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Investigation of excited-state proton transfer in 2-naphthol derivatives containing a carboxyl group in organic solvents and in methanol–water mixtures

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Abstract

Excited-state proton transfer (ESPT) in 2-naphthol derivatives with electron withdrawing substituent (–SO_2NH–) dissolved in methanol and methanol–water mixtures is investigated. The analogs studied contain also a carboxyl group, situated at various distances from the proton donating phenolic group. The method of investigation consists in measuring the steady state fluorescence spectra. The parameter $\Re$ of these spectra (ratio of fluorescence quantum yields at two defined wavelengths) given by Weller's equation was fitted to the experimental values of $\Re$ as a function of water concentration in the mixtures. From this procedure, ESPT rate constant and other parameters of this reaction were obtained. It was found that the carboxyl group contained in the molecule of 2-naphthol derivative greatly enhances ESPT reaction in methanol. This effect depends on the flexibility of a chain linking the aromatic system with the carboxyl group. ESPT reaction in five various analogs of 2-naphthol with an electron withdrawing substituent and a carboxyl group was compared. Influence of water addition and the fluorophore concentration, on ESPT was studied in detail. The effect of organic acid addition to the methanolic solution was also investigated. Influence of other organic solvents on ESPT is compared to that of methanol. Our results indicate that proton transfer to the carboxyl group and a preferential binding of other polar molecules play an essential role in the kinetics of proton transfer reaction The role of these catalytic effects is more pronounced in low polar media than in water solutions. By analogy to our model systems, conclusions concerning the proton transfer reaction in biological energy transformation systems can be drawn.
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Keywords: Naphthol; Proton transfer; Excited state

1. Introduction

Excited-state proton transfer (ESPT) is an important phenomenon, which may be applied in such domains as laser technology, signal transduction and photodynamic therapy. Therefore, it has been extensively studied for various compounds dissolved in water [1–5] as well as in alcohol [6–10] and other organic solvents [11–13]. Further reason for interest in the title phenomenon is the fact that proton transfer in membranes plays an important role in biology. It is known that impermeability of apolar layers of biological membranes for protons and transport of H^+ by specific transmembrane protein channels, is a necessary condition for proper function of biological systems [14]. Therefore, a study of ESPT in solvents of low polarity contributes to our understanding of biological systems.

ESPT reaction is characteristic for some weak acids which in their first singlet excited state reached by light absorption, undergo electronic rearrangement, giving increase of acidity of many orders of magnitude. Such substances, as naphthol derivatives, occurring in a neutral water solution in the protonated form (ROH), loose protons in their excited state (ROH^*), by a transfer to an acceptor (A). This is a nonequilibrium process, dependent on the ratio of its rate to the rate of fluorescence emission and of other excited state processes.

$$ROH^* + A \leftrightarrow [RO^{*-} \cdots AH^+] \rightarrow RO^{*-} + AH^+$$

At definite conditions, the band of the dissociated form (RO^{*-}) appears in the stationary fluorescence spectra, besides the emission of the original protonated species (ROH^*).

ESPT was thoroughly investigated for naphthols and their analogs at various conditions [5–20]. The aim of the present work is to gain information on the characteristics of the proton transfer reaction in media of low polarity by studying ESPT reaction of selected compounds in methanol

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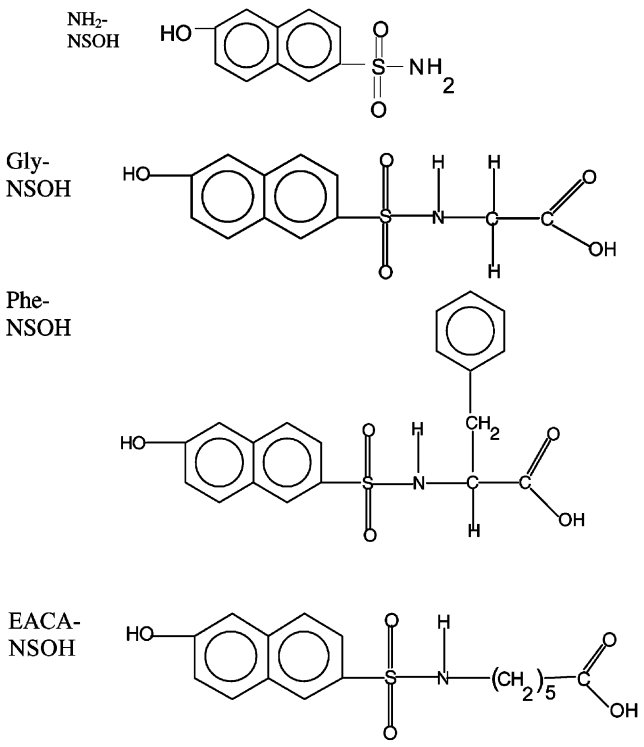
(MeOH) and other organic solvents. New contribution of the present work, to the title problem, consists in investigating ESPT in aromatic systems with electron withdrawing substituents, containing a carboxyl group linked by a spacer. From site-directed mutagenesis studies on proteins it is known that in some natural systems (e.g. the photosynthetic apparatus) in which proton transfer plays an essential role, the carboxyl groups positioned at definite sites are necessary for proper biological function [21–25]. Therefore, a study on a simple model system may be helpful in understanding biological phenomena dependent on the proton transfer. Another feature of the present work is the use of a simple approach to the spectrofluorimetric data, which may yield valuable information unavailable by other methods.

In most compounds studied by us, dissolved in alcohol, ESPT can be observed by steady state fluorescence and an addition of water greatly enhances the effect. The results obtained indicate that an intramolecular interaction between the phenolic and the carboxyl groups and formation of solvent mediated hydrogen bonds play an essential role in ESPT reaction in the compounds studied. Important conclusions concerning the mechanism of proton transfer in media of low dielectric constant and in biological systems can be drawn.



Scheme 1. The formulae of the compounds studied. The formula of LA-NSOH is analogous and is given schematically as follows: NSOH-L-I-I-L-V-P-P-F-F-OH where L, leucyl; I, isoleucyl; V, valyl; P, prolyl; F, phenylalanyl.

2. Materials and methods

6-Hydroxynaphthalene-2-sulphonamide (NH_2-NSOH), 6-hydroxynaphthalene-2 sulphon-amide of α-L-phenylalanine (Phe-NSOH), 6-hydroxynaphthalene-2-sulphonamide of glycine (Gly-NSOH), 6-hydroxynaphthalene-2-tisulphonamide of ε-amino capronic acid (EACA-NSOH), 6-hydroxynaphthalene 2-(αN) sulphonamide of linopeptide A (LA-NSOH) were synthesized as described in [4]. Linopeptide A (LA) HL-I-I-L-V-P-P-F-F-OH was a gift from Dr. M. Cebrat (Faculty of Chemistry, University of Wrocław). Formulae of the substances studied are given in Scheme 1.

The purity of these compounds was checked by elemental analysis, HPLC and verified occasionally by TLC. The solvents were of spectroscopic or HPLC grade (Aldrich) with exception of tetrahydrofurane (POCh, Poland), which was of analytical grade, and were used without further purification freshly after opening the bottle. The solutions of the compounds investigated in MeOH–water mixtures and mixtures of water with organic solvents were prepared by changing the proportion of solvents at a constant fluorophore concentration.

