0.04	6.80±0.03	34
Fluorescein isothiocyanate **II**	0.9±0.1	5.43±0.05	6.45±0.02	35
3,7-Dichlorofluorescein **III**	–	4.84±0.04	5.50±0.04	18.6
4,5-Dibromofluorescein **IV**	–	4.92±0.04	5.42±0.04	–
2,4,5,7-Tetrabromofluorescein (eosine) **V**	–	1.47±0.02	5.26±0.05	1.3
3',4',5',6'-Tetrabromofluorescein **VI**	–	5.63±0.03	6.69±0.02	–
2,4,5,7,3',4',5',6'-Octabromofluorescein **VII**	–	2.95±0.03	3.03±0.02	–

[a] $\mathrm{p}K_{\mathrm{a},0}^{a}$ values of compounds **III–VI** are located in strongly acidic medium and were not evaluated by us in the work presented.

Table 2. Optical characteristics of dyes in the 1.5×10^{-3} mol l^{-1} water solution of $n\text{-}C_{16}H_{33}N(CH_3)_2^+\text{–}(CH_2)_3\text{–}SO_3^-$, 25ºC

Compound	λ_max, nm ($\varepsilon\times10^{-3}$)		
	R^{2-}	HR^-	HR^-
I	500 (81.2)	455 (27.7); 475 (24.4)	465 (0.80); 495 (0.70)
II	502.5 (74.1)	460 (39.8)	460-465 (1.11); 490 (0.939)
III	515 (80.5)	490 (17.6); 525 (53.2)	470 (1.44); 500 (1.39)
IV	516 (69.40)	495 (16.9); 530 (42.6)	475 (1.40)
V	527 (103.0)	505 (31.1); 540 (106.0)	480-485 (9.80)
VI	526 (115.4)	470 (31.4); 500 (25.5)	–[b]
VII	516.5 (52.8)[a]; 554 (167.8)	522.5 (22.5); 565 (78.1)	–[c]

[a] Clearly expressed shoulder in the spectrum of anion. [b] Complete discoloration at pH < 3.5 and the concentration of dye 10^{-5} mol l^{-1}. [c] Complete discoloration at pH < 1 and the concentration of dye 10^{-5} mol l^{-1}.

described procedure of the simultaneous calculation of $\mathrm{p}K_\mathrm{a}^a$ and the molar absorption coefficients of H_2R molecules and HR^- monoanions was used [7–19]. Results of evaluation of factors of the apparent constants of gradual ionization are presented in Table 1. Spectral characteristics of ionic and molecular forms are presented in Table 2 and in the Figs. 3, 4.

Alterations in the absorption spectra of the ions and molecules of the indicator dyes were used for the evaluation of the completeness of binding of these equilibrium forms by micelles. Some examples are presented in the Figs. 4, 5. The shifts of absorption bands of the R^{2-} ions as compared to the spectra of water solutions are close to that of the micellar solutions of cationic surfactanst such as cetyltrimethylammonium bromide [10, 19] and *N*-cetylpyridinium chloride [8]. It permits to propose that sufficiently complete binding of dyes by micelles takes place in the case under study as well. Conclusions concerning the degree of binding were made also on the basis of spectra at the concentrations of zwitterionic amphiphile above and below 1.5×10^{-3} mol l^{-1}. Only in the case of compound **II** spectrum of the R^{2-} form showed alterations until the concentration of surfactant 0.02 mol l^{-1}.

Detailed picture of prototropic equilibrium of dyes is presented in the scheme 1. All the structures are colorated at the exclusion of discolorated lactone **D**. Because of sp^3-hybridization of the nodal carbon atom. HR^- and R^{2-} anions of dyes from this series may also have the lactonic structure which is not shown in the scheme. It is observed evidently only in the case when the substituent is nitro group [5, 20].

As is known [21], ionization of carboxylic croup in the position 2' (COOH → COO^-) insignificantly influences the long-wave absorption band of dye, what permits to model the spectra of particles such as **B** and **C** by the spectra of particles **A** and **E** of the corresponding dye [5, 7–10, 12, 19, 21]. This approach was later used also by the other workers [11, 13, 22]. Some of them [11, 13] deliver practically the same values of constants of tautomeric equilibria and

