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Ionization and tautomerism of oxyxanthene dyes in aqueous butanol

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Abstract

The protolytic equilibria of 7 oxyxanthene dyes were studied in 82 wt% aqueous *n*-butanol, a solvent with low permittivity ($\varepsilon = 20.8$) and high molar fraction of water (0.474). The thermodynamic pK_a values of fluorescein (p$K^{o}_{a0} = 1.2$, p$K^{o}_{a1} = 8.5$, p$K^{o}_{a2} = 9.3$) and eosin (p$K^{o}_{a1} = 5.0$, p$K^{o}_{a2} = 8.3$), as well as of their ethyl and decyl esters and of sulfonefluorescein were determined at 25°C in the molar concentration scale. On the basis of the absorption spectra of the substances, conclusions were made about the tautomerism of fluorescein and eosin. The values of the tautomeric equilibrium constants and of the microconstants of ionization were calculated. The results are compared with those obtained previously in other solvents.

Keywords: Oxyxanthene dyes; Fluorescein; Eosin; Decyl fluorescein; Decyl eosin; Sulfonefluorescein; Protolytic equilibria; Aqueous butanol; Ionization constants; Absorption spectra; Tautomeric equilibrium constants; Microconstants of ionization; Medium effects

1. Introduction

Oxyxanthene dyes are continuously attracting researchers due to their unique photophysical and photochemical properties [1–11]. The most widely studied and applied are fluorescein and its halogen derivatives, of which the 2,4,5,7-tetrahalogen fluoresceins are of greatest importance. The structures of fluorescein and eosin dianions (R^{2-}) are shown in Fig 1.

Recently some new dyes, for instance, thio analogues of fluorescein [9], have been reported. The application of xanthene compounds for various purposes, including fiber optics, is often connected with the use of surfactant solutions [2,7,10], polymers [3,8] or organic solvents [1,2,4–6]. Therefore, a further development of knowledge of the influence of nonaqueous media on the interconversions of the various prototropic forms of oxyxanthenes is of significance.

The protolytic equilibria of oxyxanthene dyes have been previously studied by us in media with high values of relative permittivity (ε) [11–18]. The dissociation occurs stepwise [Eqs. (1)–(3)]:

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

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

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

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The main probable structures of the ionic and molecular forms are presented in Scheme 1. A brief review of the literature and an analysis of the detailed ionization scheme of the dyes, including aminoxanthenes and amino-oxyxanthenes, can be found in the literature [11–18].

The protolytic equilibria have been examined in water [11,12], as well as in methanol and in mixtures of water with acetone, DMSO, 1,4-dioxane and ethanol [13–17]. The scheme of the protolytic equilibria (Scheme 1) also allows us to interpret the relationship between the values of the so called

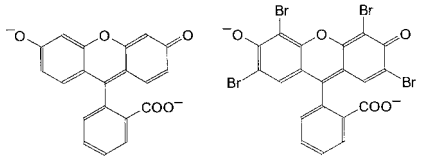
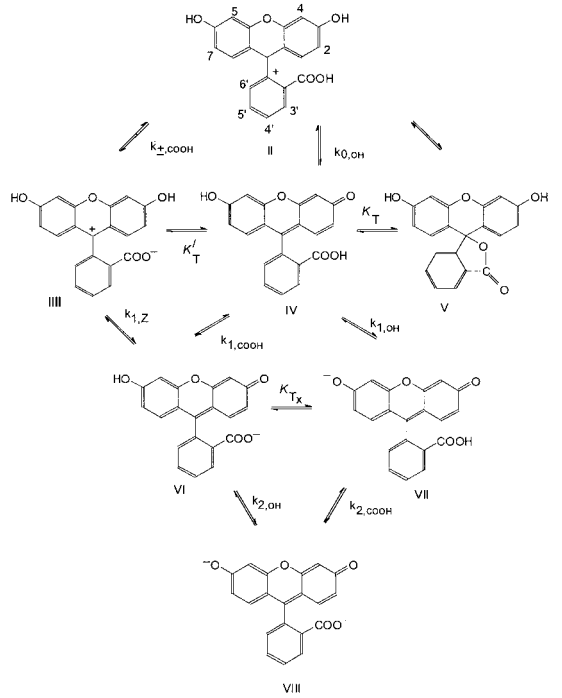


Fig. 1. The dianionic structures R^{2-} of fluorescein and of 2,4,5,7-tetrabromofluorescein (eosin).



Scheme 1. Fluorescein (**IIa–VIIIa**): 2,4,5,7 = H; eosin (**IIb–VIIIb**): 2,4,5,7 = Br. $K_T = [V]/[IV]$; $K'_T = [III]/[IV]$; $K''_T = K_T/K'_T = [V]/[III]$; $K_{T_x} = [VII]/[IV]$; $k_{\pm,COOH} = a^*_{H^+}a_{III}/a_{II}$; $k_{0,OH} = a^*_{H^+}a_{IV}/a_{II}$; $k_{1,z} = a^*_{H^+}a_{VI}/a_{III}$; $k_{1,COOH} = a^*_{H^+}a_{VI}/a_{IV}$; $k_{1,OH} = a^*_{H^+}a_{VII}/a_{IV}$; $k_{2,OH} = a^*_{H^+}a_{VIII}/a_{VI}$; $k_{2,COOH} = a^*_{H^+}a_{VIII}/a_{VII}$.

'apparent' pK_a (denoted as pK^a_a) in micellar solutions of colloidal surfactants [19–21]. In particular, it becomes possible to evaluate the so called 'microscopic' ionization constants, or 'microconstants' (k, see Scheme 1).

The spectral properties of the ethers and esters of the dyes [1,22] confirm the validity of the scheme. Analysis of recent publications [4–6,23,24] shows that the scheme is valid. The character of the spectral changes of fluorescein with pH variations [24,25], as well as the results of a potentiometric study of fluorescein dissociation [26,27], including the ratio of the constants of the stepwise dissociation (K_{a1} and K_{a2}), can be comprehended only by considering the state of the tautomeric equilibria, and the sequence of dissociation of functional groups.

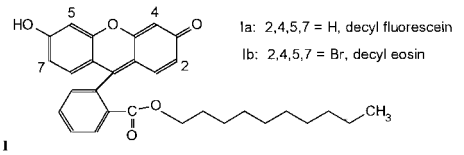
This study is concerned with the protolytic equilibria of oxyxanthene dyes in the mixed solvent: *n*-butyl alcohol (82 wt%)–water (18 wt%).

The acid-base equilibria in butanol and in aqueous butanol have been substantially less studied than those in methanol and ethanol [28,29]. This is due to the comparatively low relative permittivity of butanol ($\varepsilon = 17.4$), resulting in ion association, and with the limited miscibility of butanol and water. In the case of butanol, the approach based on the complete dissociation of ionic pairs (valid for water, methanol and ethanol), was shown to be insufficient [30].