Steady state fluorescence spectra were measured by SF1 module spectrofluorimeter (COBRABID-OPTEL Opole, Poland) connected to a computer for acquisition of data or with a Perkin-Elmer model 204 (USA) apparatus. Correction of the fluorescence spectra with respect to the detector response and the determination of the fluorescence quantum yields (Φ) was performed as described by Lakowicz [26]. The methods consist in a comparison of the spectra, at the maximum intensity of a given band, with those of standards. 2-Naphthol (POCh, Poland) three times crystallized from $CHCl_3$ in acetic acid buffer of pH 4.62 at 22 °C [27] and Phe-NSOH in aqueous HCl (0.01 M) ($\Phi_{st} = 0.25$ [4]) were used as a standard for spectra correction and for quantum yield determination, respectively.

The excited state lifetimes of the protonated form of the fluorophore were determined by means of SLM Aminco 48000S phase and modulation instrument or by means of IBH (Glasgow, UK) single photon counting apparatus.

The parameters characterizing ESPT such as proton transfer rate constant (k_{PT}) and equilibrium constant in the excited state K_a^*, cannot be determined from the steady state methods analogously as in the spectrofluorimetric titration in water solution, since these methods are based on the dependence of the fluorescence intensity on the hydronium ion concentration, which is the product of ESPT in water but not in an alcohol solution where its concentration is very near to zero.

For the determination of the rate constant of ESPT of our samples in MeOH–water mixtures, we used the method based on Eq. (1) derived by Weller which was also used in

other similar cases [28][1]

$$\frac{\Phi'/\Phi'_0}{\Phi/\Phi_0}(W_B) - (1 - W_B) = \frac{N_R}{N_0} \quad (1)$$

where Φ and Φ_0 are the fluorescence quantum yield of the protonated (ROH*) form at 363 nm in the presence and absence of a proton acceptor, respectively and Φ', Φ'_0 are analogous values for the deprotonated (RO^{*-}) form of the fluorophore at 444 nm.

W_B—the fraction of fluorophores (Eq. (1a)), which at the moment of excitation have no acceptor molecule in their vicinity (V_D) called also diffusion volume and given by Eq. (1b)

$$W_B = \exp(-V_D\, C'_A) \quad (1a)$$

$$V_D = \frac{4\pi(\gamma R_0)^2 (D_\tau)^{0.5} B (D_{\tau_0})^{0.5}}{(1-\gamma)R_0 + B(D_{\tau_0})^{0.5}} \quad (1b)$$

where C'_A is the number of acceptor molecules per cm^3, D the diffusion coefficient of reaction partners, (τ, τ_0) the excited state lifetime of the fluorophore in the given conditions and in the absence of ESPT, respectively and R_0 is the encounter distance (in cm) of reactants and $\gamma = k_R/(k_D + k_R)$, where k_D, k_R are rate constants of diffusion and separation of reactants, respectively.

In Eq. (1), N_R is the probability of ESPT and N_0 ($=1/\tau_0$) is the probability of the deactivation of the excited state. $N_R = k_2 C_A$ where k_2 is the second-order rate constant of the total reaction in (Scheme 1) and C_A is the molar concentration of water in M/dm^3.

In the text below we change notation and instead of N_R we use the definition

$$k_{PT} = k_2 C_A \quad (1c)$$

which at $C_A = 1$ gives $k_{PT} = k_2$ where k_{PT} is first-order rate constant of proton transfer from a fluorophore to infinity equal to the second-order rate constant (k_2) extrapolated to low acceptor concentration (1 M).[2]

Eq. (1) was rearranged so as to obtain the ratio of the relative fluorescence quantum yields $((\Phi'/\Phi'_0)/(\Phi/\Phi_0) = \Re)$ as a convoluted function of water concentration (C_A, C'_A)

$$\Re = \left(\frac{1 - W_B}{W_B}\right) + k_2\tau_0 \frac{C_A}{W_B} = \left(\frac{1 - W_B}{W_B}\right) + \frac{k_{PT}\tau_0}{W_B} \quad (2)$$

Φ and Φ' were determined, from the respective fluorescence band maxima, at given conditions. Φ_0 and Φ'_0 were estimated by the addition of an acid (acetic or perchloric) or alkali to obtain pure emission of the protonated (ROH*) and deprotonated (RO^{*-}) forms, respectively.

The results of Φ'_0 obtained at pH > pK_a in the ground state, were compared with the values of Φ'_0, estimated by extrapolation of the phenolate fluorescence intensity using the relation: $\Phi/\Phi_0 + \Phi'/\Phi'_0 = 1$, derived by Weller [29].

The determination of the rate of ESPT at low acceptor concentration ($C_A = 1$ M) consists in fitting of Eq. (2) to the experimental values of Φ'/Φ'_0 (for RO^{*-}) and Φ/Φ_0 (ROH*). In the fitting procedure, adjustable parameters were k_{PT}, γ, R_0. In this procedure, several approximations in Eqs. (1) and (1b) were made: B—the electrostatic interaction factor was assumed to be unity ($B = 1$), $D = (D_A + D_B)$ was approximated by $D = D_A$, D_B was neglected because of relatively high molecular weight of our analogs. The values of D_A at various water content in MeOH were taken from [30]. The values of τ (in Eq. (1b)) at given water content were calculated from relation (3) [28]

$$\tau = \tau_0 \frac{\Phi}{\Phi_0} \quad (3)$$

where τ_0 the excited state lifetime in absence of ESPT determined in separate experiments.

The parameters of Eq. (2) were fitted to the experimental results of the ratio of relative fluorescence quantum yields ($\Re$) as a function of water concentration (C_A) obtained for the compounds studied, at fluorophore concentration near to 2×10^{-6} M/dm^3. The values of k_2 obtained were equal to k_{PT} ($k_{PT} = k_2 C_A$) at $C_A = 1$.

3. Results

3.1. The determination of the ratio of relative fluorescence quantum yields ($\Re$) in methanol–water mixtures

The compounds investigated are soluble in MeOH in a broad range of concentrations giving optically clear solutions though showing light scattering detectable by our fluorimeter. In MeOH–water mixtures the fluorescence bands of the protonated form (ROH* peaked at 363 nm), and of the phenolate species (RO^{*-} at 444 nm) are observed, only slightly blue shifted (1–2 nm), with respect to the analogous bands in water solution. Therefore, we suppose that the aromatic chromophoric systems do not interact considerably because in such a case a greater shift of the fluorescence (and absorption) bands would be expected. Relatively high intensity of the scattered light, especially at solute concentration higher than 10^{-4} M/dm^3 in MeOH, is to be ascribed to an aggregation, originating from adhesion of the polar groups of our 2-naphthol derivatives (–COOH, –OH, $–SO_2NH–$).

[1] The use of Weller's method derived for a treatment of the prestationary effects in the ultra fast diffusion-controlled reactions, in some cases studied, may seem questionable. The prestationary effects are accounted for by the parameter $(1 - W_B)$. The reasons for the use of this model here are: (1) in the cases where the reaction rate is low (e.g. at low C'_A), Eq. (1a) yields $W_B = 1$ ($(1 - W_B) = 0$) which implies that no prestationary effect is present. Thus, the model covers also such cases; (2) our efforts to fit other models as static, dynamic or mixed quenching of ROH* by water, to our experimental data, gave unsatisfactory results.

[2] In recent publications [7,8,10,12] on the title problem k_{PT} is used as a symbol for proton transfer rate constant by which is meant precisely the forward reaction of the stage 1 in the reaction scheme. We use k_{PT} in the meaning given because it was used so in our previous works [4], some results of which are compared to the data of the present paper.

