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Acidobasic equilibria and spectral characteristics of acetyl and tosyl derivatives of 4,4′-diaminostilbene-2,2′-disulfonic acid

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Abstract

Acetyl and tosyl derivatives of 4,4′-diaminostilbene-2,2′-disulfonic acid and their *N*,*N*′-dimethyl analogues were prepared. The course of syntheses was checked by HPLC and products were identified with NMR spectroscopy. Chemical stability was tested in phosphate buffers. Acidobasic properties of prepared substances were studied with potentiometric titration and also with absorption and fluorescence spectrophotometry. The fluorescence quantum yields of pure acidobasic forms were measured. The tosylamino derivative was found to behave as N-acid and in aqueous alkali medium exhibits a strong fluorescence. The pK_A values were estimated. © 2002 Published by Elsevier Science Ltd.

Keywords: *N*,*N*′-dimethylamino derivatives; Acylamides and sulfonamides of 4,4′-diaminostilbene-2,2′-disulfonic acid; Acidobasic equilibria; Absorption and fluorescence spectroscopy

1. Introduction

About 80% of the total production of fluorescent whitening agents are derived from stilbene [1]. Acylation products of 4,4′-diaminostilbene-2,2′-disulfonic acid are most important monostilbene brighteners [2,3]. In regard to their great technical importance, their physico-chemical properties are frequently discussed. For this reason, acidobasic characteristics and pH dependencies of absorption and fluorescence spectra of the substances of this class could be expected to be studied. It is, therefore, rather surprising that the work [4] discussing an effect of amino nitrogen protonation of 4,4′-diaminostilbene and 4-amino-4′-trimethylammonium-stilbene hydrogen sulfate on spectral characteristics did not appear before 1996. Only the preliminary information on acidobasic equilibrium of sulfonated stilbene derivatives where the amino group is substituted with an electron-acceptor was presented on Colorchem 2000 [5]. In this paper we present the syntheses and acidobasic and spectral absorption and fluorescence characteristics of bifunctional symmetrical acetylamino and tosylamino derivatives (Table 1, substances II and III) and *N*,*N*′-dimethylamino derivatives.

2. Experimental

The substances were synthesised in aqueous medium according to Scheme 1.

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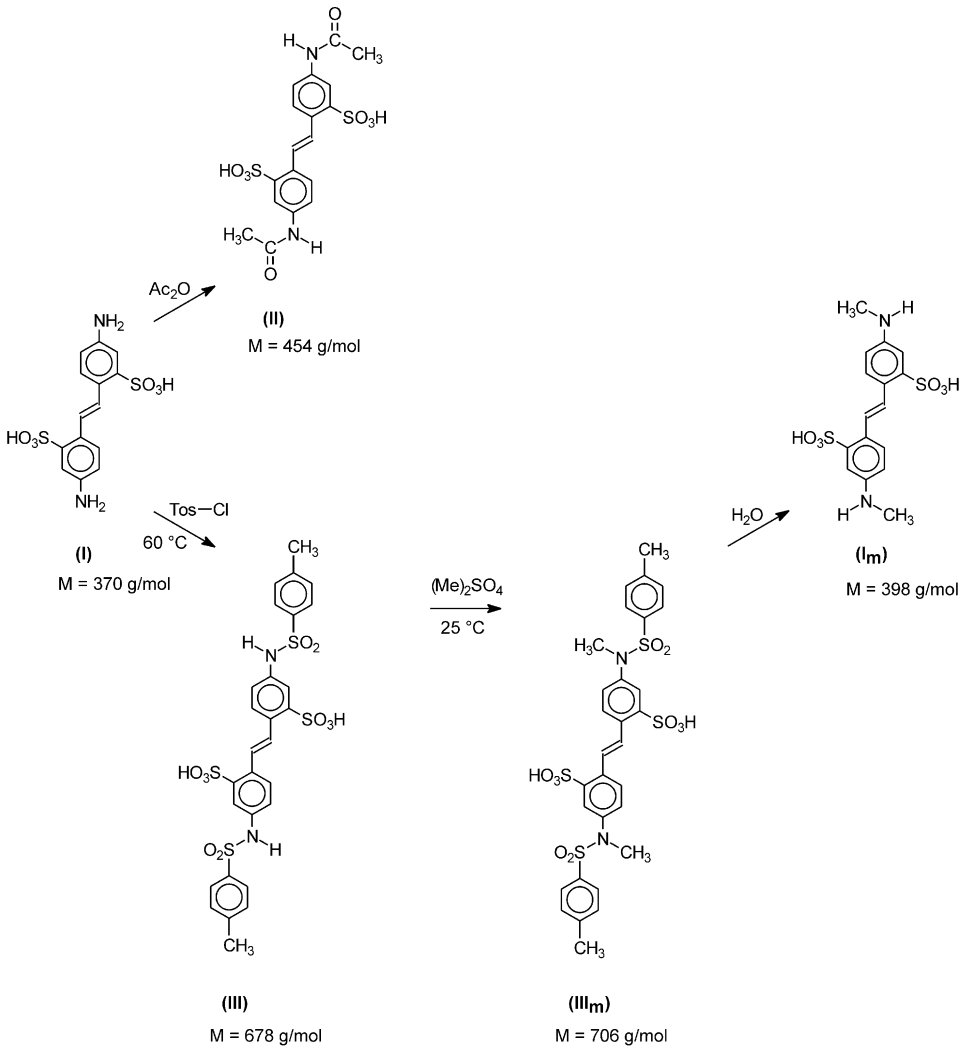


Scheme 1.