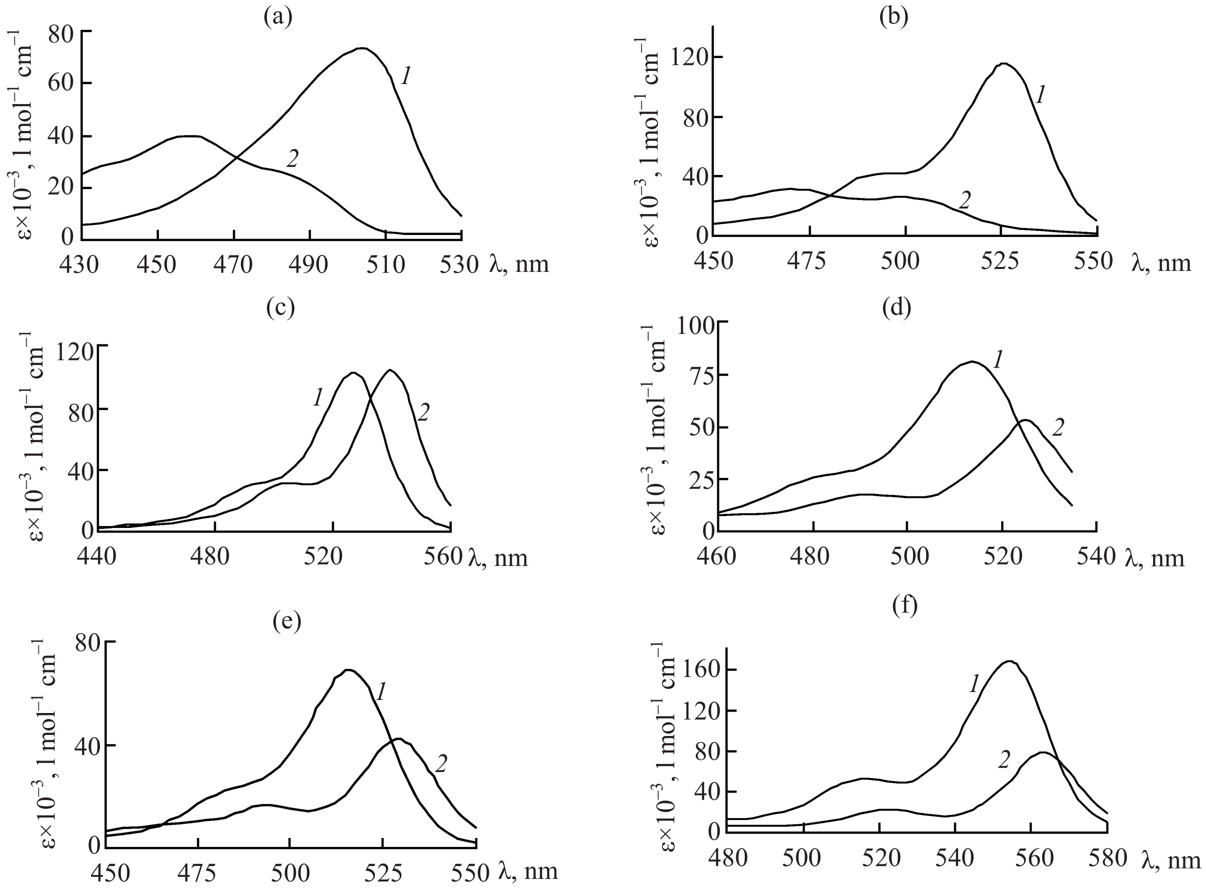


Fig. 3. Absorption spectra of anions of fluorescein dyes in the micellar solution of *N*-cetyl-*N,N*-dimethyl-3-ammonium-1-propanesulfonate: (*1*) R^{2-} and (*2*) HR^{-}. (a) Fluorescein isothiocyanate, (b) 3',4',5',6'-tetrabromofluorescein, (c) eosine, (d) 2,7-dichlorofluorescein, (e) 4,5-dibromofluorescein, and (f) 2,4,5,7,3',4',5',6' -octabromofluorescein.

