Dissociation, tautomerism and electroreduction of xanthene and sulfonephthalein dyes in *N,N*-dimethylformamide and other solvents[†]

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ABSTRACT: Protolytic equilibria in DMF of 10 dyes, fluorescein and its halogen derivatives, rhodamine B and *N,N*-diethylrhodol, and of four sulfonephthaleins were studied by visible spectroscopy. The ratios of the stepwise dissociation constants of the substances in DMF, and the changes of these constants on transferring from water to DMF and other solvents, were explained taking into account the nature of functional groups and of the state of tautomeric equilibria. From the correlations between the Gibbs energies of tautomerization of the neutral species (quinonoid $\rightleftharpoons$ colorless lactone) and the normalized Reichardt's parameter, $E_{\mathrm{T}}^{\mathrm{N}}$, the 'limiting' values of tautomerization constants of fluorescein and eosin at $E_{\mathrm{T}}^{\mathrm{N}} \to 0$ (non-polar aprotic media) were evaluated. The conversion of the neutral form of sulfonephthaleins into colorless sultones in DMF is demonstrated. The peculiarities of the reduction of the substances on a dropping mercury electrode in non-aqueous media were revealed, namely the differentiating action of DMF in the first and second stages of electroreduction of the R^{2-} anions. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: xanthene dyes; sulfonephthalein dyes; dimethylformamide; UV-visible absorption spectra; dissociation constants; tautomerism; electroreduction

INTRODUCTION

Owing to their unique optical properties, xanthene dyes are widely used, especially for optical sensing of O_2, CO_2, H_2S, sulfur-containing organic compounds and NH_3,[2,3] as pH sensors, as fiber-optical systems,[4] for rapid sequencing of biopolymers by using the fluorescence polarization technique,[5] for microfluidic enzyme immunoassay using silicon microchips,[6] etc. They are also used in spectrofluorimetric hydrodynamic voltammetry.[7] Various papers have reported the behavior of rhodamine as a guest molecule in supramolecular chemistry.[8] Throughout the last decade, these dyes have been increasingly utilized in organic solvents. Thus, fluorescein was proposed for oxygen and carbon dioxide monitoring in *N,N*-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO) solutions;[9] a recent study was devoted to the fluorescence lifetimes of fluorescein and various rhodamines in organic media.[10] Consequently, further development of knowledge about the influence of non-aqueous media on the interconversions of the various prototropic forms of these substances is of significance.

Previously we studied systematically the behavior of hydroxyxanthene[11] and aminoxanthene[11a,c,d,g,i,12] dyes (fluorescein, eosin, rhodamine, etc.) and of structurally similar sulfonephthaleins[11i,12b,13] in water, alcohols and mixtures of water with acetone, DMSO and 1,4-dioxane. We found that the problem of medium effects on acid–base equilibria of these substances centers around the tautomerism.

To obtain a more generalized and objective picture, it seemed necessary to study the equilibria in a (practically anhydrous) 'dipolar aprotic' solvent. Such solvents, also termed 'dipolar non-hydroxylic' or, according to Bordwell,[14] 'dipolar non-HBD' (HBD = hydrogen bond donor; for details, see Ref. 14) solvents, display a strong differentiating effect on the acid–base properties of the solutes, and are also of great use for electrochemical studies. A typical example is DMF.

The purpose of this work was to examine the protolytic properties of a set of typical representatives of the aforementioned dye groups in DMF and in such a manner to rationalize their behavior in solutions. In addition, some polarographic studies were carried out in this non-aqueous medium and in H_2O–C_2H_5OH.

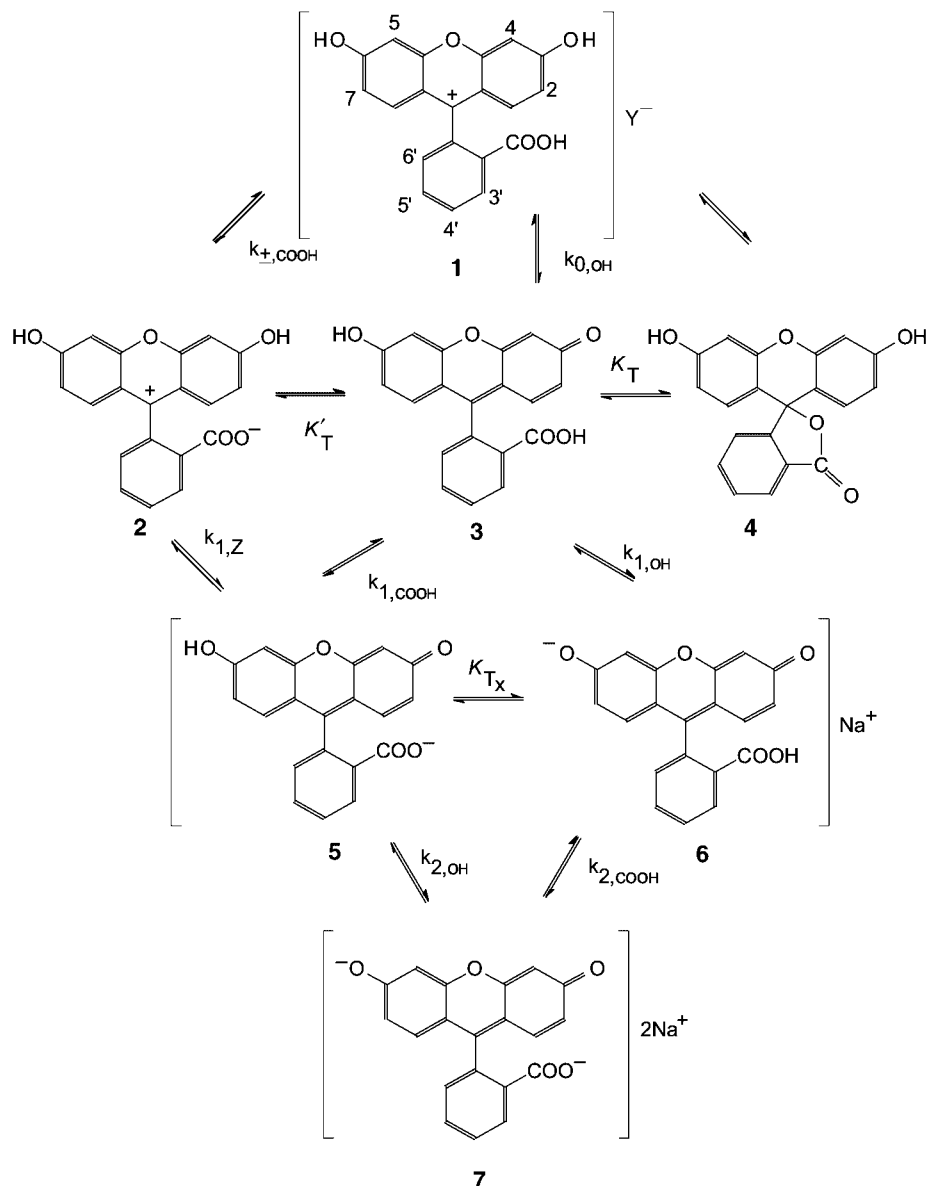The dissociation of fluorescein occurs stepwise:
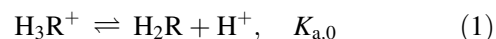
$$H_3R^+ \rightleftharpoons H_2R + H^+, \quad K_{a,0} \tag{1}$$

$$H_2R \rightleftharpoons HR^- + H^+ \quad K_{a,1} \tag{2}$$

Scheme 1. Protolytic conversions of hydroxyxanthenes. Fluorescein, **1a–7a**; 2,4,5,7-tetrabromofluorescein (eosin), **1b–7b**; and 2,7-dichlorofluorescein, **1c–7c**. $K_T = [\mathbf{4}]/[\mathbf{3}]$; $K'_T = [\mathbf{2}]/[\mathbf{3}]$; $K''_T = K_T/K'_T = [\mathbf{4}]/[\mathbf{2}]$; $K_{T_x} = [\mathbf{6}]/[\mathbf{5}]$; $k_{\pm,COOH} = a^*_{H^+} a_2/a_1$; $k_{0,OH} = a^*_{H^+} a_3/a_1$; $k_{1,Z} = a^*_{H^+} a_5/a_2$; $k_{1,COOH} = a^*_{H^+} a_5/a_3$; $k_{1,OH} = a^*_{H^+} a_6/a_3$; $k_{2,OH} = a^*_{H^+} a_7/a_5$; $k_{2,COOH} = a^*_{H^+} a_7/a_6$

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

The detailed picture of these equilibria is presented in Scheme 1.[11] In order to determine the pK_a values in DMF we have obtained the visible absorption spectra of the dyes within a wide range of acidity, which was created by HCl or by salicylate, benzoate and 5,5-diethylbarbiturate buffer solutions. The latter were prepared by mixing appropriate amounts of solutions of corresponding acids and of tetraethylammonium hydroxide. The working buffer solutions contained $0.04 \, \mathrm{mol \, dm^{-3}}$ CH_3OH, 1.5 vol.% C_6H_6 and $0.013 \, \mathrm{mol \, dm^{-3}}$ H_2O. In several cases the spectra of R^{2-} species were measured in DMF with additives of KOH + 18-crown-6, NH$(C_2H_4NH_2)_2$ or aqueous NaOH. For obtaining the visible

spectra of cationic species, addition of concentrated sulfuric acid, besides HCl, was also used as well.

In buffer solutions and in dilute HCl, the p$a^*_{H^+}$ scale was used as a measure of acidity (p$a^*_{H^+} = -\log a^*_{H^+}$); $a^*_{H^+}$ is the lyonium ion activity on a molar scale, the standard state is the hypothetical infinitely diluted solution in DMF with $a^*_{H^+} = 1$.[15] The p$a^*_{H^+}$ values were calculated by using the thermodynamic values of the dissociation constants K_{HA} of buffer acids (HA $\rightleftharpoons$ A$^-$ + H$^+$), whose concentrations were 2–3 orders of magnitude higher than those of dyes. It is well known[16–18] that in order to describe quantitatively the ionic equilibria in DMF it is necessary to take into account the possibility of incomplete dissociation of some electrolytes, known to be 'strong' in water, and also the processes of the so-called

'homoconjugation',[15–17] in particular between the components of buffer solutions:

$$A^- + HA \rightleftharpoons HA_2^- \qquad K^f_{HA_2^-} \qquad (4)$$
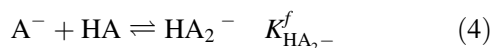
The proton-exchange processes, which may be in the simplest case described by Eqn (5), can also occur in the presence of small amounts of water in the solvent. However, we suppose that at an H_2O content of $0.013 \, mol \, dm^{-3}$ (on average) the equilibrium state is strongly shifted towards the left, to high basicity of DMF.

$$[(CH_3)_2NCHO]H^+ + H_2O \rightleftharpoons (CH_3)_2NCHO + H_3O^+ \qquad (5)$$
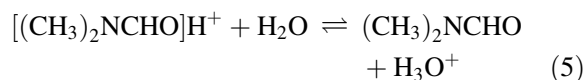
This assumption is supported by very low values of transfer activity coefficient (γ) of the proton from water to DMF. For instance, within the framework of the tetraphenylborate hypothesis, $\log\gamma_{H^+} = -2.5$.[19] That is why in DMF, as in DMSO ($\log\gamma_{H^+} = -3.1$), and in contrast to methanol ($\log\gamma_{H^+} = +1.8$), acetonitrile ($\log\gamma_{H^+} = +7.8$) and acetone, small admixtures of water can hardly influence the state of acid–base equilibria (although such H_2O concentrations are able to influence the λ_{max} values of the fluorescein dianion, R^{2-}, **7a**[9c]). An even smaller influence of alcohol and benzene additives to DMF could be expected, taking into account the comparison of organic acid dissociation in DMF with that in DMF–C_2H_5OH–benzene (79:4:17) mixture.[18c] Only in the region of very high $pa^*_{H^+}$ values (the length of the $pa^*_{H^+}$ scale in pure DMF is reported to be 31.6 units[20]) even traces of water suppress the dissociation of very weak acids. Just this effect is observed at the last stage of electroreduction of the dyes under study, when in alkaline media the tetraanion R^{4-} accepts a proton and transforms practically irreversibly into a weak CH-acid:

$$R^{2-} \xrightarrow{+e} R^{\cdot 3-} \xrightarrow{+e} R^{4-} \xrightarrow{+H^+} RH^{3-} \qquad (6)$$
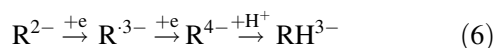
Nevertheless, the difference of the half-wavelength potentials, $E^I_{\frac{1}{2}} - E^{II}_{\frac{1}{2}}$, increases in DMF compared with water on average by 0.3 V.