4,4′-Diaminostilbene-2,2′-disulfonic acid (supplied by the Research Institute for Organic Syntheses, Pardubice-Rybitví, Czech Republic) was recrystallised from water in the form of disodium salt.

Both the course of reactions and the purity of products (III, III_m, I_m) were checked using HPLC (Thermo separation products PC 1000) with reverse-phase column (250×4 mm) Nucleosil C18 and absorption UV/vis detector (340 and 250 nm). The mobile phase consisted of acetonitrile (30–40%) and aqueous 0.15 M ammonium sulfate. In the final products, the ratio C/N was also checked by elemental analysis.

The ^1H and ^{13}C NMR spectra were recorded on an AMX 360 (Bruker) spectrometer at 360.14 and 90.57 MHz, respectively. The chemical shifts δ_H and δ_C measured in [$^2H_6$] dimethylsulfoxide solutions were related to the central signal of the solvent multiplet $\delta_H = 2.55$ ppm and $\delta_C = 39.6$ ppm, respectively. The signals in the ^{13}C NMR spectrum were assigned by means of APT (attached

Table 1
Structures of studied substances

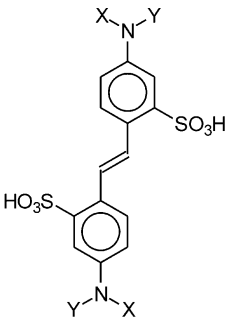
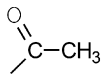
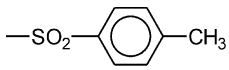
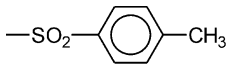
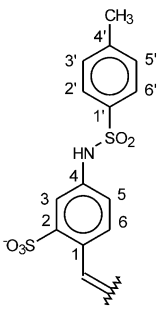
General formula	Substance	X	Y
	I	−H	−H
	II	−H	
	III	−H	
	I_m	$-CH_3$	−H
	III_m	$-CH_3$	

Table 2
^{1}H NMR chemical shifts of substance III

Numbering of carbon atoms in substance III	Signal δ_H (ppm)	Multiplicity of signal	Proton	Coupling constant J (Hz)
	2.37	Singlet	6 H, 2×(Ph-C<u>H</u>$_3$)	–
	7.18–7.21	Double doublet	2 H, 2×(H-5)	8.5 and 2.4
	7.38–7.41	Multiplet	4 H, 2×(H-3′, H-5′)	8.1
	7.46–7.48	Doublet	2 H, 2×(H-6)	8.5
	7.63–7.64	Doublet	2 H, 2×(H-3)	2.4
	7.71–7.73	Multiplet	4 H, 2×(H-2′, H-6′)	8.1
	7.88	Singlet	2 H, 2×(=C<u>H</u>–)	–
III	10.32	Broad signal	2H, 2×(NH)	–

proton test)—this method allows us to differentiate between the resonances of quaternary carbons, $C_{quat.}$, and $-CH_2-$ and carbons of =CH–, $-CH_3$ groups. Theoretical chemical shifts were calculated using ^{13}C NMR module in the ChemWindows 3.0.2 program.

Disodium salt of 4,4′-bisacetamidostilbene-2,2′-disulfonic acid (II) was prepared according to [6].

Disodium salt of 4,4′-bis(*p*-tosylamino)stilbene-2,2′-disulfonic acid (III) was prepared according to the procedure described for the synthesis of aromatic (NaO_3S–Ar–NH–SO_2–Ph–NH_2) sulfonamides [7]. To remove by-products (*p*-toluene sulfonic acid), precipitation (III) was washed with hot ethanol. ^{1}H NMR and ^{13}C NMR data are given in Tables 2 and 3, respectively.

For the synthesis of disodium salt of 4,4′-bis[*N*-methyl(*p*-tosylamino)]stilbene-2,2′-disulfonic acid (III_m), the analogy of the method for partial methylation of hydrazine with dimethylsulfate in

aqueous medium was used [8]. The crude product was purified by brief washing with cold water. ^{1}H NMR data are given in Table 4.

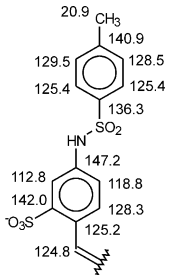
To prepare 4,4′-bis(*N*-methylamino)stilbene-2,2′-disulfonic acid (I_m), 0.005 mol of disodium salt of 4,4′-bis[*N*-methyl(*p*-tosylamino)]stilbene-2,2′-disulfonic acid (III_m) was refluxed for ca 20 min with 40 ml of diluted sulfuric acid (1:1, v/v). After hydrolysis, the reaction mixture was cooled and poured into ca 200 ml of water. To remove *p*-toluene sulfonic acid, the crude product (I_m) was washed with water and with ethanol, then dried at 50 °C. ^{1}H NMR data are given in Table 5.

The stability of substances II, III and III_m (conc. 0.0002 M in buffer at pH 2 and 12) was tested using the Ehrlich drop test and HPLC, respectively, during ca 24 h.

Potentiometric curves were measured at 295 K with a WTW 330 pH-meter using a SenTix 97 electrode (calibred for commercial buffers).

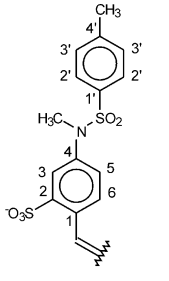
Electronic absorption spectra were measured at 295 K on a Perkin-Elmer 555 UV/vis absorption

Table 3
^{13}C NMR chemical shifts of substance III

Computed signals of substance III δ_C (ppm)	Measured =CH– and –CH_3 signals[a] δ_C (ppm)	Measured $C_{quat.}$ signals δ_C (ppm)
 III	21.2 $\underline{C}H_3$	130.9 C-1
	119.0 C-3	136.1 C-1′
	120.0 C-5	136.8 C-4′
	126.4 =$\underline{C}$H–	143.5 C-2
	126.5 C-6	146.1 C-4
	126.9 C-2′ and C-6′	
	129.9 C-3′ and C-5′	

[a] For the numbering of carbons, see Table 2.