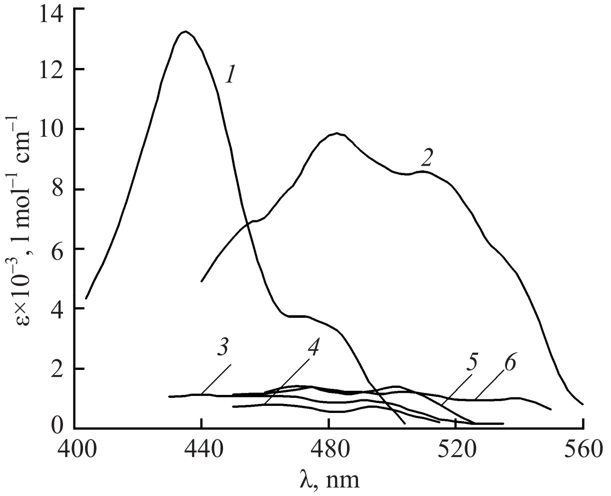


Fig. 4. Absorption spectra of neutral forms of fluorescein dyes in water: (*1*) fluorescein [21] and in the micellar solution of *N*-cetyl-*N,N*-dimethyl-3-ammonium-1-propanesulfonate (1.5×10^{-3} mol l^{-1}), (*2*) eosine, (*3*) fluorescein isothiocyanate, (*4*) fluoescein, (*5*) 2,7-dichlorofluorescein, and (*6*) 4,5-dibromofluorescein.

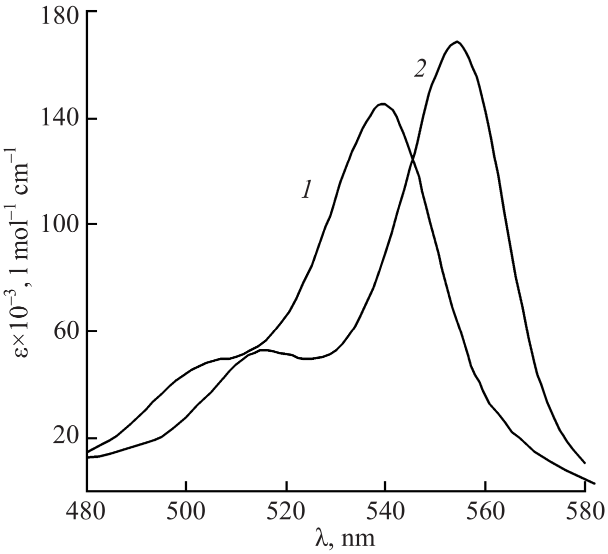
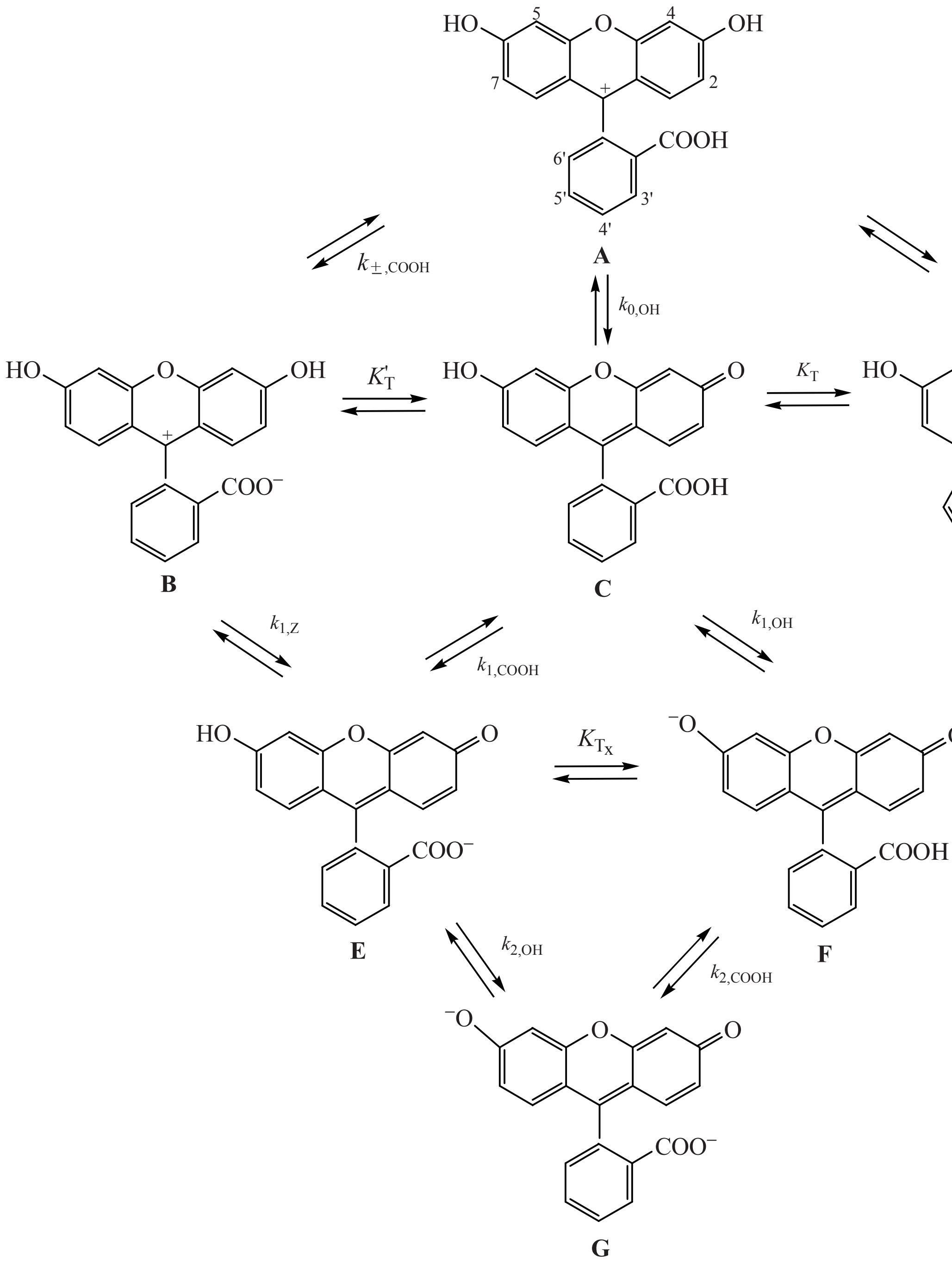


