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IONIZATION AND TAUTOMERISM OF FLUORESCEIN DYES IN MIXED MICELLAR SOLUTIONS*

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The acid-base and tautomeric equilibria of a set of oxyxanthene dyes, fluorescein and its derivatives and analogues, were studied in aqueous solutions containing a binary mixture of colloidal surfactants, cetyl pyridinium chloride (CPC, cationic surfactant) and Tween 80 (non-ionic surfactant). The apparent ionization constants, K_a^a, of the dyes have been determined spectrophotometrically at 25°C in the mixture CPC : Tween 80 = 1 : 4 (mole : mole), at bulk Cl^- concentration ca. 0.05 M. The K_a^a values of fluorescein stepwise ionization are compared with the K_a^a values of model compounds, containing the substituents SO_3H, H, $CO_2C_2H_5$ and $CO_2C_{10}H_{21}$ instead of the group CO_2H, in terms of tautomerism and depth of penetration into the micellar pseudophase. The variation of the pK_a^a values along with the changes in the CPC : Tween 80 ratio are discussed.

Introduction

Fluorescein and other oxyxanthenes belong to widely used dyes due to their unique spectral, photophysical and photochemical properties [1–6]. Therefore a further development of knowledge about the influence of non-aqueous media on the interconversions of the various prototropic forms of fluorescein is of significance. The dissociation occurs stepwise [(Eqs. (1)–(3)]:

$$H_3R^+ \rightleftharpoons H_2R + H^+, \quad K_{a0} \tag{1}$$

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

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

The most probable structures of the ionic and molecular forms of the unsubstituted fluorescein are presented in Scheme 1 [7–14]. In the previous papers we reported the results of the study of protolytic equilibria of fluorescein dyes in water [7,8,10], absolute and aqueous alcohols, 1,4–dioxane, aqueous acetone, and dimethyl sulfoxide [11,13,14], as well as in micellar solutions of cationic, non-ionic and anionic surfactants [8,9,12,14].

The scheme of the protolytic equilibria (Scheme 1) allows to interpret the relationship between the values of the so called 'apparent' pK_a (denoted as pK_a^a) in micellar solutions of colloidal surfactants [8,9,12,14–19]. In particular, it becomes possible to evaluate the 'microscopic' ionization constants, or 'microconstants', k (see Scheme 1, in which a_i denote activities) [7–14]. This allows to explain the changes in pK_a as compared with the 'aqueous' pK_a (pK_a^w), i.e., the medium effects, ΔpK_a ($= pK_a - pK_a^w$).

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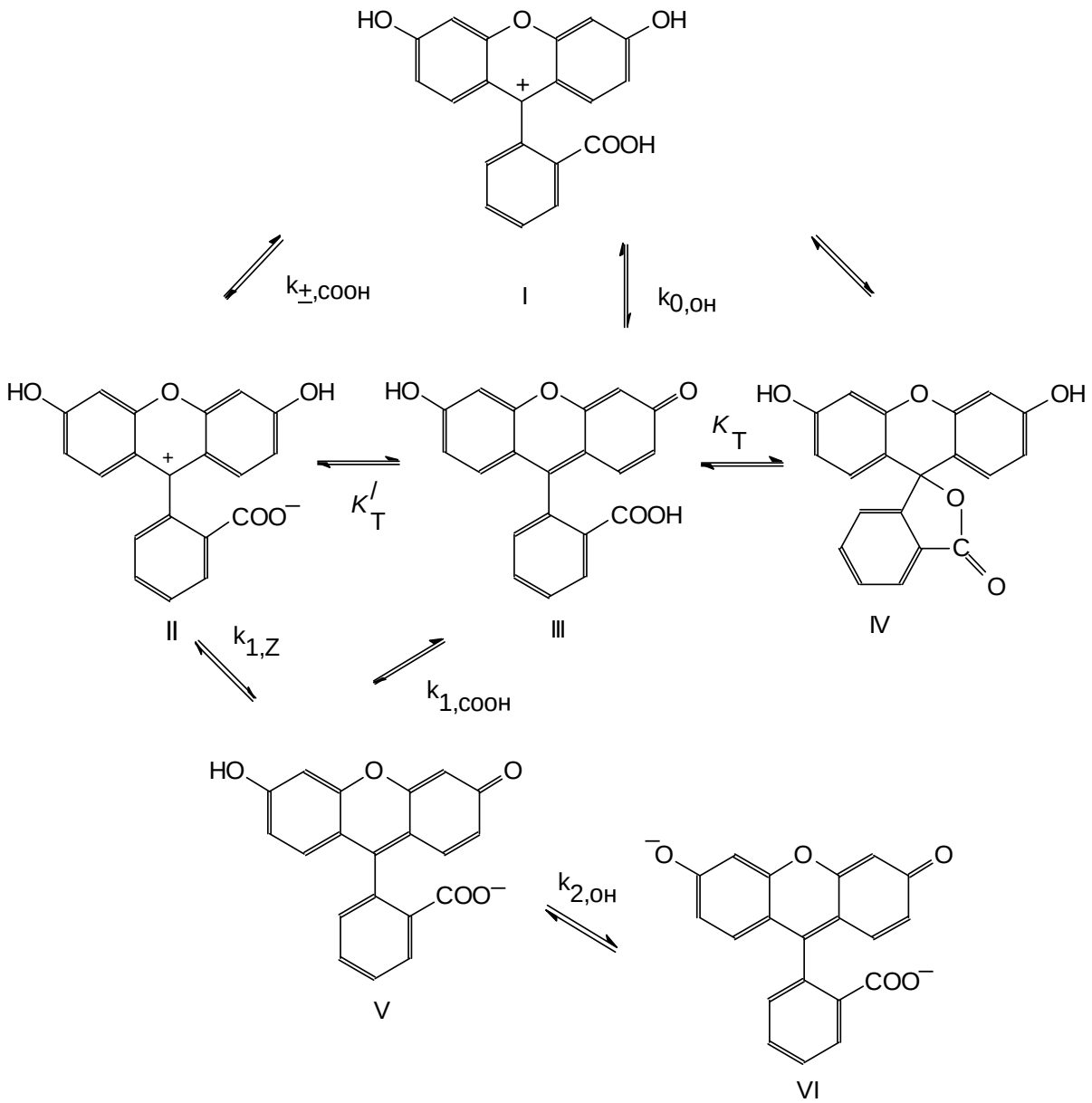
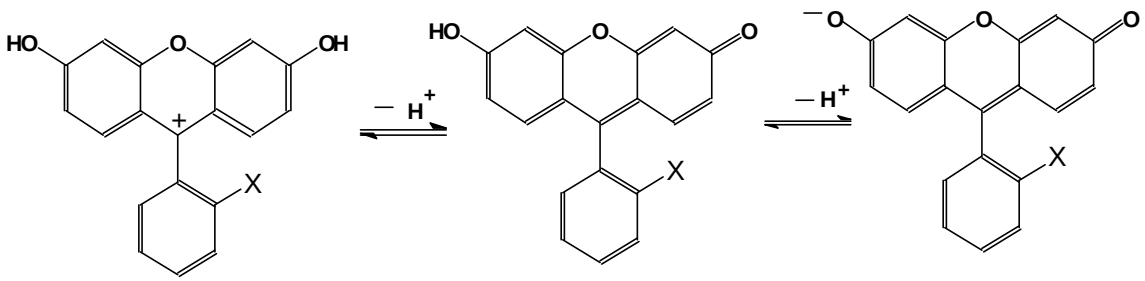