Table 4
^{1}H NMR chemical shifts of substance III_m

Numbering of carbon atoms in substance III_m	Signal δ_H (ppm)	Multiplicity of signal	Proton	Coupling constant *J* (Hz)
 III_m	2.45	Singlet	6 H, 2×(Ph-C$\underline{H}_3$)	–
	3.17	Singlet	6 H, 2×(N-C$\underline{H}_3$)	–
	7.09–7.11	Doublet	2 H, 2×(H-5)	8.3
	7.46–7.53	Multiplet	8 H, 2×(H-2′,H-3′)	8.1
	7.58–7.60	Doublet	2 H, 2×(H-6)	8.3
	7.67	Singlet	2 H, 2×(H-3)	–
	8.09	Singlet	2 H, 2×(=C$\underline{H}$–)	–

spectrophotometer in 1 cm cuvette. Fluorescence emission and fluorescence excitation spectra were measured on a Perkin-Elmer LS-5 fluorescence spectrophotometer. Fluorescence emission spectra were corrected for characteristics of the emission monochromator and the photomultiplayer response. In order to prevent *trans–cis* isomerization, the solutions were prepared in dark. In course of the measurement, practically no time dependence of fluorescence intensity was observed.

While the pH was alternated between 2 and 12, the reversibility of dissociation processes was

Table 5
^{1}H NMR chemical shifts of substance I_m

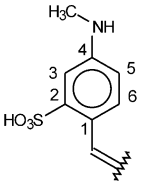
Numbering of carbon atoms in substance I_m	Signal δ_H (ppm)	Multiplicity of signal	Proton	Coupling constant J (Hz)
	3.42	Singlet	6 H, 2×(N-C<u>H</u>$_3$)	–
	7.41–7.44	Double doublet	2 H, 2×(H-5)	8.4 and 2.3
	7.74–7.75	Doublet	2 H, 2×(H-3)	2.3
	7.78–7.81	Doublet	2 H, 2×(H-6)	8.4
	8.19	Singlet	2 H, 2×(=C<u>H</u>–)	–
I_m	11.10	Broad signal	Acidic hydrogens	–

Fig. 1. Potentiometric titration curve of substance I (50 ml of 0.001 M solution of I was acidified with several drops of 0.05 M H_2SO_4 and then titrated).

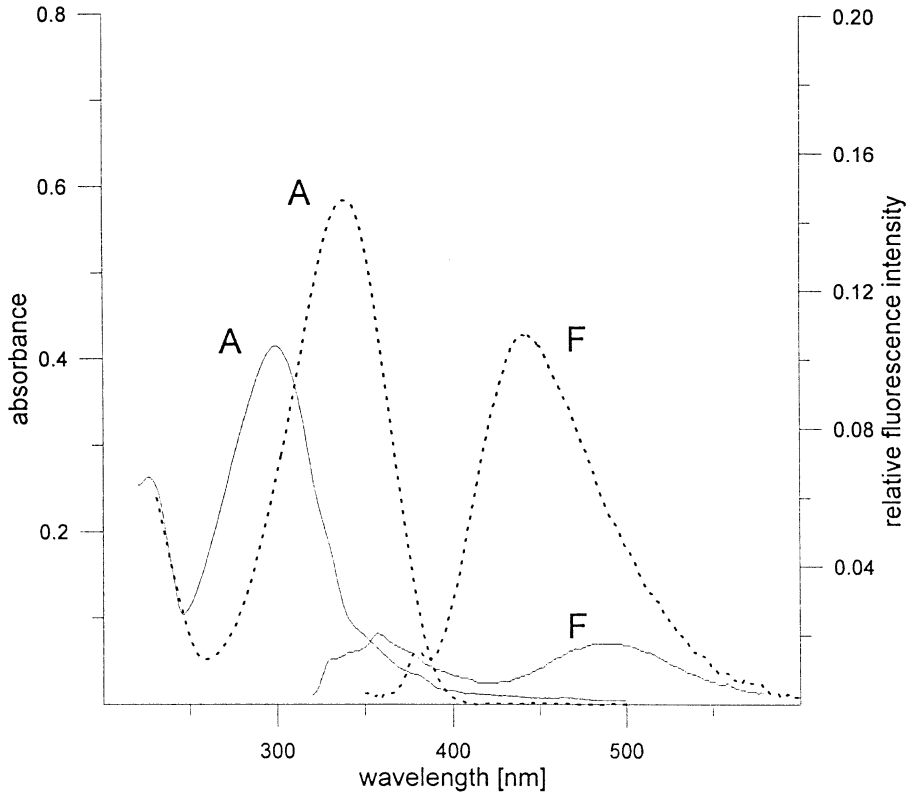
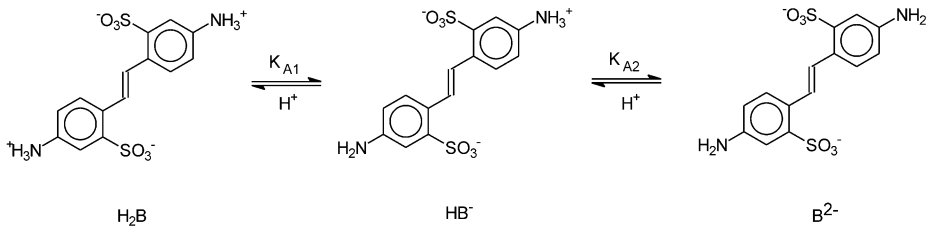


Fig. 2. The absorption (A) and fluorescence (F) spectra of substance I in dependence on pH, (—) pH 2.3, (...) pH 12.