RESULTS AND DISCUSSION

Dissociation constants and visible spectra of dyes in DMF

The visible spectroscopic determination of pK_a values of the dyes was made as described earlier.[11c–g] In the case of water–DMSO mixtures, our algorithm of pK_a calculation[11f] was confirmed by potentiometric titrations,[21] and the data on fluorescein dissociation are in agreement with results of others.[9b] The $pa^*_{H^+}$ values of buffer solutions,

used for pK_a evaluations, were calculated through with the equation[16,18b]

$$(a^*_H)^2 f^2 c_{A^-} - a^*_H f K_{HA}[c_{HA} + c_{A^-} + K^f_{HA_2^-} \\ \times (c_{A^-} - c_{HA})^2] + K_{HA}{}^2 c_{HA} = 0 \qquad (7)$$

where f is the concentration activity coefficient of the ion and c_{HA} and c_{A^-} are initial concentrations of the acid and of its anion, respectively. The values of c_{A^-} and of the ionic strength of the solution were equated to the $N(C_2H_5)_4OH$ analytical (total) concentration, which was maintained constant ($0.0016 \, mol \, dm^{-3}$). The c_{HA} values were obtained by subtraction of c_{A^-} from the total HA concentration. In HCl solutions, the ionic strength was not held constant, and the $pa^*_{H^+}$ values were determined, solving by the Newton method [Eqn (8)], which can be produced from the balance of concentrations and electrical charges:

$$(a^*_H)^3\left[f - 4f^{-1}K^f_{HCl_2^-}K_{HCl}\right] + 4(a^*_H)^2 c K^f_{HCl_2^-}K_{HCl} \\ - a^*_H\left[f^{-1}K^2_{HCl} + cf K_{HCl} + c^2 f K^f_{HCl_2^-}\right] \qquad (8) \\ + cK_{HCl}{}^2 = 0$$

If necessary, the dyes were also taken into account in the proton balance. Based on comparative analysis[1] of data, available from the literature,[15b,16–18,22] the pK_{HA} values of HCl and salicylic and benzoic acids were equated to 3.2, 8.2 and 12.3, and the $\log K^f_{HA_2^-}$ values to 2.2, 1.7 and 2.4, respectively. In the case of 5,5-diethylbarbituric acid, homoconjugation was not taken into account, because in another aprotic solvent, acetonitrile, the $K^f_{HA_2^-}$ value for this acid is 50–100-fold lower than those for salicylic and benzoic acids.[23] The value $pK_{HA} = 14.7 \pm 0.1$ was calculated for 5,5-diethylbarbituric acid, based on indicator measurements with phenol red, whose pK_{a2} was previously determined in benzoate buffers.

All the f values were calculated by using the Debye–Hückel second approach, taking the ionic parameter value as equal to 5. As for the majority of salts in DMF $pK_{dis} \leq 2$,[17a,24] the incompleteness of dissociation of $N(C_2H_5)_4OH$, $N(C_2H_5)_4A$ and $N(C_2H_5)_4HA_2$, and possible association of dye ions with oppositely charged buffer ions (including HO^-, Cl^-, and Na^+), were not taken into account within the working concentration range. In Table 1, the thermodynamic pK_a values of the dyes are compiled. Typical spectral data are presented in Figs 1 and 2 and in Table 2.

Now let us consider the interrelations among the pK_a values of stepwise dissociation of the dyes in DMF and medium effects, ΔpK_a, at transfer from water to DMF. The ΔpK_a value of the given dissociation stage (e.g. $HB^z \rightleftharpoons B^{z-1} + H^+$) is connected with the transfer activity coefficients, γ, by the following equation:

$$\Delta pK_a = pK_a - pK_a^w = \log\gamma_{H^+} + \log(\gamma_B/\gamma_{HB}) \qquad (9)$$

Table 1. pK_a values of the dyes in DMF[a,b]

Substance	$pK_{a,0}$	$pK_{a,1}$	$pK_{a,2}$
Fluorescein	0.3 ± 0.1^c	14.6 ± 0.3	14.8 ± 0.3
N,N-Diethylrhodol	3.2 ± 0.2	12.6 ± 0.1	—
Rhodamine B	6.5 ± 0.1	—	—
2,7-Dichlorofluorescein	$<-1^d$	10.4 ± 0.2	13.2 ± 0.1
Eosin	—[e]	7.1 ± 0.1	12.4 ± 0.3
Ethyleosin	—[e]	3.6 ± 0.1^f	—
Bromophenol blue	<0	2.9 ± 0.3	7.6 ± 0.1
Bromocresol purple	—[g]	—[g]	11.8 ± 0.2
Phenol red	$<-1^h$	4.2 ± 0.4	14.2 ± 0.1
Thymol blue	-0.5 ± 0.1^h	4.1 ± 0.1	15.2 ± 0.1

[a] The solvent contained $0.013\,mol\,dm^{-3}$ H_2O, $0.04\,mol\,dm^{-3}$ CH_3OH and 1.5 vol.% of benzene.
[b] The scale $mol\,dm^{-3}$.
[c] Calculated from measurements in the $pa^*_{H^+}$ region 1.68–2.87.
[d] The values of $\log([H_2R]/[H_3R^+])$ in 1.8, 3.6, and $5.4\,mol\,dm^{-3}$ H_2SO_4 solutions in DMF are 1.57, 1.15 and 0.32, respectively.
[e] The $pK_{a,0}$ value is in the region of very high acidity.
[f] Calculated from measurements in the $pa^*_{H^+}$ region 2.43–2.87.
[g] The pK_a value was not determined.
[h] In 'concentration' scale; HCl solutions in DMF.

It is evident that the medium effects for stepwise dissociation constants of fluorescein, $K_{a,0}$, $K_{a,1}$, and $K_{a,2}$, differ substantially. While the ratio $K_{a,0}/K_{a,1} = 2 \times 10^{14}$ in DMF is 12 orders of magnitude higher than in water ($K^w_{a,0}/K^w_{a,1} = 204$), the ratio $K_{a,1}/K_{a,2}$ drops from 224 in water to 1.6 in DMF. Moreover, taking into account the accuracy of pK_a determination (Table 1), one can assume that in DMF $K_{a,1} \approx K_{a,2}$. Thus, leveling of the acidic strength of the first and second steps takes place, although DMF is well known as a strongly differentiating solvent.[16,17,18c,20,24] Our data for the system water–DMSO are of similar character.[11f] The same conclusion can be made on the basis of fluorescein spectra in DMF mixture with N,N-dimethyl-p-toluidine:[9a] with variation of the $N(C_4H_9)_4OH$: CO_2 ratio the intensity of the band near $\lambda_{max}(R^{2-})$ decreases owing to the dye conversion into
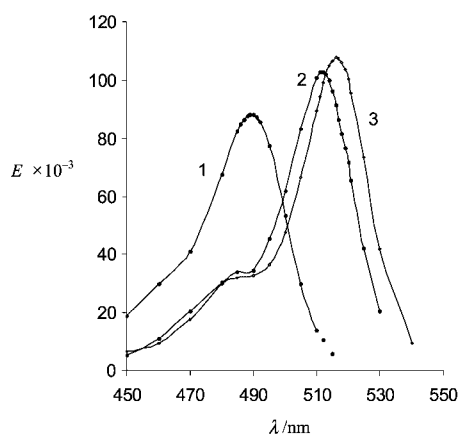

Figure 1. Visible absorption spectra of fluorescein dianion, R^{2-}, in water, pH 10 (1), in acetonitrile with KOH + dicyclohexyl-18-crown-6 (2) and in DMF with KOH + 18-crown-6 (3)
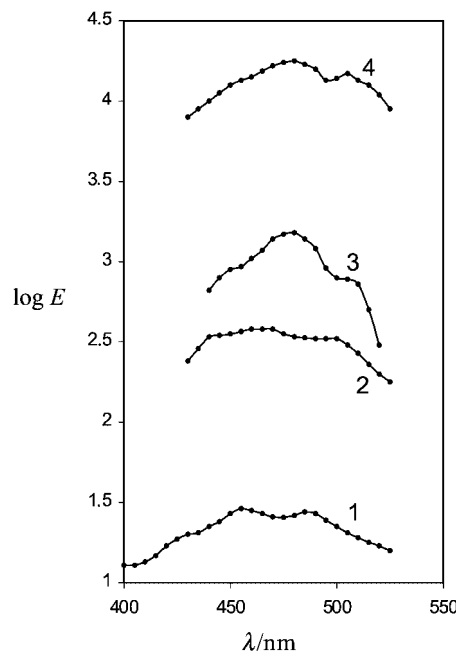
Figure 2. Visible absorption spectra of neutral forms of fluorescein (1), 2,7-dichlorofluorescein (2), eosin (3) and ethyleosin (4) in DMF

H_2R molecules, represented mainly by the colorless lactone **4a**. Interestingly, the spectrum, typical for the HR^- species of fluorescein (with $\lambda_{max} \approx 450$–$455$ and 470–$475\,nm$), does not appear in the mentioned system under any conditions.

Note that the spectrum of fluorescein dianion R^{2-} (**7a**) in acetonitrile, available from the literature, possesses an abnormally low molar absorptivity ($E_{max} \approx 5.5 \times 10^3\,dm^3\,mol^{-1}\,cm^{-1}$ at $\lambda_{max} = 513\,nm$).[26] A partial transformation of intensely colored ion **7a** into the anion-lactone could have been expected, notwithstanding our earlier data, according to which the phenomenon is observed only for fluorescein dyes with nitro groups in positions 2 and 7.[11h] However, our experiments demonstrated that in CH_3CN, in alkaline media, created by addition of KOH (0.005–$0.01\,mol\,dm^{-3}$) in the presence of dicyclohexyl-18-crown-6 ether (0.006–0.01 $mol\,dm^{-3}$), the fluorescein spectrum possesses an usual, for the dianion R^{2-}, value of $E_{max} = 103 \times 10^3\,dm^3\,mol^{-1}\,cm^{-1}$ ($512\,nm$), and Beer's law is valid. Interestingly, addition of 20% (by volume) of DMF to acetonitrile has practically no influence on the position of the maximum in the R^{2-} spectrum, while the introduction of only 1% of water decreases λ_{max} to $505\,nm$. Probably, in the work of Hirano,[26] the traces of acidic admixtures that are practically inevitable in acetonitrile caused almost complete conversion of the initial sample Na_2R, with concentration $10^{-5}\,mol\,dm^{-3}$ in unbuffered medium, into the practically colorless form H_2R. It can be shown that the conversion of ca 90% of fluorescein in the neutral form, with the simultaneous existence of ca 5% of

Table 2. Values of λ_{max} (nm) [$E_{max} \times 10^{-3}$ (dm^3 mol^{-1} cm^{-1}) in parentheses] and tautomerization constants of dyes in DMF

Substance	Anion R^{2-} [a]	Cation	Neutral from	K_T
Fluorescein	520.5 (116)[b,c,d,e,f]	≈450 (55)	455 (0.029)	1033
Eosin	529.4 (122)[g,h]	≈545	480 (1.5)	15
2,7-Dichlorofluorescein	526 (89.6)[i]	≈450 (78)[j]	470 (0.38)	≈70
N,N-Diethylrhodol	517 (21.3)[k]	525 (42.7)	525 (0.773)	32
Rhodamine B	—	560 (95.7)	537 (0.945)	100[l]

[a] In the case of hydroxyxanthenes, the R^{2-} spectra, represented in this table, were measured in DMF in the presence of 0.05–0.4 mol dm^{-3} of NH(C$_2$H$_4$NH$_2$)$_2$.
[b] In DMF with KOH + 18-crown-6 the values are 516.5 (108).
[c] In the presence of 2 vol.% H$_2$O, at 0.004 mol dm^{-3} NaOH the values are 515.5 (104).
[d] In NEt$_4$OH solutions, in the presence of 0.04 mol dm^{-3} CH$_3$OH and 1.5 vol.% benzene, the values are 514 (90).
[e] In water the values are 490 (88).
[f] According to literature data,[25] in DMF with addition of 0.1–0.5 mol dm^{-3} triethylamine the values are 519 (106 ± 5), and in DMF with KOH addition (1.7 × 10^{-4} mol dm^{-3}) λ_{max} = 517.6.[9c]
[g] In NEt$_4$OH solutions, in the presence of 0.04 mol dm^{-3} CH$_3$OH and 1.5 vol.% benzene, the values are 529 (98).
[h] In water the values are 515 (96.7).
[i] In water the values are 501.5 (75).
[j] In 7–9 mol dm^{-3} H$_2$SO$_4$ solutions in DMF.
[k] R$^-$.
[l] K_T''.

the dye in form of R^{2-} anion, even in the presence of small amounts of HR$^-$ ion,[26] gives evidence for the inversion of the constants of stepwise dissociation in CH$_3$CN (p$K_{a,1}$ − p$K_{a,2}$ ≥ 1). Our experiments demonstrated that if acidification of the solution in CH$_3$CN, containing R^{2-} ions, leads to a substantial (e.g. two-fold) intensity decrease, the spectra coincide after normalizing. This proves the co-existence of two species (R^{2-} → H$_2$R), while the output of HR$^-$ in solution is negligible. In our experiments with benzoate buffer solution (pa$_{H^+}^*$ ≈ 20, according to Kolthoff *et al.*[23]), the intensity of fluorescein absorption in CH$_3$CN decreases by at least two orders of magnitude compared with the R^{2-} spectrum. For eosin dianion, the published E_{max} value[26] is also one order of magnitude smaller than ours (Table 2), probably also due to acidic admixtures in acetonitrile.

The visible absorption spectra of R^{2-} ions of both fluorescein and eosin depend to some extent on the nature of the base added to DMF (Table 2). It seems to be caused by association with alkylammonium ions, having one or several NH groups. In dipolar aprotic solvents, such association is known to be much more probable[27] than in the case of cations Na$^+$ and especially [Na-crown]$^+$ and N(C$_2$H$_5$)$_4^+$. On the other hand, the association of R^{2-} anions with cations in DMF and DMSO results in a small bathochromic shift and hyperchromic effect. This was shown by Shakhverdov,[28] who studied the interaction of eosin and erythrosin with triply charged cations of lanthanides; the absorption and emission spectra demonstrate that the association constants in such cases may be of the order of 10^6.[28] The λ_{max} value of the fluorescein ion R^{2-} in *N,N*-dimethylacetamide (DMAC), available from the literature (520 nm),[29] is close to that registered by us in DMF (Table 2), while the relatively low E_{max} value (29 × 10^3), obtained in unbuffered medium,[29] can be

explained by incomplete conversion of the dye into the R^{2-} ion, as in the case of acetonitrile (see above).

Tautomerism of molecules and ions of xanthenes and comparison of pK_a values in different solvents

In order to interpret the pK_a values, it is necessary to refer to the detailed picture of acid-base equilibrium (Scheme 1), which was proposed and used earlier.[11] The principal extrathermodynamic assumption, about the identity (or proximity) of visible absorption bands of the species **1** and **2**, of **3** and **5** and (after correction to the λ_{max} shift) of **6** and **7** of the given dye,[11] together with the undoubted fact of decoloration after formation of lactone **4** (due to sp^3 hybridization of the central carbon atom), allows us to estimate the composition of equilibrium mixtures of tautomers, designated by the formulae H$_2$R and HR$^-$.