In aqueous butanol with a molar fraction of water $x_{H_2O} = 0.474$, the formation of ionic associates does not, as a rule, practically manifest itself in studies of the protolytic equilibria at low ionic strength. At the same time, possibilities to investigate the behaviour of dyes in a water–alcohol mixture with low relative permittivity ($\varepsilon = 20.8$) are opened up. Such an ε value is markedly lower than that of methanol ($\varepsilon = 32$), and even somewhat lower than that of ethanol ($\varepsilon = 25$). As the chosen water content is close to the solubility of H_2O in butanol at 25°C, the results are relevant to the understanding of the nature of the dyes functioning as two-phase indicators [1].

Another reason for such a study is that the pK^a_a values of hydrophobic (as a rule, possessing a hydrocarbon tail) dyes, situated on the interface of surfactant micelles, phospholipid liposomes, droplets of microemulsions, etc., are usually being compared

with the pK_a values of the corresponding water-soluble (without a long hydrocarbon tail) dyes in water (pK_a^w) or in aqueous–organic mixtures [2].



Ia: 2,4,5,7 = H, decyl fluorescein
Ib: 2,4,5,7 = Br, decyl eosin

I

Therefore, it is of interest to clarify the influence of a long hydrocarbon chain on the pK_a value. As suitable dyes for this purpose decylfluorescein (**Ia**) and decyleosin (**Ib**) were chosen. In 82% BuOH both ethyl and decyl derivatives are sufficiently soluble for pK_a determinations.

2. Experimental

2.1. Materials

The samples of fluorescein, eosin and other oxyxanthenes were obtained as previously noted [11–21]. They were purified by column chromatography [14]. Decyl fluorescein and decyleosin were synthesised as described in the literature [1]. The latter dyes were characterised by ^{1}H NMR and elemental analysis. No decolourization (i.e. lactone formation) occurred in organic solvents, which is evidence for esterification of the carboxylic groups. The purity of all dyes was also comfirmed using thin layer chromatography (TLC). (Silufol plates).

The following were used as components of standard buffer solutions: salicylic acid, sodium salicylate, potassium biphthalate, picric acid, lithium picrate, benzoic acid, lithium benzoate, phenol, potassium hydroxide and lithium hydroxide. They were purified according to conventional methods. Potassium and lithium chlorides were purified by recrystallization. The stock alcoholic solutions of HCl were prepared by saturation of the solvent with gaseous HCl. The latter had been obtained by the action of conc. H_2SO_4 upon potassium chloride and dried before use. The stock solutions of C_4H_9ONa were prepared by dissolving sodium in butanol. Diethylbarbituric acid and $HClO_4$ were of analytical grade.

The standard aqueos sodium hydroxide solution was prepared using CO_2-free water. Butanol was purified using standard procedures. The water content was checked by using the titration according to K. Fisher.

2.2. Measurements

E.m.f. measurements were performed at 25.00 ± 0.05°C on a P 37-1 potentiometer and pH-121 pH meter. The glass electrode ESL-63-07 with a wide limit of pH-functioning in the mixed solvent was used as an indicator electrode, and an AgCl|Ag electrode as a reference electrode. For the determination of $pa^*_{H^+} = -\log a^*_{H^+}$ (where $a^*_{H^+}$ is the proton activity standardized to the infinite dilution in the used mixed solvent [30,31]) the e.m.f. measurements were used in a cell with the liquid junction shown in Eq. (4):

$$\text{Glass electrode / buffer solution in 82 wt \% butanol} \;\|\; \text{aqueous KCl, 1 mol kg}^{-1} \;|\; \text{AgCl} \;|\; \text{Ag} \qquad (4)$$

A set of buffer solutions in the given solvent, used for calibration of the cell, was created for 82% butanol. The $pa^*_{H^+}$ values (Table 1) were obtained electrometrically with the help of the US NBS method, as described earlier for other butanol–water systems [30]. The cell without liquid junction depicted in Eq. (5) was used:

$$\text{Electrode, reversible to H}^+\text{ions in the solvent } S \;|\; \begin{matrix}\text{Buffer, LiCl}\,(m)\\ \text{in the solvent } S\end{matrix} \;|\; \text{AgCl} \;|\; \text{Ag}, \qquad (5)$$

where m is the concentration of LiCl; $m = 0.001$–0.01 mol kg^{-1}. The peculiarities of the creation of standard buffer solutions in 82% butanol will be described in our further publications.

Table 1
The $pa^*_{H^+}$ values of buffer solutions used for cell calibration (82 wt% BuOH, 25°C, molal scale of concentrations)

Buffer systen (mol kg^{-1})	$pa^*_{H^+}$
Picric acid (0.1) + lithium picrate (0.01)	2.49
Salicylic acid (0.015) + sodium salicylate (0.015)	5.49
Potassium bipthalate (0.01)	6.24
Benzoic acid (0.01) + lithium benzoate (0.01)	7.06
Phenol (0.02) + lithium hydroxide (0.01)	12.15

High $pa^*_{H^+}$ values, created with the help of diethylbarbituric buffers, were calculated using the pK_a value of this acid at the corresponding ionic strength [15].

Electronic absorption spectra of dye solutions were measured in the visible region using a spectrophotometer SP-46 (Russia). The appropriate acidities were created by mixing aliquots of NaOH solutions with the following acids: salicylic, benzoic and diethylbarbituric. Acidic and alkaline solutions of fluorescein, sulfonefluorescein and eosin were prepared by using dilute $HClO_4$ and NaOH (or NH_3) solutions instead of buffer systems (in absolute butanol: HCl and C_4H_9ONa, respectively).

The ionic strength (I, molar scale of concentrations) of the solutions was, as a rule, constant: in buffer solutions the analytical concentration of NaOH was equal to 0.01 M, while in $HClO_4$ solutions appropriate amounts of NaCl stock solutions were added to maintain the total $I =$ 0.01 M. Some examples of absorptivity variations caused by $pa^*_{H^+}$ changes are given in Figs. 2–4.

During the investigation of esters of oxyxantenes, it was necessary to consider the possibility of $-CO-O-C_nH_{2n+1}$ hydrolysis in alkaline media. The working concentrations of the dyes were 6×10^{-6} to 1×10^{-4} M.

Whereas the developed $pa^*_{H^+}$ scale is expressed in molal (or so-called 'practical') scale of concentration (moles per kg of the solvent), the obtained pK_a values were recalculated into the molar scale of concentration (M). The value $pK_a = 9.90$ of veronal was determined spectrophotometrically by using Bromocresol Purple as an indicator and benzoic acid as a standard substance.