Scheme 1. Most probable structures of fluorescein in solutions; $K_T = [IV]/[III]$; $K_T^{/} = [II]/[III]$; $K_T^{//} = K_T / K_T^{/} = [IV]/[II]$; $k_{\pm,COOH} = a_{H^+} a_{II} / a_I$; $k_{0,OH} = a_{H^+} a_{III} / a_I$; $k_{1,Z} = a_{H^+} a_V / a_{II}$; $k_{1,COOH} = a_{H^+} a_V / a_{III}$; $k_{2,OH} = a_{H^+} a_{VI} / a_V$.

If the dyes are used as pK_a – probes situated on the interface of surfactant micelles, phospholipid liposomes, droplets of microemulsions, then long hydrocarbon tails are usually introduced into their structure to ensure complete binding of all the species to the pseudophase. However, the influence of the tail itself as well as the influence of the deeply–penetrated position of the dye, caused by the long hydrocarbon portion, may be of significance. In the previous paper [13] we compared the pK_a values of hydrophobic (possessing a long hydrocarbon tail) fluorescein dyes with that of 'common', i.e. water-soluble analogues, in a mixture of water with n-butanol. For more detailed comparison the following set of model compounds is suitable:



VII VIII IX

Scheme 2. X = SO_3^- (sulfonefluorescein; **VIIa–IXa**); H (6–hydroxy–9–phenyl fluorone; **VIIb–IXb**); $CO_2C_2H_5$ (ethyl fluorescein; **VIIc–IXc**); $CO_2C_{10}H_{21}$ (decyl fluorescein; **VIId–IXd**). In the case of sulfonefluorescein the protonation of the SO_3^- group in solution can occur only in very acidic media.

Earlier [8,9,12,14] the protolytic equilibria of these dyes were studied in micellar solutions of individual surfactants. According to the electrostatic model, for the 'medium effect', i.e. ΔpK_a^a, of acid-base indicators completely bound to the micelles Eq. (4) is generally accepted [8,12,14–19]:

$$\Delta pK_a^a = pK_a^a - pK_a^w = \log\frac{\gamma_B}{\gamma_{HB}} + \log\frac{f_B^m}{f_{HB}^m} - \frac{\Psi F}{2.3RT}, \qquad (4)$$

where pK_a^w is the thermodynamic pK_a value in water, and γ_B and γ_{HB} are the activity coefficients of transfer of corresponding species from water to the pseudophase, f^m are the concentration activity coefficients of the species, Ψ is the electrical potential of the Stern layer, F is the Faraday constant, R is the gas constant, T is absolute temperature (298.15 K). Due to the high electrolyte concentration in the Stern region, where the indicator molecules are located, it is often supposed that $f_B^m \approx f_{HB}^m$.

The present paper is devoted to the equilibria in mixed surfactant systems. Mixed micelles have been intensively studied for decades [20–22]. However, only relatively simple indicator dyes were as a rule used till now in such systems [16,17,22]. Therefore, it is of interest to clarify the behaviour of the above mentioned set of fluorescein dyes in mixed surfactant solutions, namely in the system water + cationic surfactant (cetyl pyridinium chloride, CPC) + non-ionic surfactant (Tween 80; polyoxyethylene sorbitan monooleate).

Experimental

Materials

Fluorescein was purified by re–precipitation from the aqueous solution with hydrochloric acid, and then by chromatography. The dyes ethyl– and n-decyl fluorescein (synthesized by Dr. V.I. Alekseeva and co-workers), and sulfonefluorescein (synthesized by Dr. V.Kh. Grif) were used previously [8,12,13,19]. The sample of 6–hydroxy–9–phenyl fluorone was prepared by Dr. D.V. Samoylov. The purity of all the dyes was checked by means of spectrophotometry and T.L.C. (Silufol plates). CPC (Minkhimprom, USSR) and Tween 80 (Sigma) were used as received. To create the required pH values, analytical-grade hydrochloric acid, sodium hydroxide, acetic and phosphoric acids and sodium chloride were used. The standard aq. sodium hydroxide solution was prepared using CO_2 –free water and kept protected from the atmosphere.

Measurements

The pH values of solutions were checked by means of potentiometry by using cells with a liquid junction, glass electrode ESL-63-07, and a silver / silver chloride reference electrode, according to the compensation scheme on a potentiometer P 363/ 3 and pH meter-millivoltmeter pH-121. All the solutions were prepared and pH measurements performed at 25.0 ± 0.1 °C. The spectra of the dye solutions were measured by using SP-46 spectrophotometer. The working dye concentrations were as a rule ca. 10^{-5} M, in the case of fluorescein

at pH 3–5 – near 10^{-4} M. All the spectrophotometric experiments were performed at 25 ± 1 °C. The emission spectra were registered with the Hitachi F-4010 apparatus.

The ionic strength (I, molar scale of concentrations) of the solutions was, as a rule, constant: in the buffer solutions appropriate amounts of NaCl stock solutions were added to maintain the total I = 0.05 M. The 'true' ionic strength was somewhat higher due to the presence of CPC. However, such small deviations from I = const display practically no influence on the equilibrium state [12,17].

Results and discussion

Determination of ionization constants

For fluorescein the K_{a1}^{a} and K_{a2}^{a} values are close. At fixed λ the dependence A vs. pH (pH = $-\log a_{H^+}$) can be described by Eq. (5) [10-13]:

$$A = \frac{A_{H_2R}(a_{H^+})^2 K_{a0}^{a} + A_{HR^-} a_{H^+} K_{a0}^{a} K_{a1}^{a} + A_{R^{2-}} K_{a0}^{a} K_{a1}^{a} K_{a2}^{a}}{(a_{H^+})^2 K_{a0}^{a} + a_{H^+} K_{a0}^{a} K_{a1}^{a} + K_{a0}^{a} K_{a1}^{a} K_{a2}^{a}} \qquad (5)$$

In this case only the A_{H_2R} and $A_{R^{2-}}$ values can be measured directly at the appropriate acidity. The examples of absorptivity variation caused by pH changes are depicted in Fig.1.