The spectrum of the singly charged fluorescein anion HR$^-$ in DMF, like those in other solvents,[11] reveal its 'carboxylate' structure **5a**. Hence the last dissociation stage is **5a** → **7a**. Among three tautomers of the neutral form H$_2$R, the colorless lactone **4a** predominates. Further, the spectrum of H$_2$R species of fluorescein (Fig. 2) gives evidence of some small amount of quinonoid tautomer **3a**, while the band that could have been attributed to zwitterion **2a** is not observed. The constant of the tautomeric equilibrium, K_T, characterizing the degree of conversion of the quinonoid, or 2-(6-hydroxy-3-oxo-3*H*-xanthene-9-yl) benzoic acid (**3a**) into the lactone, or 3',6'-dihydroxyspiro[isobenzofuran-1(3*H*), 9'(9*H*)- xanthene]-3-one (**4a**), appeared to be equal to 1033 in DMF. This value differs from the data given in literature (K_T = 770).[30] The deviation is caused, in our

view, by the presence of some HR^- ions while measuring the H_2R visible absorption spectrum in the cited work.[30] Similar discrepancy with the results of Fompeydie and Levillain[30] were noted earlier for the system water–DMSO.[11d,f] From Scheme 1 the following relationships can easily be derived:

$$pK_{a,0} = pk_{0,OH} - \log(1 + K_T + K'_T) \quad (10)$$
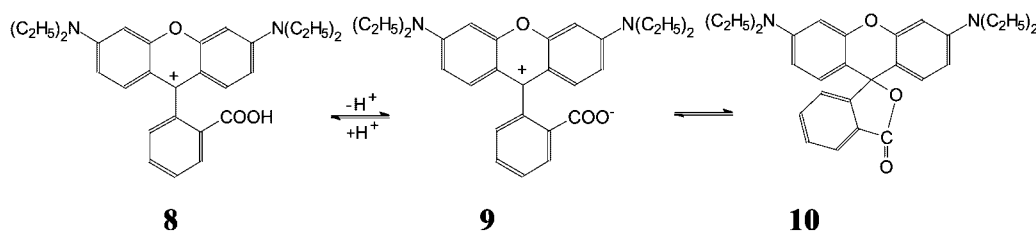
$$pK_{a,1} = pk_{1,COOH} + \log(1 + K_T + K'_T) \quad (11)$$

$$pK_{a,2} = pk_{2,OH} \quad (12)$$

The knowledge of the K_T value allows us to estimate the microscopic dissociation constants, k (Table 3). Based on the spectral data, $K_T \gg K'_T$ can be assumed; the values of these constants are commensurable only in water or aqueous solutions with modest additives of organic co-solvents. As in other solvents, the $pk_{1,COOH}$ value obtained (11.6) is lower than the pK_a value of benzoic acid (12.3).

The dissociation of rhodamine B cation occurs according to

$$HR^+ \rightleftharpoons R + H^+ \quad K_{a,0} \quad (13)$$

The cation HR^+ exists in solutions as structure **8**, while the form R is actually an equilibrium mixture of the zwitterion **9** and colorless lactone **10**.[11a,c,d,i,12,31,32]

The equilibrium $9 \rightleftharpoons 10$ is an example of an intramolecular Lewis acid–base reaction.[14a] Using our K''_T values for water, acetone[12a,b] and for trichloromethane saturated with water (K''_T 90)[12b] and also the data for alcohols,[32] we obtained the following dependence of $\log K''_T$ on the normalized Reichardt parameter, E_T^N:[14]

$$\log K''_T = 3.80 - 6.20 E_T^N$$
$$n = 15, \ r = -0.96, \ s = 0.34 \quad (14)$$

Processing the previously published data[12c] for the system water–DMSO within the range of DMSO molar fractions from 0.221 to 0.844 results in

$$\log K''_T = 6.69 - 9.095 E_T^N$$
$$n = 14, \ r = -0.97, s = 0.16 \quad (15)$$

Extrapolation to pure water leads to $K''_T = 0.004$, which agrees satisfactorily with our earlier estimates.[12a,b] The reason for the successful description of the tautomeric equilibrium data by a single-parametric equation seems to be the nature of the E_T^N function, which reflects both the H-donor ability and polarity of the solvent.[14] Thus, a compromise is made between the viewpoints on the exceptional role of H-bonding on the one hand, and on the electrostatic conceptions of the zwitterion nature on the other.[12a,b,14a,32] At the same time, simultaneous handling of the data for alcohols and water–DMSO mixtures aggravates the correlation coefficient ($r = -0.81$). Probably, some effects, while insignificant in alcohols, be-

8   **9**   **10**

The estimation of the tautomeric equilibrium constant $K''_T = [\mathbf{10}]/[\mathbf{9}]$, made on the assumption that the maximum molar absorptivities of the structures **9** and **8** are equal, results in the value $K''_T = 100$. The latter is much higher than that in water ($K''_T = 0.005-0.01$),[12a,b] higher than in 91 mass% DMSO ($K''_T = 59$)[11d,12c] and lower than in acetone ($K''_T = 250$).[12a,b] The value $E_{max} = 23 \times 10^3$, reported for rhodamine B in DMAC ($\varepsilon = 39$),[29] is rather high for such an aprotic solvent. Through calculation of the equilibrium in this solvent it was shown earlier[12b] that the colored cation HR^+ (**8**) is present in unbuffered solutions in DMAC. Also, the λ_{max} values of the dye in DMAC and DMF without addition of any base, 559 nm[29] and 560 nm,[31] respectively, also correspond to cation **8**, and not to zwitterion **9** (Table 2).

come important in mixtures of water with aprotic solvents.

Visible absorption spectra of structures **8** and **9** differ markedly in DMF compared with water and alcohols (Fig. 3). The data from the NMR spectra and electronic absorption and emission spectra[11d,12c] confirm that the interaction between the xanthene chromophore of the zwitterion and the carboxylate group takes place both through the inductive mechanism and through the solvent. In media with high fractions of aprotic solvents (DMSO, acetone, 1,4-dioxane), under conditions of sparing solvation of COO^- by H-bond formation, the spectrum of the zwitterion, registered on the background of the colorless lactone, differs dramatically from the cationic spectrum.[11d,12b,c] Gradual, regular variations of the
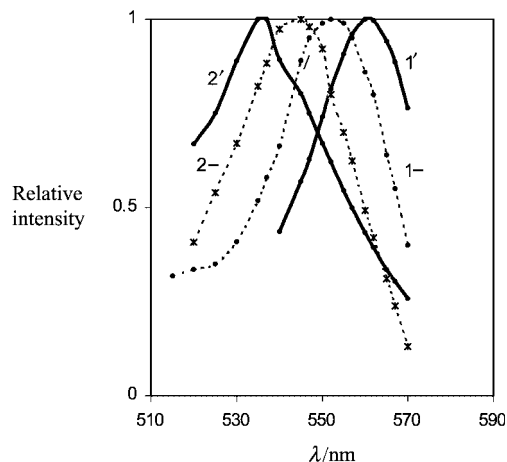
Figure 3. Normalized visible absorption spectra of the cationic species HR^+ (1, 1′) and of the netural form (2, 2′) of rhodamine B in methanol (1, 2) (from Ref. 1) and in DMF (1′, 2′)

neutral form spectra with increase in $(CH_3)_2CO^{12b}$ and $DMSO^{11d,12c}$ content in water–organic mixtures permit us to consider unlikely the appearance of any other particles besides **9** and **10**. However, such strong alterations of the zwitterion **9** spectrum suggest that the assumption that $E_{max}(\mathbf{8}) = E_{max}(\mathbf{9})$, used for estimation of tautomerization constants, is less exact than in the case of alcoholic media. If we regard tautomer **9** as an 'intramolecular ion pair', then in aprotic DMF, poorly solvating the COO^- group, this structure is rather a contact (intimate) or tight associate than a solvent-separated or loose one. This difference manifests itself in the visible spectra (Fig. 3).

Having the estimate of K_T'', it is easy to calculate the $pk_{\pm,COOH}$ value, which corresponds to equilibrium $\mathbf{8} \rightleftharpoons \mathbf{9} + H^+$:

$$pK_{a,0} = pk_{\pm,COOH} - \log(1 + K_T'') \quad (16)$$

The increase in the $pk_{\pm,COOH}$ value by 5.3 units on going from water to DMF (Table 3) confirms zwitterionic structure **9** of the colored tautomer of the neutral form R, because the charge type $+/\pm$ allows us to expect a much greater decrease in acid strength with such a variation of the solvent than in the case of traditional cationic dyes with charge type $+/0$. In fact, the $pk_{0,OH}$ value of fluorescein changes from 3.1 in water to 3.3 in DMF (Table 3).

The adequacy of the detailed scheme (Scheme 1) can be indirectly proved by utilizing the dissociation constants of model compounds and microconstants of fluor-

escein itself. Using the above $pk_{0,OH}$ value and putting $pk_{\pm,COOH}$ and $pk_{1,COOH}$ values of fluorescein equal to $pk_{\pm,COOH}$ of rhodamine B and to pK_a of benzoic acid, respectively, it is possible to estimate the K_T' value through Eqn (17), following from Scheme 1:

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

These assumptions, especially the second one, can cause essential errors when estimating K_T'. Nevertheless, they clearly demonstrate that the K_T' value is certainly very low, namely ca 10^{-5}. The $pk_{1,Z}$ value is about 6.4. As the constants K_T'' and K_T are interrelated ($\log K_T'' = \log K_T - pk_{0,OH} + pk_{\pm,COOH}$),[11a,11c] we can estimate $K_T'' \approx 10^8$ by using the values $\log K_T = 3.0$, $pk_{0,OH} = 3.3$ and $pk_{\pm,COOH} = 8.5$. This K_T value confirms the conclusion regarding the negligibility of the **2a** fraction in the mixture (**2a** $\rightleftharpoons$ **3a** $\rightleftharpoons$ **4a**) deduced from visible absorptions.

Scheme 1 allows us to explain the ΔpK_a values. In DMF for fluorescein $\Delta pK_{a,0} = -1.8$, $\Delta pK_{a,1} = 10.2$ and $\Delta pK_{a,2} = 8.0$. From Eqns (10)–(12), the following relation can be derived:

$$\Delta pK_{a,0} = \Delta pk_{0,OH} - \Delta\log(1 + K_T + K_T') \quad (18)$$

$$\Delta pK_{a,1} = \Delta pk_{1,COOH} + \Delta\log(1 + K_T + K_T') \quad (19)$$

$$\Delta pK_{a,2} = \Delta pk_{2,OH} \quad (20)$$

The $\Delta pk_{0,OH}$ and $\Delta pk_{1,COOH}$ values are 0.2 and 8.1, respectively. Hence it is evident that the dramatic decrease in $pK_{a,0}$ in DMF compared with the value in water, and also 'leveling' of the acidic strength of the first and second steps ($pK_{a,1} \approx pK_{a,2}$, Table 3), are strongly caused by the sharp K_T increase, as can be predicted by the equations

$$\Delta(pK_{a,1} - pK_{a,0}) = \Delta pk_{1,COOH} - \Delta pk_{0,OH} + 2\Delta\log(1 + K_T + K_T') \quad (21)$$

$$\Delta(pK_{a,2} - pK_{a,1}) = \Delta pk_{2,OH} - \Delta pk_{1,COOH} - \Delta\log(1 + K_T + K_T') \quad (22)$$

The coefficient 2 in Eqn (21) explains the strong increase in the $(pK_{a,1} - pK_{a,0})$ difference for fluorescein on transferring from water to organic media.

Being a 'hybrid' of fluorescein and rhodamine B, the dye N,N-diethylrhodol exists in DMF in the form of H_2R^+, HR and R^-, and the molecules HR may exist as three tautomers,[11c] among which the colorless lactone **13** predominates.
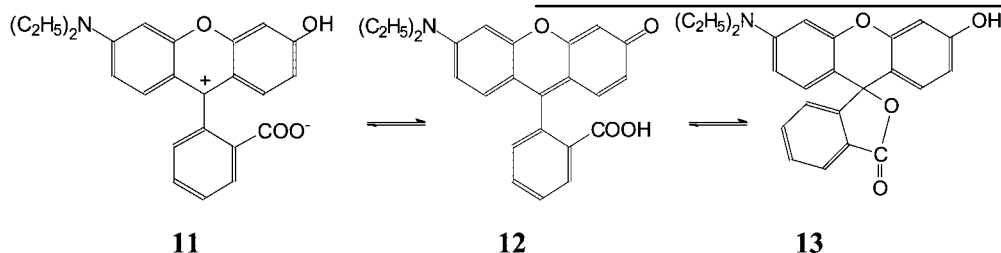

11   **12**   **13**

Table 3. Values of pK_a and tautomeric equilibria constants and pk values of xanthenes in different solvents

| Substance	Constant	Water[a]	Methanol[b]	Ethanol[c]	91% DMSO[d]	90% acetone[e]	DMF
Fluorescein	$pK_{a,0}$	2.14	3.1	3.3	−0.51	0.92	0.3
	$pK_{a,1}$	4.45	10.6	11.7	10.33	12.5	14.6
	$pK_{a,2} = pk_{2,OH}$	6.80	11.5	12.6	8.98	11.2	14.8
	K_T	6.0	54	59	587	1900	1033
	$pk_{0,OH}$	3.10	4.8	5.1	2.2	4.2	3.3
	$pk_{1,COOH}$	3.49	8.8	9.9	7.6	9.2	11.6
	$\Delta pk_{0,OH}$[f]	0	1.7	2.0	−0.9	1.1	0.2
	$\Delta pk_{1,COOH}$	0	5.3	6.4	4.1	5.7	8.1
	$\Delta pk_{2,OH}$	0	4.7	5.8	2.2	4.4	8.0
Benzoic acid	pk_{COOH}	4.20	9.4	10.25	8.05	9.75	12.3
Rhodamine B	$pK_{a,0}$	3.22	7.4	8.7	5.60	6.47	6.5
	K_T''	0.008	0.1	0.28	59	16.6	100
	$pk_{\pm,COOH}$	3.22	7.5	8.8	7.4	7.7	8.5
	$\Delta pk_{\pm,COOH}$	0	4.3	5.5	4.2	4.5	5.3
Eosin	$pK_{a,1}$	2.81	6.9	7.7	3.9	6.4	7.1
	$pK_{a,2} = pk_{2,COOH}$	3.75	9.2	9.8	7.81	10.0	12.4
	K_T	1.8	6.4	10	9.0	16.5	15
	$pk_{1,OH}$	2.4	6.0	6.7	2.9	5.2	5.9
	$\Delta pk_{1,OH}$	0	3.6	4.3	0.5	2.8	3.5
	$\Delta pk_{2,COOH}$	0	5.4	6.2	4.1	6.25	8.6
ε		78	32	25	56	24	37
E_T^N		1.000	0.762	0.654	0.50	0.57	0.404

[a] From Refs 11a,e–g, 12a,b.
[b] From Ref. 11a.
[c] From Ref. 11i.
[d] From Ref. 11d,f.
[e] From Ref. 11b,g,12a.
[f] $\Delta pk = pk - pk$ (in water).

