

Solvatochromic Fluorescence of 8-(Phenylamino)-1-naphthalene-ammoniumsulfonate (8,1 ANS) in 1,4-Dioxane/Water Mixtures, revisited

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Z. Naturforsch. **48a**, 709–712 (1993); received January 11, 1993

The correlation of the solvatochromic fluorescence properties of 8-(phenylamino)-1-naphthalene-ammoniumsulfonate (8,1 ANS) in 1,4-dioxane/water mixtures with $E_T(30)$ and Δf values of the respective solvents shows that besides the polarity the nature of the solvent mixture determines the fluorescence intensity I and the Stokes shift $\Delta \tilde{\nu}$. Different amounts of hydrogen-bonding are suggested to be responsible for the different slopes of the correlation line plots.

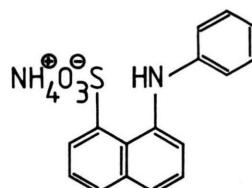
Key words: Solvatochromic fluorescence 8-(phenylamino)-1-naphthalene-ammoniumsulfonate (8,1 ANS); Semi-empiric polarity parameters 1,4-dioxane/water mixtures.

Introduction

Environmental factors often strongly influence the fluorescence of appropriately qualified molecules [1, 2]. The decrease in fluorescence intensity and the bathochromic shift of the emission wavelength of 8-(phenylamino)-1-naphthalene-ammoniumsulfonate (8,1 ANS) with increasing polarity of its environment is well known and its application as a fluorescence probe in biochemical studies is well documented [3–7].

Kosower and co-workers have investigated the influence of the solvent polarity on the emission energies E_f (calculated from the respective emission wavelength) and the fluorescence quantum yields of differently substituted 8,1 ANS molecules using different solvent mixtures of rising polarity [8], the characteristic feature in each case being a bilinear dependence of E_f on the solvent polarity parameter $E_T(30)$. The semi-empirical solvent polarity parameter $E_T(30)$, as introduced by Dimroth and Reichardt, is defined as the molar transition energy of the long-wavelength, solvatochromic UV/Vis-absorption band of a specific pyridinium-N-phenoxide betaine dye, caused by an intramolecular charge-transfer absorption [9–11].

The two-slope-behavior let Kosower conclude that the fluorescence of the 8,1 ANS molecule arises from two different excited states. According to this theory



8,1-ANS

Fig. 1. 8-(phenylamino)-1-naphthalenesulfonic acid ammonium salt (8,1 ANS).

the slopes represent the solvatochromic behavior of a non-planar “S1np” state and a more sensitive “S1ct” charge transfer state of the molecule, respectively.

Inspired by this papers we worked on the application of 8,1 ANS for the detection of environmental pollution and as a dye for staining thin-layer chromatograms for qualitative and quantitative analyses [12–14]. During this work we noticed that besides polarity, p_{Ka} -values of the environment – or the solvent applied – are apparently important for the solvatochromic fluorescence characteristics of 8,1 ANS. To find out more about the influence of protons on the fluorescence of 8,1 ANS we were interested in comparing the $E_T(30)$ dependence with the influence of some other polarity parameters, e.g. the orientation polarizability Δf [15]. The results reveal that for a complete description of the phenomena the specific properties of the 1,4 dioxane/water mixtures have to be taken into account.

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Experimental

8,1 ANS was bought from Aldrich and purified by repeated chromatography on silica gel using toluene/ethanol 1:1 as eluant until the thin-layer chromatogram (TLC) showed one spot. 1,4-dioxane (Aldrich; Gold Lable, >99%) and dionized water were distilled under nitrogen prior to use. The absorption spectra were recorded on a Philips PU 8720 UV/VIS Spectrophotometer, the fluorescence spectra on a Perkin-Elmer MPF-3L fluorescence photometer. All measurements were carried out at 20 °C using a 10 µmolar solution of 8,1 ANS in the respective solvent mixture. The fluorescence intensities were related to those of a sample dissolved in pure 1,4-dioxane which was set equal to 1000. The corresponding $\Delta\bar{v}$ and Δf values were calculated according to [2]:

$$\Delta\bar{v} = 1/\lambda_{\text{abs}} - 1/\lambda_{\text{em}},$$

$$\Delta f = (\varepsilon - 1)/(2\varepsilon + 1) - (n^2 - 1)/(2n^2 + 1).$$

Dielectric constants (ε) were taken from literature or by interpolation of these values [14]. Refractive indices were measured with a Zeiss Abbe refractometer.

Results

Langhals et al. [17] first determined the influence of solvent polarity on the longest wavelength absorption band maximum (λ_{abs}) of 8,1 ANS. In contrast to the results of Kosower et al. [8], they found a distinct effect. Solutions of the dye in 1,4-dioxane/water mixtures exhibited a significant hypochromic shift of the absorption band maximum with rising amounts of water, which could be described by the general equation $E_T = E_D \cdot \ln(c_p/c^* + 1) + E_T^0$.