The band of dianion VI has λ_{max} = 490–491 nm, with molar absorptivity, $E_{R^{2-}}$, equal to 85×10^3 dm^3 mol^{-1} cm^{-1}. As a first approximation, the fluorescein ionization constants, K_{a1}^{a} and K_{a2}^{a}, were obtained by using the procedure described earlier [11]. The spectrum at pH 2.90 was taken as the H_2R spectrum, and the pK_{a1}^{a} value was calculated within the pH range 4.16–5.07, at λ = 485 nm, where the extinction of the HR^- and R^{2-} species is relatively similar. Thus estimated pK_{a1}^{a} value was then utilized for pK_{a2}^{a} calculations at λ = 500 nm, pH 5.38–6.04. The iterative procedure leads to the values pK_{a1}^{a} = 6.03 and pK_{a2}^{a} = 6.70. Finally, the CLINP programme [13] was used; the data for 28 working solutions with various pH and wavelength λ = 460 nm and 500 nm (Fig.1) were utilized in the calculations with the result given in Table 1.

Table 1. The pK_a^a values of fluorescein dyes in mixed micellar solutions: 20% cetylpyridinium chloride – 80% Tween 80 (mole : mole), total surfactant concentration 0.003 M; I = 0.05 M; 25 °C

Dye	pK_{a0}^{a}	pK_{a1}^{a}	pK_{a2}^{a}
fluorescein [a]	0.18 ± 0.02	5.83 ± 0.10	6.68 ± 0.04
sulfonefluorescein (X = SO_3^-) [b]	— [c]	2.48 ± 0.12	6.54 ± 0.02
6–hydroxy–9–phenyl fluorone (X = H)[d]	2.03 ± 0.05	6.25 ± 0.02	—
ethyl fluorescein (X = $CO_2C_2H_5$) [e]	2.36 ± 0.01	6.05 ± 0.01	—
decyl fluorescein (X = $CO_2C_{10}H_{21}$) [f]	1.50 ± 0.05	6.87 ± 0.04	—

[a] In water: pK_{a0}^{w} 2.14; pK_{a1}^{w} 4.45; pK_{a2}^{w} 6.80 [7]; in 82 wt.% n-butanol: pK_{a0} 1.18; pK_{a1} 8.5; pK_{a2} 9.3 [13]. [b] In water: pK_{a1}^{w} 3.22; pK_{a2}^{w} 6.76 [8,11]; in 82 wt.% n-butanol: pK_{a1} 4.39; pK_{a2} 9.46 [13]. [c] The spectrophotometric method is unsuitable for determination of this value ($SO_3H \rightarrow SO_3^-$); however, the protonation of the sulfonate group probably occurs in very acidic media. [d] In wa-

ter: pK_{a0}^{w} 3.10, pK_{a1}^{w} 6.28 [25,26]. [e] In water: pK_{a0}^{w} 2.94, pK_{a1}^{w} 6.31; in 82 wt.% n-butanol: pK_{a0} 2.68, pK_{a1} 8.44 [13]. [f] In 82 wt.% n-butanol: pK_{a0} 2.53, pK_{a1} 8.56 [13].

Having the pK_{a1}^{a} and pK_{a2}^{a} values, it was then possible to calculate the molar absorptivities of HR^- at various wavelengths, in such a way obtaining the spectrum of HR^- (Fig.2). Thus estimated E_{HR^-} values are ca. 30% smaller than in other solvent systems [7–13]. Besides, near pH 6, the absorbances in phosphate buffer solutions are somewhat higher than those in acetate buffers with the same pH. As a rule, such discrepancies were not registered earlier while studying equilibria in cationic micellar systems. This may be regarded as an evidence of more complicated detailed equilibrium scheme as compared with the one commonly used by us. In particular, the NaCl contribution to the value I = 0.05 M of the buffer mixtures is 0.04 M in the case of acetate buffer solutions, while 0.03 M – in the case of phosphate ones. Therefore we are not going to attach particular significance to such a relatively low E_{HR^-} value.

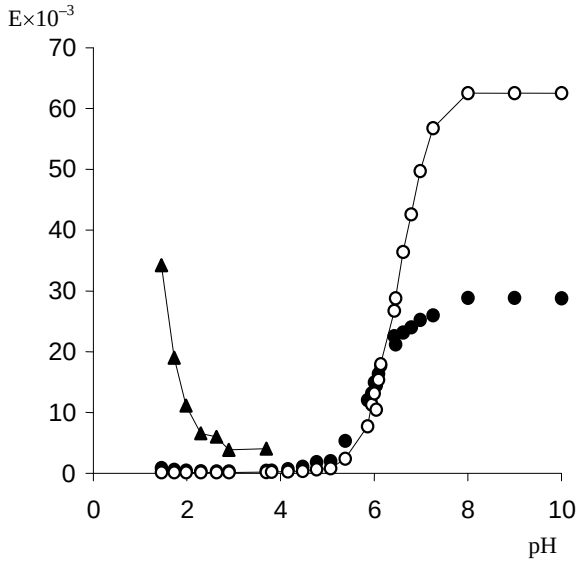


Fig.1. The relationship of fluorescein absorbtivities with pH in the CPC – Tween 80 system; λ 460 nm (full circles), λ 500 nm (empty circles) and 440 nm (triangles; these values are multiplied by 10); ionic strength 0.05 M.

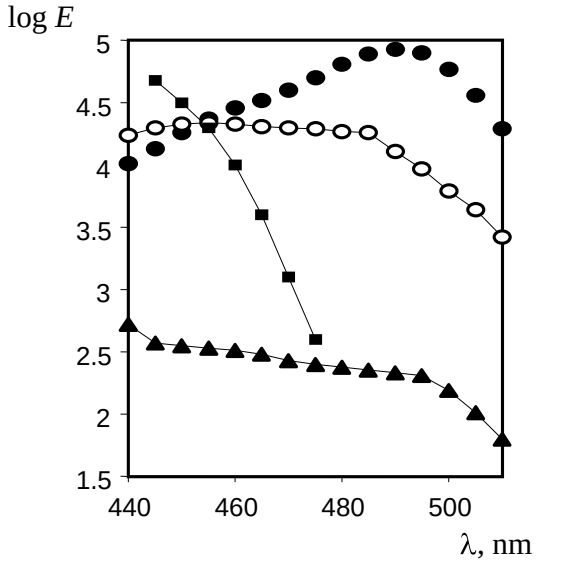


Fig.2. Absorption spectra of fluorescein species in mixed micellar solutions of CPC (0.0006 M) and Tween 80 (0.0024 M): dianion R^{2-}, **VI** (full circles); monoanion HR^- (**V**), calculated from equilibrium data (empty circles); neutral H_2R (triangles); I = 0.05 M (NaCl), except the spectrum of cation H_3R^+, **I** (squares), obtained in aqueous HCl (1 M) without surfactants [7].

The pK_{a0}^{a} value of fluorescein was obtained isolated from the other steps, within the pH range 1.46–3.30 (HCl + NaCl), because in a more acidic region the ionic strength would be higher than 0.05 M.

The $E_{H_3R^+}$ value was determined in aqueous HCl [7], without surfactants, in order to avoid the possible precipitation of CPC; the bands of oxyxanthene cations in bulk phase and in mixed micelles are very similar (see below).