The fraction of colored tautomers is about 3% of the total of *N*,*N*-diethylrhodol molecules, the quinonoidal structure **12** and not the zwitterionic structure **11** being more probable in the given case. Then, a sufficiently reliable estimate, $pk_{1,COOH} = 11.1$, can be obtained, while the $pk_{0,OH}$ value (4.7) is higher than that for fluorescein (3.3), owing to the strong electron-donor properties of the $N(C_2H_5)_2$ group. The sharp increase in the $K_{a,0}/K_{a,1}$ ratio in DMF (2.5×10^9) compared with that in water (5.6×10^2)[11c] is caused mainly by the same reasons as for fluorescein, namely, by the strong shift of the tautomeric equilibrium towards lactone **13** and greater weakening of the neutral carboxylic acid than the cationic one: $\Delta pk_{1,COOH} > \Delta pk_{0,OH}$.

In the case of eosin (2,4,5,7-tetrabromofluorescein), the monoanion HR^- is represented by the 'phenolate' tautomer **6b**. Protonation of the ion R^{2-} $(\lambda_{max} = 529\,nm)$ leads first to a slight (10–15 nm) bathochromic shift, accompanied by insignificant intensity changes (**7b** → **6b**); the spectrum becomes similar to that of the ethyleosin monoanion **15** $(\lambda_{max} = 545\,nm)$. With further $pa_{H^+}^*$ decrease, a sharp fall in intensity takes place; in HCl solutions the spectrum of H_2R is registered, being unaffected within a wide $pa_{H^+}^*$ range. This spectrum is substantially lower in intensity than the spectrum of the neutral form **14** of ethyleosin, because the latter is unable to form a lactonic (colorless) structure (Fig. 2).

Following the earlier described procedure,[11a–f] we obtained the estimate $K_T = 15$, which is close to the value

of 14 reported by others.[30] Thus, for eosin the dissociation by steps (2) and (3) can be reflected as (**3b** ⇌ **4b**) ⇌ **6b** ⇌ **7b**, and the $pK_{a,1}$ and $pK_{a,2}$ values are equal to $pk_{1,OH} + \log(1 + K_T)$ and $pk_{2,COOH}$, respectively.
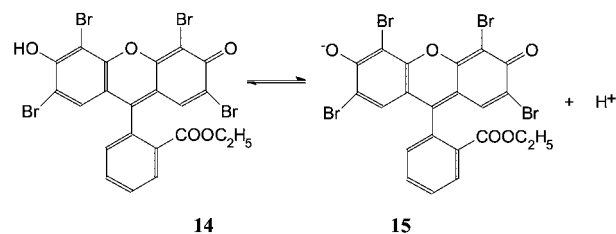
As a result, owing to the greater weakening of the acidic strength of the carboxylic group in comparison with the hydroxylic group, and also to the comparatively small rise in the K_T value (Table 3), the ratio $K_{a,1}/K_{a,2}$ of eosin on transferring from water to DMF does not decrease, in contrast to the fluorescein case, but increases dramatically from 8.7 to 2×10^5.

The dyes can be regarded as derivatives of benzoic acid. Comparing the $pk_{\pm,COOH}$ of rhodamine B with $pk_{1,COOH}$ of *N*,*N*-diethylrhodol and fluorescein and $pk_{2,COOH}$ of eosin, which in DMF are equal to 8.5, 11.1–11.6 and 12.4, respectively, it is possible to demonstrate how the charge of the xanthene moiety (+1, 0 or −1) influences the acidity of the carboxylic group. This impact can be realized through both inductive and 'field' mechanisms. The above pk_{COOH} values are ranged in

accord with the aforementioned sequence of charges, which agrees with the semi-quantitative estimates made using the Bjerrum–Kirkwood–Westheimer approach. The additional negative (positive) charge leads to a pk increase (decrease), δpk:

$$\delta pk = \frac{e^2 N_A}{2.30RT \times 4\pi \times 8.854 \times 10^{-12}} \times \frac{1}{\varepsilon_{eff}\rho} = \frac{24.7}{\varepsilon_{eff}\rho} \tag{23}$$

where ε_{eff} is the 'effective' dielectric constant, e is the elemental electrical charge, N_A is Avogadro's number, R is the gas constant, T is the absolute temperature (293 K) and ρ is the distance between the charged and the dissociating groups (in nm). More refined models are discussed by Vereshchagin.[33] The $(pk_{1,COOH} - pk_{\pm,COOH})$ value exceeds the difference $(pk_{2,COOH} - pk_{1,COOH})$. This trend can be observed not only in DMF, but also in mixtures of water with DMSO and acetone, in alcohols (Table 3) and in 82 mass% n-butanol.[11g] The probable reason is the effective positive charge on the central carbon atom not only in structures **8** and **9**, but also in structures **3** and **5–7**.

The comparison of the protolytic parameters of the dyes in DMF with the values obtained by us earlier in other solvents (Table 3) reveals certain regularities. First, practically always the following sequence of medium effects for the given solvent is observed:

$$\Delta pk_{0,OH} < \Delta pk_{1,OH} < \Delta pk_{2,OH} < \Delta pk_{1,COOH} < \Delta pk_{2,COOH} \tag{24}$$

This agrees well with classical conceptions:[14a,15a] in alcohols, DMF, DMSO and acetone, the ΔpK_a values for cationic acids are lower than those for neutral species. The ΔpK_a for the latter, are lower than for anionic species (when the ionizing group is the same). On the other hand, the ΔpK_a values for carboxylic acids on the whole are higher than those for phenols. Since the dissociation of hydroxylic groups in hydroxyxanthenes (and also in sulfonephthaleins) leads to the appearance of anions with strongly delocalized charge, it results in a further decrease in ΔpK_a. Further, the $\Delta pk_{\pm,COOH}$ value of rhodamine B is as a rule lower than the $\Delta pk_{1,COOH}$ values (fluorescein, benzoic acid, etc.). A simplified approach, treating the zwitterion as two separate charges,[15a] suggests the similarity of $\Delta pk_{\pm,COOH}$ and $\Delta pk_{1,COOH}$. However, in DMF, and also in other aprotic media, the poorly solvated COO^- group can strongly influence the charge distribution within structure **9** (see above).

Both the ΔpK_a values and the $K_{a,0}/K_{a,1}$ and $K_{a,1}/K_{a,2}$ ratios in various solvents are caused by the nature of dissociating groups, as well as by the tautomerization constants [Eqns (18)–(22)]. The medium effect for the $pK_{a,0}$ value of rhodamine B is expressed by

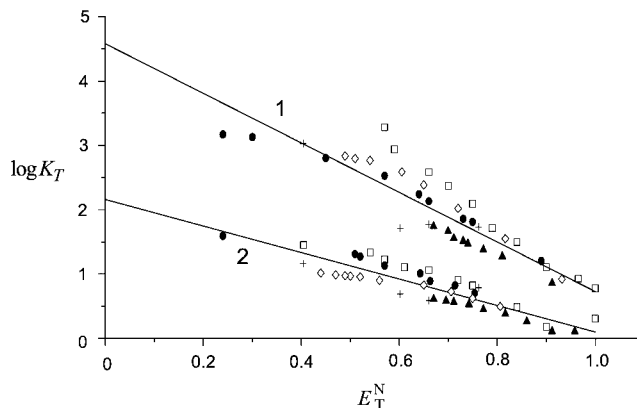$$\Delta pK_{a,0} = \Delta pk_{\pm,COOH} - \Delta \log(1 + K''_T) \tag{25}$$

Figure 4. Dependence of the logarithm of the tautomerization constants of fluorescein (1) and eosin (2) on the normalized Reichardt parameter E_T^N. ($\square$) The system water–acetone; ($\diamond$) water–DMSO; ($\bullet$) water-1,4-dioxane; ($\blacktriangle$) water–ethanol; ($+$) non-aqueous solvents

The fundamental difference between the $\Delta pK_{a,0}$ values in DMF of rhodamine B (3.3) and that of fluorescein (−1.8) is thus caused by the above-mentioned much sharper rise in the $pk_{\pm,COOH}$ value compared with the $pk_{0,OH}$ value. The inversion of the dissociation constants of fluorescein ($K_{a,1}/K_{a,2} < 1$) is strongly determined by the high K_T values in aprotic solvents and in their mixtures with water. The K_T value of fluorescein is always higher than that of eosin.

It is now possible to draw some conclusions concerning the solvent influence upon tautomerism of the neutral form of oxyxanthenes. Such attempts usually lead to dividing of solvents into aprotic types and the H-bond donors.[30] Searching for a mostly universal correlation with solvent properties, we observed that the logarithms of the tautomerization constants or Gibbs energies ($\Delta G_{taut} = -2.303RT\log K_T$, kJ mol^{-1}) in solvents of different nature correlate to a certain degree with the E_T^N parameter,[14] which reflects both the polarity of the solvent molecules and their ability to be H-bond donors (Fig. 4). The following correlations are obtained:

Fluorescein:

$$\Delta G_{taut} = -(26.1 \pm 0.2) + (21.59 \pm 0.01)E_T^N$$
$$n = 39, r = 0.90, s = 2.1 \tag{26}$$

Eosin:

$$\Delta G_{taut} = -(12.3 \pm 0.1) + (11.74 \pm 0.01)E_T^N$$
$$n = 43, r = 0.89, s = 0.92 \tag{27}$$

Here, not only the data for water, methanol, ethanol, n-butanol, and DMF were used, but also for mixtures of water with DMSO, acetone, 1,4-dioxane, and ethanol.[1,11f,34] Within each water–organic mixture, the correlation coefficient is much higher (on average $r = 0.98$). In mixed solvents, the preferential solvation of dyes is possible. Probably the ratio of organic molecules to water

in the solvation shell of the betaine dye 2,6-diphenyl-4-(2,4,6-triphenylpyridinium-1-yl) phenolate, used for developing the E_T^N scale,[14] is close to that in the oxyxanthene microenvironments. The gradual nature of K_T changes near 100% acetone, DMSO and ethanol suggests that uneven re-solvation does not take place here.

The correlations allow us to estimate approximately the 'limiting' values of K_T at $E_T^N = 0$ for fluorescein and eosin as 4×10^4 and 1.4×10^2, respectively. A zero value of E_T^N corresponds, in particular, to tetramethylsilane.[14] Direct experimental determination of K_T values in media of such low polarity is hindered owing to the extremely low solubility of the dyes under study. At the same time, extrapolation to the gas phase ($E_T^N = -0.11$)[14] results in values of 1×10^5 for fluorescein and 2.4×10^2 for eosin.

2,7-Dichlorofluorescein lies between unsubstituted fluorescein and its 2,4,5,7-tetrahalo derivatives with regard to both pK_a values and H_2R tautomerism (Fig. 2, Table 2). In non-aqueous solutions, the fractions of the carboxylate (**5c**) and phenolate (**6c**) tautomers of HR^- can be commensurable for this dye.[11a,d,e] On R^{2-} protonation in DMF a slight (ca 5 nm) red shift takes place. Such a shift is never registered for fluorescein; it indicates the appearance of the tautomer **6c**. However, the $pK_{a,2}$ values of eosin and 2,7-dichlorofluorescein differ by 0.8 units (Table 1), which reflects the incompleteness of the equilibrium shift (**5c** $\rightleftharpoons$ **6c**) to the right.

In the case of ethyleosin, the $pK_{a,1}$ value, obtained in HCl solutions, corresponds to $pk_{1,OH}$. In solvents studied previously, this value is lower than the $pk_{1,OH}$ value of eosin, but the difference is not so great as in DMF (2.3 units, see Tables 1 and 3). It can possibly be caused by some specific interactions between HCl and dyes anions. As a result, the difference between the $pK_{a,2}$ value of bromophenol blue and the $pK_{a,1}$ value of ethyleosin increases from 2.3 in water to 4.0 in DMF. On the other hand, in DMF the $pK_{a,2}$ value of fluorescein (14.6) is higher than that phenol red (14.2), though in the majority of solvents studied the situation is the reverse.