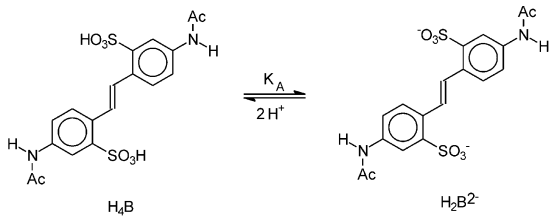


Scheme 2. Acidobasic forms of substance I in aqueous solution.

investigated by the measurement of spectral characteristics in solutions with constant concentration of studied substances.

Fluorescence quantum yields ($q_{\mathrm{fl.}}$) were determined from ratio of area under fluorescence emission spectrum and absorbance (A) measured at excitation wavelength according to formula:

$$q_{\mathrm{fl.}} = K \times \frac{n^2}{A} \int_{\tilde{\nu}_1}^{\tilde{\nu}_2} I \times \mathrm{d}\tilde{\nu};$$



Scheme 3. Acidobasic forms of substance II in aqueous solution.

n is optical refractive index, I is corrected fluorescence intensity, $\tilde{\nu}_1$ and $\tilde{\nu}_2$ (1/cm) are limit wavenumbers of an fluorescence band. Calibration constant (K) was determined using quinine sulfate in 0.5 M H_2SO_4 ($q_{fl.} = 0.54$, $n = 1.33$) as a standard [9].

3. Results and discussion

Relatively simple shape of potentiometric titration curve of substance I (Fig. 1) contrasts with more complicated reversible dependence of absorption and fluorescence emission spectra on pH (Fig. 2).

When pH decreases from 12 to 2, the absorption maximum (Fig. 2) shows hypsochromic shift from 340 to 300 nm. Fluorescence spectra in alkaline and neutral solution (Fig. 2) are represented by one simple band with maximum at 440 nm. In acid solution, fluorescence intensity decreases and new fluorescence bands appear at 360 and 490 nm. The short-wavelength band with vibrational structure corresponds to fluorescence spectrum of non-substituted stilbene. Received fluorescence data show the substance I exists in form of three species within the pH range 2–12 (Scheme 2 and Table 6).

Di-protonated H_2B form of I (Scheme 2) with fluorescence maximum at 360 nm (Fig. 2) exists in an equilibrium with monoprotonated HB^- form with fluorescence maximum at 490 nm in acid medium. Pure nonprotonated B^{2-} form (fluorescence maximum at 440 nm) exists in solution at pH > 7. Unlike absorption spectrum in neutral and alkaline buffer (pure B^{2-} form), absorption spectrum in acid solution is a superposition of spectra of H_2B and HB^- and B^{2-} species. The maximum

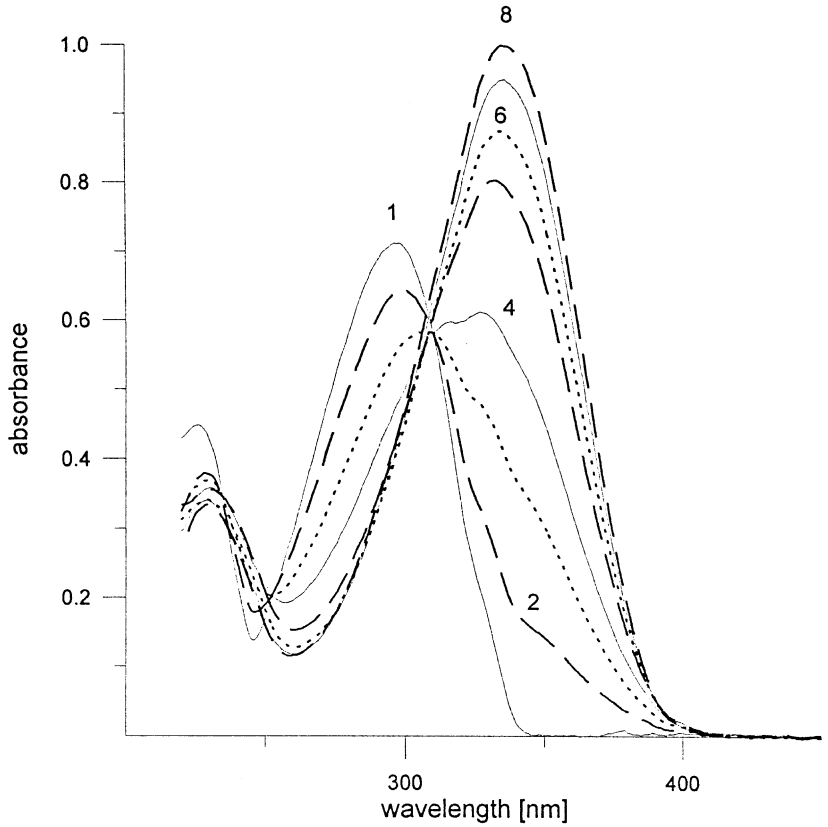


Fig. 3. Dependence of absorption spectra of I on pH. Curve 1: pH 0.7; 2: pH 2.6; 3: pH 3.1; 4: pH 3.5; 5: pH 4.1; 6: pH 4.6; 7: pH 5.0; 8: pH 6.0.