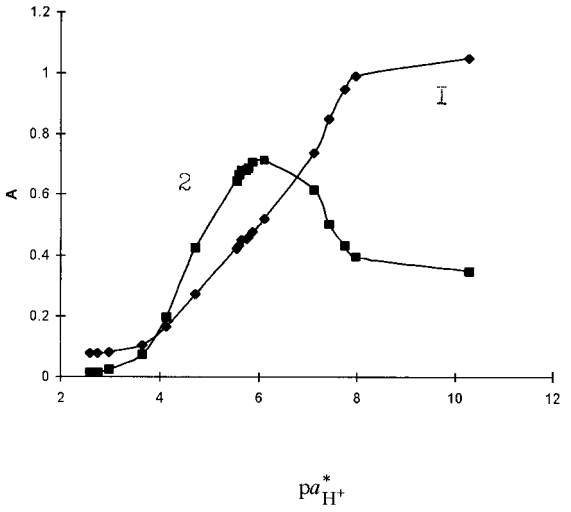


Fig. 2. The relationship of eosin absorbances with $pa^*_{H^+}$ in 82 wt% butanol; λ 505 nm (1) and 545 nm (2); ionic strength 0.01 M.

3. Results and discussion

3.1. Determination of ionization constants

The pK_a values for isolated ionization steps were calculated according to the standard Eq. (6):

$$pK_a = pa^*_{H^+} + \log\frac{A_B - A}{A - A_{HB}} \quad (6)$$

where $A_B = E_B Cl$, $A_{HB} = E_{HB} Cl$ and A is the absorbance at the current $pa^*_{H^+}$ value. Here, E_i

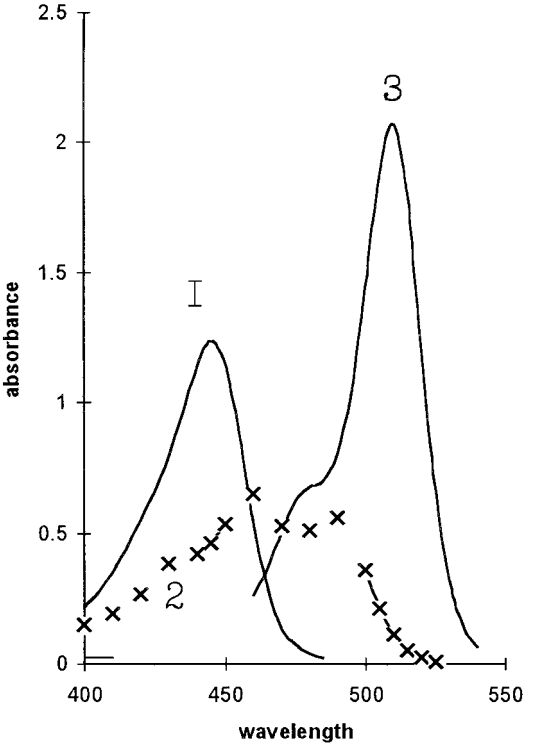


Fig. 3. Absorption spectra of various species of decyl fluorescein in 82 wt% butanol: cation H_2R^+, **IXa** (1), neutral HR, **Xa** (2) and anion R^-, **XIa** (3).

denotes the molar absorptivities of the corresponding species (protonated, HB^{z+1}, and deprotonated, B^z). The wavelengths near the absorption maximum (e.g. $\lambda = 445$ and 510 nm, Fig. 3) were used as analytical positions. For some oxyxanthenes the K_{a1} and K_{a2} values are close. In the most general case, and for fluorescein in particular, at fixed λ the dependence A vs $pa^*_{H^+}$ can be described by Eq. (7):

$$A = \frac{A_{H_3R^+}(a^*_{H^+})^3 + A_{H_2R}(a^*_{H^+})^2 K_{a0} + A_{HR^-} - a^*_{H^+} K_{a0} K_{a1} + A_{R^{2-}} K_{a0} K_{a1} K_{a2}}{(a^*_{H^+})^3 + (a^*_{H^+})^2 K_{a0} + a^*_{H^+} K_{a0} K_{a1} + K_{a0} K_{a1} K_{a2}} \quad (7)$$

In this case only the $A_{H_3R^+}$ and $A_{R^{2-}}$ values can be measured directly at the appropriate acidity. However, as a first approximation, the fluorescein spectra at $pa^*_{H^+}$ ca. 5 may be taken as the H_2R spectrum. In the case of eosin (Fig. 2), the pK_{a1} and pK_{a2} values differ sufficiently enough to be easily defined by using the iterative procedure [32].

The determination of the pK_{a1} and pK_{a2} values of fluorescein proved to result in major difficulties.

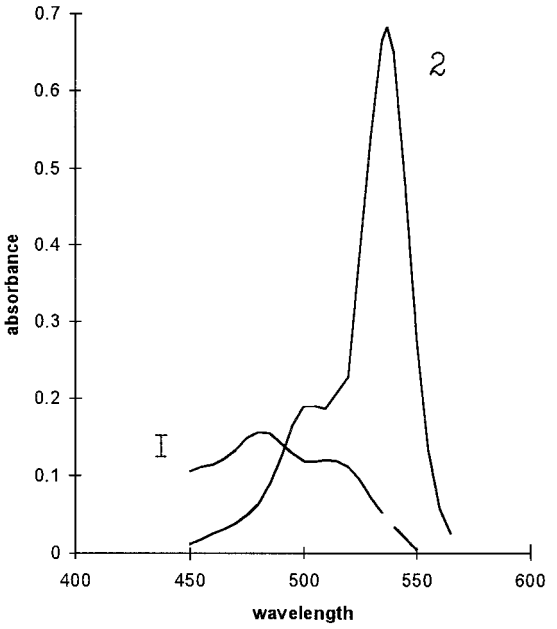


Fig. 4. Absorption spectra of various species of decyl eosin in 82 wt% butanol: neutral HR, **Xb** (1) and anion R^-, **XIb** (2).

The CLINP programme used earlier [15], allows only the calculation of the sum $(pK_{a1} + pK_{a2}) = 16.80 \pm 0.01$ (molar scale of concentrations, $I = 0.01$ M), but not the individual pK_{a1} and pK_{a2} values, although the data for 15 wavelengths (440–510 nm) were utilized in the calculations.

At the same time, the treatment of Albert and Serjeant's test data for benzidine ($H_2B^{2+} \Longleftrightarrow HB^+ \Longleftrightarrow B$) [32] by using the mentioned programme at $\lambda = 300$ nm leads to the values $pK_{a1} = 3.57 \pm 0.01$; $pK_{a2} = 4.70 \pm 0.02$; $A_{HB} = 0.490$, being in good agreement with the most precise results [32].

Therefore, another approach developed earlier specifically for fluorescein, and also considering the peculiarities of its spectra [17,21], was applied, resulting in the values: $pK_{a1} = 8.20 \pm 0.11$, $pK_{a2} = 8.48 \pm 0.17$. It can be noted that the sum $(pK_{a1} + pK_{a2}) = 16.68$ is in satisfactory agreement with the value mentioned above.

The low molar absorptivity of H_2R allows us to determine the pK_{a0} in relatively dilute solutions of $HClO_4$.