Taking into account the aprotic and dipolar nature of DMF, a strong differentiating action on the last step of sulfonephthalein dissociation could be expected.[13b] In fact, the ΔpK_a values vary from 3.4 (bromophenol blue) to 6.2 (phenol red). The slope of the dependence [Eqn (28)] is 1.55 and is between those for 90 mass% aqueous acetone (1.43) and pure acetonitrile (1.97):[13b,23,35]

$$pK_{a,2} \text{ (in DMF)} = 1.44 + 1.55 pK_{a,2}^w$$
$$n = 4, r = 0.99, s = 0.42 \quad (28)$$

In another dipolar aprotic solvent, N-methylpyrrolidin-2-one ($\varepsilon = 32$),[36] the $pK_{a,2}$ values of three sulfonephthaleins and the $pK_{a,0}$ value of rhodamine B are 1.0 ± 0.3 units lower than those in DMF. The $pK_{a,2}$ values describe the equilibria between the yellow monoanions HR^- and deeply colored R^{2-} dianions (see Scheme 2). In DMF,

the values of λ_{max} (nm) for the sulfonephthalein dianions R^{2-} (**21**) are 578 (phenol red), 601 (bromophenol purple), 602 (bromophenol blue) and 620 (thymol blue). Hence here the $pK_{a,2}$ value corresponds $pk_{2,OH}$ (Scheme 2).

Tautomerism of sulfonephthaleins

The $pK_{a,1}$ values of sulfonephthaleins (Table 1, Scheme 2) appeared much more difficult to interpret than $pK_{a,2}$. Tautomers **17g** and **19e** in the solid state and in solution are identified by vibrational spectroscopy.[13c] In aqueous solutions, sulfonephthalein indicators are characterized by two color transitions: from red (structures **16** or **17**) to yellow (structures **18** or **20**), and then from yellow to blue (red in the case of unsubstituted phenol red), **21**.[13a,37] In acetonitrile[23,35,37] and in water–acetone mixtures with high acetone content,[13a] there are three color transitions: from red **16** to colorless or to practically colorless (sultone **19** with admixture of zwitterion **17** or/and quinonoid **18**), and further to yellow monoanion **20** and to deeply colored dianion **21**. In some publications, another structure of the colorless species of phenol red and thymol blue in water–acetone mixtures is suggested.[38] Basing on NMR spectroscopic data, the authors presumed the existence of a C—H bond at the central carbon atom.[38] However, such a triarylmethane structure can appear only as a result of reduction (with hydrogen, or through electroreduction, see below) of the initial dye, and not because of pH variation.

From Scheme 2, the following relationships can be written for $pK_{a,0}$ and $pK_{a,1}$:

$$pK_{a,1} = pk_{1,SO_3H} + \log(1 + K_T + K_T')$$
$$= pk_{1,Z} + \log[1 + K_T'' + (K_T')^{-1}] \quad (29)$$

$$pK_{a,0} = pk_{0,OH} - \log(1 + K_T + K_T')$$
$$= pk_{\pm,SO_3H} - \log[1 + K_T'' + (K_T')^{-1}] \quad (30)$$

Within the $pa_{H^+}^*$ range where sulfonephthalein equilibria are described with the constants $K_{a,0}$ and $K_{a,1}$, a decrease in the absorption intensity by 1–1.5 orders of magnitude compared with the bands of HR^- and R^{2-} is observed (Fig. 5).

In acidic solutions in another aprotic solvent, DMSO, we could not detect the formation of colorless sultones. This result agrees with the opinion of Kolthoff *et al.*[37] IR spectra in DMSO, obtained by us, confirm such a conclusion. For example, in concentrated phenol red solutions (Fig. 6) the IR spectrum remains practically unaffected by addition of an equivalent amount of trifluoroacetic acid, which makes the possibility of the existence of marked amounts of HR^- ions under these
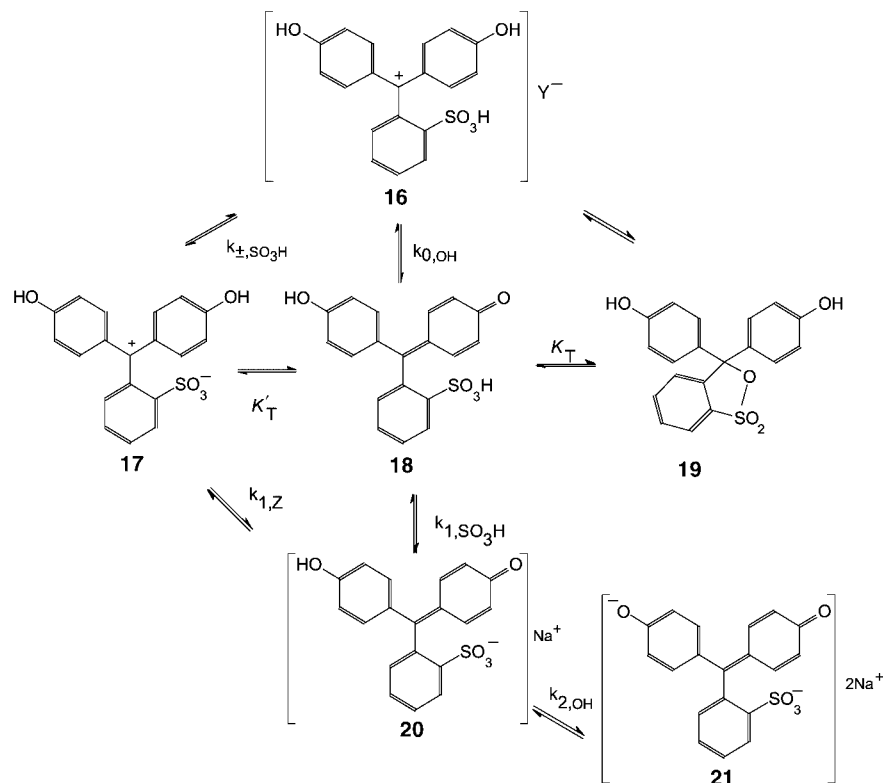
Scheme 2. Protolytic conversions of sulfonephthaleins. Phenol red (phenolsulfonephthalein), **16d–21d**; bromophenol blue (3,3′,5,5′-tetrabromophenolsulfonephthalein), **16e–21e**, bromocresol purple (3,3′-dibromo-5,5′-dimethylphenolsulfonephthalein), **16f–21f**; and thymol blue (3,3′-diisopropyl-6,6′-dimethylphenolsulfonephthalein), **16g–21g**. $K_T = [\mathbf{19}]/[\mathbf{18}]$; $K_T'' = [\mathbf{17}]/[\mathbf{18}]$; $K_T''' = K_T/K_T' = [\mathbf{19}]/[\mathbf{17}]$; $k_{\pm,SO_3H} = a_{H^+}^* a_{17}/a_{16}$; $k_{0,OH} = a_{H^+}^* a_{18}/a_{16}$; $k_{1,Z} = a_{H^+}^* a_{20}/a_{17}$; $k_{1,SO_3H} = a_{H^+}^* a_{20}/a_{18}$; $k_{2,OH} = a_{H^+}^* a_{21}/a_{20}$.
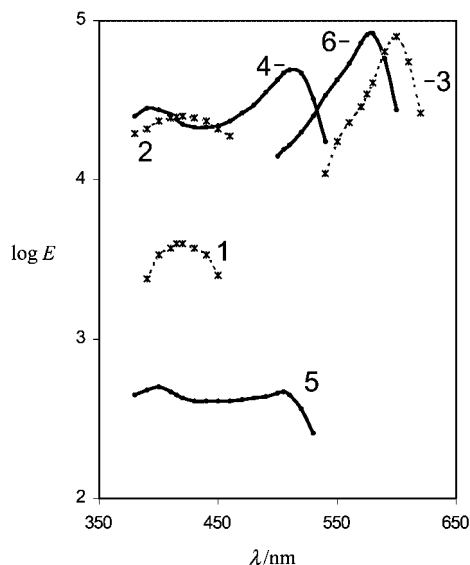

Figure 5. Visible absorption spectra of ionic and molecular forms of bromophenol blue (1–3) and phenol red (4–6) in DMF: the species H_2R (1, 5), HR^- (2), R^{2-} (3, 6) and H_3R^+ (4). Spectra (1) and (5) remain unchanged within the c_{HCl} ranges 0.68–1.18 and 0.01–0.2 mol dm^{-3}, respectively

conditions unlikely. Hence curves 1 and 2 in Fig. 6 represent the spectrum of the neutral form H_2R. The bands at $\nu = 1612–1605$, 1590–1583, 1512–1505 and 1435 cm^{-1} must be attributed to vibrations of the aro-

matic rings, 1336–1328 cm^{-1} to vibrations of the C—aryl bond,[13c,39,40] the band at 1194–1185 cm^{-1} to antisymmetric vibrations of the SO_3^- group and 1277–1272 cm^{-1} to valence vibrations of the ordinary C—O bond. Deformational vibrations C–OH also cause absorption in the region 1430 cm^{-1}. Such an attribution of the bands proves, that the form H_2R of phenol red in DMSO possesses zwitterionic structure **17d**, as in the solid state.[13c] KOH addition leads to a successive conversion, **17d** → **20d** → **21d**. On addition of 1 equiv. of alkali (curve 3), an intense band at $\nu = 1624$ cm^{-1} appears, which can be attributed to valence vibrations of C=O of structure **20d**. On addition of a second equivalent of KOH (curve 4), its intensity markedly decreases, and the band at 1277–1272 cm^{-1} disappears. The conjugated moiety of the dianion **21d** is symmetrical and does not include C=O and C—O$^-$ as such. The bands at $\nu = 1617$, 1574, 1459, 1387 and 1374 cm^{-1} reflect the vibrations of aromatics and of the C—aryl bond. The band at 1155 cm^{-1}, caused by antisymmetric SO_3^- vibrations, naturally remains unchanged.

In the solid state, the H_2R molecules of bromophenol blue exist in the form of a sultone **19e**:[13c] absorption of uncharged aromatics is less intense, while the bands at $\nu = 1350$ and 1194 cm^{-1} correspond to valence vibrations of S=O. However, we are unable to draw conclusions regarding the structure of the form H_2R of
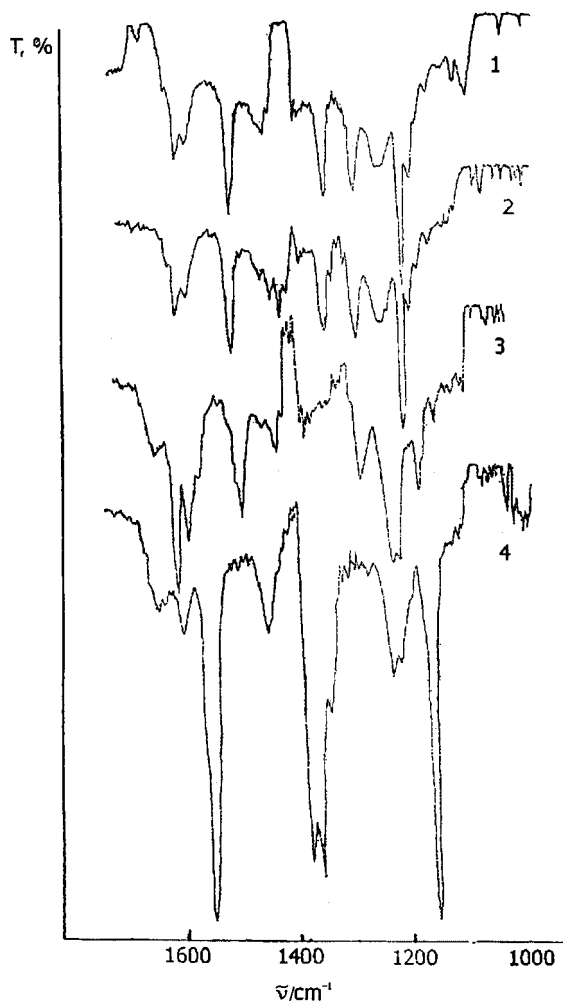
Figure 6. IR spectra of phenol red (0.03 mol dm^{-3}) in DMSO: 0.03 mol dm^{-3} CF$_3$COOH (1), in pure solvent (2), 0.03 mol dm^{-3} KOH (3), 0.06 mol dm^{-3} KOH (4)

bromophenol blue and of other bromo derivatives in solution because of their practically complete dissociation in DMSO even with substantial CF$_3$COOH addition. On the other hand, although in DMF the electronic spectra indicate transformation of the dyes into colorless species, the strong absorption of the solvent in the region 1400–1700 cm^{-1} hinders the application of IR spectroscopy. Even in alcohols, where the color transitions are in general like those in water, some of the dyes, and especially bromophenol blue, at certain $pa^*_{H^+}$ values undergo reversible decoloration in methanol and in n-butanol. This testifies to the formation of sultone **19** also in these media.[1,13a] In methanol and ethanol, the tautomers **19d** and **g** of phenol red and thymol blue are practically absent from solutions, and the p$K_{a,1}$ value probably coincides with p$k_{1,Z}$ (Scheme 2). In this context, the behavior of sulfonephthaleins within the acidic region in DMF is unusual, namely phenol red and thymol blue demonstrate an even greater tendency to form colorless

sultones than bromophenol blue. The intensity of their visible absorption is extremely low: $E_{max}(H_2R) = 0.5 \times 10^3$ (phenol red) and 5.0×10^3 (thymol blue). In the case of phenol red, the spectrum changes only slightly within the HCl concentration range 0.01–0.2 mol dm^{-3} (Fig. 5). Expecting, as a first approximation, the spectra of tautomers **17** and **18** to coincide with those of cation **16** and of monoanion **20**, respectively, we found, that the fractions of the tautomers **17d** and **18d** are small and commensurable, whereas the colorless tautomer **19d** with the fraction 98–99% predominates. Hence the tendency for ring closure in DMF is a general feature of the molecules H$_2$R of xanthenes and sulfonephthaleins.