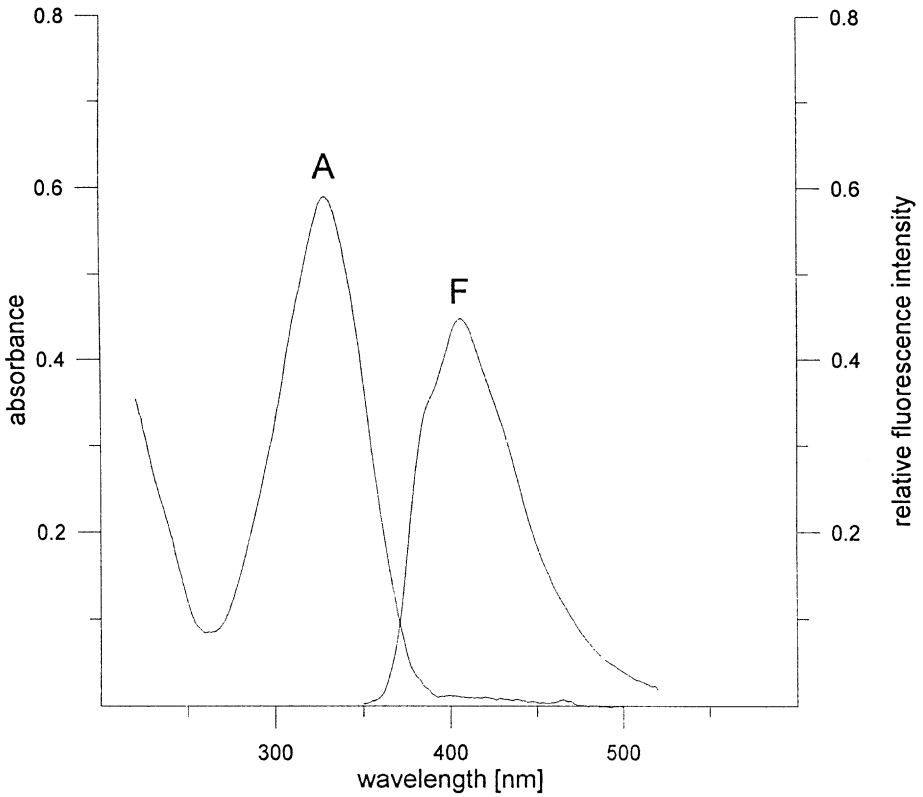


Fig. 4. The absorption (*A*) and fluorescence (*F*) spectra of substance II in the buffer at pH 7.

of absorption band of pure H_2B was found at 300 nm (curve 1 in Fig. 3). The complicated system of absorption curves (Fig. 3) does not allow us to determine easily the absorption coefficient of the HB^- form. Therefore, it is difficult to evaluate ionisation constants K_{A1} and K_{A2}, spectrophotometrically. In addition, a weak intramolecular interaction between amino groups causes pK_{A1} and pK_{A2} to be very close to each other (within about pH 3–4) and, therefore, it is not possible to detect them on the titration curve (Fig. 1).

In the case of substance II, the simple shape (the same as shown on Fig. 1) of the potentiometric titration curve and the independence of absorption and fluorescence spectra on pH (in Fig. 4, the spectra only at pH 7 are shown) indicate that the acidobasic equilibrium is simple (Scheme 3). The constant positions of absorption and fluorescence bands of II in the pH range 2–12 are a consequence of electron-acceptor acetyl substitution on the amino groups.

Another situation comes with the tosylamino derivative III. Potentiometric titration curves and dependencies of absorption/fluorescence spectra on pH are shown in Figs. 5 and 6, respectively. The equilibrium (pK_{A1} in Fig. 5 and K_{A1} in Scheme 4) in acid range corresponds to proton dissociation from sulfo groups. Since the consumption of 0.1 M HCl between two points of inflection (Fig. 5) corresponds to equation $2H^+ + X^{2-} = H_2X$, there are three acidobasic forms. The first one (at pH≈5) corresponds to H_2B^{2-} (Scheme 4); due to the electron-withdrawing effect of tosyl substituent and I-effect of sulfo group, the second form (maximal concentration at pH≈8.3) may correspond to the HB^{3-}, and the third form (at pH≈10) is a B^{4-}. Since the absorption maximum does not depend on pH in the acid range, the proton addition on