Fig. 5. Absorption spectrum of 2,4,5,7,3',4',5',6'-octabromofluorescein R^{2-} form in (*1*) water and (*2*) in the micellar solution of *N*-cetyl-*N,N*-dimethyl-3-ammonium-1-propanesulfonate (1.5×10^{-3} mol l^{-1}), pH 12 (NaOH).

Scheme 1.



K_T = [**D**]/[**C**], K'_T = [**B**]/[**C**]; $K''_T = K_T/K'_T$ = [**D**]/[**B**]; K_T = [**F**]/[**E**]; $k_{\pm,COOH} = a^*_{H^+}a_{\mathbf{B}}/a_{\mathbf{A}}$; $k_{0,OH} = a^*_{H^+}a_{\mathbf{C}}/a_{\mathbf{A}}$; $k_{1,COOH} = a^*_{H^+}a_{\mathbf{E}}/a_{B}$; $k_{1,Z} = a^*_{H^+}a_{\mathbf{E}}/a_{\mathbf{B}}$; $k_{1,OH} = a^*_{H^+}a_{\mathbf{F}}/a_{\mathbf{C}}$; $k_{2,OH} = a^*_{H^+}a_{\mathbf{G}}/a_{\mathbf{E}}$; $k_{2,COOH} = a^*_{H^+}a_{\mathbf{G}}/a_{\mathbf{F}}$.

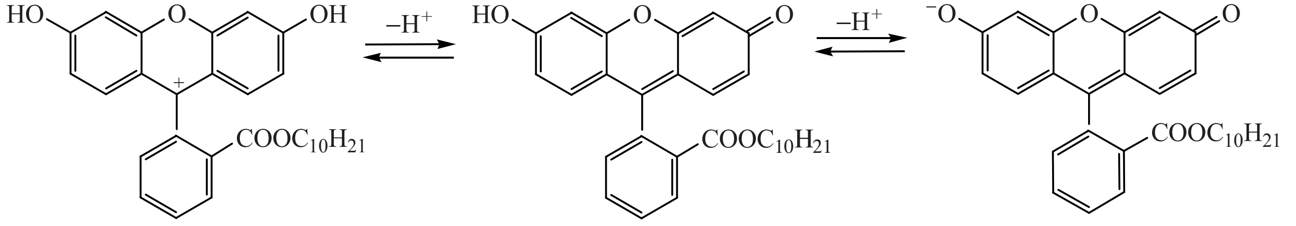
ionization microconstants of fluorescein in water as were obtained by us [9, 21]. Absence of reference to our works can be considered the independent confirmation of our results published long before.

For all the studied dyes in micellar solutions of sulfobetaine amphiphile tautomeric equilibrium of neutral form (H_2R) in the micelles is strongly shifted to the side of colorless tautomer **D** as compared to water solution (Figs. 1, 4). It is explaned by complete binding of H_2R form by micelles. As is known [5, 7–10, 12, 14, 19] formation of lactone ring is facilitated in nonaqueous media.