Table 1
Fluorescence quantum yields and lifetimes of ROH* form in MeOH and in water

Substance	In MeOH			In H_2O[a]			
	Φ_0	Φ_0'	τ_0 (ns)	Φ_0	Φ_0'	τ_0 (ns)	τ_0' (ns)
NH_2-NSOH	0.18	0.3	4.89			7.6	
Phe-NSOH	0.17	0.28	4.90	0.25	0.5	7.17	10.23
Gly-NSOH	0.18	0.31	4.91			7.16	
EACA-NSOH	0.16	0.27	4.76	0.22	0.38	7.13	
LA-NSOH	0.09	0.15	2.35				

[a] Taken from [4].

3.1.1. The fluorescence quantum yields and lifetimes

The fluorescence quantum yields (Φ_0) and excited state lifetimes (τ_0), in absence of ESPT of the protonated (ROH*) form obtained by addition of an acid and the fluorescence quantum yields (Φ_0') of the phenolate (RO^{*-}) by addition of alkali for compounds investigated in MeOH and in water are given in Table 1.

The lifetime results obtained by phase and modulation technique by analyzing the decay within the band of the ROH* form in MeOH, suggest monoexponential decay of the compounds studied at the given experimental conditions.

For EACA-NSOH in MeOH, excited at 320 nm following results were obtained: by the single photon counting data analyzed at 365 nm $\tau_1 = 4.64$ ns with amplitude $\alpha_1 = 0.95$; and $\tau_2 = 2.9$ ns α_2: 0.05; (χ^2: 1.04) and by analysis at 445 nm; $\tau_1 = 6.10$ ns ($\alpha_1 = 8.5$) and $\tau_2 = 6.38$ ns ($\alpha_2 = -7.5$); (χ^2: 1.26). By analogy to 2-naphthol [26], the decay component with the lifetime $\tau_1 = 4.6$ ns should be ascribed to the protonated form (ROH*) of our fluorophore and the component with the negative amplitude ($\tau_2 = 6.38$ ns) is to be associated with the deprotonated form (RO^{*-}). The decay component characterized by $\tau = 6.1$ ns, obtained by analysis at 445 nm, is probably identical with that of τ_1 (4.6 ns) perturbed by some side effects.

The values of $\Re$ (Eq. (2)) in MeOH–H_2O mixtures were obtained using the data of Φ_0, Φ_0' together with the data of τ obtained from relation (3) and the results of τ_0 given in Table 1. For EACA-NSOH and Phe-NSOH Φ_0 and Φ_0' increase initially with an addition of water at $C_A < 10$ and then decrease slightly at higher water content (Table 2). For other compounds studied analogous results were obtained. The variation of Φ_0 and Φ_0' with water addition (Table 2) do not influence essentially our measurements since $\Re$ is unchanged under the condition that Φ_0'/Φ_0 is constant which was fulfilled for all compounds studied within our experimental error (10%).

Table 2
Fluorescence quantum yields of ROH* (Φ_0) and RO^{*-} (Φ_0') forms in MeOH–H_2O mixtures for three 2-naphthol derivatives

C_A (M/dm^3)	EACA-NSOH		Phe-NSOH		NH_2-NSOH	
	Φ_0	Φ_0'	Φ_0	Φ_0'	Φ_0	Φ_0'
2.7	0.18	0.28	0.14	0.28	0.18	0.28
5.56	0.19	0.39	0.19	0.41	0.19	0.39
11.11	0.19	0.37	0.19	0.41	0.19	0.40
27.77	0.19	0.39	0.20	0.44	0.20	0.41

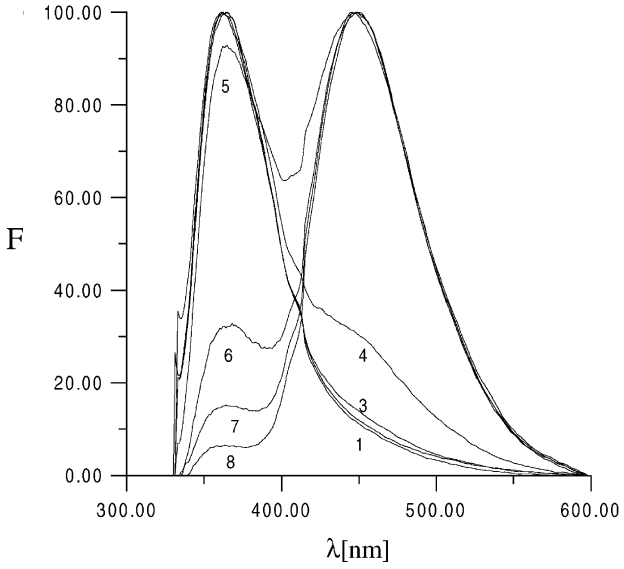


Fig. 1. The influence of water addition to the methanolic solution of Phe-NSOH on the fluorescence spectra where final water concentration (C_A in M/dm^3) is as follows: (1) 0, (2) 0.23, (3) 0.56, (4) 2.23, (5) 5.56, (6) 11.11, (7) 16.67, (8) 27.78 (F: relative fluorescence intensity corrected and normalized to 100).

The dependence of the fluorescence spectra of Phe-NSOH on water concentration in MeOH solution is shown in Fig. 1. It is visible that the phenolate fluorescence at 444 nm increases with a rise of water content, giving an increase of $\Re$ value. From these data and analogous results for other investigated compounds the values of Φ and Φ' were obtained, needed for determination of $\Re = (\Phi'/\Phi_0')/(\Phi/\Phi_0)$. Thus, Φ was calculated from the intensity at 363 nm and Φ' from that at 444 nm from which was subtracted some residual value (7–9% of the maximum) due to a contribution of the ROH* emission at 444 nm.

3.1.2. The fitting of Eq. (2) to the experimental results of $\Re$

The calculated values of $\Re$ as a function of water concentration (C_A) for NH_2-NSOH and EACA-NSOH (solid lines) are shown in Fig. 2 together with the experimental data of $\Re$.

At water content $C_A > 30$ M/dm^3 large scatter of the experimental points is due to very low intensity of ROH* fluorescence and low signal to noise ratio. Therefore, in the fitting procedure we used only the experimental results of $\Re$ obtained in the range of $1 < C_A < 30$ M/dm^3. Using the adjustable parameters (k_{PT}, γ, R_0) thus obtained, the values of $\Re$ at $C_A > 30$ could be estimated.

For other compounds studied analogous results (not shown) were obtained. It can be stated that Eq. (2) is successfully fitted to the experimental data ($\Re_e$), which must be treated as an exponential function of water concentration.

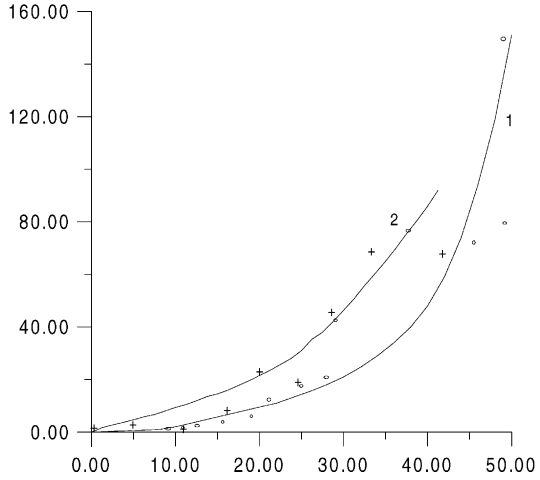


Fig. 2. The dependence of calculated $\Re$ values for NH_2-NSOH (1) and EACA-NSOH, (2) on the molar concentration of water (C_A). Experimental points are shown (o for 1 and + for 2).