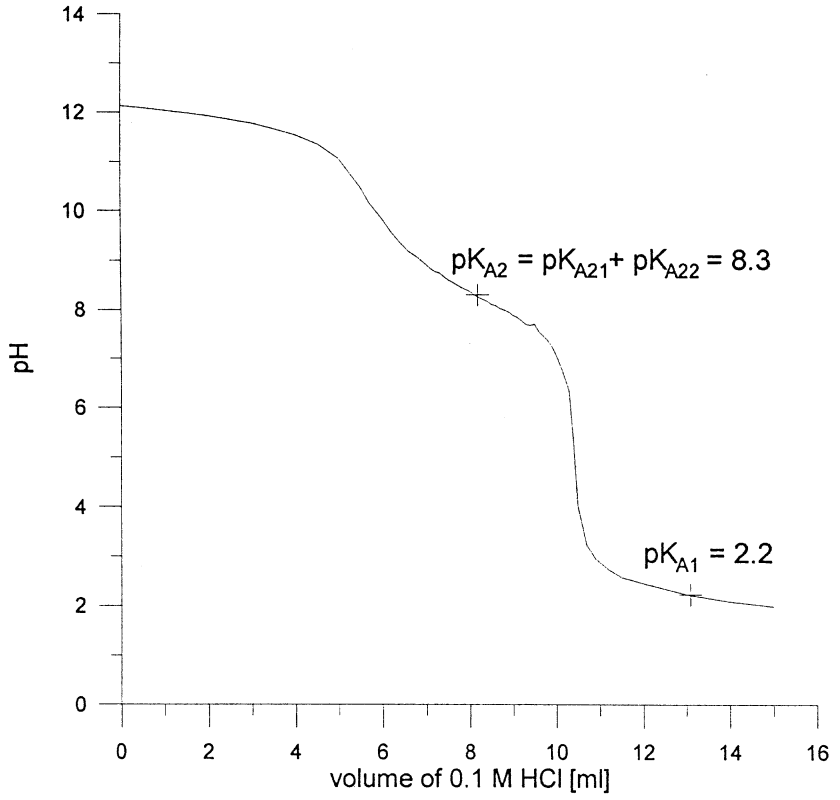
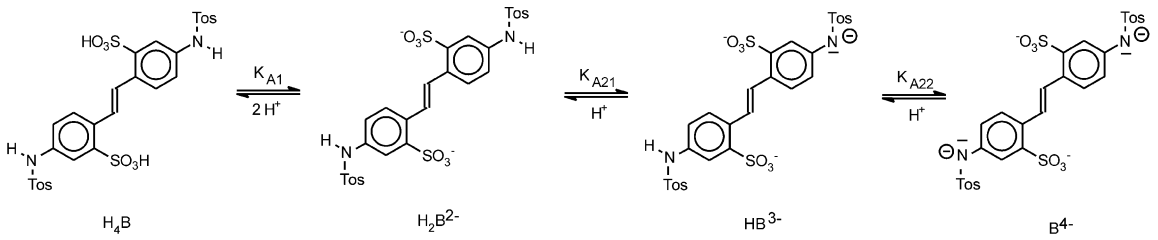


Fig. 5. Potentiometric titration curve of substance III (25 ml 0.01 M of III was alkalised with several drops of 0.1 M NaOH and then titrated).



Scheme 4. Acidobasic forms of substance III in aqueous solution.

amido nitrogen does not occur (similar to the aqueous solution of substance II). Unlike fluorescence spectra either in acid or in alkaline solution (Fig. 6), the spectrum in neutral solution is composed of two fluorescence bands (maximum at 400 nm and shoulder at 500 nm). This result shows, probably, that the equilibrium of several forms of substance III is set up in the neutral solution. The fluorescence band at 400 nm corresponds probably to the H_2B^{2-} species (Scheme 4) and the long-wavelength shoulder to hypothetic HB^{3-} originating from mono-deprotonation of H_2B^{2-}. In the alkaline range, B^{4-} with high fluorescence intensity does exist (Fig. 6 and Table 6). It would mean that substance III behaves as N-acid in aqueous alkaline solution. In a similar way as in the case of I, the complicated system of absorption curves (Fig. 7) does not allow us to determine, easily, the

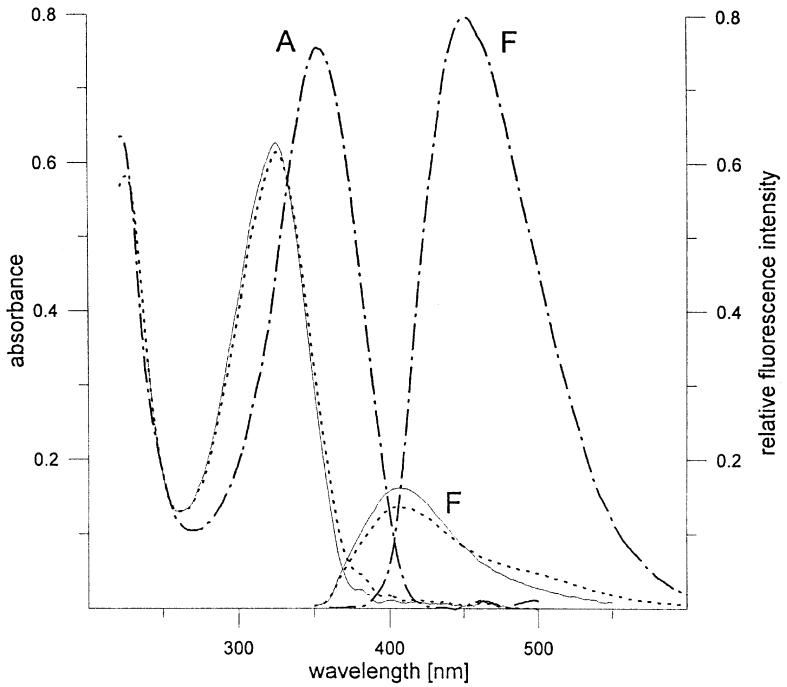


Fig. 6. The absorption (*A*) and fluorescence (*F*) spectra of substance III in dependence on pH, (—) pH 2, (. . .) pH 7, (-·-·-) pH 12.