At the exclusion of compounds **VI, VII** $\varepsilon(H_2R)$ values significantly differ from zero. Character of corresponding spectra (Fig. 4) permit to conclude that the prevailing lactone form **D** exists together with the quinoid tautomer **C**. Highly polar tautomer **B** contributing 22% into the total number of fluorescein molecules H_2R in water [21] is not observed at all in the micellar solutions. It is in general characteristic of the nonaqueous surrounding [5, 7–10, 12, 19]. Really, relationship (5) follows from the Scheme 1.

$$\log K'_{\mathrm{T}} = \mathrm{p}k_{0,\mathrm{OH}} - \mathrm{p}k_{\pm,\mathrm{COOH}}. \qquad (5)$$

Values of $k_{0,\mathrm{OH}}$ and $k_{\pm,\mathrm{COOH}}$ fluorescein micro-constants may be considered equal to the values of ionization constants of cations of *n*-decylfluorescein and rhodamine dyes found recently by the workers [6, 23] in the micellar solutions of given amphiphile at the ion force 0.05 mol l^{-1} (NaCl + HCl). For example, $\mathrm{p}K^a_{\mathrm{a},0}$ value for *n*-decylfluorescein determining the transformation of cation **H** to the molecule **I** is 1.63 ($\mathrm{p}K^a_{\mathrm{a},1}$ value being equal to 6.07) [16].



H **I** **J**

Values of $\mathrm{p}K^a_{\mathrm{a}.0}$ for rhodamine **B** ($X^1 = X^2 = C_2H_5$) and *N,N'*-di-*n*-octadecylrhodamine (X^1 = H, X^2 = n-$C_{18}H_{37}$) correspond to the transformation of cation **K** to the zwitterion **L**. They are equal to 3.58 and 3.60 respectively [16, 20].

Hence, for fluorescein $K'_{\mathrm{T}} \sim 0.01$, and the part of tautomer **B** is two factors of ten lower than that of tautomer **C**, and still more lower than the part of the preferred tautomer **D**. In the absence of tautomer B the constant of tautomeric equilibrium (**C** $\rightleftarrows$ **D**) may be evaluated from the relationship (6).

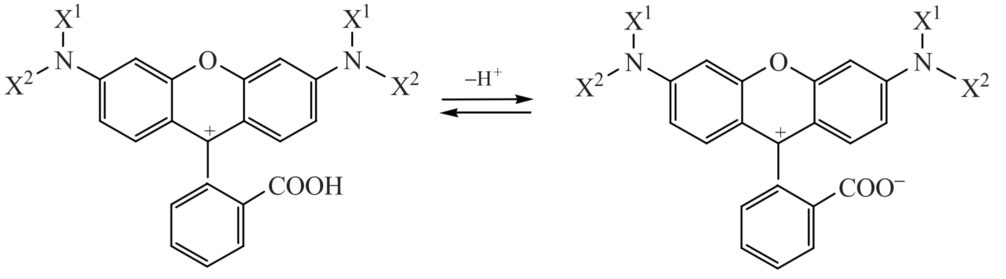
$$1/K_{\mathrm{T}} = \varepsilon(H_2R)/[\varepsilon(\mathbf{E}) - \varepsilon(H_2R)]. \qquad (6)$$

Values of ε(HR) for the compounds **I**, **II**, and **VI** were used as their ε(**E**) (see Table 2). For the compound **V** its ε(**E**) was accepted to be equal to 23×10^3. This meaning was used recently in the equilibrium studies in nonaqueous media [5, 7, 10]. Finally, for the dye **III** ε(**E**) value was accepted to be equal to ε(HR) for this compound in water solution, 28.3×10^3 [5] where the tautomer **E** is the main component.

Shift of the Eq. (1) to the strogly acidic pH range at the introduction of halogen atoms in the xantene ring is caused by strong decrease in $\mathrm{p}k_{0,\mathrm{OH}}$ value, and at the transfer from water to nonaqueous medium (including the micellar solution) by strong increase in K_{T} meaning. As it is seen from the Eq. (7) following from Scheme 1, both effects diminish $\mathrm{p}K_{\mathrm{a},0}$ value.

$$\mathrm{p}K_{\mathrm{a},0} = \mathrm{p}k_{0,\mathrm{OH}} - \log(1 + K_{\mathrm{T}}). \qquad (7)$$

Now we transfer to considering the tautomerism of monocharged anions HR^-. As is known, in the case of



K **L**

narrow absorption bands (particles of the types **F** and **J**), ionization of carboxylic group reveals in the absorption spectra. It causes the hypsochromic shift of maximum point by several nanometers [5, 7–10, 12, 14]. Character of the spectra (Fig. 3c) shows that HR^- form of eosine **V** in the micellar solution exists mainly as the phenolic tautomer **F**. Hence, $pK^a_{a,2}$ values are in line with $k_{2,COOH}$ (Scheme 1), and $pK^a_{a,1}$ is equal to $[pk_{1,OH} + \log(1 + K_T)]$. Hence, $pk_{1,OH}$ of eosine is equal to 1.1. Comparison of it with the $pK^a_{aa,1}$ value 6.07 for the quinoid structure **I'** shows the effect of halogen substituents usual for the xantene series [5, 7, 8, 10, 12].