The refinement of E_{H_2R} values (Fig.2) was carried out for fluorescein at pH 2.90 by using the spectra of the cation and the anions [13], to avoid any influence of traces of intensely coloured ions (H_3R^+, HR^- and R^{2-}) on the spectra of the neutral forms.

The pK_a^a values for practically isolated ionization steps (2,3) of sulfonefluorescein and (6,7) for 6−hydroxy−9−phenyl fluorone and alkyl fluoresceins were calculated according to the

$$H_2R^+ \rightleftharpoons HR + H^+, \qquad K_{a0} \tag{6}$$

$$HR \rightleftharpoons R^- + H^+, \qquad K_{a1} \tag{7}$$

standard procedure [13,17−19]. The wavelengths near the absorption maximum (e.g. λ = 445-450 nm and 510 nm, Figs.3,4) were used as analytical positions.

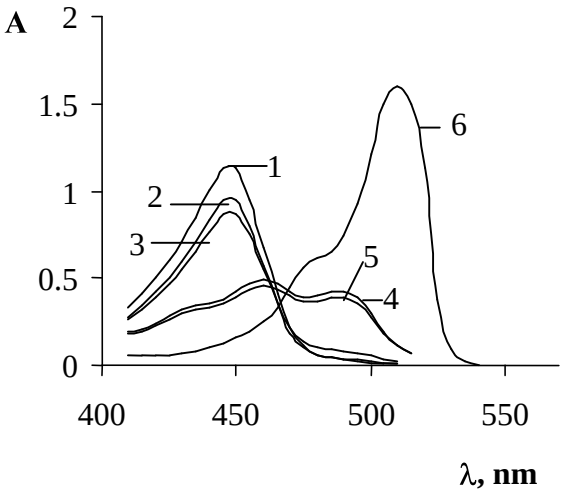


Fig.3. Absorption spectra of decyl fluorescein in micellar solutions of Tween 80 (0.05 M, I = 0.05 M (NaCl): cation H_2R^+, **VIId** (1); pH = 0.7 (2); pH = 1.3 (3); pH = 4.5 (4), pH = 4.7 (5), the both spectra are close to the spectra of HR species, **VIIId**; pH = 10, anion R^-, **IXd** (6).

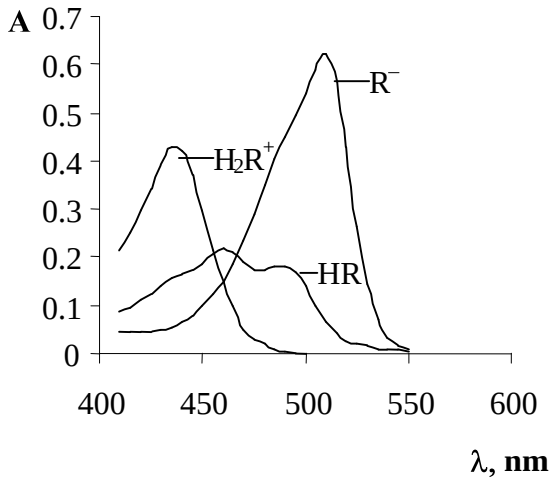


Fig.4. Absorption spectra of 6–hydroxy–9–phenyl fluorone in mixed micellar solutions of CPC (0.0006 M) and Tween 80 (0.0024 M): cation H_2R^+, **VIIb**; neutral HR, **VIIIb**; anion R^-, **IXb**; I = 0.05 M (NaCl), except the cationic spectrum.

The pK_a^a values are compiled in Tables 1,2. The pK_{a0}^a values (and the pK_{a1}^a value of sulfonefluorescein) were determined in HCl + NaCl solutions, while pK_{a1}^a values (and the pK_{a2}^a value of sulfonefluorescein) − in phosphate buffers. Visible absorption spectra of the species of some dyes are presented in Figs.2−4; the λ_{max} values are given in Table 3.

Table 2. The pK_a^a values of decyl fluorescein in mixed micellar solutions; total surfactant concentration 0.003 M; I = 0.05 M; 25 °C

Molar fraction of CPC in CPC − Tween 80 Mixture	pK_{a0}^a	pK_{a1}^a
1.00 [a]	0.79 ± 0.10	4.92 ± 0.07
0.60	—	5.38 ± 0.04
0.40	—	5.72 ± 0.01
0.20	1.50 ± 0.05	6.87 ± 0.04
0.00 [b,c]	2.03 ± 0.12	7.19 ± 0.07

[a] From Ref. 23. [b] From Ref. 19,23. [c] Mean values within the range of Tween 80 concentrations 7×10^{-4} to 0.05 M.

Table 3. The λ_{max}, nm values of fluorescein dyes in mixed micellar solutions: 20% cetylpyridinium chloride − 80% Tween 80 (mole: mole), total surfactant concentration 0.003 M; I = 0.05 M; 25 °C [a]

Dye	I and VII	VI and IX
fluorescein	435–440 (437) [b]	490–491 (491) [c]
sulfonefluorescein (X = SO_3^-)	440 (440) [d]	500 (495) [e]
6–hydroxy–9–phenyl fluorone (X = H)	436 (437) [f]	509–510 (492) [g]
ethyl fluorescein (X = $CO_2C_2H_5$)	440 (437–438)	510 (490.5)
decyl fluorescein (X = $CO_2C_{10}H_{21}$)	440 [h]	510 (495) [i]

[a] The values in parenthesis refer to water. [b] In CPC micellar solutions: 438 nm; in CTAC − 4 M Cl^- system: 444–445 nm. [c] In CPC micellar solutions: 504–505 nm; in CTAC − 4 M Cl^- system: 500 nm. [d] In CTAC − 4 M Cl^- system: 452 nm. [e] In CTAC − 4 M Cl^- system: 512 nm. [f] In CPC micellar solutions: 440 nm. [g] In CPC micellar solutions: 515 nm. [h] In micellar solutions of cationic and non-ionic surfactants: 447 nm and 448 nm, respectively. [i] In micellar solutions of cationic and non-ionic surfactants: 513 nm and 510 nm, respectively.

Binding of dyes by micelles

The choice of the mixture CPC (20%) + Tween 80 (80%) as a basic one in the present investigation has been determined by the attempt to ensure as far as possible a sufficient binding of dye species, and at the same time to essentially deviate from cationic 'homomicelles', studied earlier in detail [8,12,14]. However, the absorption spectra of cationic species I, VIIb,c, as well as of the zwitterionic H_2R form of sulfonefluorescein, VIIa, are slightly changed as compared with the spectra in aqueous solutions (Table 3). Thus, their binding to the pseudophase cannot be guarantied. On the other hand, the λ_{max} value of the cation VIId, which is certainly completely bound, is practically the same as that of VIIb,c. Hence, one can conclude that for cations the λ_{max} values are ca. 440 nm, and the spectral changes are not so pronounced as for anions.