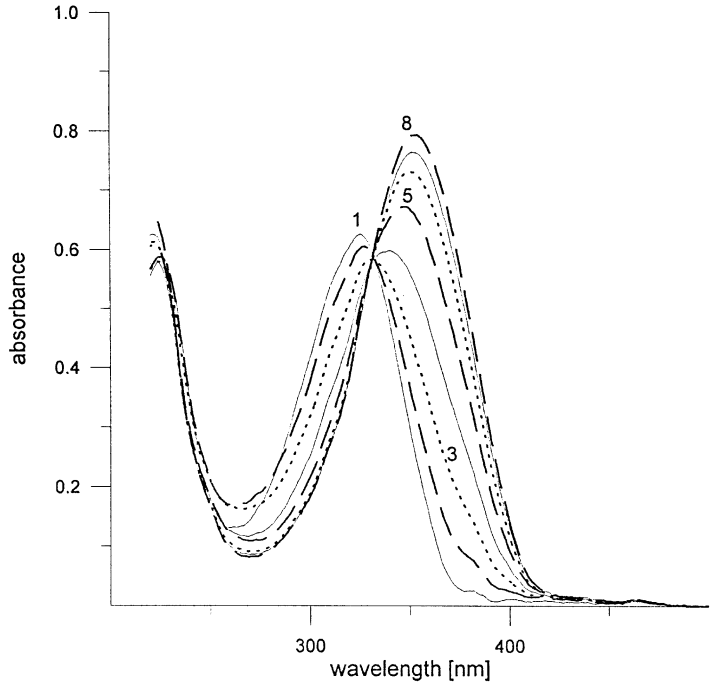
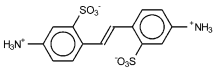
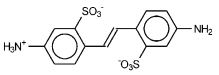
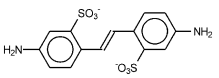
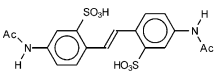
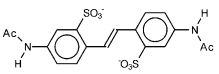
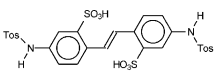
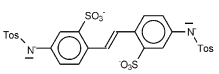
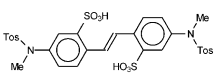
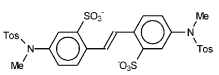
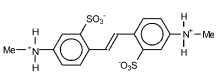
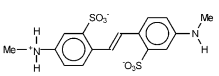
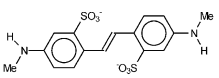


Fig. 7. Dependence of absorption spectra of III on pH. Curve 1: pH 1.7; 2: pH 7.2; 3: pH 7.7; 4: pH 8.2; 5: pH 8.7; 6: pH 9.2; 7: pH 9.7; 8: pH 10.7.

Table 6
Spectral characteristics of different acidobasic forms of studied substances in phosphate buffers

Substance (form)	Formula	The pH range of occurrence	Absorption maximum $\lambda_{max.}$ (nm)	Absorption coefficient $\varepsilon_{max.}\times10^{-3}$ (l/mol.cm)	Fluorescence maximum $\lambda_{max.}$ (nm)	Fluorescence quantum yield $q_{fl.}$ (%)
I (H_2B)		2	300	21±1	360	–
I (HB^-)		3 < pH < 4	–	–	495	1.00±0.04[a]
I (B^{2-})		> 7	338	29±1	442	2.30±0.06[b]
II (H_4B)		< 3	326	32±2	408	6.00±0.15
II (H_2B^{2-})		> 7	326	33±2	408	6.00±0.15
III (H_4B)		< 3	325	33±1	408	1.96±0.07
III (B^{4-})		> 10	354	40±1	450	9.00±0.20
III_m (H_2B)		< 3	320	25.4±0.6	408	2.61±0.05
III_m (B^{2-})		> 7	320	25.4±0.6	408	2.61±0.05
I_m (H_2B)		2	296	–	360	–
I_m (HB^-)		3 < pH < 4	–	–	507	1.59±0.03[a]
I_m (B^{2-})		> 7	353	29.6±0.5	457	2.95±0.09

[a] Measured at pH≈3, fluorescence excited within 350 to 370 nm.
[b] Compare with $q_{fl.}$ = 1.8% in Ref. [10].

absorption coefficient of the HB^{3-} form and, therefore, it is difficult to find ionisation constants K_{A21} and K_{A22} spectrophotometrically. A weak intramolecular interaction between amino groups also causes pK_{A21} and pK_{A22} to be very close to each other (within about pH 8–9) and it is not possible to differentiate them on the titration curve (Fig. 5).

The titration curve of III_m has only one point of inflexion (the same as shown on Fig. 1) and neither absorption nor fluorescence spectra depend on pH (Fig. 8). It means the substance III_m exhibits the same acidobasic and spectral properties as II.

The shape of the titration curve of substance I_m is the same as for substance I—only one point of

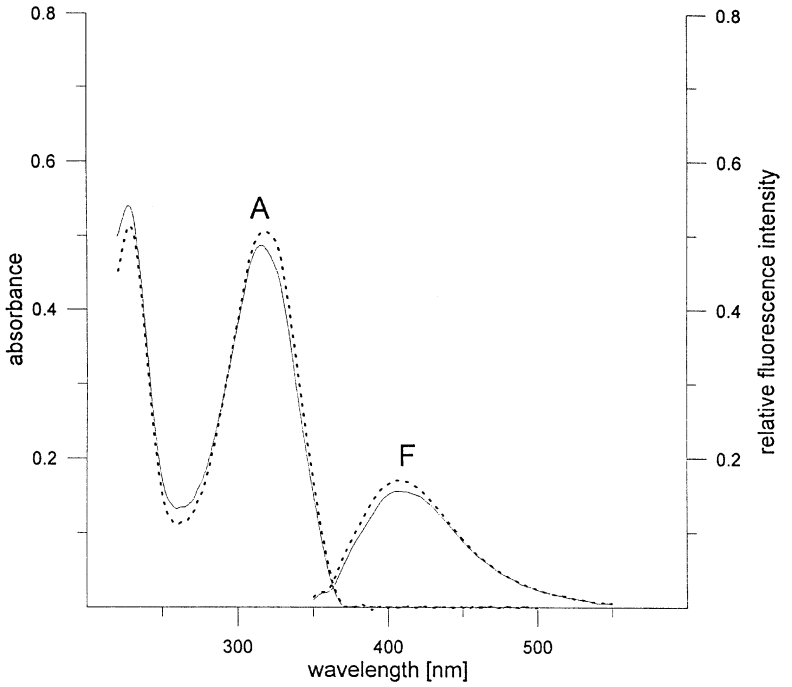


Fig. 8. The absorption (*A*) and fluorescence (*F*) spectra of substance III_m in dependence on pH, (...) pH 2, (—) pH 12.