The thermodynamic values pK^0_a, presented in Table 2, can be obtained by using Eq. (8):

$$pK^o_a = pK_a + \log f_{HB} - \log f_B \quad (8)$$

The Debye–Hückel equation was used for calculations of ionic activity coefficients f_i (Eq. 9).

$$\log f_i = -\frac{Az_i^2\sqrt{I}}{1 + B\mathring{a}\sqrt{I}} \quad (9)$$

where A and B are constants, $\mathring{a}$ is the ionic parameter, believed by us to be equal to 5×10^{-10} m,

Table 2
The pK^o_a values of flourescein dyes in 82 wt% butanol (25°C; molar scale of concentrations)

Dye	pK^o_{a0}	pK^o_{a1}	pK^o_{a2}
Fluorescein	1.18 ± 0.05	8.5 ± 0.1	9.3 ± 0.2
Sulfonefluorescein	–	4.39 ± 0.06	9.46 ± 0.10
Ethyl fluorescein	2.68 ± 0.02	8.44 ± 0.07	–
Decyl fluorescein	2.53 ± 0.02	8.56 ± 0.03	–
Eosin	–	4.95 ± 0.09	8.33 ± 0.04
Ethyl eosin	–	3.71 ± 0.05	–
Decyl eosin	–	3.86 ± 0.04	–

$A = 3.737$; $B = 0.639 \times 10^{10}$ m^{-1}. The ionic strength of the solutions used, as a rule, equals 0.01 M. The f_i values for neutral molecules are assumed to be equal to unity. The formation of ionic associates $H_3R^+Cl^-$, $H_3R^+ClO_4^-$, Na^+HR^-, $(Na^+)_2R^{2-}$, $H_2R^+Cl^-$, $H_2R^+ClO_4^-$ and Na^+R^- does not seem probable, due to low concentrations of the dyes (10^{-5}–10^{-6} M).

3.2. Estimation of visible absorption spectra of H_2R and HR^- species of fluorescein and eosin and the tautomeric equilibria

Having the K_{a1} and K_{a2} values, it was then possible to calculate the molar absorptivities of HR^- at various wavelengths, and in such a way obtaining the spectra of these species [Eq. (10)]:

$$E_{HR^-} = E + (E - E_{H_2R})a^*_{H^+}K_{a1}^{-1} + (E - E_{R^{2-}})(a^*_{H^+})^{-1}K_{a2}, \quad (10)$$

where E is the 'apparent' absorptivity at the current $pa^*_{H^+}$ value: $E = AC^{-1}l^{-1}$. The interval $pK_{a1} \leqslant pa_{H^+} \leqslant pK_{a2}$ was used. The refinement of E_{H_2R} values was carried out for fluorescein and eosin (at $pa^*_{H^+}$ ca. 5 and 1.5, respectively) with the help of Eq. (11):

$$E_{H_2R} = E + (E - E_{H_3R^+})a^*_{H^+}K_{a0}^{-1} + (E - E_{HR^-})(a^*_{H^+})^{-1}K_{a1} \quad (11)$$

to avoid any influence of traces of intensely coloured ions (H_3R^+, HR^- and R^{2-}) on the spectra of the neutral forms.

The spectral data thus obtained, as well as the results for aqueous and butanolic solutions, are presented in Table 2 and in Figs. 5 and 6. The values of tautomerization constants K_T (Scheme 1), given in the same table, were obtained by using absorption spectra, as described earlier [11–21]. As the K_T values, expressed in the scale of concentrations, were obtained at low ionic strength, they were regarded as thermodynamic values.

According to the main extrathermodynamic assumption, taken as a basis for studying tautomerism, the spectra of species of types **IV** and **VI** (Scheme 1) are similar, and the E_{max} values may be

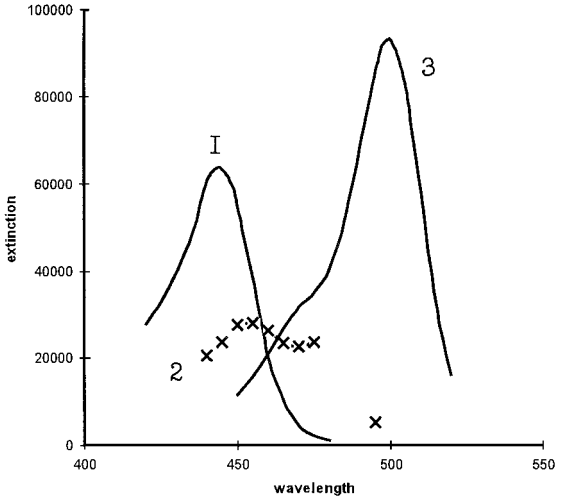


Fig. 5. Absorption spectra of fluorescein ions in 82 wt% butanol: cation H_3R^+, **IIa** (1), monoanion HR^-, **VIa** (2) and dianion R^-, **VIIIa** (3). The spectrum (2) is calculated from the equilibrium data as described in the text.

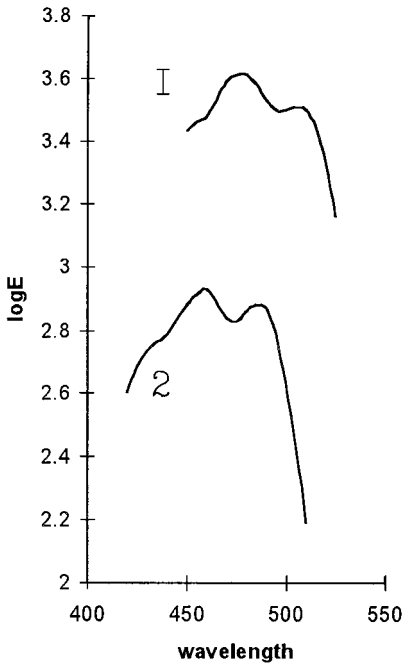
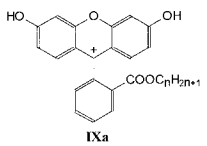


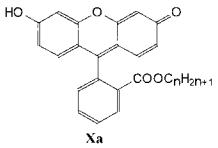
Fig. 6. Molar absorptivities ($\log E_{H_2R}$ scale) of the neutral forms of eosin (1) and fluorescein (2) in 82 wt% butanol.

taken as equal. The same is the case for the species of types **III** and **II**. The ionization of the carboxylic group in the 2′ position (COOH→COO^-) effects only the negatively charged oxyxanthene chromophore, leading to blue shift of the **VIII** band as compared with the **VII** band.

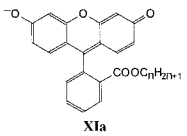
This is confirmed by comparing the absorption spectra of fluorescein (Fig. 5) and decyl fluorescein (Fig. 3). The cationic, neutral and anionic species of fluorescein esters are shown below as structures **IXa**, **Xa** and **XIa**, respectively. The ratio $A^{max}_{H_2R^+}/A^{max}_{HR}$ for decyl fluorescein (1.9) coincides with that for ethyl fluorescein. The value $A^{max}_{R^-}/A^{max}_{HR} = 3.2$ also is the same for both dyes, esters of fluorescein.