The neutral forms of the studied oxyxanthene dyes (except the zwitterion VIIa) are poorly soluble in water, so their stability in the presence of micelles gives evidence for their solubilization (here: binding to the pseudophase). Besides, strong decolourization of fluorescein neutral form, as compared with aqueous solutions (see below) is caused by the tautomeric equilibria shift, reflecting the principal changes in the microenvironment (the decolourization in micellar medium is reversible, the coloured ionic species appear in solutions after further pH changes).

The absence of precipitates of R^{2-} $(CP^+)_2$, R^- CP^+ testifies the complete solubilization of the dye anions (here 'solubilization' means binding to micelles independently of the location character within the pseudophase), as they are observed in dilute CPC solutions in water, in the presence of the dyes. Such hypothesis is supported by the red shifts of the absorption maxima of R^- anions (IXb-d) against their bands in water (Table 3).

The absorption maximum of fluorescein dianion R^{2-} in the studied mixed surfactant systems stays unchanged as compared with aqueous solutions; the same is the case with the emission maximum (514 nm). However, both kinds of spectra, absorption and fluorescence, somewhat differ for aqueous and micellar media in intensity; in addition, re–distribution of intensity on the left and right of maximum.

For the system CPC + TX 100 with total surfactants concentrations 0.001 M to 0.004 M and fraction of CPC from 30% to 60% the absorption maxima of fluorescein R^{2-} ion varies within the range 497 nm − 503 nm, differing markedly from the 'aqueous' value 491 nm. The emission maximum under these conditions is ca. 523 nm.[§]

[§] *From unpublished results obtained in collaboration with S.V Malevaniy*

Note, that the fluorescein dianion R^{2-} stays practically non-bound by the non-ionic micelles. Maybe, in the mixed micelles this anion is located in the peripheral part of the micelles. The sulfonefluorescein dianion can be somewhat more hydrophobic, as the monoanion is somewhat more extractable into the chloroform phase in form of ionic pair HR^- $As(C_6H_5)_4^+$, as compared with that of fluorescein [24].

However, the pK_{a2}^a values of fluorescein and sulfonefluorescein are close (Table 1). The pK_{a2}^a value of fluorescein is 0.14 units higher; in pure CPC micelles (0.05 M Cl^-) the difference is 0.08 [8], while in cetyl trimethylammonium chloride (CTAC) system (with 4.00M Cl^-) it equals 0.17 [12]. At the same time the changes in the λ_{max} values of sulfonefluorescein R^{2-} anion are more expressed, and the anionic species are expected to be completely bound. Thus, we assume that the fluorescein dianion R^{2-} is also completely bound.

In the further discussion the binding of all the dye species, except cations I, VIIb,c, is expected to be complete.

Tautomerism of fluorescein and the microscopic ionization constants, k

The absorption spectra (Fig.2) confirm that the anion HR^- of fluorescein exists as a carboxylate tautomer (V). As for the H_2R spectrum of fluorescein, it differs from the spectra of HR molecules of alkyl fluoresceins and of HR^- ion of sulfonefluorescein only in intensity. This is a consequence of the tautomeric equilibrium shift toward the colourless lactone IV. According to the main extrathermodynamic assumption, taken as a basis for studying tautomerism, the spectra of species of types III and V (Scheme 1) are similar, and the E_{max} values may be taken as equal. In the H_2R spectrum (Fig.2) there are no distinct signs of the zwitterionic species II, which is expected to have a spectrum close to that of cation I. For fluorescein the zwitterion II appears only in water or in mixed solvents with a small ratio of organic co-solvents [7,9,11,13]. The fraction of H_2R existing as the tautomer III is found to be equal to 0.0167 by using absorption spectra, as described earlier [7–14]. Thus the value of tautomerization constant of fluorescein, K_T (Scheme 1), in mixed micelles is 59, which indicates the substantial predominance of the lactonic structure IV relative to the quinonoid structure III.

Such a K_T value of fluorescein is close to those in alcohols, e.g. in methanol (here the relative permittivity, ε, is 32, and the Reichardt parameter, E_T^N, is equal to 0.762), where K_T equals 54, or in n-butanol (ε = 17.7, E_T^N = 0.602), where K_T equals 54 [13].

The ionization of the carboxylic group in the $2'$ position ($CO_2H \rightarrow CO_2^-$) effects only the negatively charged oxyxanthene chromophore, leading to blue shift of the VI band as compared with the IXb–IXd bands [13]. The positions of the absorption bands (Table 3) confirm this regularity in the systems under study; however, the different penetration depth of the species VI and IXb–IXd can contribute to this effect as well.

In the general case, the following relationships are valid for the pK_a^a values of fluorescein [Eqs.(8)–(10)]:

$$pK_{a0}^a = pk_{0,OH}^a - \log(1+K_T+K_T^{/}) \quad (8)$$

$$pK_{a1}^a = pk_{1,COOH}^a + \log(1+K_T+K_T^{/}) \quad (9)$$

$$pK_{a2}^a = pk_{2,OH}^a \quad (10)$$

Evidently, $K_T >> K_T^{/}$. Knowledge of the ratio of H_2R tautomer allows to estimate the values of the microscopic ionization constants, k [7–14]. The value $pk_{0,OH}^a$ = 1.96 agrees with the pK_{a0}^a values of 6–hydroxy–9–phenyl fluorone and ethyl fluorescein better, than with that of the decyl derivative (Table 1). It can be a result of incomplete binding of relatively hydrophilic cations I, VIIb,c as compared with the cation VIId. Besides, deeper penetration of decyl

fluorescein into the micellar pseudophase can be the reason for the low pK^a_{a0} value of this dye, because the dissociation of cationic acids [Eqs.(1),(6)] are known to increase at the addition of organic co-solvent to aqueous solutions. Typical dependences of ΔpK_a for a cationic and a neutral acid on the fraction of ethanol and acetone are depicted in Fig.5 for the case of 6–hydroxy–9–phenyl fluorone.

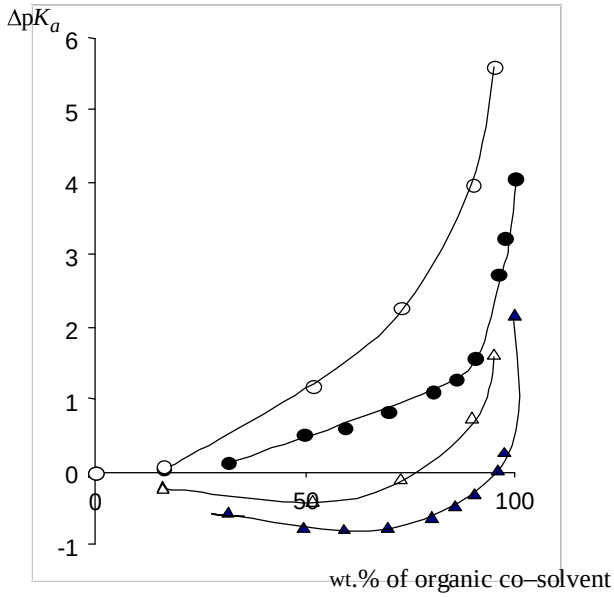


Fig.5. The dependences of ΔpK_a (= $pK_a - pK^w_a$) values of 6–hydroxy–9–phenyl fluorone on the composition of water–ethanol and water–acetone mixtures; triangles: ΔpK_{a0}; circles: ΔpK_{a1}; full: H_2O–C_2H_5OH; empty: H_2O–$(CH_3)_2CO$ [25,26].