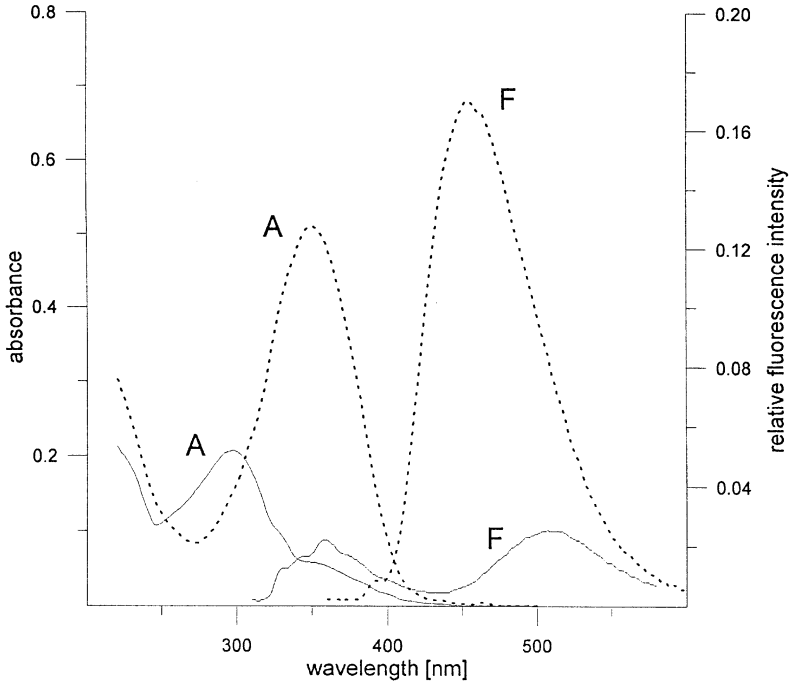


Fig. 9. The absorption (*A*) and fluorescence (*F*) emission spectra of substance I_m in dependence on pH, (—) pH 2.3, (...) at pH 12.

inflexion corresponds to simple acidobasic equilibrium. Similarly, the dependencies of absorption and fluorescence spectra of I_m on pH (Fig. 9) are analogous to the same dependencies of substance I. No spectral changes occur in the pH range within 7–12. These results confirm the existence of the same acidobasic equilibria for substances I and I_m (Scheme 2, Table 6).

4. Conclusion

The comparison of experimental data for substance I with published data for the nonsulfonated analogue shows that an introduction of sulfo groups does not considerably affect its spectral properties. An acylation of the amino group (e.g. substance II) prevents protonation of nitrogen in the acid range ($2 < pH < 7$). In alkaline range ($7 < pH < 12$), a relatively low electron-withdrawing effect of the acetyl group of substance II is not sufficient to split off a proton from the amido nitrogen.

In the case of substance III, the electron-withdrawing effect of the tosyl substituent (higher than acetyl of II) enables the splitting off a proton from the amido nitrogen in aqueous alkaline solution and the substance behaves as an N-acid (equilibrium $H_2B^{2-} \overset{K_{A2}}{\rightleftharpoons} 2H^+ + B^{4-}$, $pK_{A2} \approx 8.3$ at 295 K). This proton dissociation results in an increase of fluorescence intensity.

As we expected, methyl derivative III_m does not exhibit any N-acid properties and its fluorescence intensity does not depend on pH in the used range. Due to the electron-withdrawing effect of tosyl substituent (similarly as II and III) no proton addition on amido nitrogen of III_m occurs in the acid range.

Finally, in accordance with expectation, the substance I_m shows analogous spectral dependencies on pH as substance I.

References

[1] Gold H. In: Venkataraman K, editor. The chemistry of synthetic dyes, vol. 5. New York and London: Academic Press, 1971, p. 556.
[2] Zahradník M. The production and application of fluorescent brightening agents. Chichester: John Wiley, 1982.
[3] Zollinger H. Color chemistry: syntheses, properties and applications of organic dyes and pigments, 2nd ed. Weinheim, New York, Basel, Cambridge: VCH, 1991.
[4] Yujiong Zhao, Khodorkovsky V, Cohen J, Priel Z. J Photochem Photobiol A: Chemistry 1996;99:23–8.
[5] Machalický O, Vaněrka J. Study of *N*-methylaminostilbene-2,2′-disulfonic acids as reactive fluorescent probes: synthesis, photochemical and acidobasic properties. In: 8th International Conference on Dyes and Pigments, Colorchem 2000, Špindlerův Mlýn, Czech Republic, 2000, P39.
[6] Chattawax FD. J Chem Soc 1931:2495–6.
[7] Grossley ML, Northley EH, Hultquist ME. J Am Chem Soc 1938;60:2217–22.
[8] Blatt AH. Organic syntheses, coll, vol. 2. NY: John Wiley & Sons, 1943.
[9] Birks JB. Fluorescence quantum yield measurements. Washington (DC): National Bureau of Standards, Special Publication No. 466, 1977.
[10] Smit KJ, Ghiggino KP. J Polym Sci, Part B: Polymer Physics 1991;29:1397.