IXa



Xa



XIa

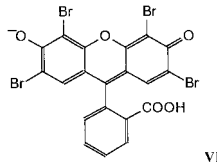
The band of the anion **XIa** is shifted by ca. 10 nm to the red as compared with that of **VIIIa**. The ratio $A^{max}_{R^-}/A^{max}_{HR}$ for decyl eosin (4.7) approximately agrees with that for ethyl eosin (4.2).

As for the H_2R spectrum of fluorescein, it differs from the spectra of molecules of alkyl fluoresceins only in intensity; this is a consequence of the tautomeric equilibrium shift toward the colourless lactone **Va**.

In the H_2R spectra (Fig. 6) there are no distinct signs of zwitter-ionic species (**III**). For eosin, the structure **IIIb** is not characteristic, due to high acidity of the hydroxy groups, while for fluorescein the zwitter-ion **IIIa** appears only in water or in mixed solvents with a small ratio of organic cosolvents [15,17].

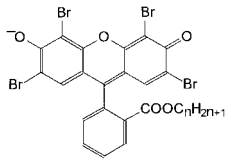
3.3. Tautomeric equilibria of eosin

The absorption spectrum shows that the anion HR^- of eosin exists as a phenolate tautomer (**VIIb**):



VIIb

in accordance with earlier predictions of Kölbel [33], and of Scheibe and Brück [34], and with our own results obtained for various solvents [12–15,17–21]. Indeed, in the H_2O–butanol mixture studied in the present work, the λ_{max} values of the anions **XIb**



XIb

were 535–537 nm. This is close to the λ_{max} value of the HR^- ion of eosin (533 nm). Then, the following relations between the values of the 'total' ionization constants, K^0_a, and of the 'microscopic' ionization constants, k (see Scheme 1), are valid (Eqs. 12 and 13):

$$pK^o_{a2} = pk_{2,COOH} \quad (12)$$

$$pK^o_{a1} = pk_{1,OH} + \log(1 + K_T) \quad (13)$$

The absorption spectrum of the neutral form H_2R of eosin is practically identical with those of HR forms of its esters (see e.g. Fig. 4), except for the intensity: the E_{max} value of eosin is 6 times lower than that of ethyl eosin. As in other solvent systems studied [13,14,16,19,21], the shift of the tautomeric equilibria (**IVb**⇌**Vb**) toward the right is evident. By using the K_T value it is possible to calculate the $pk_{1,OH}$ value (Table 4).

As can be seen in Table 2, lengthening of the hydrocarbon tail results in only a small influence on the ionization of the hydroxy groups: the $pk_{1,OH}$ values are 0.1–0.15 units higher, and the $pk_{0,OH}$ is 0.15 units lower for the compounds with the $C_{10}H_{21}$ group compared with those with the C_2H_5 group. These small effects are evidence of the somewhat more 'alcoholic' microenvironments of dyes with long hydrocarbon chains, due to preferential solvation in a mixture with $x_{BuOH} = 0.526$, $x_{H_2O} = 0.474$. On the whole, however, the corresponding pk values are very close

and we can then conclude that lengthening of the hydrocarbon tails of the esterified dyes has only a relatively small influence on the pK_a values in the mixed solvent ($x_{H_2O} = 0.474$). Probably, differences in microenvironments of the functional groups are slight in the systems under study. Another possible reason may be the relatively large distance of the tails from the functional groups.

However, the $pk_{1,OH}$ value of eosin is significantly higher (by 0.6 units) than that of ethyl eosin, while the difference between the $pk_{0,OH}$ values of fluorescein and ethyl fluorescein is negligible. This may be caused by the steric effects of the 2′-substituents on the pK_a value of 2,4,5,7-tetrabromo derivatives. It can be noted that the λ_{max} values of the species **VIIb** and **XIb** also do not exactly coincide, the difference being 2–4 nm.

3.4. Tautomeric equilibria of fluorescein

The absorption spectra (Fig. 5) show that the anion HR^- of fluorescein exists as a carboxylate tautomer (**VIa**). The K_T (Table 3) indicates the substantial predominance of the lactonic structure **Va** relative to the quinonoid structure **IVa**. However, the K_T values of fluorescein in solvents with similar permittivity, e.g. in 90% acetone or 64% 1,4-dioxane ($\varepsilon = 24$), are even markedly higher ($K_T = 2 \times 10^3$ and 1.7×10^2, respectively) [16,18], than in the butanolic solvent systems studied. This illustrates the principal role of the H-bonding ability of the media; moreover, in 91% DMSO ($\varepsilon = 56$) the K_T value is also very high ($K_T = 6 \times 10^2$) [13,17], and in methanol ($\varepsilon = 32$) the K_T value (54) [14], is much closer to those in butanol and in 82% BuOH. Thus the results of this investigation agree with those reported [17], and the K_T values correlate with the E_T^N parameter. The $\log K_T$ values for the above mentioned solvents, including butanol and 82% butanol, correlate with E_T^N with only a rather low correlation coefficient ($r = 0.81$), probably due to the limited number of solvents ($n = 7$, including water). However, this polarity parameter, reflecting also the influence of H-bonding [38], allows us to interpret the influence of solvent effects on the tautomeric equilibria better than the ε values.

In the general case, the following relationships are valid for the pK_a^o values of fluorescein [Eqs. (14)–(16)]:

$$pK_{a0}^o = pk_{0,OH} - \log(1 + K_T + K_T') \quad (14)$$

$$pK_{a1}^o = pk_{1,COOH} + \log(1 + K_T + K_T') \quad (15)$$

$$pK_{a2}^o = pk_{2,OH} \quad (16)$$

As a rule, K_T is much greater than K_T' in organic solvents (in water $K_T/K_T' = 3$) [11]. Knowledge of

Table 3
Spectral characteristics of oxyxanthene dyes in the water–butanol system and values of the tautomerization constant K_T =[lactone]/[quinonoid]

Dye	Solvent	λ_{max}, nm ($E_{max} \times 10^{-3}$) of the species:				K_T
		H_3R^+	HR^-	R^{2-}	H_2R	
Fluorescein	Water (Ref. [11])	437 (54.3)	454–474 (32.7–33.8)	490.5 (88.0)	437 (13.9) 470–485 (3–4)	6.04[a]
Fluorescein	82% Butanol	444 (63.6)	458 (28.0) 486 (24.8)	499 (92.8)	458 (0.838) 486 (0.750)	32
Fluorescein	Butanol	445 (60)	460, 480	505 (94)	455 (0.567) 475	52
Eosin	Water (Ref. [12])	≈454 (44.5)	517–519 (81.9)	515 (96.7)	480–485 (8.5)	1.8
Eosin	82% Butanol	–	533 (63.2)	523 (104.0)	475 (4.08) 505 (3.17)	4.9
Eosin	Butanol		535 (93)	528 (101)	475 (4.08)	5.0