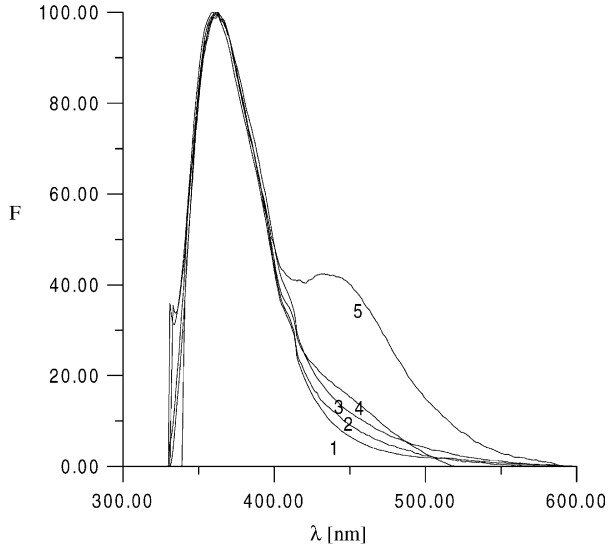


Fig. 3. The fluorescence spectra in MeOH at the fluorophore concentration near to 10^{-6} M/dm^3 of (1) NH_2-NSOH, (2) Phe-NSOH, (3) Gly-NSOH, (4) LA-NSOH, (5) EACA-NSOH (F: relative fluorescence intensity corrected and normalized to 100).

3.1.3. Rate constant of ESPT (k_{PT}) obtained from fitting Eq. (2) to the experimental data of $\Re$

From fitting of Eq. (2) to the experimental $\Re$ values the parameters (k_{PT}, R_0 and W_B) characterizing ESPT in the compounds studied were obtained (Table 3).

It can be seen that the analogs studied greatly differ with respect to k_{PT}, R_0 and W_B, this last effect being a consequence of a variation of R_0 value. The values of k_{PT} for low water concentration range (1 M/dm^3), obtained by our fitting procedure for EACA-NSOH and LA-NSOH (Table 3) are comparable to ^{1}S deactivation rate ($k_S = 1/\tau_0$),[3] which is near to 1.5×10^8 s^{-1} for most of our 2-naphthol analogs (see Table 1). For another 2-naphthol derivatives studied (Gly-NSOH, Phe-NSOH and NH_2-NSOH) k_{PT} is much lower than k_S. This finding is in accord with the fact that marked phenolate fluorescence with maximum at 444 nm at low water concentration (near to 1 M) is clearly observable only for indicated analogs (EACA-NSOH and LA-NSOH).

Highest values of k_{PT} (Table 3) are found by means of our procedure for EACA-NSOH and LA-NSOH—the analogs with the carboxyl group connected to the naphthol system by a flexible atomic chain (see Scheme 1). It should be added that higher k_{PT} for LA-NSOH than that for EACA-NSOH, in spite of lower $\Re_e$ values (Fig. 3), is due to lower excited state lifetime for the nonapeptide derivative (see Table 3 and Eq. (2)).

Intermediate rates of ESPT characterize the phenylalanyl and glycyl derivatives of 2-naphthol 6 sulphonate in which the carboxyl group is linked to the aromatic system by a shorter but less flexible chain composed of a sulphonamide group and α-amino acid triad N–C_α–C′. Lowest value of k_{PT} is obtained for NH_2-NSOH that has no carboxyl group.

These results suggest that the carboxyl group catalyses ESPT in 2-naphthol analogs studied, dissolved in MeOH water mixtures of low water content. At higher C_A values proportionally higher k_{PT} results can be obtained. Using parameters of Table 3 for Phe-NSOH at $C_A = 27.77$ M/dm^3 the value of $k_{PT} = 1.6 \times 10^9$ can be obtained (Eq. (1c)) which is near to the experimental result in water reported previously [4]. In water solution the value of k_{PT} for Phe-NSOH is very similar to that of EACA-NSOH [4].

The fact that at low water content the analogs studied differ with respect to $\Re$ and at high water percent they show similar results of $\Re$ is due to various curvature of the plot of $\Re$ on C_A caused by various values of W_B (see Eqs. (1a) and (1b)). Without water addition W_B is equal to 1 for all compounds studied and by increasing water content W_B decreases to a various degree for different analogs (Table 3). The fact that the 2-naphthol analogs studied differ with respect to k_{PT} value at low water content (C_A) and have practically the same k_{PT} at high C_A, cannot be easily explained on the ground of the model used (see Eqs. (1) and (2)) and suggests that some effects not accounted for in the model play a role here.

For zero-water concentration ($C_A = 0$) Eq. (2) yields $\Re = 0$ while from the results presented in Section 3.2, it can be seen, that for EACA-NSOH and other analogs with a carboxyl group, some nonzero value of $\Re$ was observed in alcohol solution. Inconsistency of the comparison of k_{PT} values in water and in alcohol water mixtures with a prediction from Eqs. (1) and (2) may be due to some effects concerning ESPT in 100% alcohol and at very low water content.

[3] $\tau_0 = 1/(k_f + k_q)$, where k_f is the radiative rate and k_q is the rate of radiationless processes other than ESPT.

Table 3
The fit of the parameters of Eq. (2) to the experimental[a] results of $\Re_e = (\Phi'/\Phi'_0)/(\Phi/\Phi_0)$ as a function of water content in MeOH for the substances studied at concentration (C_B) $10^{-7} < C_B < 3 \times 10^{-6}$

Sample	k_{PT} (s^{-1})[b]	γR_0 (cm)[b]	W_B[a]	S[c]	χ^2[d]	r[e]	u[f]
NH_2-NSOH	3.02×10^7	6.21×10^{-6}	1–0.27	114.88	17.20	14	0.6
Phe-NSOH	5.98×10^7	4.05×10^{-6}	1–0.63	13.77	2.94	9	0.6
Gly-NSOH	7.98×10^7	4.20×10^{-6}	1–0.59	9.09	2.31	7	0.6
EACA-NSOH	1.325×10^8	2.50×10^{-6}	1–0.95	46.56	14.22	9	0.8
LA-NSOH	1.65×10^8	5.0×10^{-8}	1–0.99	12.23	2.68	6	0.6

[a] The range of the experimental values: between $C_A = 0$ and 30 M/dm^3, respectively.
[b] Symbols explained by Eq. (1); γ was 0.9 in all cases except LA-NSOH where it was 0.5.
[c] Standard deviation $S = \sum(\Re_e - \Re_c)^2$ where $\Re_c$ is the value of $\Re$ calculated by Eq. (2).
[d] $\chi^2 = \sum((\Re_e - \Re_c)^2 \Re_c)$.
[e] Number of degrees of freedom.
[f] Trust level needed for acceptance of the fit.