For recording the fluorescence spectra of 8,1 ANS in the respective mixtures, we chose the excitation energies as the closest integer wavelength with respect to the corresponding absorption maxima. Our measurements of the corresponding emission wavelength of the dye in the applied solvent mixtures as well as the polarity data of these media are summarized in Table 1.

These parameters were used to analyse the relationship of the fluorescence intensity I and the Stokes' shift $\Delta\bar{v}$ with the two polarity parameters $E_T(30)$ and Δf , respectively. The results are shown in Figures 2a–d. They display its well known bathochromic shift and

Table 1. Polarity data of 1,4-dioxane/water mixtures and the excitation and emission wavelength of 8,1 ANS in the respective solvent.

H ₂ O ^a (cl/l)	$E_T(30)$ ^b kcal/mol	$\Delta f \cdot 10^2$ ^c	λ_{exc} ^d nm	λ_{em} ^d nm
0	36.0	2.36	377	462
0.75	38.1	2.97	377	462
1.10	39.0	3.90	377	462
1.46	40.0	4.34	377	462
2.00	41.5	5.26	377	463
2.66	41.8	6.57	376	463
3.00	42.1	8.24	376	464
4.80	43.5	11.08	376	465
6.50	45.0	13.91	376	467
7.80	45.7	15.61	375	469
8.50	46.0	16.27	375	470
11.20	47.0	19.56	375	472
15.00	47.9	21.91	374	474
18.20	48.6	23.43	373	475
21.00	49.2	24.57	373	475
26.00	50.2	26.06	373	476
29.10	50.7	26.68	372	477
36.50	51.9	27.81	371	478
37.60	52.1	27.95	371	478
44.00	53.0	28.60	370	480
51.50	54.0	29.20	370	482
57.00	55.0	29.58	369	486
62.00	56.0	29.96	368	487
69.00	57.0	30.36	368	492
81.00	59.0	31.01	360	499
90.00	61.0	31.48	358	507

^a Percentage of water by volume mixed with 1,4-dioxane.

^b From [8] and [12] or by linear interpolation.

^c From (2). ^d ± 2 nm.

the simultaneous decrease in fluorescence intensity. This is not only true for the Stokes' shifts but also for the relative fluorescence intensity.

The general aspects of the correlation are obviously very similar for both polarity parameters. In contrast to the plots presented by Kosower et al., however, our measurements offer at least a trilinear relationship in case of the $E_T(30)$ dependences as indicated by the correlation lines. With the three-slope-behavior – especially obvious from the plot I versus $E_T(30)$ – one found the intersections of the correlation lines to represent mixtures with a water content of about $c_p = 1.39 \pm 0.32$ mol/l and $c_p = 28.60 \pm 1.39$ mol/l, respectively, for both dependencies.

In the case of the Δf plots no linear interpretation fitted the data points. One can see from the curved line, however, that it consists of three different parts, indicated by two inflection points at an water content of approximately 3 mol/l and 23 mol/l.

On a closer inspection it even seems more likely that the trilinear interpretation of the $E_T(30)$ results