In the case of compounds **I**, **II**, and **IV** (Figs. 1, 3a, 3b) the carboxylate structure **E** is preferred. That is why $K^a_{a,2}$ values coinciding with $k_{2,OH}$ for this dye (Scheme 1) are considerably close (Table 1). All of them are a half factor of ten lower than the ionization constants of quinoid **I'** what is undoubtedly connected with the presence of negatively charged substituent in the position 2' of monoanion **E**. In the case of dye **II** binding of R^{2-} form with micelles is incomplete (see above), and it may be the reason of underestimation of $pK^a_{a,2}$ value. $pK^a_{a,1}$ in this case is equal to $[pk_{1,COOH} + \log(1 + K_T)]$. From that it follows that for fluorescein **I** $pk_{1,COOH}$ is 4.4, and for fluorescein isothiocyanate **II** it is 3.9.

Position of the tautomeric equilibrium $\mathbf{E} \rightleftarrows \mathbf{F}$ in the series of substituents is determined by the presence or the absence of halogen atoms in the xantene ring. From Scheme 1 relationship (8) follows:

$$K_{T_x} = k_{1,OH}/k_{1,COOH} = k_{2,OH}/k_{2,COOH}. \tag{8}$$

In the case of compounds **III**, **IV**, **VII** from the absorption spectra (Figs. 3d–3f, Table 2) it follows that that the parts of tautomers **E** and **F** are comparable, and $K_{a,1}$ and $K_{a,2}$ constants are close. Molecules of two first compounds contain only two halogen substituents in the xantene ring. Due to that $pk_{1,OH}$ value will lie between 6.07 for the molecule **I'** and 2.1 for the tautomer **C** of eosine **V.** Hence, they will be close to the above-presented $pk_{1,COOH}$ values.

Interpretation of the equilibrium parameters of octabromofluorescein **VII** is more complicated. It may be really expected that $pk_{1,OH}$ of this compound will be close to the corresponding value for eosine **V,** but K_T value must be much higher because of practically complete discoloration of neutral form (Table 2). From the other side $pk_{1,COOH}$ and $pk_{2,COOH}$ values must be much lower as compared to eosine because of introduction of four bromine atoms in the residue of phthalic acid. That is why $pK_{a,1}$ and $pK_{a,2}$ of compound **VII** are close in value, and both these meanings are significantly lower than in 2,7-dichlorofluorescein **III** and 4,5-dibromofluorescein **IV.**

In general relationship between the ionization constants pK, microconstants k and tautomeric equilibrium constants K_T and K_{T_x} is determined by the Eqs. (9), (10) following from Scheme 1:

$$\begin{aligned} pK_{a,1} &= pk_{1,COOH} + \log(1 + K_T) - \lg(1 + K_T\ ) \\ &= pk_{1,OH} + \log(1 + K_T) - \log(1 + K_T^{-1}), \end{aligned} \tag{9}$$

$$pK_{a,2} = pk_{2,COOH} + \log(1 + K_T^{-1}) = pk_{2,OH} + \log(1 + K_{T_x}). \tag{10}$$

Relationships between the first and the second ionization constants are the partial cases of these equations.

Anions of these dyes are probably located near the ammonium fragment of sulfobetaine micelles analogously to their location in cationic micelles.

But pK^a_a values are much higher than in the micellar solutions of cationic surfactants at the same ion force of the continuous water phase. They are much closer to corresponding values at ion force 4 mol l^{-1}, that is under the conditions of almost complete shielding of the surface charge (Table 3). Hence, in spite of high value of dipole moment of the hydrophilic part of amphiphile, the aggregates of the latter may be considered most likely as the micelles of cationic surfactant with the covalently fixed equivalent of counterions (SO_3^- groups), and on the whole their effect on the bound molecules and ions of dyes mostly resembles the effects of neutral micelles.