The value $pk^a_{1,COOH}$ for fluorescein was found to be equal to 4.05. The pK^a_{a1} and pK^a_{a2} values of sulfonefluorescein correspond to $pk^a_{1,Z}$ and $pk^a_{2,OH}$ of fluorescein, respectively. For alkyl fluoresceins $pK^a_{a0} = pk^a_{0,OH}$, $pK^a_{a1} = pk^a_{1,OH}$. The ionization constants of model compounds can be used for proving the validity of the scheme of fluorescein ionization. From Scheme 1 Eq. (11) can be derived:

$$\log K^{/}_{T} = pk^a_{1,Z} - pk^a_{1,COOH} \qquad (11)$$

The estimation of $K^{/}_{T}$ can be made, if we take the $pk^a_{1,Z}$ value being equal to the pK^a_{a1} value of sulfonefluorescein: $\log K^{/}_{T}$ = 2.48 − 4.05 = −1.57. Consequently, the conclusion about the absence of the zwitterionic tautomer II, made on the base of spectral data, is reliable: its fraction is 4×10^{-4}. On the other hand, the pk values at 25 °C are in semi-quantitative agreement with the Bjerrum − Kirkwood − Westheimer approach [Eq. (12)]:

$$\delta pk = 243/\varepsilon_{eff} r \ , \qquad (12)$$

in which δpk is the difference between the pk values of acids with and without an additional charged group, ε_{eff} is the 'effective' permittivity, and r is the distance between the charged and ionizing groups (in $\overset{o}{A}$). Some more refined relations are available from literature as well [27]. Therefore, the pk values of the hydroxy groups of oxyxanthenes are markedly higher if a negatively charged group is present in the $2^{/}$ – position. This regularity appeared to be valid both in organic solvent systems and in micellar solutions of cationic surfactants. So, in the mixture of n–butanol (82 wt.%) with water (18 wt.%): $pk_{0,OH}$ = 2.7, $pk_{1,Z}$ = 4.4, δpk = 1.7, and $pk_{1,OH}$ = 8.4, $pk_{2,OH}$ = 9.4 , δpk = 1.0 [13]. In micellar solutions of CPC and CTAC, at [Cl^-] = 0.05 M and 4.00 M, the pK^a_{a2} values of fluorescein and sulfonefluorescein are always higher than the pK^a_{a1} values of 6–hydroxy–9–phenyl fluorone, ethyl fluorescein, and decyl fluorescein [8,12],[¶] the differences being 0.33 to 0.83.

Similar relations could have been expected in mixed micelles as well. And really, the pK^a_{a2} values of fluorescein and sulfonefluorescein are 0.29–0.63 units higher than the pK^a_{a1} values of 6–hydroxy–phenyl fluorone and ethyl fluorescein. However, the pK^a_{a1} value of decyl fluorescein is with 6.87 markedly higher not only than the correspondent values of 6–

[¶] *Some of these data are not yet published*

hydroxy−phenyl fluorone and ethyl fluorescein, but even than the pK_{a2}^{a} values of fluorescein and sulfonefluorescein (Table 1). Taking into account the complete binding of neutral and anionic dye species, that can be explained only by different microenvironments, which, in turn, are caused by various penetration depth of dyes with different hydrophobicity.

The pK_a values referring to equilibria [VIIb−d $\rightleftharpoons$ VIIIb−d + H^+, Eqn. (6)] are rather close to each other not only in water but also in non−aqueous media, e.g. in 82 wt.% n-butanol (Table 1). The same is the case with the equilibria [VIIIb−d $\rightleftharpoons$ IXb−d + H^+, Eqn. (7)]; here coincidence is observed in cationic micelles as well [in CTAC − 4 M Cl^- system the pK_{a1}^{a} are equal to 6.67, 6.59, and 6.61, respectively (to be published)].

Hence, the lengthening of the hydrocarbon tail results in significant changes just in mixed surfactant systems, where the hydrophilic portion of micelles is more bulky and dissimilar as compared with that in cationic micelles. Interestingly, in micellar solutions of Tween 80 the pK_{a1}^{a} value of 6−hydroxy−9−phenyl fluorone [26] with 7.22 again practically coincides with that of decyl fluorescein at the same bulk ionic strength (Table 2).

Analysis of medium effects in mixed micelles

In the case of systems with tautomerism, traditional interpretation of ΔpK_a^a in terms of the charge type and of the nature of the ionizing group is to be completed by taking into account the tautomeric equilibria shifts. Thus for fluorescein the following relationships [Eqs. (13)−(15)] are valid:

$$\Delta pK_{a0}^{a} = \Delta pk_{0,OH}^{a} - \Delta \log(1 + K_T + K_T^{/}) \quad (13)$$

$$\Delta pK_{a1}^{a} = \Delta pk_{1,COOH}^{a} + \Delta \log(1 + K_T + K_T^{/}) \quad (14)$$

$$\Delta pK_{a2}^{a} = \Delta pk_{2,OH}^{a}. \quad (15)$$

The medium effects, ΔpK_{a0}^{a} and ΔpK_{a1}^{a}, are equal to −1.96 and +1.38, respectively, while for microscopic constants: $\Delta pk_{0,OH}^{a} = -1.14$, $\Delta pk_{1,COOH}^{a} = +0.56$. So, the changes in the experimentally determined macroscopic constants in micellar media are strongly controlled by the tautomeric equilibria shifts. For fluorescein and sulfonefluorescein $\Delta pk_{2,OH}^{a}$ equal to −0.12 and −0.22, respectively, while the $\Delta pk_{1,Z}^{a}$ value for sulfonefluorescein equals −0.74. Such results are in agreement with the charge types (Fig.5) and with the nature of the ionizing group (addition of organic co−solvent to water leads as a rule to stronger increase in pK_a values of carboxylic acids than in those of phenols). Note, that in the case of neutral and anionic acids the 'non-aqueous', or 'organic' microenvironment and the positive charge of the micellar surface [$\Psi > 0$, Eq.(4)] cause counter−acting effects, while in the case of cationic acids both reasons lead to decrease in $pk_{0,OH}^{a}$.

The direct comparison with organic solvents is hindered. Though in such systems the influence of the electrical surface charge is absent; however, the values ΔpK_a contain the term $\log \gamma_{H^+}$ [$\Delta pK_a = \log \gamma_{H^+} + \log (\gamma_B / \gamma_{HB})$], contrary to Eq.(4). Here γ are the activity coefficients of transfer from water to the given solvent.