3.2. ESPT in alcohol and at water concentration ($C_A \ll 1$ M/dm^3)

Fluorescence spectra of 2-naphthol derivatives (concentration: 1×10^{-6} to 3×10^{-6} M/dcm^3) in MeOH are shown in Fig. 3. The procedure for the determination of k_{PT} described above cannot be used in this case. It can be stated only that the rate of ESPT of naphthol derivatives studied in alcohol solution, if it is not equal to zero, must be near to that obtained at low water concentration (1 M/dm^3, Table 3). In the spectrum of EACA-NSOH a marked phenolate emission band with maximum at 444 nm is visible, in the spectrum of LA-NSOH it is less conspicuous, in other cases it is difficult to discern and for NH_2-NSOH probably it is absent at all.

The spectra in Fig. 3 suggest that the rate of ESPT in alcohol solution without water increases in the series of compounds studied, on going from Phe-NSOH to EACA-NSOH analogously as in MeOH–water mixtures. However, for elucidation of the mechanism of proton transfer in alcohol, the changes of the fluorescence by dilution of samples must be taken into account.

An inspection of the fluorescence spectra, of our analogs, at higher (10^{-4}) solute concentrations (not shown), leads to the conclusion that ESPT reaction in MeOH in our samples, is strongly enhanced by decreasing the concentration of a fluorophore. The dependence of the ratio of the intensity of RO^{*-} fluorescence band at 444 nm to that of the ROH^* at 363 nm (F_{444}/F_{363}) on the fluorophore concentration, for the substances studied, is shown in Fig. 4.

In the concentrations range of the fluorophores $10^{-4} < C_B < 10^{-3}$ (M/dcm^3), in the spectra of the naphthol derivatives studied in alcohol solution, practically no phenolate fluorescence with maximum at 444 nm can be discerned. Conspicuous phenolate emission appears in the spectrum of EACA-NSOH at concentration $C_B < 10^{-4}$ M/dm^3, in LA-NSOH at $C_B < 5 \times 10^{-5}$ M/dm^3 and in the spectra of Gly-NSOH and Phe-NSOH at C_B near to 10^{-6} M.

An apparent increase of the emission component at 444 nm for NH_2-NSOH, with dilution, may result from a decrease of the precision of the fluorescence measurements, due to a decrease of the signal-to-noise ratio at very low

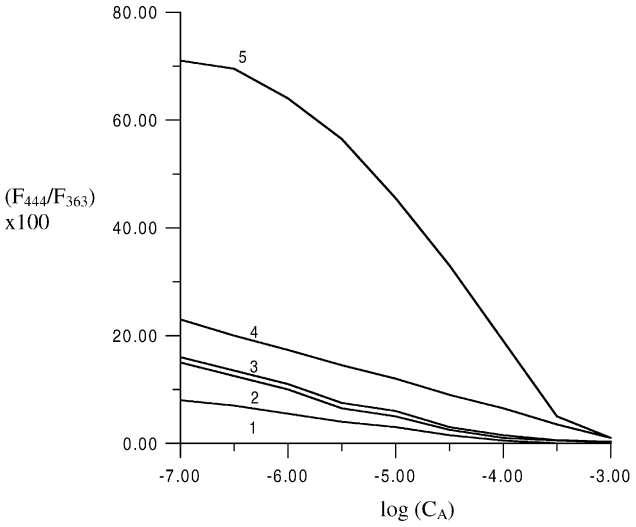


Fig. 4. The dependence, of the ratio of fluorescence intensity at 444 nm to that at 363 nm on log of the fluorophore concentration for (1) NH_2-NSOH, (2) Phe-NSOH, (3) Gly-NSOH, (4) LA-NSOH, (5) EACA-NSOH.

fluorophore concentration. This last conclusion is supported by a comparison of the fluorescence spectra of NH_2-NSOH in alcohol with and without addition of an acid, which are practically identical.

The changes of the scattered light intensity with a sample dilution for EACA-NSOH and Phe-NSOH are shown in Table 4.

Table 4
The intensity of elastic scattered light at 320 nm in relative units (the fluorescence intensity at 363 nm in MeOH of a given compound assumed as 100)

Compound	The intensity of the scattered light			
	5×10^{-5}[a] (0)[b]	1.4×10^{-6}[a] (0)[b]	1.4×10^{-6}[a] (0.07)[b]	1.4×10^{-6}[a] (0.55)[b]
EACA-NSOH	250	90	20	3
Phe-NSOH	260	75	16	5

[a] C_B (M/dm^3).
[b] C_A (M/dm^3).

Table 5
The ratio ($\Re$) of RFQY values of the phenolate (Φ'/Φ'_0) and the protonated form (Φ/Φ_0) at very low water concentration (C_A)

Compound[a]	$\Re = (\Phi'/\Phi''_0)/(\Phi/\Phi_0)$				
	0[b]	0.07[b]	0.11[b]	0.22[b]	0.55[b]
EACA-NSOH	0.46	0.26	0.29	–	0.58
LA-NSOH	0.11	–	–	–	0.17
Gly-SOH	0.10	–	0.09	0.14	–
Phe-NSOH	0.10	–	0.11	0.12	0.17
NH_2-NSOH	0	–	0	0.04	0.02

[a] $C_B = 1.4 \times 10^{-6}$ M/dm^3.
[b] C_A (M/dcm^3).

A comparison of Table 4 and Fig. 4 shows that diluting a sample from C_B values of 5×10^{-5} to 1.4×10^{-6} M/dm^3 leads to a decrease of light scattering and an increase of the phenolate fluorescence. Assuming that the intensity of the scattered light is proportional to the degree of the solute aggregation, it is suggested by these data, that at fluorophore concentration 5×10^{-5} M/dm^3 the solute aggregation hinders ESPT, probably by preventing the proton to escape and the dilution of a sample hinders the aggregation and enhances the ESPT. At solute concentration $C_B = 1.4 \times 10^{-6}$ M/dm^3, the scattered light intensity is lowered but not negligible. It seems, therefore, that some type of aggregates exist even at $C_B = 1.4 \times 10^{-6}$ M/dm^3 which enables ESPT reaction to proceed.

An addition of water to MeOH solutions of the compounds studied at concentration 1.4×10^{-6} M/dm^3 leads initially to lowering of the phenolate fluorescence (Table 5) and a decrease of light scattering.

This means that at fluorophore concentration (1.4×10^{-6} M/dm^3) the aggregates in which ESPT reaction takes place, are destroyed by small water addition, what inhibits initially ESPT. It is suggested by these results that an effect responsible for the appearance of ESPT in some compounds studied in 100% alcohol, is a special type of aggregation present at low fluorophore concentration (C_B). This aggregation process must be different from that present at high C_B.

In the mechanism of ESPT in alcohol, the carboxyl group in the fluorophore molecule must play an essential role, since in the analog without a carboxyl group (NH_2-NSOH), practically no ESPT in 100% MeOH can be observed.

3.3. The quenching by geminate protons

It has been shown by Pines et al. [31] that the proton to be separated from the excited fluorophore in ESPT, quenches the fluorescence of the ionized form, by a collision with the excited product (RO^{*-}). In such a case the intensity of RO^{*-} fluorescence excited directly (Φ_{dir}), at pH value higher than the ground state pK_a of the fluorophore, is higher than that excited indirectly (Φ_{indir}), observed at pH much lower than the pK_a, even if ESPT is much faster than other excited state processes [32].

In consequence of this finding, a possibility must be taken into account that ESPT takes place in alcohol solutions of all analogs studied in the present work, but its main observable effect: the phenolate emission, cannot be noticed in NH_2-NSOH and is scarcely visible in Phe and Gly analogs because of the quenching by geminate protons. In the analogs with larger and more flexible substituent in the aromatic system the quenching might be inhibited by steric hindrance.