In general it may be stated that the essential scheme of equilibria of the xantene dyes [5, 7–10, 12, 20, 21, 23] is true in this case also. Comparison with the water phase data is not always possible. Limited solubility of the molecular forms of some dyes in water, in detail of compound **VII** complicates the reliable evaluation of pK_a in this standard solution. Because of that only the values for the compounds **I**, **III**, and **V** may be compared.

First of all it may be concluded that significantly different alterations in the pK_a values of various dyes as well as in the $pK_{a,1}$ and $pK_{a,2}$ of one and the same compound at the transfer from water to the micelles of the zwitterionic amphiphile under study take place. Hence, for fluorescein the $(pK_{a,2} - pK_{a,1})$ difference is

Table 3. pK_a^a values for the compounds **I**, **III**, and **V** in the micellar solutions of surfactants [5, 8, 10, 19], and thermodynamic pK_a values in water [5, 21]

Solvent	Fluorescein **I**		2,2-Dichlorofluorescein **III**		Eosine **V**	
	p$K_{a,1}$	p$K_{a,2}$	p$K_{a,1}$	p$K_{a,2}$	p$K_{a,1}$	p$K_{a,2}$
N-cetyl-*N,N*-dimethyl-3-ammonium-1-propanesulfonate solution, 0.05 mol l^{-1} NaCl	5.95	6.80	4.84	5.50	1.47	5.26
N-cetylpyridinium chloride solution, 0.05 mol l^{-1} KCl	3.60	5.54	3.58	3.70	0.5	2.82
Cetyltrimethylammonium bromide solution, 0.03 mol l^{-1} NaCl	4.48	5.81	–	–	–	–
Cetyltrimethylammonium chloride solution, 4.0 mol l^{-1} KCl	6.41	7.17	5.50	5.79	1.83	5.76
Water	4.45	6.80	4.00	5.19	2.81	3.75

0.85, while in water solution this value is 2.35. At the same time for eosine the corresponding values are 3.79 and 0.94 respectively. Such differential action is explained by more sharp increase in K_T for fluorescein as compared to eosine, and also by the different ionization order of COOH and OH groups in the case of unsubstituted dye and its 2,4,5,7-tetrabromoderivative (Scheme 1).

EXPERIMENTAL

N-Cetyl-*N,N*-dimethyl-3-ammonium-1-propanesulfonic acid preparation (Sigma, main substance content >99%) was used without additional purification. Preparations of dyes **I-III**, and **V** were the same as in the recent studies. [5, 7–10, 12, 19]. Compounds **IV**, **VI**, and **VII** were let to us by the deceased prof. A.V. El'tsov. Characteristics of compounds **VI**, **VII** were published by him and the coworkers [1]. Varied pH values were created by means of HCl, NaOH, and the phosphate, the acetate, and the borate buffer solutions. Starting solutions of acetic acid were prepared by dilution of glacial acetic acid. Phosphoric acid was of the "chemically pure" grade. Borax was purified by double crystallization. For preparing the buffer solutions and standardization of acid solutions CO_2-protected NaOH solution was used. Its concentration was established with the use of potassium biphthalate. Sodium chloride of "chemically pure" grade was purified by crystallization. Starting solutions of surfactants and dyes were prepared by dissolution of batches in the corresponding volumes of water.

Control of pH in the continuous phase was carried out at 25.0±0.1ºC by means of the ESL-63-07 glass electrode in the chain with the transfer (liquid connection by means of 1 mol l^{-1} KCl solution) graduated by means of the standard buffer solutions (pH 1.68, 4.01, 6.86, and 9.18). Operated solutions were prepared by taking the aliquots of standard solutions at constant temperature (25±0.1ºC). Constant value of ion force (0.05 mol l^{-1}) was provided by the addition of NaCl. Contributions of acetate, phosphate, and borate buffer solutions into the summary ion force were 0.01, 0.01–0.02 and 0.0125 mol l^{-1} respectively. pH values below 3.5 were created by HCl–NaCl mixtures in hydrogen chloride solutions with pH <1.3 The ion force was not maintained constant.

Absorption spectra of dyes were obtained on a SF-46 device in 1 cm and 5 cm cuvettes at 25ºC. Operating solutions were practically clear. Spectra were measured against the reference solutions containing all the same components at the exclusion of dyes. For each dye the number of solutions with different pH values was 13–15 with the step about 0.2 pH units. Twenty to twenty five analytical wavelengths were used, and the spectral range depended on the specific features of the chromophore system of dye. For example for fluorescein **I** the spectral range was 420–520 nm, while for octabromofluorescein **VII** it was 480–580 nm.