The p$K_{a,1}$ value of bromophenol blue in DMF was determined in HCl solutions within the $pa^*_{H^+}$ range 0.94–3.40. Within the range of HCl concentrations 0.7–1.2 mol dm^{-3} the spectrum remains unchanged, which allows it to be attributed to the H$_2$R form. This spectrum is similar to that of monoanion HR$^-$ (structure **20e**; λ_{max} 415 nm, $E_{max} = 25 \times 10^3$), except for the much lower intensity in the case of H$_2$R ($E_{max} = 4.0 \times 10^3$). This allows the form H$_2$R to be regarded as an equilibrium mixture of two tautomers (**18e** $\rightleftharpoons$ **19e**). Equating the E_{max} of structure **18e** with that of **20e**, one can obtain $K_T = 5$. In 2.4–4.7 mol dm^{-3} H$_2$SO$_4$ solutions in DMF, slight absorption at 520 nm appears. This is apparently caused by traces of the cation H$_3$R$^+$ of bromophenol blue (**16e**). In the case of bromocresol purple, the tendency for conversion of the neutral form into the colorless tautomer **19f** is less evident.

As can be seen from Eqns (29) and (30), the shift of the tautomeric equilibrium towards the sultone **19** decreases the p$K_{a,0}$ value compared with p$k_{0,OH}$ and p$k_{\pm,SO_3H}$, and increases the p$K_{a,1}$ value as compared with p$k_{1,Z}$ and pk_{1,SO_3H}.

The p$K_{a,0}$ values of sulfonephthaleins are estimated only approximately (Table 1); judging from available information,[41] the H_0 scale in this region of HCl concentrations in DMF is close to $-\log c_{HCl}$. Let us consider in detail the p$K_{a,1}$ values. Both literature data[42a] and our results (Table 1) demonstrate that the dependence of the p$K_{a,1}$ value of thymol blue on the composition of H$_2$O–DMF mixture passes through a minimum, and then rises (in parentheses the mass% of DMF is given): 1.60 (0), 1.31 (20), 1.03 (40), 1.02 (60), 1.04 (70), 1.17 (80), 4.1 (100). Whereas in water–DMF mixtures p$K_{a,1}$ is equal to p$k_{1,Z}$, in pure DMF the term $\log[1 + K_T'' + (K_T')^{-1}]$ plays a significant role. At the same time, in the system water–DMSO (up to 80 mass% of the organic co-solvent) the p$K_{a,1}$ value decreases monotonically down to 1.1;[42b] in 91 mass% DMSO we obtained an even smaller value, p$K_{a,1} = 0.86$, by means of spectrophotometry. Evidently, both the low stability of the sultone **19** and the strong dissociation of the SO$_3$H group of the quinonoid **18** in DMSO are caused by low p$k_{\pm,SO_3H}$ and pk_{1,SO_3H} values. The pK_a values of sulfonic and sulfo acids (including the ion HSO$_4^-$) are known to be low in this solvent.[37]

Because for bromophenol blue in DMF $K_T' \ll K_T$, and $K_T = 5$, the pk_{1,SO_3H} value equals 2.1 [see Eqn (29)]. The following equation, resulting from Scheme 2, explains the differences in the behavior of bromophenol blue on the one hand, and of phenol red and thymol blue on the other:

$$\log K_T' = pk_{0,OH} - pk_{\pm,SO_3H} = pk_{1,Z} - pk_{1,SO_3H} \quad (31)$$

Evidently, the acidic properties of the SO_3H group depend only slightly on the substituents in the phenolic rings, whereas the $pk_{0,OH}$ and $pk_{1,Z}$ values drop sharply in the case of derivatives with halogens in positions 3, 3′, 5 and 5′. Hence it becomes clear why the zwitterionic structure **17**, typical for the unsubstituted phenol red and its alkyl derivatives, does not appear in the case of bromophenol blue. For the latter the form H_2R exists as an equilibrium mixture (**18d** $\rightleftharpoons$ **19d**), with the predominance of the colorless sultone in many organic solvents. This is also confirmed by polarographic data obtained in DMF.

Peculiarities of electroreduction of xanthene and sulfonephthalein dyes on a dropping mercury electrode in DMF

The use of organic solvents, including DMF, allows the eliminatation of adsorption effects.[43] Even in the case of eosin we were unable to register adsorption waves. As a criterion of medium acidity in DMF, the pa_H^* scale was used; some measurements were carried on in 91 mass% aqueous ethanol as well. To interpret the results by means of the above pK_a values of depolarizers, their well-known difference from the 'polarographic' dissociation constants[43b] is taken into account; the influence of the double electrical layer corresponds to the increase in proton concentration in the space near the Hg droplets. The number of electrons, n, participating in the reduction process in buffered media was determined by using the well-known equation[43a]

$$E_{\frac{1}{2}} = E_0 - \frac{2.3mRT}{\alpha nF} pa_H^* \quad (32)$$

For reversible processes, the $\alpha = 1$ and the coefficient $\partial E_{\frac{1}{2}}/\partial pa_H^*$ shows the number of protons, m [(if $m = n$, then at $20\,^{\circ}C$ $\partial E_{\frac{1}{2}}/\partial pa_H^* = -58\,mV/(pa_H^*$ units)]. In the case of irreversible processes, the value of $\partial E_{\frac{1}{2}}/\partial pa_H^*$ does not exactly correspond to the number of reacting protons. In DMF, the polarograms were mainly run within the region $pa_H^* = 7.6–14.2$, at ionic strength $0.0024\,mol\,dm^{-3}$, and also in $0.0024\,mol\,dm^{-3}$ $N(C_2H_5)_4OH$ solution. In 91% ethanol, buffers with $pa_H^* = 4.3–9.5$ and ionic strength $0.0075\,mol\,dm^{-3}$ were used, in addition to $0.0075\,mol\,dm^{-3}$ LiOH solutions. In the both solvents, highly acidic media were created with H_2SO_4 (up to $0.75\,mol\,dm^{-3}$). The colored structures may be regarded as carbcation derivatives:
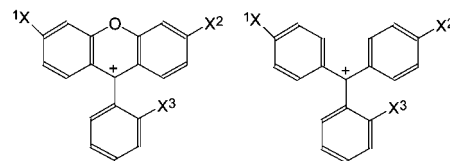
1–3, 5–9, 11, 12, 14, 15   **16–18, 20, 21**

Hence, for fluorescein **1a**, $X^1 = X^2 = OH$, $X^3 = COOH$; **2a**, $X^1 = X^2 = OH$, $X^3 = COO^-$; **3a**, $X^1 = OH$, $X^2 = O^-$, $X^3 = COOH$; **5a**, $X^1 = OH$, $X^2 = O^-$, $X^3 = COO^-$; **7a**, $X^1 = X^2 = O^-$, $X^3 = COO^-$, and for phenol red **16d**, $X^1 = X^2 = OH$, $X^3 = SO_3H$; **17d**, $X^1 = X^2 = OH$, $X^3 = SO_3^-$; **18d**, $X^1 = OH$, $X^2 = O^-$, $X^3 = SO_3H$; **20d**, $X^1 = OH$, $X^2 = O^-$, $X^3 = SO_3^-$; **21d**, $X^1 = X^2 = O^-$, $X^3 = SO_3^-$. Analogously, taking into account the conventional character of graphical formulae, rhodamine B, N,N-diethylrhodol, halogen derivatives of fluorescein and of phenol red, in addition to sulfonefluorescein (**22–24**) can be depicted.
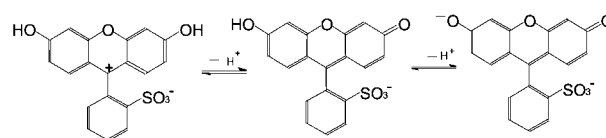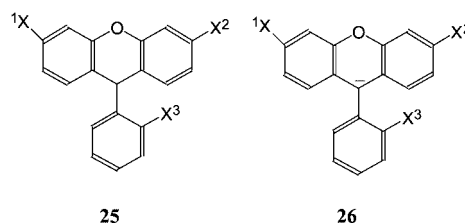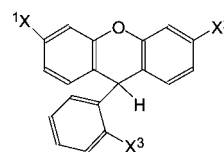

22   **23**   **24**

Three types of xanthene reduction products, radicals, carbanions and CH-acids, are illustrated by structures **25**, **26** and **27**, respectively.

25   **26**

27

It is easy to imagine analogous structures for sulfonephthaleins. Hence the complete redox system is here much more complicated than those for hydrocarbons (Hoiytink), quinone/hydroquinone (Fetter) and chalcones (Stradinš, Vanags).[43a] Up to 25 various structures, including 18 reduction products, are to be taken into account in the case of fluorescein, although the probabilities of their existence differ greatly. Hence a carbanionic structure is possible only in the case of fluorescein tetraanion R^{4-} (**26**, with $X^1 = X^2 = O^-$, $X^3 = COO^-$), this product

irreversibly transforming into RH^{3-} in the presence of proton donors (by placing the symbol H not before, but after the symbol R, we emphasize the distinguishing feature of this CH-acid as compared with OH-acids H_2R and HR^-). The literature on electroreduction of fluoresceins[44,45] and sulfonephthaleins[46] is vast.

The morphology of polarograms in DMF and in 91% C_2H_5OH is similar. In the case of two separate stages of electroreduction both waves are of diffuse nature, the first being reversible, and the second irreversible. The third wave, observable only in the case of bromo derivatives of phenol red in unbuffered DMF in $N(C_2H_5)_4ClO_4$ background (e.g. for bromocresol purple it is a wave with $E_{\frac{1}{2}} = -2.08$ V), is caused by the splitting of the C—Br bond. In alkaline medium, this wave shifts anodically and disappears. The reduction of the pyrone ring of xanthenes is not reliably identified.

In the acidic pa_H^* region, we registered two-electron, irreversible and pa_H^*-independent waves for xanthenes and sulfonephthaleins. For instance, in the case of fluorescein:

$$H_3R^+ + 2e + H^+ \rightarrow H_3RH \qquad (33)$$
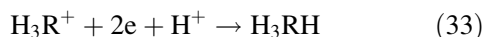
Here, H_3RH corresponds to structure **27**, with $X^1 = X^2 = OH$, $X^3 = COOH$. Hence the regularity, revealed for aqueous solutions,[44–46] is confirmed in non-aqueous solutions. Such a nature of waves does not contradict the possibility of reduction through the disproportionation mechanism,[45,46] while dimerization accompanied by C—C bond formation is less probable.

For the cations of fluorescein, **1a**, diethylrhodol and rhodamine B, **8**, the half-wave potentials in DMF (-0.64, -0.38 and -0.56 V) and in 91% ethanol (-0.64, -0.45 and -0.52 V) are similar. In water, for fluorescein and rhodamine B cations $E_{\frac{1}{2}} = -0.50$ and -0.48 V, respectively. For rhodamine B at pH 9.18, a two-electron wave is registered as well (with $E_{\frac{1}{2}} = -0.885$ V). The $i_{\lim}$ value does not change compared with that at pH 1.68; the shift of $E_{\frac{1}{2}}$ value towards the cathodic region by 0.40 V reflects the influence of the carboxylate negative charge in structure **9** as compared with cation **8**.

For phenol red and thymol blue in 0.75 mol dm^{-3} H_2SO_4 the $E_{\frac{1}{2}}$ values in aqueous ethanol (-0.39 and -0.41 V) are lower than in DMF (-0.29 and -0.30 V); probably in alcoholic media the fractions of the dyes converted into cations **16d** and **g**, are small, and the neutral (zwitterionic) structures **17d** and **g** predominate. In the case of bromophenol blue, the cation **16e** does not appear at such an acid concentration in DMF, and therefore the $E_{\frac{1}{2}}$ values are -0.34 and -0.39 V in aqueous ethanol and in DMF, respectively. Under these conditions, the $i_{\lim}$ value in DMF decreases compared with data obtained at higher pa_H^*. This seems to be a consequence of the formation of sultone, which is difficult to reduce, because in DMF, judging from spectral data, in contrast to 91% C_2H_5OH, the structure **19e** appears. For sulfone-fluorescein in DMF, the irreversible wave ($n = 2$) with $E_{\frac{1}{2}} = -0.44$ V in acidic media probably corresponds to the process H_2R (structure **22**) $+ 2e + H^+ \rightarrow H_2RH^-$ (**27**, $X^1 = X^2 = OH$, $X^3 = SO_3^-$), although the protonation of the SO_3^- group at $pa_H^* < 3$ cannot be excluded. Note that for fluorescein and rhodamine B a decrease in the limiting current is registered on going from H_2SO_4 solutions to media with higher pa_H^*. For fluorescein, the $i_{\lim}$ value decreases by 40%. These waves are apparently caused by conversion of dyes into lactones **4a** and **10**, which are difficult to reduce in DMF, similarly to the situation in aqueous solutions.[44d]

Then the waves became pa_H^*-dependent and remained as such up to relatively high pa_H^* values. The n values are not whole numbers ($2 > n > 1$), which gives evidence for the probability of disproportionation, with both intra- and inter-radical proton transfer. Fluorescein, eosin, sulfone-fluorescein and sulfonephthaleins behave in a similar manner. In 91% C_2H_5OH, for phenol red and thymol blue a single wave remains up to pa_H^* 9.5, whereas for bromphenol purple and bromophenol blue, dyes with lower pK_a values, only up to pa_H^* 6.3 and 4.3, respectively. The situation in DMF is analogous.

At very high pa_H^* values the dyes exist as completely deprotonated species. In this region, dianions R^{2-} give two one-electron waves, as in aqueous solutions;[44,45] the first wave is reversible (Table 4). For rhodamine B in 91%

Table 4. Half-wave potentials ($-E_{\frac{1}{2}}$), ± 0.01 V, in alkaline media[a]

| Depolarizer (in form of R^{2-} species)	$-E_{\frac{1}{2}}$(I) (V)		$-E_{\frac{1}{2}}$(II) (V)		$E_{\frac{1}{2}}$(I)$-E_{\frac{1}{2}}$(II) (V)		
	91% C_2H_5OH	DMF	91% C_2H_5OH	DMF	H_2O[b]	90% C_2H_5OH	DMF
Phenol red	0.56	0.67	1.31	1.78	0.46	0.75	1.11
Bromocresol purple	0.74	0.78	1.48	1.63	0.56	0.74	0.85
Bromophenol blue	0.72	0.85	1.37	1.60	0.64	0.65	0.75
Rhodamine B (as R)	0.76	0.65	1.34	1.43	—[c]	0.58	0.78
Fluorescein	0.75	0.86	1.28	1.48	0.25	0.53	0.62
Eosin	0.71	0.78	1.29	1.43	0.5	0.58	0.65
Sulfone fluorescein	0.65	0.62	1.57	1.65	—	0.92	1.03

[a] Against the saturated calomel electrode.
[b] Except rhodamine B, all the data from the literature.[1]
[c] One wave at pH 9.2; in more alkaline solutions rhodamine B was not studied.