The values of Φ_{indir}/Φ_{dir} for the analogs studied, obtained by excitation at the isosbestic wavelength (294.1–297.5) are given in Table 6. At water content 50% (27.78 M/dm^3) or higher, where the phenolate emission band dominates the spectrum, the quotient of Φ_{indir}/Φ_{dir} for the analogs with a carboxyl group is near to 1, which gives the rate of quenching by geminate protons (k'_q) negligible as compared to k_{PT}. For water content lower than 50% the contribution of the protonated (ROH*) form to the spectra is considerable, what disables a simple determination of Φ_{indir} from the experimental data. Therefore, for the determination of Φ_{indir} we used an extrapolation procedure assuming that $\Phi/\Phi_0 + \Phi'/\Phi'_0 = 1$ (see Section 2) and substituting the value of Φ'_0 thus obtained for Φ_{indir}.

Table 6
The quenching by geminate protons in MeOH and MeOH–water mixtures of various water concentrations (C_A)

Sample	Φ_{indir}/Φ_{dir}	$(\Phi_{dir}/\Phi_{indir}) - 1$	k_{PT} (s^{-1})[a]	k'_q (s^{-1})[b]
Phe-NSOH in a mixture with H_2O of C_A 2.77	0.599	0.672	1.65×10^8	1.13×10^8
Phe-NSOH in a mixture with H_2O of C_A 5.56	0.743	0.345	3.32×10^8	1.15×10^8
Phe-NSOH in a mixture with H_2O of C_A 11.11	0.682	0.467	6.64×10^8	3.1×10^8
Phe-NSOH in a mixture with H_2O of C_A 27.7	0.97	0.031	1.66×10^9	5.1×10^7
EACA-NSOH in MeOH	0.65	0.53	1.325×10^8	7.0×10^7
EACA-NSOH in a mixture with H_2O of C_A 0.55	0.68	0.47	3.67×10^8	1.72×10^8
EACA-NSOH in a mixture with H_2O of C_A 5.56	0.71	0.400	7.37×10^8	2.95×10^8
EACA-NSOH in a mixture with H_2O of C_A 27.7	0.917	0.09	3.68×10^9	3.31×10^8
NH_2-NSOH in a mixture with H_2O of C_A 2.77	0.627	0.594	8.36×10^7	4.96×10^7
NH_2-NSOH in a mixture with H_2O of C_A 5.56	0.623	0.606	3.36×10^8	2.03×10^8
NH_2-NSOH in a mixture with H_2O of C_A 27.7	0.598	0.673	8.39×10^8	5.65×10^8

[a] The values calculated from Eq. (1c): $k_{PT} = k_2 C_A$.
[b] Calculated according to [31] by approximate formula: $k_q = k_s((\Phi_{dir}/\Phi_{indir}) - 1)$. By further approximation, it was assumed that k_s [31] is equal to k_{PT}. A detailed kinetic scheme of excited state processes with quenching by geminate protons is given in [31], see also [7,10].

The results of Table 6 show that the quenching by geminate protons, with rate constant k'_q influences considerably the excited state processes of our analogs in MeOH–water mixtures of low water content. In 100% alcohol and at water content (C_A) < 21 M/dm^3, for most analogs studied, the ratio of k'_q/k_{PT} is near to 0.5 and at higher C_A it decreases. In low water percent the effect is comparable for the compounds investigated, including NH_2-NSOH, indicating that by low water addition the quenching by geminate protons is similar for the compounds investigated. This conclusion is supported by the fact that excited state lifetimes and fluorescence quantum yields (Φ_0, Φ'_0) in MeOH are similar for all compounds studied (Table 1). These data caused us to reject the possibility that the observed differences in the fluorescence spectra of the 2-naphthol analogs studied by us are due to a various quenching by geminate protons. For NH_2-NSOH the value of k'_q increases even at C_A > 21 M/dm^3 in contrast to other analogs studied. The difference between NH_2-NSOH and the analogs with a carboxyl group, is probably due to the fact that in the analogs with the carboxyl group its ionization at higher water content hinders the quenching by geminate protons.

3.4. The influence of acetic acid on ESPT in methanol

If the differences in the rate of ESPT between analogs studied are to be contributed to the influence of the carboxyl group, the problem arises, at which conditions the carboxyl group can attract a proton released from the excited phenol. To elucidate this question we studied in detail the influence of an addition of acetic acid to the methanolic solution of EACA-NSOH.

The effect of an addition of glacial acetic acid, on the fluorescence spectra of EACA-NSOH at concentration 1.4×10^{-6} M is shown in Fig. 5. No shift in the ROH* fluorescence band position due to acetic acid addition was detected though a small increase of Φ_0 (of 10–20%) was observed.

An addition of a small quantity of acetic acid, to MeOH, lower than the concentration of the fluorophore, gives only a small decrease of the phenolate emission. At the acid concentration comparable to that of the fluorophore an increase of Φ/Φ_0 and a parallel decrease of Φ'/Φ'_0 takes place. At the acid concentration about two times higher than that of the fluorophore intermediate values of Φ/Φ_0 and Φ'/Φ'_0 are attained, which do not change considerably at some acid concentrations range (plateau: 4.5 < log[C] < 5.5). Further acid addition gives a decrease of Φ'/Φ'_0 to 0 and an increase of Φ/Φ_0 to 1.

The picture obtained suggests that at sufficient organic acid concentration in MeOH, a complex of our 2-naphthol analogs with acetic acid is formed, in which the protonation reaction is much faster than deactivation of the excited state in absence of ESPT. The first inflection point at log[CH_3COOH] near to −4.5 may be due to formation of 1:1 complex with the fluorophore and the second inflection at about −5.7 to a complex of higher acetic acid content.

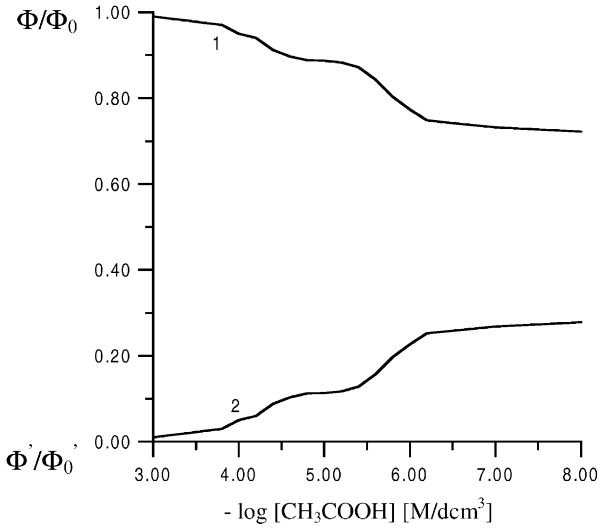


Fig. 5. The dependence of Φ/Φ_0 (ROH* emission—upper curve (1)) and Φ'/Φ'_0 (RO*− band—lower curve (2)) for EACA-NSOH, on the negative logarithm of acetic acid concentration.

It should be noted that in MeOH–water mixtures the effect of the acid addition is lower. Probably at high water content no complex between acetic acid and our fluorophore is formed. From the results of this section it must be concluded that the undissociated carboxyl group of an organic acid in methanolic solution can function only as a proton donor to the excited phenolate and by no means as a proton acceptor.

3.5. ESPT in aprotic organic solvents

Fluorescence spectra of EACA-NSOH in various aprotic organic solvents are compared to those in MeOH in Figs. 6 and 7.