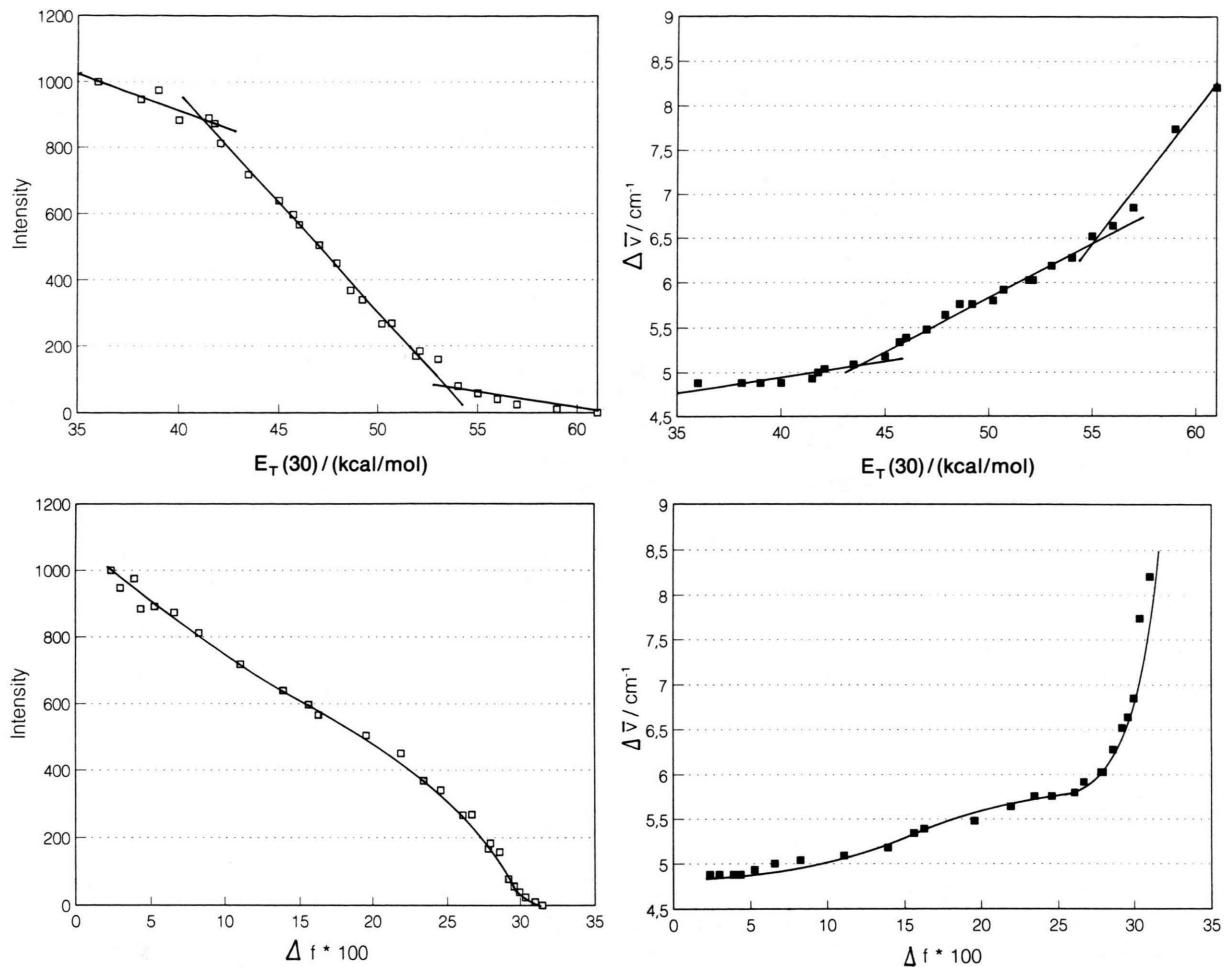


Fig. 2a–d. Plots of the fluorescence intensity I (given in arbitrary units $\pm 5\%$) and Stokes' shift $\Delta\bar{v}$ of 8,1 ANS in 1,4-dioxane/water mixtures versus the polarity parameters $E_T(30)$ and Δf .

are only an approximation. A curved line might meet the relationship of the fluorescence properties of 8,1 ANS and solvent polarity far better than linear curves.

Discussion

Our investigations concerning the solvatochromic fluorescence properties of 8,1 ANS show that the polarity of the media used can be described by $E_T(30)$ -values as well as by the orientation polarizability (Lippert's parameter). Plots of fluorescence intensity I or Stokes' shift $\Delta\bar{v}$, respectively, versus these parame-

ters displayed in general the same functional course. However, concerning the $E_T(30)$ dependences the trilinear interpretation means a striking difference to the strict two-slope plots presented by Kosower et al. [8, 9]. On the other hand our results seem to be in accordance with the investigations of Langhals [17] on the polarity of binary solvent mixtures. As a general rule his plots of several semi-empirical polarity parameters versus $\ln(c_p)$ showed always a straight line and a non-linear curve (which Langhals proved both to be covered by a single equation). In case of 1,4-dioxane/water mixtures, however, the plot additionally exhibited a second linear correlation line, starting at a water content of 22 mol/l corresponding to an $E_T(30)$ -value of 51.9 kcal/mol. In case of the $E_T(30)$ -values our re-

sults of the solvent dependence of the solvatochromic fluorescence properties of 8,1 ANS paralleled these investigations. Whereas the orientation polarizability can be looked at as a parameter that is calculated from constants that describe the more static polar influences, semi-empirical $E_T(30)$ -values take dynamic processes like hydrogen-bonding into account. The latter dominates the overall polarity of the solvent mixtures. Thus, it can be concluded that the three-slope-behavior of the 8,1 ANS plots displayed a characteristic interaction of the 1,4-dioxane/water mixtures used with specific properties of the dye. We suggest different amounts of hydrogen bonding of the mixtures to be the most probable explanation of the phenomena. It was discussed earlier that protons have an important influence on the fluorescence of 8,1 ANS [4, 5] and, concerning environmental effects on this molecule, Ainsworth and Flanagan distinguish between the terms "hydrophobic" and "non-polar" [18]. In an early paper, dealing with glycerol solutions of the closely related 6,2 ANS and some of its N-alkyl

derivatives even Kosower et al. postulated excited state protonation to account for their fluorescence characteristics [19].

We suggest that the most apolar mixtures have more or less the solvent structure of 1,4-dioxane with enclosed isolated water molecules; at a water concentration of approximately 1–2 mol/l possibly water–water hydrogen bonding may appear besides water–1,4-dioxane hydrogen bonding and finally, at concentrations above 22 mol/l the structure of liquid water may dominate the structure of the mixture. Concurrent with this the ability to protonate the S1 excited state of 8,1 ANS rises. Thus, supported by literature, our results focus on the fact that the solvatochromic fluorescence properties of 8,1 ANS do not solely depend on the polarity of the environment but are mainly influenced by the proton activities of the solvents used. Measurements of the fluorescence properties of 8,1 ANS in pure solvents of rising polarity discriminated between protic and aprotic should show a distinct linear correlation of its own [20].

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