C_2H_5OH, two one-electron waves are observed at pa_H^* > 9.5. The $-E_{\frac{1}{2}}$ values decrease from water to 91% ethanol for all the substances studied, and from water to DMF for all the substances except bromophenol blue. Evidently, the main reason is stronger solvation of anions in water due to H-bonds, except the most hydrophobic ones. The $E_{\frac{1}{2}}$ differences, caused by variations of the liquid junction, seem to be less significant. Interestingly, although the visible absorption bands of ions **24** and **7a** practically coincide, and are shifted by ca 60 nm towards the blue as compared with the band of the ion **21d**, the $E_{\frac{1}{2}}(I)$ values of the R^{2-} ion of sulfonefluorescein are much closer to those of phenol red than to those of fluorescein (Table 4).

The reason for the pa_H^* independence of the second (irreversible) wave is that the protonation occurs after the addition of the second electron to the anion-radical. In DMF, taking into account the initial concentrations of the depolarizer ($0.001\ mol\ dm^{-3}$), and also those of water, methanol and $N(C_2H_5)_4OH$ (0.013, 0.04 and $0.0024\ mol\ dm^{-3}$, respectively), the state of the equilibrium $R^{4-} + H_2O \rightleftharpoons RH^{3-} + OH^-$ can be expected to be strongly shifted towards the right. The corresponding rough estimations can be made by using the constants available in literature for equilibria ($Ar_3C^- + H_2O \rightleftharpoons Ar_3H + HO^-$) in another aprotic dipolar solvent, DMSO, using the pK_a values of uncharged CH-acids triphenylmethane, methanol, xanthene and phenyl-xanthene (30.6, 29.0, 30.0 and 27.9, respectively).[47] Even more impossible is the existence of R^{4-} species in aqueous ethanol.

Hence, in strongly alkaline medium the EEC mechanism [Eqn (6)] is proved, whereas at lower pa_H^* values other mechanisms take place, which was defined by Compton *et al.*[45a] as DISP1 and DISP2 (the latter is probable in more acidic media). For instance, after the electroreduction of fluorescein dianion R^{2-} to ion-radical the protonation of the latter can occur ($R^{3-} + H^+ \rightarrow HR^{2-}$). Further disproportionation [Eqn (34)] actually became irreversible owing to negligible dissociation of the very weak CH-acid:

$$2HR^{2-} \rightleftharpoons HR^- + RH^{3-}\ (or\ R^{2-} + HRH^{2-}) \quad (34)$$
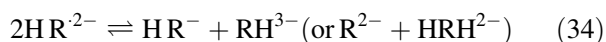
Here HR^{2-} corresponds to structure **25** ($X^1 = O^-$, $X^2 = OH$, $X^3 = COO^-$) and HRH^{2-} to structure **27** with the same substituents.

Photoreduction of hydroxyxanthenes has been studied by several workers in aqueous media.[48] For ion-radical R^{3-} of eosin, the visible absorption band with $\lambda_{max} = 430\ nm$, $E_{max} = 38 \times 10^3\ dm^3\ mol^{-1}\ cm^{-1}$, was reported.[48d] The attribution of the band with $\lambda_{max} = 430\ nm$ to the corresponding ion-radical of fluorescein[48b] was criticized by other authors; Lindqvist[48a] reported the values $\lambda_{max} = 394\ nm$, $E_{max} = 50 \times 10^3$ for the ion-radical HR^{2-} of fluorescein. The ESR spectra of fluorescein dyes in aqueous media can be found in the literature;[44i,45,48c] the ESR signal of fluorescein R^{3-} ion-

radical in $1\ mol\ dm^{-3}$ KOH–$1\ mol\ dm^{-3}$ KCl, obtained by using a 'bubble' electrode,[44i] is in agreement with earlier data.[45a]

In aqueous solutions, some authors[44b,d] reported the 'polarographic' pK_a value, describing the process ($HR^{2-} \rightarrow R^{3-} + H^+$), being equal to 9.2–9.5, and compared it with the value 9.5, obtained by Lindqvist[48a] from photochemical data. However, in general case the true K_a values must differ from the 'polarographic' dissociation constants (see above). Besides, the value $pK_a = 9.5$ reported by Lindqvist corresponds to another dissociation step ($H_2R^- \rightarrow HR^{2-} + H^+$), while the dissociation of the radical HR^{2-} occurs at pH > 13.[48a] The value $pK_a = 10.5$, estimated spectroscopically for the dissociation of the semireduced methyl ether of methylfluorescein ($HR^- \rightarrow R^{2-} + H^+$) in 50 vol.% ethanol,[48c] seems to agree better with Lindqvist's data.

Our study of electroreduction in organic media revealed an interesting regularity, namely, in highly alkaline medium the first and second waves are drawn apart, without exception for all the dyes studied, on transferring from water to 91% C_2H_5OH and then to DMF [in Table 4 the values $E_{\frac{1}{2}}(I) - E_{\frac{1}{2}}(II)$ are given], and the waves become more distinct. Such an expressed differentiating action of the solvent with regard to the stages of one-electron reduction [Eqn (6)], regularly strengthening from aqueous ethanol to aprotic DMF, may seem to be strange, as we are dealing with an aprotic medium containing $0.04\ mol\ dm^{-3}$ CH_3OH. In water, the proton is also added here only after the second electron. We suggest the following explanation. The factors influencing the $E_{\frac{1}{2}}$ values, such as solvation of substituents, character of dye diffusion, steric effects and, in water, adsorption on mercury droplets, are of approximately the same character both for the depolarizers in their oxidized form (i.e. for the initial anions) and for anion-radicals. In contrast, the decisive factor is the increased stability of anion-radicals with strongly delocalized charge, such as R^{3-} (**25**, with $X^1 = X^2 = O^-$, $X^3 = COO^-$ or SO_3^-), in organic media, and particularly, in DMF, due to dispersion interactions with solvent molecules. Furthermore, even if the rise in local $N(C_2H_5)_4^+$ concentration near the electrode causes ion association, it is reasonable to assume that it occurs similarly for anions of different dyes.

Polarograms of sulfonephthaleins obtained in unbuffered medium in DMF, in $N(C_2H_5)_4ClO_4$ background, are complicated through the simultaneous presence of various dye species. In the case of phenol red and thymol blue, together with two-electron waves with $E_{\frac{1}{2}} = -(0.25–0.4)$ V, one-electron waves with $E_{\frac{1}{2}} = -(1.6–1.7)$ V were also registered. These two-electron waves apparently belong to zwitterions **17d** and **g**, while the one-electron waves are probably the second waves of neutral (**17d** and **g**) or monoanionic species (**20d** and **g**). In the case of bromo derivatives under the same conditions $E_{\frac{1}{2}} = -(0.25–0.6)$ V: introduction of bromine atoms makes the reduction of tautomers (**18e** and **f**) easier.

It must be noted that the reduction of sulfonephthaleins at low pH in aqueous solutions, was explained based on structures of type 17.[46b] However, for bromo derivatives the quinonoid structure 18 is more typical, as shown by our spectral studies. Moreover, the neutral species seem to be absent from the aqueous solutions at pH 2.5 at all, and the monoanionic species HR⁻ (20) are more probable here. For bromocresol purple in water, this was stressed in a further study;[44d] however, the disproportionation mechanism was not taken into account. On the other hand, the presence of bromophenol blue in water in the form of sultone 19e, even at pH 1.81, assumed by some authors for explanation of reduction mechanism,[44c] seems to be less probable.

CONCLUSIONS

The detailed dissociation scheme proposed previously for the explanation of protolytic equilibria of xanthenes in water, alcohols and water–organic mixtures is valid for DMF also. The ratios of the stepwise dissociation constants of the dyes in DMF are controlled by the charge type of acid–base pairs, by the nature of the dissociating group, by the strong differentiating impact of the solvent studied, and by the essential influence of the latter on the state of tautomeric equilibria. The monoanion HR⁻ of fluorescein exists in solutions as 'carboxylate' tautomer 5a, whereas in the case of eosin the 'phenolate' tautomer 66 predominates. If we regard the zwitterionic structure of rhodamine B, 9, as an intramolecular ion pair, then in aprotic DMF, where the carboxylate anion is poorly solvated, this associate is rather a contact (intimate) one, whereas in methanol it can be classified as solvent-separated. The mentioned differences are reflected in the electronic absorption. At the same time, the colorless lactonic tautomer 10 predominates in the equilibrium mixture 9 ⇌ 10.

For the tautomerization equilibria [3 (quinonoid) ⇌ 4 (lactone)] of fluorescein and eosin, a linear correlation between the ΔG_{taut} and E_T^N values is revealed, although with modest correlation coefficients, for ca 40 mixed and anhydrous solvents of varied nature. This allows to estimate the 'limiting' ΔG_{taut} values by extrapolation to $E_T^N \to 0$. The neutral forms H₂R of sulfonephthaleins, being unable to form internal esters (sultones) in DMSO, easily convert into this colorless tautomer in DMF. In the case of bromophenol blue and other bromo-substituted sulfonephthaleins, the sultone is equilibrated with quinonoid tautomer, whereas for neutral forms of the unsubstituted phenol red and the alkyl-substituted dye thymol blue, the existence of the zwitterionic tautomer is also possible. Hence the stepwise dissociation of phenol red in DMF occurs in the sequence [17d ⇌ 18d ⇌ 19d (predominating)] ⇌ 20d ⇌ 21d whereas in the case of bromophenol blue only the following equilibria are described quantitatively: (18e ⇌ 19e) ⇌ 20e ⇌ 21e.

In organic media (DMF, 91 mass% aqueous ethanol), the existence of lactones and sultones manifests itself in an essential fall in the limiting current during the electroreduction process. In acidic, neutral and slightly alkaline pa_H^* regions, two-electron irreversible waves are observed, in accord with the up-to-date reduction scheme, including the disproportionation processes. In highly alkaline medium, two one-electron waves are registered (EEC mechanism); the first wave is reversible. The difference between the half-wave potentials, $E_{\frac{1}{2}}(I) - E_{\frac{1}{2}}(II)$, in organic media, especially in DMF, increases in comparison with aqueous solutions, mainly due to additional stabilization of the anion-radicals $R^{\cdot 3-}$ in an aprotic solvent.

EXPERIMENTAL

The fluorescein and eosin samples used were purified by column chromatography. The purity of these and other xanthenes was checked by TLC and by means of excitation spectra. The sample of N,N-diethylrhodol of high purity kindly provided by Dr V. I. Alekseeva (NIOPiK, Moscow). The purity of sulfonephthaleins (Minkhimprom, USSR) was examined using their absorption spectra and pK_a values in water. Bromphenol blue and thymol blue were additionally recrystallized, and the content of sulfur was checked by the Schöniger method. DMF was purified with Al₂O₃ and distilled under vacuum with benzene. The water content (average 0.025%) was checked using Karl Fischer titration (potentiometric procedure). Acetonitrile, carefully purified and dried over P₄O₁₀ directly before use, was a gift from Dr S. M. Kiyko. Our experiments demonstrated that acidic admixtures which appear in CH₃CN within a year after purification can be titrated with alkali, using fluorescein as indicator. DMSO was purified by vacuum distillation over NaOH, and then over zeolites of NaA type. Salicylic and benzoic acids were purified by recrystallization. Stock solutions of HCl were prepared by absorption of gaseous hydrogen chloride by dried DMF, and then standardized against CO₂-free sodium hydroxide. Potassium hydroxide, used to obtain alkaline media in DMF, DMSO and acetonitrile, and H₂SO₄ were of analytical grade. Bis(2-aminoethyl)amine, NH(C₂H₄NH₂)₂, and trifluoroacetic acid were purified by distillation. The samples of 18-crown-6 and dicyclohexyl-18-crown-6 ether (DCH-18-crown-6), cis-anti-cis (isomer B) (purity >99%), were purchased from the Institute of Organic Chemistry, Novosibirsk, and the Institute of General and Inorganic Chemistry, Moscow, respectively. N(C₂H₅)₄OH solution was prepared according to the standard procedure.[27] The sample of N(C₂H₅)₄ClO₄ (analytical grade), used as salt background in polarographic studies, was purified by recrystallization from aqueous ethanol.

Electronic absorption spectra were measured with SP-46 (of USSR origin) and Hitachi U 3210 instruments at

20 °C, immediately after preparation of the solutions, at working concentrations of the dyes near to 10^{-5} mol dm^{-3}; under conditions of lactone and sultone formation the concentrations were higher. The IR spectra were measured on a Specord IR-75 instrument (CaF$_2$ cells, 0.005 cm) against solvent blanks; these experiments were carried out in collaboration with Dr Yu. N. Surov and Dr R. Salinas Mayorga.