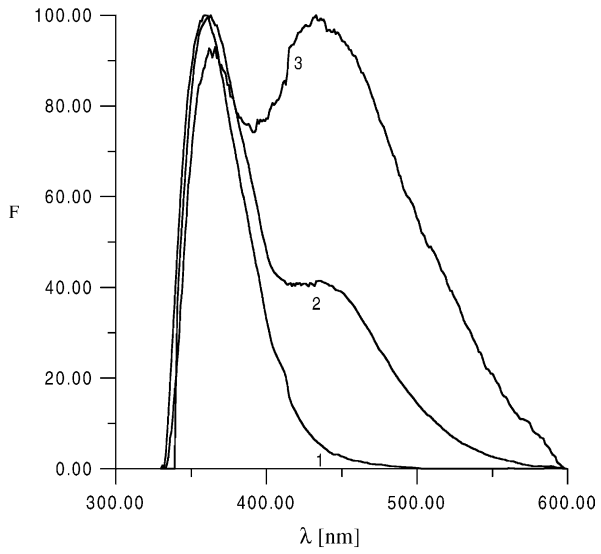


Fig. 6. Corrected and normalized fluorescence spectra of EACA-NSOH ($C_B = 2.6 \times 10^{-6}$ M) in (1) in acetonitrile, (2) in MeOH, and (3) in DMF ($C_B = 1.3 \times 10^{-6}$ M).

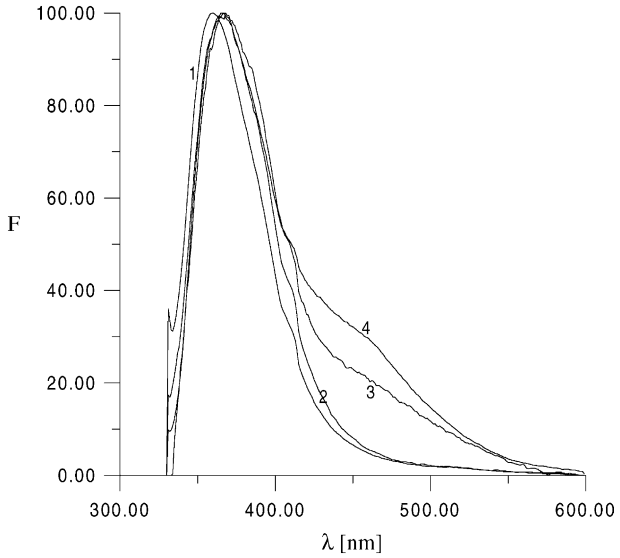


Fig. 7. Corrected and normalized fluorescence spectra ($10^{-6} < C_B < 1.4 \times 10^{-6}$ M) of (1) NH_2-NSOH in MeOH, (2) NH_2-NSOH in DMF, (3) Gly-NSOH in DMF, (4) LA-NSOH in DMF.

It can be seen that the phenolate fluorescence and consequently also the rate of ESPT is highest in dimethylformamide (DMF) and practically equal to zero in acetonitrile (AN). In tetrahydrofurane (and MeOH) intermediate intensities of RO^{*-} fluorescence are found.

A comparison of Figs. 3 and 6 demonstrates that the relative intensity of the phenolate band of EACA-NSOH is much higher in DMF than that in MeOH. Analogously, for Gly-NSOH (Fig. 7, curve 3) and Phe-NSOH (not shown) in DMF, a more pronounced RO^{*-} fluorescence band is found, that that in MeOH.

The bandwidth of the protonated (ROH^*) form in AN seems to be lower than in other solvents. ROH^* band position is shifted to the red on going from AN to MeOH, THF and DMF. The shape of the fluorescence spectrum of NH_2-NSOH in DMF and THF is practically identical with that in MeOH (Fig. 8) though the peak position is shifted in DMF to the red by 3 nm.

Table 7
Parameters characterizing the solvents used

Solvent	ε	α	β
MeOH	32.7	0.62	0.93
AN	37.5	0.19	0.31
DMF	36.7	0	0.69
THF	7.4	0	0.55

The properties of the solvents investigated: static dielectric constant (ε [33]), the ability of hydrogen bond formation as proton donor (α [34]) and acceptor (β [34,36])) are compared with correspondent properties of MeOH in Table 7. From a comparison of Table 7 with Figs. 6 and 7, it is visible that solvents of very similar dielectric constant near 30 (AN, DMF, MeOH) differ essentially in their influence on ESPT. Therefore, the differences in the rate of ESPT, in 2-naphthol analogs studied, observed in solvents of intermediate polarity cannot be ascribed solely to solvent polarity effects.

However, it must be noticed here that in nonpolar solvents such as $CHCl_3$ and hexane no ESPT reaction can be found in analogous compound: 2-hydroxy-naphthalene-6-sulphonamide of dodecylamine (results not shown). Therefore, it must be predicted that decreasing medium polarity below $\varepsilon = 10$ will hinder ESPT reaction in 2-naphthol derivatives studied. The results of Table 7 indicate that a rise of proton acceptor ability enhances significantly the rate of ESPT and an increase of the proton donating ability inhibits this reaction. For such an effect to appear, the carboxyl group is necessary since no phenolate fluorescence was observed for NH_2-NSOH in DMF or THF. Some other features of ESPT reaction found in MeOH solution are paralleled in the aprotic solvents: unexpected enhancement of the reaction rate with

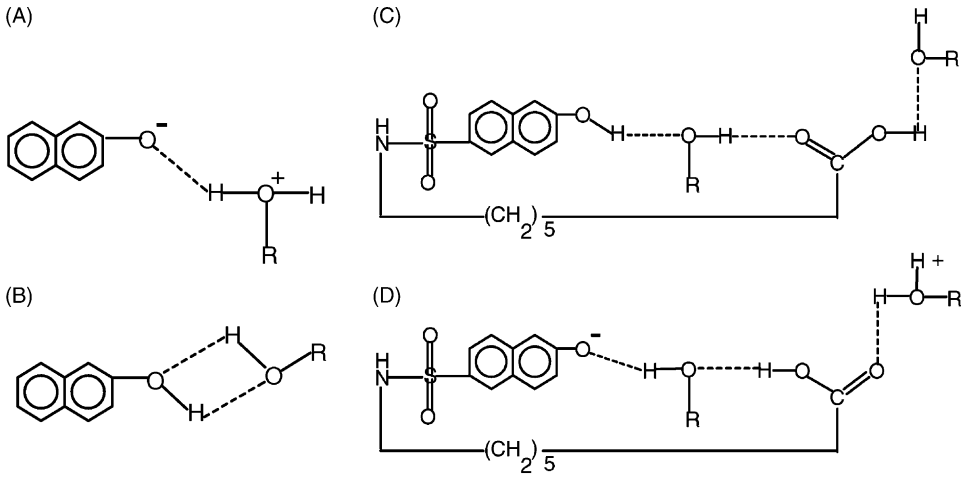


Fig. 8. Some possible types of interactions between the excited naphthol derivative and a solvent: (A) direct proton transfer to solvent (B) reprotonation by double proton transfer (C, D) charge separation in EACA-NSOH catalyzed by the carboxyl group.

a decrease of a fluorophore concentration and considerable aggregation of solute molecules detected by light scattering.