The $\Delta pk_{0,OH}^{a}$ values for 6−hydroxy−9−phenyl fluorone, ethyl fluorescein, and decyl fluorescein are −1.07, −0.58, and −1.44 (in the last case the pk in water is equated to that of ethyl fluorescein), while $\Delta pk_{1,OH}^{a}$ for the mentioned dyes are: −0.03, −0.26, and +0.56. This demonstrates a possibly more 'non−aqueous' microenvironment of the dye with long hydrocarbon chain, due to deeper penetration depth. The pK_{a1}^{a} values differ by 0.62−0.82 units, which certainly demonstrates the distinction of the $\log(\gamma_{R^-} / \gamma_{HR})$ values, while the difference in the pK_{a0}^{a} values can reflect the incompleteness of binding of cations H_2R^+ (VIIb,c) to

micelles as well. On the other hand, the essentially dissimilar depth of penetration of dyes may result also in the distinction of the local electrical potentials of their microenvironments. Maybe, the neutral form VIIId is particularly deeply penetrated within the micellar pseudophase, while the anion IXd is located like that of less hydrophobic dyes (IXb,c). Note, that λ_{max} values of the mentioned anions coincide (Table 3). The absorption bands of neutral species VIII are less solvent–sensitive.

In the case of fluorescein the shift of the tautomeric equilibria (II, III $\rightarrow$ IV) toward the right, and the K_T rise in micelles result in an additional decrease in pK^a_{a0}, and in an increase in pK^a_{a1}, as compared with medium effects for the model compounds. So, the difference $(pK^a_{a1} - pK^a_{a0})$ is 5.65 (Table 1), while for 6–hydroxy–9–phenyl fluorone and ethyl fluorescein the corresponding values are 3.18–3.37 and 4.22–3.69, respectively. In the case of decyl fluorescein the difference is 5.37, indicating a more 'organic', i.e. a more 'dehydrated' microenvironment (see above). In 82 wt.% n-butanol these values for fluorescein, ethyl fluorescein, and decyl fluorescein are 7.3, 5.8, and 6.0, respectively [13]. This leads to a pronounced difference between the acidity strength of the H_3R^+ and the H_2R species of fluorescein [Eq. (16)]:

$$\Delta(pK^a_{a1} - pK^a_{a0}) = (pK^a_{a1} - pK^a_{a0}) - (pK^w_{a1} - pK^w_{a0}) = \\ = \Delta pk^a_{1,COOH} - \Delta pk^a_{0,OH} + \Delta 2\log(1 + K_T + K_T^{/}). \quad (16)$$

In contrast, the nature of the medium effects for $pk^a_{2,OH}$ and $pk^a_{1,COOH}$, and of the shift of the tautomeric equilibria toward the lactone (IV), results in the leveling of the acidity strength of H_2R and HR^-:

$$\Delta(pK^a_{a2} - pK^a_{a1}) = (pK^a_{a2} - pK^a_{a1}) - (pK^w_{a2} - pK^w_{a1}) = \\ \Delta pk^a_{2,OH} - \Delta pk^a_{1,COOH} - \Delta\log(1 + K_T + K_T^{/}). \quad (17)$$

Evidently, the values $\Delta(pK^a_{a1} - pK^a_{a0})$ = 3.3 and $\Delta(pK^a_{a2} - pK^a_{a1})$ = −1.5 are independent of the Ψ value. In 82 wt.% n-butanol these values for fluorescein are 5.0 and −1.4, respectively [13].

To explain the pK^a_a values of decyl fluorescein in the CPC – Tween 80 system, presented in Table 2 and depicted in Fig.6, it is necessary to clarify the composition of the mixed micelles.

Analysis of the dependence of pK^a_a on the composition of the binary surfactant mixture

According to Rubingh [28], the molar fraction of the first (here – cationic) surfactant, y, with c.m.c. = C_1, in the mixed micelles, is connected with its total molar fraction in the binary mixture, x, by means of Eq. (19).

$$y^2 \ln\frac{C^* x}{C_1 y} = (1 - y)^2 \ln\frac{C^*(1 - x)}{C_2 (1 - y)}. \quad (19)$$

Here C_2 is the c.m.c. value of the second surfactant, while C^* is the critical concentration for the mixed micelles. The equation is valid if the total concentration of each surfactant is below its c.m.c. (C_1 and C_2). If this is not the case, then the excess of the given surfactant must be within the micellar pseudophase even at weak interaction with the co-surfactant.

However, the c.m.c value of CPC at $[Cl^-]$ = 0.05 M, available from literature, is $\leq 8\times10^{-5}$ M, and that of Tween 80, in accord with various authors, is within the range $(1.2 - 5)\times10^{-5}$ M. Thus the working concentrations of both surfactants essentially exceed their c.m.c. Hence, we assume that in the case under study y $\rightarrow$ x. It also must be noted, that the non-ionic surfactant used by us is certainly a mixture of oligomers, and the mean molecular weight of Tween 80 is unknown. Thus the real composition of mixed micelles is beyond the possibility of exact calculation.

Under conditions of complete binding of acid-base indicators, according to the electrostatic model, the pK_a^a value is connected with the thermodynamic 'aqueous' value, pK_a^w, and the electrical potential of the Stern layer according to Eq. (4). For the surface charge density, σ, the rather evident Eq. (20) can be derived:

$$\sigma = \frac{\alpha y \bar{n} F}{s_{mic} N_A} , \qquad (20)$$

where $\bar{n}$ is the mean aggregation number of the mixed micelles, α is the degree of the micellized CPC dissociation [$(C_{16}H_{33}NC_5H_5^+Cl^-)_{mic} \rightleftharpoons (C_{16}H_{33}NC_5H_5^+)_{mic} + (Cl^-)_{water}$], s_{mic} – the micellar surface area, N_A – the Avogadro number. On the other hand, for a flat double electrical layer the following well–known expression is valid [17]:

$$\sigma = (8000 c \varepsilon \varepsilon_0 RT)^{1/2} \mathrm{sh}\frac{\Psi F}{2RT}, \qquad (21)$$

where c is the concentration of the electrolyte, M; $\varepsilon_0 = 8.854\times10^{-12}$ Fm^{-1}. Taking ε to be equal to 78.4, Eq. (21) can be rewritten as:

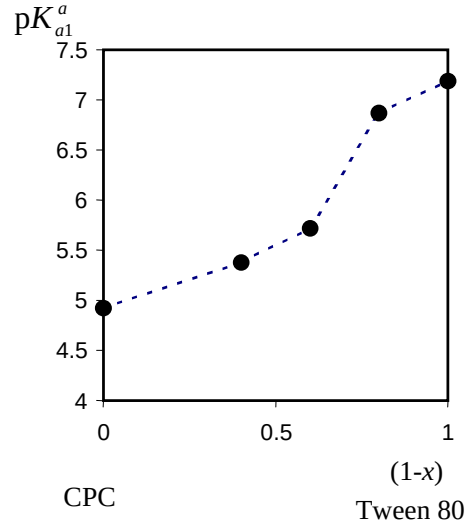


Fig.6. The dependence of pK_{a1}^a value of decyl fluorescein (**VIIId** $\rightleftharpoons$ **IXd** + H^+) on the composition of the CPC – Tween 80 system; ionic strength of buffer solutions: 0.05 M; *x* – molar fraction of CPC in the binary surfactant mixture.