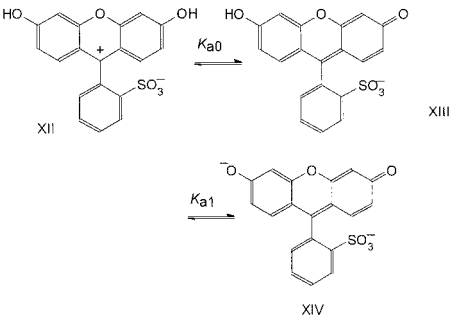
[a] $K_T' = 1.97$; $K_T'' = 3.08$ (data from Ref. [11]).

the ratio of H_2R tautomers allows us to estimate the values of the microscopic ionization constants, k (Table 4). The $pk_{0,OH}$ value agrees with the pK^o_{a0} values of alkyl fluoresceins (Tables 2 and 4). The $pk_{1,COOH}$ value (7.0) is somewhat lower than the pK^0_a value of benzoic acid (7.42, calculated from the $pa^*_{H^+}$ values of buffer solutions) and the same is true for other solvents [14,16,17]. Taking into account a high error in the $pk_{2,OH}(= pK^o_{a2})$ of fluorescein, the agreement of this value with the pK^o_{a2} value of sulfonefluorescein (Table 2) is satisfactory. The ionization scheme of the last named dye is given in Scheme 2; the pK^o_{a1} and pK^o_{a2} values correspond to $pk_{1,Z}$ and $pk_{2,OH}$, respectively:

On the other hand, the ionization constants of model compounds may be used for proving the validity of the scheme of fluorescein ionization. From Scheme 1, Eq. (17) can be derived:

$$\log K'_T = pk_{0,OH} - pk_{\pm,COOH} = pk_{1,Z} - pk_{1,COOH} \quad (17)$$

An attempt of estimation of K'_T may be made, if we take the $pk_{0,OH}$ value being equal to pK^o_{a0} of ethyl fluorescein, the $pk_{\pm,COOH}$ value to that of rhodamine B (5.65, from unpublished studies), the $pk_{1,Z}$ to the pK^o_{a1} value of sulfonefluorescein and the $pk_{1,COOH}$ value-to the pK^o_a value of benzoic acid. Some of these assumptions may be rather approximate, but they undoubtedly show that the K'_T value is very low, ca. 10^{-3}. Thus, a sharp decrease in K'_T is apparent as compared with water ($K'_T = 3$) [11]. Consequently, the conclusion about the absence of the zwitter-ionic tautomer IIIa, made on the base of spectral data, is reliable.



Scheme 2. Sulfonefluorescein structures: H_2R (**XII**), HR^- (**XIII**) and R^{2-} (**XIV**).

Table 4
Ionization microconstants of the dyes and medium effects (Δpk) in various solvents

Substance, pk	pk values					
	82 wt% butanol ($\varepsilon = 21$)	64 wt% dioxane[a] ($\varepsilon = 24$)	90 wt% Me_2CO[c] ($\varepsilon = 24$)	100% methanol[d] ($\varepsilon = 32$)	$\Delta pk = pk - pk^w$ ($H_2O \rightarrow 82\%$ butanol)	Charge type
Fluorescein $pk_{0,OH}$	2.7	3.0	4.2	4.8	−0.4	+/0
Ethyl fluorescein, $pk_{0,OH}$	2.7	2.7[b]	3.9[b]	5.2[e]	−0.4	+/0
Rhodamine B, $pk\pm$,COOH	5.6	6.1	7.7	7.5	2.4	+/±
Fluorescein, $pk_{1,COOH}$	7.0	7.2	9.2	8.9	3.5	0/−
Ethyl fluorescein, $pk_{1,OH}$	8.4	8.1[b]	10.3[b]	10.0[f]	2.1	0/−
Ethyl eosin, $pk_{1,OH}$	3.7	4.0	4.7	5.5	1.8	0/−
Eosin, $pk_{1,OH}$	4.2	4.1	5.2	6.0	1.8	0/−
Sulfonefluorescein, $pk_{1,Z}$	4.4	4.6	–	5.3	1.2	±/−
Sulfonefluorescein, $pk_{2,OH}$	9.5	9.6	–	11.3	2.7	−/=
Fluorescein, $pk_{2,OH}$	9.3	9.5	11.2	11.5	2.5	−/=
Eosin, $pk_{2,COOH}$	8.3	8.6	10.0	9.2	4.6	−/=

[a] Data from Ref. [16].
[b] Data for 6-hydroxy-9-phenyl fluorone.
[c] Data from Refs. [35–37].
[d] Data from Ref. [14].
[e] For 6-hydroxy-9-phenyl fluorone $pk_{0,OH} = 5.1$.
[f] For 6-hydroxy-9-phenyl fluorone $pk_{1,OH} = 10.2$.

Furthermore, the estimate $K_T/K'_T = K''_T \approx 3 \times 10^4$ may be made by using Eq. (18):

$$\log K''_T = \log K_T - pk_{0,OH} + pk_{\pm,COOH} \quad (18)$$

The pk values are in semi-quantitative agreement with the Bjerrum–Kirkwood–Westheimer Eq. (19):

$$\delta pk = \frac{e^2 N_A}{2.302RT \times 4\pi \times 8.854 \times 10^{-12}} \times \frac{1}{\varepsilon_{eff} r} = \frac{243}{\varepsilon_{eff} r} \quad (19)$$

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, e is the elementary charge, R is the gas constant, T is absolute temperature (298.15 K) and r is the distance between the charged and ionizing groups (in Å). Therefore, the pk values of the hydroxy groups are markedly higher if a negatively charged group is present in the 2′-position: $pk_{0,OH} = 2.7$ and $pk_{1,OH} = 8.4$, while $pk_{1,Z=4.4}$ and $pk_{2,OH} = 9.3-9.5$. The charge (positive or negative) of the xanthene nucleus has a similar influence on the acidity strength of the carboxylic group: $pk_{\pm,COOH} = 5.6$, $pk_{1,COOH} = 7$ and $pk_{2,COOH} = 8.3$.

Finally, the negligibility of the **VIIa** fraction of the fluorescein monoanion HR^- can be demonstrated by Eq. (20):

$$\log K_{T_x} = pk_{1,COOH} - pk_{1,OH} = pk_{2,COOH} - pk_{2,OH} \quad (20)$$

Applying the above $pk_{1,COOH}$ and $pk_{2,OH}$ values, and also using the above data about the influence of additional charge on the pk values for the estimation of $pk_{2,COOH}$ and $pk_{1,OH}$, we can obtain $\log K_{T_x} \leqslant -1$. A similar result is obtained while using the $pk_{1,OH}$ value of ethyl fluorescein and $pk_{2,COOH}$ value of eosin as the corresponding values of fluorescein.