3.5.1. ESPT in EACA-NSOH dissolved in acetonitrile–water mixtures

The band position of the original (ROH*) excited form of EACA-NSOH in AN is shifted to the red with increasing water content. No phenolate emission is present in the spectra in AN but an addition of water causes the appearance of the phenolate fluorescence near 443 nm (results not shown). The amount of water required for this effect to appear is higher ($C_A = 5\,M/dm^3$) than that in MeOH. Fitting of the parameters of Eq. (2) to the experimental data yields $k_{PT} = 1.97 \times 10^7\,s^{-1}$, $R_0 = 6.64 \times 10^{-6}$ cm and $\gamma = 0.9$. The quality of the fit ($\chi^2 = 22.77$ for 15 experimental points) is worse than for methanolic solutions. Using various ranges of the experimental data for the fit, leads to the conclusion, that the error due to a change of the solvent properties on water addition, is higher in the case of AN than in MeOH. This effect may be ascribed to a stronger interaction of water with AN than with MeOH what is also confirmed by the experimental results and calculations of [19].

4. Discussion and conclusions

It is visible from our results that the rate of ESPT, in 2-naphthol derivatives in media of low polarity, is greatly enhanced by the presence of a carboxyl group in the molecular environment of a proton donor. This finding seems to be in a contradiction to the results of Tolbert and Haubrich [8] and of Solntsev et al. [10] who studied CN substituted 2-naphthol analogs. It can be concluded from the works cited that in 2-naphthol analogs, investigated in the present work, having 6-SO_2NH– substituent characterized by a lower electron withdrawing strength than 5-CN group, ESPT though thermodynamically allowed, would not be observed in MeOH, by the steady-state fluorescence methods. In accord with this analogy 2-naphthol-6-sulphonamide (NH_2-NSOH), containing no carboxyl group shows no phenolate emission in 100% alcohol. The appearance of ESPT in a MeOH solution of the analogs containing a carboxyl group seems unexpected in this context.

The hypothesis that the charge separation in ESPT, of some 2-naphthol analogs, is catalyzed by the carboxyl group, may seem questionable, because—it was shown in Section 3.4—acetic acid added to MeOH plays a role of a proton donor and not of a proton acceptor to the excited phenol. This apparent contradiction probably will be solved, by taking into account the ability of alcohols and other organic solvents, to form mixed hydrogen bonds in solutions, with water and polar groups of dissolved compounds [19].

By an analogy to the complex of EACA-NSOH with acetic acid in MeOH (Section 3.4), it can be supposed, that the carboxyl group of EACA-NSOH is attracted to the phenolic system, within this molecule, though a direct contact between these groups seems improbable on the ground of a simple molecular modeling. Hydrogen bonding, however, between the phenol and carboxyl groups, mediated by solvent, is possible. Some probable interactions in EACA-NSOH between the excited phenol, the carboxyl group and a hydroxylic solvent, are depicted in Fig. 8.

A solvent mediated hydrogen bond, between the phenolic and the carboxyl group, in the conformer in which the distance between these groups is optimal, may disable reprotonation (such as in Fig. 8B) and enable double proton transfer, after excitation of the phenolic group. Double proton transfer in such a system of hydrogen bonds in EACA-NSOH molecule, leading to charge separation, is represented in Fig. 8C and D. Proton transfer by such a chain system may be thought to be characterized by rather high activation barrier [35,37], which would result in the ESPT rate too low, for its observation during the lifetime of the excited state. It has been shown however [38–42], in the case of double proton transfer in the formic acid dimer, that even small distortion of the structure of the proton donor and acceptor greatly reduces the height of the barrier. It is probable that the arm linking aromatic system with the carboxyl group in the molecule of EACA-NSOH (Fig. 8C) leads to a strain in the system of proton donor and acceptor bonds. Based on this consideration, we can explain the catalytic effect of the carboxyl group in ESPT in EACA-NSOH by double proton transfer in a system of distorted hydrogen bonds.

In LA-NSOH similar catalytic effect of the carboxyl group, to that in EACA-NSOH, may occur. For other analogs studied, containing a carboxyl group: Gly-NSOH and Phe-NSOH, a similar catalytic effect of the carboxyl group, consisting in a distortion of the structure of a proton donor and acceptor and their molecular environment, may take place in aggregates present in organic solvents of low water content. In aprotic solvents (DMF, THF) the lack of a reprotonation effect (Fig. 8B) is probably responsible for a higher intensity of the phenolate fluorescence (Figs. 6 and 7, Section 3.5) as compared to that in MeOH. Solvent mediated hydrogen bonding between OH and COOH groups must also play some role in this case.

The mechanism catalyzing the process of the proton separation in ESPT consists in the stacking of the carboxyl and the phenolic groups in an alcohol solution. Such an interaction may lead to two opposite effects: rapid protonation of the excited phenolate in the complex of EACA-NSOH with acetic acid and deprotonation of the excited phenolic group of EACA-NSOH in an intramolecular process in absence of the acid. The difference between these two cases, shifting the direction of proton transfer, consists in intramolecular strain in the structure in the last case.

The adhesion of polar groups: carboxyl and phenolic, in solvents of intermediate polarity is inhibited by an addition of water. This is manifested by the disappearance, of the effect of the protonation of RO^{*-} by acetic acid, on water

addition to MeOH (Section 3.4). In the presence of water another catalytic mechanism appears in the compounds studied, consisting in proton transfer by chains of hydrogen bonded water molecules. Both these mechanisms depend on the catalytic role of the carboxyl group in our 2-naphthol analogs, and probably at low water concentration in MeOH they act parallel.

ESPT in 2-naphthol analogs with electron withdrawing substituent, containing a carboxyl function, dissolved in low polar solvents, may be a good model for proton translocation in proteins. It is supposed that proton transfer in the biological systems is enabled by the changes of their pK_a values, caused by protein conformational transitions. It is supposed [14,23–25] that proton transfer in the biological systems is enabled by the changes of the pK_a values of the carboxyl groups, caused by protein conformational transitions. The changes of pK_a would lead to the shift of the function of a given carboxyl, from a proton donor to a proton acceptor, enabling cyclic repetition of the process. It is possible that even without changes of pK_a, some role here plays a mechanism postulated above for ESPT in 2-naphthol analogs, containing a carboxyl group. Such catalytic mechanism, consisting in formation of a chain of hydrogen bonds connected with a strain in the structure, may be an essential factor in macromolecular systems, because of a competition between various possible routes of proton translocation.

A mechanism similar to that proposed for 2-naphthol analogs studied in the present work, is operating in the green fluorescent protein (GFP) from *Aequorea victoria* [17]. A chain of hydrogen bonds conducting protons consists of the phenolic group of excited chromophore, hydroxyl groups of Ser and Thr residues and carboxyl group of Glu 222 that acts as a proton acceptor. Interrelationship of the pK_a values of Glu 222 and of the phenolic group of the chromophore, leads to drastic increase of the basicity of the carboxyl (pK_a = 12), by ionization of the phenolic group.

In bacteriorhodopsin (BR) proton transfer from the excited Shiff base, formed by retinal chromophore, proceeds via successive protonation of the carboxyl groups of Asp 85 and Asp 96, which change cyclic, their pK_a values in response to variations in protein conformation [23,43].

In carbonic anhydrase and cytochrome C oxidase, in contrast to two preceding cases, proton translocation, through protein hydrophobic core, is driven by redox reactions taking place during electron transport. [44,45]. Other examples of proton transfer mechanisms in biological systems have been described [46,47].

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