ACKNOWLEDGMENTS

The authors express their gratitude to the deceased prof. A.V. El'tsov for lending the preparations of brominated fluorescein derivatives **IV**, **VI**, and **VII**.

REFERENCES

1. El'tsov, A.V., Smirnova, N.P., Ponyaev, A.I., Zakharenko, V.S., and Yartsev, A.I., *Zh. Obshch. Khim.*, 1989, vol. 59, no. 11, p. 2587.
2. Haugland, R.P., *Handbook of Fluorescent Probes and Research Products*, 9th ed., Molecular Probes, Inc., Eugen, OR 2002.

3. Nagano, T. and Yashimura, T., *Chem. Rev.*, 2002, vol. 102, no. 4, p. 1235.
4. Balzani, V., Ceroni, P., Gestermann, S., Gorka, M., Kaufmann, C., and Vogtle, F., *Tetrahedron*, 2002, vol. 58, p. 629.
5. Mchedlov-Petrosyan, N.O., *Vestnik Khar'kovskogo Natsional'nogo Universiteta*, *Ser. Khim.,* 2004, no. 626, p. 221.
6. Lavis, L.D., Rutkovski, T.J., and Raines, R.T., *Anal. Chem*., 2007, vol. 79, no. 17, p. 6775.
7. Mchedlov-Petrosyan, N.O., Kukhtik, V.I., and Egorova, S.I., *Zh. Obshch. Khim*., 2006, vol. 76, no. 10, p. 1677.
8. Mchedlov-Petrosyan, N.O. and Kleshchevnikova, V.N., *Zh. Obshch. Khim*., 1990, vol. 60, no. 4, p. 900.
9. Mchedlov-Petrosyan, N.O. and Salinas Mayorga, R., *J. Chem. Soc., Faraday Trans*., 1992, vol. 88, no. 20, p. 3025.
10. Mchedlov-Petrosyan, N.O. and Kleshchevnikova, V.N., *J. Chem. Soc., Faraday Trans*., 1994, vol. 90, no. 4, p. 629.
11. Tamura, Z., Morioka, T., Maeda, M., and Tsuji, A., *Bunseki Kagaku*, 1994, vol. 43, p. 339.
12. Mchedlov-Petrosyan, N.O., Kukhtik, V.I., and Bezugly, V.D., *J. Phys. Org. Chem*., 2003, vol. 16, no. 7, p. 380.
13. Klonis, N. and Sawyer W.H., *J. Fluorescence*, 1996, vol. 6, no. 3, p. 147.
14. Kibblewhite, J., Drummond, C.J., Grieser, F., and Thistlethwaite, P.J., *J. Phys. Chem*., 1989, vol. 93, no. 21, p. 7464.
15. Duxbury, D.F., *Chem. Rev*., 1993, vol. 93, no. 1, p. 381.
16. Mchedlov-Petrosyan, N.O., *Pure Appl. Chem*., 2008, vol. 80, no. 7, p. 1459.
17. Herrmann, K.W., *J. Colloid Int, Sci*., 1966, vol. 22, no. 4, p. 352.
18. Bunton, C.A., Mhala, M., and Moffat, J.R., *J. Phys. Chem*., 1989, vol. 93, no. 2, p. 854.
19. Bryleva, E.Yu., Vodolazkaya, N.A., Mchedlov-Petrosyan, N.O., Samokhina, L.V., Matveevskaya, N.A., and Tolmachev, A.V., *J. Colloid Int. Sci*., 2007, vol. 70, no. 8, p. 1343.
20. Samoilov, D.V., Mchedlov-Petrosyan, N.O., Martynova, V.P., and El'tsov, A.V., *Zh. Obshch. Khim*., 2000, vol. 70, no. 8, p. 1343.
21. Mchedlov-Petrosyan, N.O., *Zh. Analit. Khim*., 1979, vol. 34, no. 6, p. 1055.
22. Orte, A., Crovetto, L., Talavera, L.M., Boens, N., Alvarez-Pez, J.M., *J. Phys. Chem., A*, 2005, vol. 109, no. 5, p. 734.
23. Mchedlov-Petrosyan, N.O., Vodolazkaya, N.A., Yakubovskaya, N.A., Grigorovich, A.V., Alekseeva, V.I., and Savvina, L.P., *J. Phys. Org. Chem*., 2007, vol. 20, p. 332.