3.5. Microconstants of ionization and interpretation of medium effects

For the 'medium effect', i.e. ΔpK^o_a, Eq. (21) is generally accepted:

$$\Delta pK^o_a = pK^o_a - pK^{ow}_a = \log \gamma_{H^+} + \log \frac{\gamma_B}{\gamma_{HB}} \quad (21)$$

where pK^{ow}_a is the pK^o_a value in water and γ are the transfer activity coefficients of corresponding species from water to the given solvent. In the case of systems with tautomerism, traditional interpretation 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 ($pK^{ow}_{a0} = 2.14$, $pK^{ow}_{a1} = 4.45$, $pK^{ow}_{a2} = 6.80$), the following relationships [Eqs. (22)–(24)] are valid:

$$\Delta pK^o_{a0} = \Delta pk_{0,OH} - \Delta \log(1 + K_T + K'_T) \quad (22)$$

$$\Delta pK^o_{a1} = \Delta pk_{1,COOH} + \Delta \log(1 + K_T + K'_T) \quad (23)$$

$$\Delta pK^o_{a2} = \Delta pk_{2,OH}, \quad (24)$$

and the shift (**IIIa**, **IVa**→**Va**) in 82% butanol results in an additional decrease in pK^o_{a0}, and in an increase in pK^o_{a1} compared with medium effects for the model compounds. So the difference ($pK^o_{a1} - pK^o_{a0}$) changes from 2.31 to 7.3, while for ethyl fluorescein the corresponding values are 3.2 and 5.8. The medium effects for the microscopic constants of all the dyes under study agree with well-known relationships [28,29,31,38,39]. The Δpk values for the acid-base couples with the charge type +/0 are markedly smaller than those for the charge type 0/−, and still smaller than those for the type −/=. For each of the two last named charge types $\Delta pk_{COOH} > \Delta pk_{OH}$ (Table 4).

Additional contribution to the changes in ($pK^o_{a1} - pK^o_{a0}$) makes the shift of the tautomeric equilibria (quinonoid⇌lactone) toward the right. This leads to a pronounced difference between the acidity strength of the H_3R^+ and the H_2R species of fluorescein [Eq. (25)]:

$$\Delta(pK^o_{a1} - pK^o_{a0}) = \Delta pk_{1,COOH} - \Delta pk_{0,OH} + \Delta 2 \log(1 + K_T + K'_T) \quad (25)$$

In contrast, the nature of the medium effects for $pk_{2,OH}$ and $pk_{1,COOH}$, and of the shift of the

tautomeric equilibria toward the lactone (**Va**), results in a levelling of the acidity strength of H_2R and HR^- [Eq. (26)]:

$$\Delta(\mathrm{p}K^0_{a2} - \mathrm{p}K^0_{a1}) = \Delta\mathrm{p}k_{2,\mathrm{OH}} - \Delta\mathrm{p}k_{1,\mathrm{COOH}} - \Delta\log(1 + K_\mathrm{T} + K'_\mathrm{T}) \quad (26)$$

In the case of eosin, the different influence of butanol was apparent, viz., the $(\mathrm{p}K^0_{a2} - \mathrm{p}K^0_{a1})$ value was 1.0 and 3.4 in water and in 82% butanol, respectively. The reason for this is, firstly, a sharper increase in $\mathrm{p}k_{2,\mathrm{COOH}}$ as compared with $\mathrm{p}k_{1,\mathrm{OH}}$, and secondly, a less sharp increase in K_T than in the fluorescein case [Eq. (27)]:

$$\Delta(\mathrm{p}K^0_{a2} - \mathrm{p}K^0_{a1}) = \Delta\mathrm{p}k_{2,\mathrm{COOH}} - \Delta\mathrm{p}k_{1,\mathrm{OH}} - \Delta\log(1 + K_\mathrm{T}) \quad (27)$$

So, the changes in the experimentally determined macroscopic constants in 82% butanol are controlled by the tautomeric equilibria shifts, while the medium effects for microconstants ($\Delta\mathrm{p}k = \mathrm{p}k - \mathrm{p}k^w$, Table 4) are in accord with respect to the charge types of acid-base couples and the nature of ionizing groups [28,29,31,38,39].

The $\Delta\mathrm{p}k$ values in anhydrous methanol display the same character, but are markedly higher than in 82% butanol (Table 4). The difference varies within the range 0.9–2.6, in the mean: 1.8 ± 0.3.

Such changes seem to be, to a large extent, caused by proton exchange occurring within the relatively narrow range of water concentrations [28–31,39]. In a simplistic way, the process can be represented as in Eq. (28):

$$H_2O + C_nH_{2n+1}OH_2^+ \rightleftharpoons H_3O^+ + C_nH_{2n+1}OH \quad (28)$$

Numerous extrathermodynamical estimates of $\log\gamma_{H^+}$ show a sharp increase in the last-named parameter within the interval from ca. 90 wt% alcohol to absolute alcohol [28,29,31,39]. This confirms the existence of the proton in a hydrated state at water content more than 10 wt% [28–31,39]. Hence, in accordance with Eq. (21), the additional pk increase while changing from 80–90 wt% alcohol to absolute alcohol is inevitable.

Using the value $\mathrm{p}K^o_{a0} = 3.9$ in pure butanol [30], it was possible to obtain the values $\mathrm{p}k_{0,\mathrm{OH}} = 5.6$, $\Delta\mathrm{p}k_{0,\mathrm{OH}} = 2.5$ for butanol, while in methanol $\Delta\mathrm{p}k_{0,\mathrm{OH}} = 1.7$ (Table 4). Other true pk values in butanol are not yet available.

The thermodynamic $\mathrm{p}K^o_a$ values of fluorescein, sulfonefluorescein, eosin, rhodamine B, ethyl fluorescein and ethyl eosin in 82 wt% butanol are close to those in 64 wt% 1,4-dioxane [16], being on average 0.2 units lower (in 64 wt% dioxane 6-hydroxy-9-phenylfluorone was used instead of ethyl fluorescein). The most significant deviation, 0.95 units, is observed for $\mathrm{p}K^o_{a1}$ of fluorescein (9.45 in 64% dioxane and 8.5 in 82% butanol). This can be explained by the more pronounced shift of the tautomeric equilibrium of the neutral form H_2R toward the lactone in aqueous dioxane, as compared with 82% butanol (the K_T values equal 173 and 32, respectively). The pk values in 82% butanol average 0.15 units lower than the corresponding values in 64% dioxane (Table 4).

The values $\mathrm{p}K^o_{a1} = 5.24$ and $\mathrm{p}K^o_{a2} = 8.57$ for eosin in 64 wt% dioxane [16] agree with those in 40% dioxane ($\mathrm{p}K_{a1} = 4.45$, $\mathrm{p}K_{a2} = 6.02$; the ionic strength is not mentioned) [40]. For 4′-iodoacetamidoeosin [2] in 40% dioxane, the $\mathrm{p}K^o_{a2}$ value is the same, 6.0, but $\mathrm{p}K^o_{a1} = 3.5$, probably due to the influence of the substituent in the phthalic residue. The results of Amat-Guerri and co-authors [41] for eosin in 50% dioxane ($\mathrm{p}K_{a1} = 3.75$, $\mathrm{p}K_{a2} = 6.25$ at ionic strength 0.1) after recalculation into the conventional $\mathrm{p}a^*_{H^+}$ scale and to zero ionic strength ($\mathrm{p}K^o_{a1} = 4.2$, $\mathrm{p}K^o_{a2} = 7.3$) did not contradict our earlier data [16].