For electrochemical studies, a PU-1 system (of USSR origin) was used. The characteristics of the dropping electrode were as follows: $m = 4.2$ mg s^{-1}, $\tau = 3.2$ s^{-1} at $h = 70$ cm. A saturated calomel electrode was used as a reference electrode; the liquid junction with saturated KCl contained 3% of agar-agar. Oxygen was removed by passing an argon stream through the solutions studied. All the polarographic measurements were carried out at 20 ± 2 °C, except in the studies of temperature dependences (in these experiments, the temperature was increased up to 50 °C). The $i - E$ curves of dyes were measured in 91 mass% aqueous ethanol and in DMF in buffer solutions (salicylate, benzoate, diethylbarbiturate buffers, and in aqueous ethanol in acetate buffers also). LiOH in 91% C$_2$H$_5$OH and N(C$_2$H$_5$)$_4$OH in DMF were used as strong bases, added to the corresponding weak acid. In order to avoid essential changes in the structure of the double electrical layer along with variations in p$a^*_{H^+}$, the concentrations of the cations in the buffer mixtures were kept constant. The concentrations of depolarizers, i.e. dyes, $(8 \times 10^{-4}$–3×10^{-3} mol dm$^{-3})$ were two orders of magnitude lower than those of buffer components. To create acidic media, HCl and H$_2$SO$_4$ solutions were used. In the absence of buffer mixtures in DMF, tetraethylammonium perchlorate served as the background. Each polarographic curve was obtained 2–3 times, with satisfactory reproducibility. Information about the electrochemical behavior of the dyes was obtained from the dependences i versus E, i_{lim} versus c, i_{lim} versus $h_{Hg}^{1/2}$, $\log[i/(i_d - i)]$ versus E, $E_{\frac{1}{2}}$ versus p$a^*_{H^+}$ and $\log i_{lim}$ versus $1/T$.[43] The $E_{\frac{1}{2}}$ values were determined with confidence interval ± 0.01 V. For the analysis of diffusion-limited waves, the Ilkovič equation was applied.

REFERENCES

1. Kukhtik VI. PhD Thesis, Kharkov, 1996.
2. (a) Choi MF, Hawkins P. *Talanta* 1995; **42**: 483–492; 987–997; (b) Choi MF, Hawkins P. *Anal. Chim. Acta* 1997; **344**: 105–110; (c) Choi MF, Hawkins P. *Sens. Actuators B* 1997; **38–39**: 390–394.
3. Preininger C, Mohr GJ, Klimant I, Wolfbeis OS. *Anal. Chim. Acta* 1996; **334**: 113–123.
4. (a) Kibblewhite J, Drummond CJ, Grieser F, Thistlethwaite PJ. *J. Phys. Chem.* 1989; **93**: 7464–7473; (b) Fuh M-RS, Burgess LW, Hirschfeld T, Christian GD, Wang F. *Analyst.* 1987; **112**: 1159–1163.
5. Yan Y, Myrick ML. *Anal. Chim. Acta* 2001; **441**: 87–93.
6. Yakovleva J, Davidsson R, Lobanova A, Bentsson M, Eremin S, Laurell T, Emneus J. *Anal. Chem.* 2002; **74**: 2994–3004.
7. Compton RG, Winkler J, Riley DJ, Bearpark SD. *J. Phys. Chem.* 1994; **98**: 6818–6825.
8. (a) Liu Y, Chen Y, Liu SX, Guan XD, Wada T, Inoue Y. *Org. Lett.* 2001; **3**: 1657–1660; (b) Yuan DQ, Koga K, Kourogi Y, Fujita K. *Tetrahedron Lett.* 2001; **42**: 6727–6729; (c) Zhang YJ, Cao WX, Xu J. *Chin. J. Chem.* 2002; **20**: 322–326.
9. (a) Choi MF, Hawkins P. *Anal. Chem.* 1995; **67**: 3897–3902; (b) Choi MF, Hawkins P. *J. Chem. Soc., Faraday Trans.* 1995; **91**: 881–885; (c) Choi MF, Hawkins P. *Spectrosc. Lett.* 1994; **27**: 1049–1063.
10. Magde D, Rogas GE, Seybold PG. *Photochem. Photobiol.* 1999; **70**: 737–744.
11. (a) Mchedlov-Petrossyan NO, Vasetskaya LV. *Zh. Obshch. Khim.* 1989; **59**: 691–703; (b) Mchedlov-Petrossyan NO, Arias Kordova E, Schapovalov SA, Rappoport IV, Egorova SI. *Z. Chem.* 1990; **30**: 442–443; (c) Mchedlov-Petrossyan NO, Alekseeva VI, Gretsov YuV, Kukhtik VI. *Zh. Obshch. Khim* 1991. **61**: 217–225; (d) Mchedlov-Petrossyan NO, Salinas Mayorga R, Surov YuN. *Zh. Obshch. Khim.* 1991; **61**: 225–233; (e) Mchedlov-Petrossyan NO, Rubtsov MI, Lukatskaya LL. *Dyes Pigm.* 1992; **18**: 179–198; (f) Mchedlov-Petrossyan NO, Salinas Mayorga R. *J. Chem. Soc., Faraday Trans.* 1992; **88**: 3025–3032; (g) Mchedlov-Petrossyan NO, Tychina ON, Berezhnaya TA, Alekseeva VI, Savvina LP. *Dyes Pigm.* 1999; **43**: 33–46; (h) Samoylov DV, Mchedlov-Petrossyan NO, Martynova VP, Eltsov AV. *Zh. Obshch. Khim.* 2000; **70**: 1343–1357; *Chem. Abstr.* 2001; **135**: 327880g; (i) Mchedlov-Petrossyan NO. *Zh. Obshch. Khim.* 2003; **73**: 288–295.
12. (a) Mchedlov-Petrossyan NO. *Zh. Fiz. Khim.* 1985; **59**: 3000–3004; (b) Mchedlov-Petrossyan NO. *Kharkov Univ. Bull. Chem.* 1991; (359): 18–32; (c) Mchedlov-Petrossyan NO, Fedorov LA, Sokolovski SA, Surov YuN, Salinas Mayorga R. *Izv. Akad. Nauk SSSR, Ser. Khim.* 1992; 512–521.
13. (a) Mchedlov-Petrossyan NO, Vasetskaya LV, Koval EV, Lyubchenko IN. *Dokl. Akad. Nauk USSR* 1985; **284**: 394–398; (b) Mchedlov-Petrossyan NO, Lyubchenko IN. *Zh. Obshch. Khim.* 1987; **57**: 1371–1378; (c) Mchedlov-Petrossyan NO, Surov YuN, Trofimov VA, Tsivadze AYu. *Teor. Eksp. Khim.* 1990; **26**: 688–698.
14. (a) Reichardt C. *Solvents and Solvent Effects in Organic Chemistry* (2nd edn). VCH: Weinheim, 1988; (b) Reichardt C. *Chem. Rev.* 1994; **94**: 2319–2358.
15. (a) Bates RG. *Determination of pH* (Russian translation). Khimiya: Leningrad, 1972; (b) Aleksandrov VV. *Acidity of Non-aqueous Solutions.* Vyshcha Shkola: Kharkov, 1981.
16. Bykova LN, Petrov SI. *Usp. Khim.* 1972; **41**: 2065–2086; *Russ. Chem. Rev.* 1972; **41**: 975–990.
17. (a) Kolthoff IM, Chantooni MK. *J. Phys. Chem.* 1972; **76**: 2024–2034; (b) Chantooni MK, Kolthoff IM. *J. Phys. Chem.* 1973; **77**: 527–533.
18. (a) Evstratova KI, Kochergina AA, Kupina NA, Beloserskaya VV. *Elektrokhimiya* 1976; **12**: 677–682; (b) Evstratova KI. *Zh. Anal. Khim.* 1980; **35**: 1682–1691; (c) Evstratova KI. Doctoral Dissertation, Leningrad, 1981.
19. Kalidas C, Hefter G, Marcus Y. *Chem. Rev.* 2000; **100**: 819–852.
20. Bykova LN, Petrov SI. *Zh. Vses. Khim. Ova.* 1984; **29**: 541–547.
21. Frolov VYu, Babashov MA, Kudryavtsev SG. *Zh. Fiz. Khim.* 1992; **66**: 2970–2974.
22. Mollin J, Pavelek Z, Navratilova J, Recmanova A. *Collect. Czech. Chem. Commun.* 1985; **50**: 2670–2678.
23. Kolthoff IM, Chantooni MK, Bhowmik S. *Anal. Chem.* 1967; **39**: 315–320.
24. Alexander R, Parker AJ, Sharp JH, Waghorne WE. *J. Am. Chem. Soc.* 1972; **94**: 1148–1158.
25. Martin MM. *Chem. Phys. Lett.* 1975; **35**: 105–111.
26. Hirano K. *Bull. Chem. Soc. Jpn.* 1983; **56**: 850–854.
27. Gyenes I. *Titration in Non-aqueous Media* (Russian translation). Mir: Moscow, 1971.
28. Shakhverdov TA. *Opt. Spektrosk.* 1975; **39**: 786–789.
29. Hirano K. *J. Chem. Soc. Jpn. Chem. Ind. Chem.* 1974; 1823–1828.
30. Fompeydie D, Levillain P. *Bull. Soc.chim. Fr.* 1980; 459–465.
31. Bigelow RW. *J. Phys. Chem.* 1977; **81**: 88–89.
32. Hinckley DA, Seybold PG, Borris DP. *Spectrochim. Acta Part A* 1986; **42**: 747–754.
33. Vereshchagin AN. *Inductive Effect.* Nauka: Moscow, 1987.
34. Mchedlov-Petrossyan NO. Doctoral Dissertation, Kharkov, 1992.
35. Kolthoff IM, Bhowmik S, Chantooni MK. *Proc. Natl. Acad. Sci. USA* 1966; **56**: 1370–1376.
36. Breant M, Auroux A, Lavergne M. *Anal. Chim. Acta.* 1976; **83**: 49–57.

37. Kolthoff IM, Chantooni MK, Bhowmik S. *J. Am. Chem. Soc.* 1968; **90**: 23–28.

38. (a) Murakhovskaya AS. *Zh. Anal. Khim.* 1986; **41**: 629–638; (b) Murakhovskaya AS, Stepanyants AU. *Zh. Fiz. Khim.* 1989; **63**: 3131–3135.

39. Freedman HH. In *Carbonium Ions*, vol. 4, Olah GA, Schleyer PR (eds). Wiley–Interscience: New York, 1973; Chapter 28, 1501–1578.

40. Mchedlov-Petrossyan NO, Surov YuN, Egorova SI, Salinas Mayorga R, Arias Cordova E. *Dokl. Akad. Nauk SSSR* 1991; **317**: 152–157.

41. Kislina IS, Sysoeva SG, Temkin ON. *Izv. Akad. Nauk, Ser. Khim.* 1996; 1025–1027.

42. (a) De AL, Atta AK. *J. Chem. Soc., Perkin Trans. 2* 1986; 1367–1370; (b) De AL, Atta AK. *Can. J. Chem.* 1986; **64**: 1521–1526.

43. (a) Mayranovski SG, Stradin YaP, Bezugliy VD. *Polarography in Organic Chemistry*. Khimiya: Leningrad, 1975; (b) Agasyan PK, Zhdanov SI (eds). *Voltammetry of Organic and Inorganic Substances*. Nauka: Moscow, 1985; (c) Bezugliy VD, Faizullayev O. In *Titrimetric Methods of Analysis of Non-aqueous Solutions*, Bezugliy VD (ed). Khimiya: Moscow, 1986; 193–249.

44. (a) Nagase Y, Ohno T, Goto T. *J. Pharm. Soc. Jpn. 1953*; **73**: 1033–1039; (b) Gollmick FA, Berg H. *Ber. Bunsenges. phys. Chem.* 1965; **68**: 713–715; (c) Issa RM, Abdel-Hamid FM, Hasanein AA. *Electrochim. Acta* 1969; **14**: 561–567; (d) Bannerjee NR, Vig SK. *Indian J. Chem.* 1971; **9**: 444–448; (e) Issa IM, Samahay AA, Issa RM, Ghoneim MM. *Electrochim. Acta* 1972; **17**: 1195–1202; (f) Issa IM, Issa RM, Ghoneim MM, Temerk YM. *Electrochim. Acta.* 1973; **18**: 265–270; (g) Bannerjee NR, Negi AS. *Indian J. Chem.* 1973; **11**: 672–677; (h) Eltsov AV, Smirnova NP, Bobrovnikov MN, Stratonnikova EA. *Zh. Obshch. Khim.* 1988; **58**: 635–641; (i) Bagchi RN, Bond AM, Scholz F, Stösser R. *J. Electroanal. Chem.* 1988; **245**: 105–112.

45. (a) Compton RG, Mason D, Unwin PR. *J. Chem. Soc., Faraday Trans.* 1988; **84**: 483–489, and references cited therein; (b) Compton RG, Mason D, Unwin PR. *J. Chem. Soc., Faraday Trans.* 1988; **84**: 473–482; (c) Compton RG, Mason D, Unwin PR. *J. Chem. Soc., Faraday Trans.* 1988; **84**: 2057–2068; (d) Compton RG, Coles BA, Pilkington BG. *J. Chem. Soc., Faraday Trans. 1* 1988; **84**: 4347–4357.

46. (a) Senne JK, Marple LW. *Anal. Chem.* 1970; **42**: 1147–1150; (b) Kudirka PJ, Nicholson PS. *Anal. Chem.* 1972; **44**: 1786–1794; (c) Jain MN, Shivhare MM, Singh M. *Indian J. Chem.* 1982; **21A**: 198–200; (d) Liu YN, Yu ZM, Wan EK. *Chin. J. Appl. Chem.* 1985; **2**: 81–85.

47. Bordwell FG, Drucker GE, Andersen NH, Denniston AD. *J. Am. Chem. Soc.* 1986; **108**: 7310–7313.

48. (a) Lindqvist L. *Ark. Kemi*. 1960; **16**: 79–138; (b) Krüger U, Memming R. *Ber. Bunsenges. phys. Chem.* 1974; **78**: 670–692; (c) Niizyma S, Kikuchi K, Kokubun H. *J. Photochem.* 1987; **36**: 51–62; (d) Jones G, Chatterjee S. *J. Phys. Chem.* 1988; **92**: 6862–6864.