$$\sigma = 7.30\times10^{-3} c^{1/2} \mathrm{sh}\frac{\Psi}{51.36} . \qquad (22)$$

Here σ denotes the number of elementary charge units pro ($\mathrm{\AA}$)^2, Ψ is expressed in mV. Using Eqs. [(4), (20)–(22)] and denoting the quantity [$pK_a^w + \log(\gamma_B/\gamma_{HB})$] as pK_a^i, one can obtain the following expression for the pK_a^a value of the indicator, completely bound to mixed CPC + Tween 80 micelles:

$$pK_a^a = pK_a^i - 0.8686\,\mathrm{arcsh}\frac{137\alpha y \bar{n} F}{c^{1/2} s_{mic} N_A} \cong pK_a^i - 0.8686\,\mathrm{arcsh}\frac{9.82\times10^{-17}\alpha x \bar{n}}{s_{mic}} . \qquad (23)$$

More complicated relations, reported for a spherical double electrical layer [29], lead to qualitatively similar relations [17]. Evidently, the main matter causing the increase in pK_a^a is the decrease in x (i.e. the 'dilution' of CPC with non-ionic co-surfactant). However, even if K_a^i, the so–called 'intrinsic' equilibrium constant, can be expected to be unchanged within the whole x range ($0 \le x \le 1$), no changes in the parameters $\bar{n}$ and s_{mic} along with variation in x can be excluded. The α value certainly increases at $x \to 0$ [17]. In addition, this automatically causes some slight variations even in [Cl^-]. Some peculiarities of mixed cationic – non-ionic micelles, e.g. specific interactions between the cationic head groups and oxyethylene chains [30] may probably be of significance as well.

At $1 \ge x \ge 0.5$ the pK_{a1}^a becomes only ca. 0.8 units higher, while at $0.5 \ge x \ge 0$ the further increase is ca. 1.5 units. First of all, it is caused by the very nature of the dependence of pK_a^a vs. x [Eq.(23)]. So, for the indicator bromophenol blue ($HR^- \rightleftharpoons R^{2-} + H^+$), even sharper rise in pK_{a2}^a was reported by us earlier [17] in a rather similar system CPC – Brij 35, 0.1 M Cl^- : at x = 1, 0.2, and 0: pK_{a2}^a = 1.73, 2.91, and 5.00, respectively.

Contrary to it, while going from pure CPC micelles to pure Tween 80 micelles, the changes in the pK^a_{a0} value of decyl fluorescein are significantly smaller than those in pK^a_{a1}: 1.24 and 2.27 units, respectively (Table 2).

Finally, if the location of all the species of decyl fluorescein is expected to be similar, thus referring to the same effective Ψ and σ values, the difference ($pK^a_{a1} - pK^a_{a0}$), being equal to ($pK^i_{a1} - pK^i_{a0}$), is independent of all the aforementioned parameters except the γ values. The values of ($pK^a_{a1} - pK^a_{a0}$) in Tween 80 (x = 0) and in CPC (x = 1) are 4.13 and 5.16. Hence, the value 5.37 at x = 0.2 demonstrates that the solvating properties of the mixture CPC (20%) + Tween 80 (80 %) are from this viewpoint closer to those of pure CPC micelles.

Conclusions

1. The protolytic equilibria of fluorescein, as well as four related dyes used as model compounds, are studied in mixed surfactant solutions. The 'apparent' pK^a_a values are determined in the system CPC + Tween 80 (1 : 4). The complete binding both of all the anionic species and of the neutral molecules to the micelles is rather probable. The pronounced shift of the tautomeric equilibrium state of the neutral form H_2R (quinonoid $\rightleftarrows$ lactone) toward the colourless lactone is registered.

2. The values of the 'microscopic' ionization constants (k) of fluorescein obtained with the help of the tautomerization constants, agree satisfactorily with those of model compounds. Some deviations can be explained by different depth of penetration of the species into the pseudophase, which leads to a certain dissimilarity of microenvironments.

3. Contrary to the situation in water–organic mixtures, the lengthening of the hydrocarbon tail in the dyes molecules in the 2′ position, from H and $CO_2C_2H_5$ to $CO_2C_{10}H_{21}$ results in prominent changes in the corresponding pK^a_a values, indicating the more decisive role of penetration depth as compared to the preferential solvation of the ionizing functional groups in mixed solvents.

4. The medium effects for microconstants ($\Delta pk = pk - pk^w$) are such as were expected with respect to the charge types of acid-base couples and the nature of the ionizing groups. Moreover, the micellar pseudophase displays a strong differentiating action at relatively moderate Δpk values. The ratios of the constants K^a_{a0}, K^a_{a1}, and K^a_{a2} of fluorescein are governed by the state of tautomeric equilibria (quinonoid $\rightleftarrows$ lactone).

5. The character of pK^a_a variations for decyl fluorescein ($HR \rightleftarrows R^- + H^+$), occurring along with changes in the cationic : nonionic surfactant ratio agrees in general features with the theoretical predictions. However, notwithstanding the complete binding of all the species of decyl fluorescein, the pK^a_{a0} and pK^a_{a1} values display different alterations at transfer from CPC to Tween 80. This demonstrates the significance of the solvating peculiarities of cations, neutral molecules, and anions in micellar media of various types.

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Kharkov University Bulletin. 2002. №549. Chemical Series. Issue 8(31). Н.О.Мчедлов-Петросян, А.В.Тимий, Н.А.Водолазкая. Ионизация и таутомерия флуоресцеиновых красителей в смешанных мицеллярных растворах.

Исследованы кислотно-основные и таутомерные равновесия серии оксиксантеновых красителей, флуоресцеина и его производных и аналогов, в водных растворах, содержащих бинарные смеси коллоидных ПАВ, цетилпиридиний хлорида (ЦПХ, катионное ПАВ) и Твина 80 (неионное ПАВ). Кажущиеся константы ионизации красителей, K_a^a, определены спектрофотометрически при 25°С в смеси ЦПХ : Твин 80 = 1 : 4 (моль : моль), при концентрации ионов Cl^- в объемной фазе около 0.05 М. Значения K_a^a ступенчатой ионизации флуоресцеина сопоставлены со значениями K_a^a модельных соединений, содержащих заместители SO_3H, H, $CO_2C_2H_5$ и $CO_2C_{10}H_{21}$ вместо группы CO_2H; обсуждение проведено в терминах глубины погружения красителей в мицеллярную псевдофазу. Обсуждено также варьирование значений pK_a^a при изменении соотношения ЦПХ : Твин 80.