In general, direct comparison of pk values in different mixed solvents has problems. Let us assume that the γ values may be represented as in Eq. (29) [39]:

$$\ln\gamma_i = \frac{\Delta G^{el}_{tr}(\mathrm{i}) + \Delta G^{n}_{tr}(\mathrm{i})}{RT} \quad (29)$$

in which ΔG^{el}_{tr} and ΔG^{n}_{tr} are, respectively, the electrostatic and 'non-electrostatic' contributions to the Gibbs energy of transfer. This approach

results in a well known equation [31,38,39], which is often written without the nonelectrostatic term; we find it justified to call it the Brönsted-Izmailov equation (Eq. 30):

$$\Delta \mathrm{p}k = \frac{e^2 N_A}{4.605RT \times 4\pi \times 8.854 \times 10^{-12}} \times \left[\frac{1}{r_{H^+}} + \frac{z_B^2}{r_B} - \frac{z_{HB}^2}{r_{HB}}\right] \times \left[\frac{1}{\varepsilon} - \frac{1}{\varepsilon_w}\right] + \frac{\Delta G_{tr}^n(H^+) + \Delta G_{tr}^n(B) - \Delta G_{tr}^n(HB)}{2.302RT} \quad (30)$$

in which z is the charge of the corresponding species, ε and ε_w are the relative permittivities of the given solvent and of water, respectively; SI units are used. The ΔG_{tr}^n values reflect the effects caused by preferential solvation, molecular complex formation, ion-dipole interactions or H-bonding, etc.

In 90 wt% acetone, the relative permittivity is the same as in the above-mentioned 64 wt% dioxane ($\varepsilon = 24$), but the ionization constants differ substantially from those in 82 wt% butanol. In this water–acetone mixture, the $\Delta \mathrm{p}k$ values (Table 4) are on average 1.6 ± 0.3 units higher.

However, the molar fraction of the organic cosolvent in 90 wt% $(CH_3)_2CO$ is high, and x_{H_2O} equals only 0.264 (while in 82 wt% butanol $x_{H_2O} = 0.474$, in 64 wt% dioxane $x_{H_2O} = 0.733$). On the other hand, the $\mathrm{p}K_a^o$ values of oxyxanthenes in 82 wt% butanol are on average 0.6 units higher than those in 54 wt% $(CH_3)_2CO$, a solvent with $x_{H_2O} = 0.733$ and $\varepsilon = 46$.

The $\mathrm{p}K_a^o$ values of the studied dyes average 0.5 units lower in 82 wt% butanol than in 78 wt% acetone, x_{H_2O} being equal to 0.474 in both solvents. Only the $\mathrm{p}K_{a1}^o$ value of fluorescein in aqueous acetone is 2.1 units higher, and $\mathrm{p}K_{a0}^o$ is 0.8 units lower than in 82 wt.% butanol, probably due to higher K_T value (ca. 500) in 78 wt% acetone [see Eqs. (22) and (23)].

Thus, notwithstanding much the higher ε value ($\varepsilon = 31$) in water–acetone mixture, the $\mathrm{p}K_a^o$ values are higher than those in aqueous butanol with the same molar fraction of water. Hence the contribution of the solvation term ΔG_{tr}^n [Eq. (30)] is evident.

A water–dioxane mixture possessing $\varepsilon = 20.8$ contains 68 wt% $C_4H_8O_2$; here, according to literature and our data, the pk values should be ca. 0.5 units higher than in 64 wt% dioxane. Hence in 82 wt% butanol the pk values are ca. 0.75 units lower than in the water-dioxane mixture with the same ε value despite $x_{H_2O} = 0.697$ in the latter case.

Thus, notwithstanding the low relative permittivity ($\varepsilon = 20.8$), 82 wt% butanol effects the pk values of the dyes as a whole less than aqueous acetone or 1,4-dioxane with even lower molar fraction of water or/and with higher ε value. Evidently a decisive role is due to the specific solvation of the molecules and ions of the dyes by the OH groups of the alcohol. This makes the influence of butanol similar to that of methanol, ethanol and, to some extent, to that of water. In contrast, solvation by solvents which are no donors of H-bonding, e.g. acetone and dioxane, is of an essentially different nature. This agrees with the much sharper shift of the tautomeric equilibria (quinonoid⇌lactone) toward the right in these solvents as compared with 82% butanol and absolute butanol.

4. Conclusions

The protolytic equilibria of oxyxanthene dyes in 82 wt% aqueous *n*-butanol ($\varepsilon = 20.8$; $x_{H_2O} = 0.474$) have, on the whole, the same character as in other previously studied mixed water-organic solvents. The monoanion HR^- of unsubstituted fluorescein exists as a 'carboxylate' structure, while that of eosin exists as a 'phenolate' one. The state of tautomeric equilibria (quinonoid⇌lactone) in aqueous and anhydrous butanol, as well as in some other solvents, is governed more by the value of the E_T^N parameter than by the value of the relative permittivity, ε.

The values of the 'microscopic' ionization constants (k) obtained with the help of the tautomerization constants, agree satisfactorily with those of model compounds, viz., of dyes with esterified carboxylic groups.

It has been shown that lengthening of the hydrocarbon tail of the esterified dyes from C_2H_5

to $C_{10}H_{21}$ results in only a relatively small influence on their pK_a values in the mixed solvent ($x_{H_2O} = 0.474$). Possible reasons for this may be a similar character of the preferential solvation of the functional groups by butanol and/or the remoteness of the tails from the functional groups.

The thermodynamic pK_a^o values of fluorescein, sulfonefluorescein, eosin, ethyl fluorescein and ethyl eosin in 82 wt% butanol are close to those in 64 wt% 1,4-dioxane, being, on average, 0.2 units lower. The most significant deviation (0.85 units for pK_{a1}^o of fluorescein) can be explained by more pronounced shift of the tautomeric equilibrium of the neutral form H_2R toward the lactone in aqueous dioxane as compared with 82% butanol.

In 82% butanol the medium effects for microconstants ($\Delta pk = pk - pk^w$) are as expected with respect to the charge types of acid-base couples and the nature of the ionizing groups. The pk values in anhydrous methanol are markedly higher than in 82% butanol (the difference varies within the range 0.9 to 2.6, in the mean: 1.8 ± 0.3).

Comparative analysis of the microconstants' medium effects (Δpk) made for water–butanol, water–dioxane and water-acetone mixed solvents with $\varepsilon = 21$–24 and $x_{H_2O} = 0.26$–0.73, illustrates, parallel with the impact of relative permittivity, the substantional role of specific solvation. Notwithstanding the low relative permittivity ($\varepsilon = 20.8$), 82 wt% butanol effects the values of the dyes, as a whole, less than aqueous acetone or 1,4-dioxane with even lower molar fraction of water or/and with higher ε value. Evidently a decisive role is due to the solvation of the molecules and ions of the dyes by the OH groups of the alcohol.

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