Fluorescence of 8-(Phenylamino)-1-naphthalene-ammonium-sulfonate in Solvents of Different Polarity

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The solvatochromic fluorescence properties of 8-(phenylamino)-1-naphthalene-ammoniumsulfonate (**1**) were investigated in 22 pure solvents of rising polarity. The measurements of both Stokes' shifts and intensities discriminated between protic and aprotic solvents. Fairly good linear correlation plots of the dependence could be obtained on neglection of the values of tert.-butanol, DMF and DMSO.

Key words: Solvatochromic fluorescence; 8-(Phenylamino)-1-naphthalene-sulfonate (8,1 ANS); Protic solvents; Aprotic solvents.

1. Introduction

The decrease of the fluorescence intensity and the bathochromic shift of the emission wavelength of 8-(Phenylamino)-1-naphthalene-ammoniumsulfate (8,1 ANS-NH_4) (**1**) with increasing polarity of its environment are well known and have been used for biochemical analyses [1].

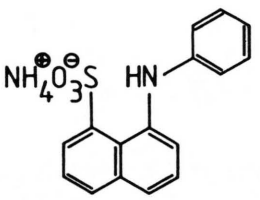

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Fig. 1. 8-(Phenylamino)-1-naphthalene-ammoniumsulfonate (**1**).

Kosower and others intensively investigated this behaviour [2]. Looking for correlations of the fluorescence parameters of **1** with the solvent polarity, they mostly used homogeneous 1,4-dioxane/water mixtures of a defined $E_T(30)$-value (the empirical solvent polarity parameter introduced by Reichardt and Dimroth [3, 4]). Contrary to Kosower's views, the solvatochromic fluorescence of **1** has recently been found to be dependent on the hydrogen-bonding properties of the solvent [5, 6]. Few data, however, have been reported on the fluorescence behaviour of **1** in pure solvents [5, 7, 8]. As far as we know, no systematic investigation of the fluorescence of **1** in pure solvents of rising polarity has been performed. Thus, to support the latest theory, we investigated the fluorescence intensity and Stokes' shift of **1** dissolved in pure solvents of defined $E_T(30)$-values.

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2. Experimental

1 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. The solvents (Aldrich, Merck, Fluka) were used without further purification when their purity was $\geq 99\%$, otherwise they were distilled under N_2. 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 using a 10 μmolar solution of **1** in the respective solvent. One sample was used to determine the maximum of the long-wavelength absorption band as well as the maximum of the emission band. With a second sample, the fluorescence intensity was measured as the hight of the emission band maximum. All measurements were carried out at 20 °C except for methylacetamide, where the temperature was 40 °C. The fluorescence intensities were related to that of a sample dissolved in pure 1,4-dioxane, which was set equal to 1000. The corresponding $\Delta\bar{\nu}$ values were calculated according to [9]: $\Delta\bar{\nu} = 1/\lambda_{Abs} - 1/\lambda_{Em}$.

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Fig. 2. Plot of the fluorescence intensity of **1** versus the polarity parameter $E_T(30)$ of different solvents. For numbers see Table 1. ⊠ = aprotic solvents, ▲ = protic solvents.

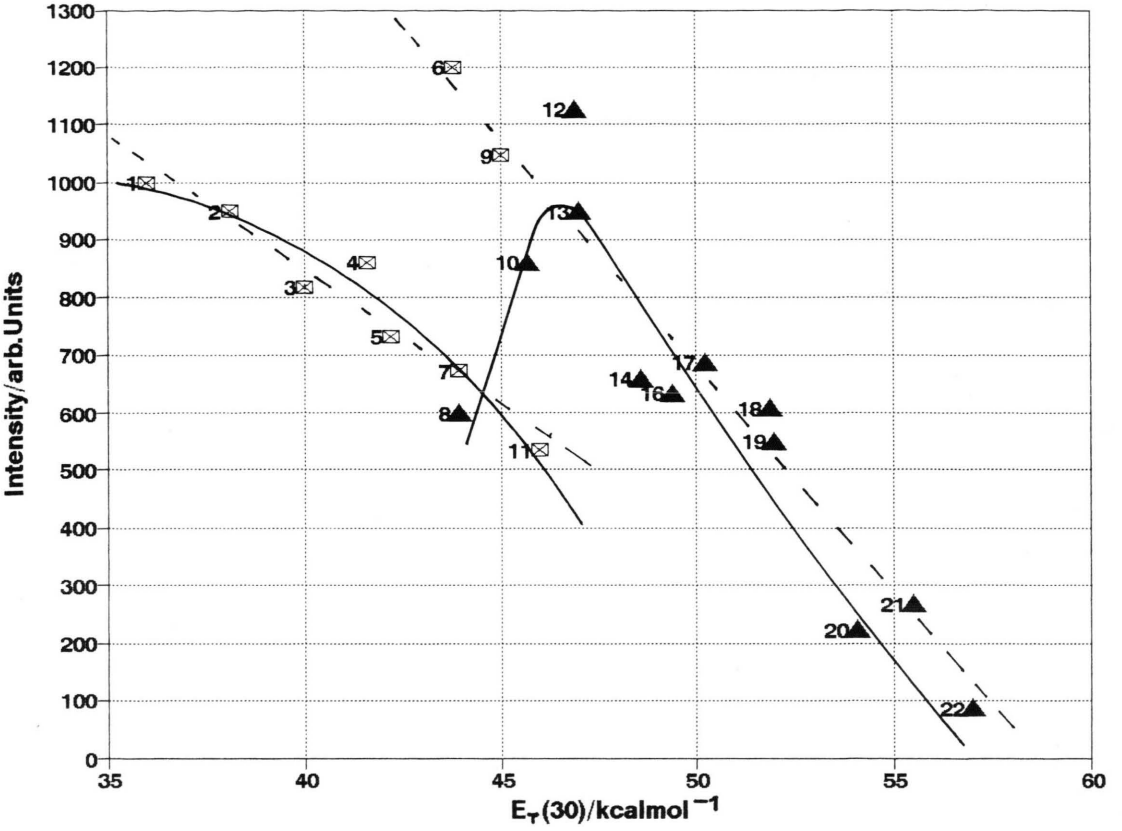
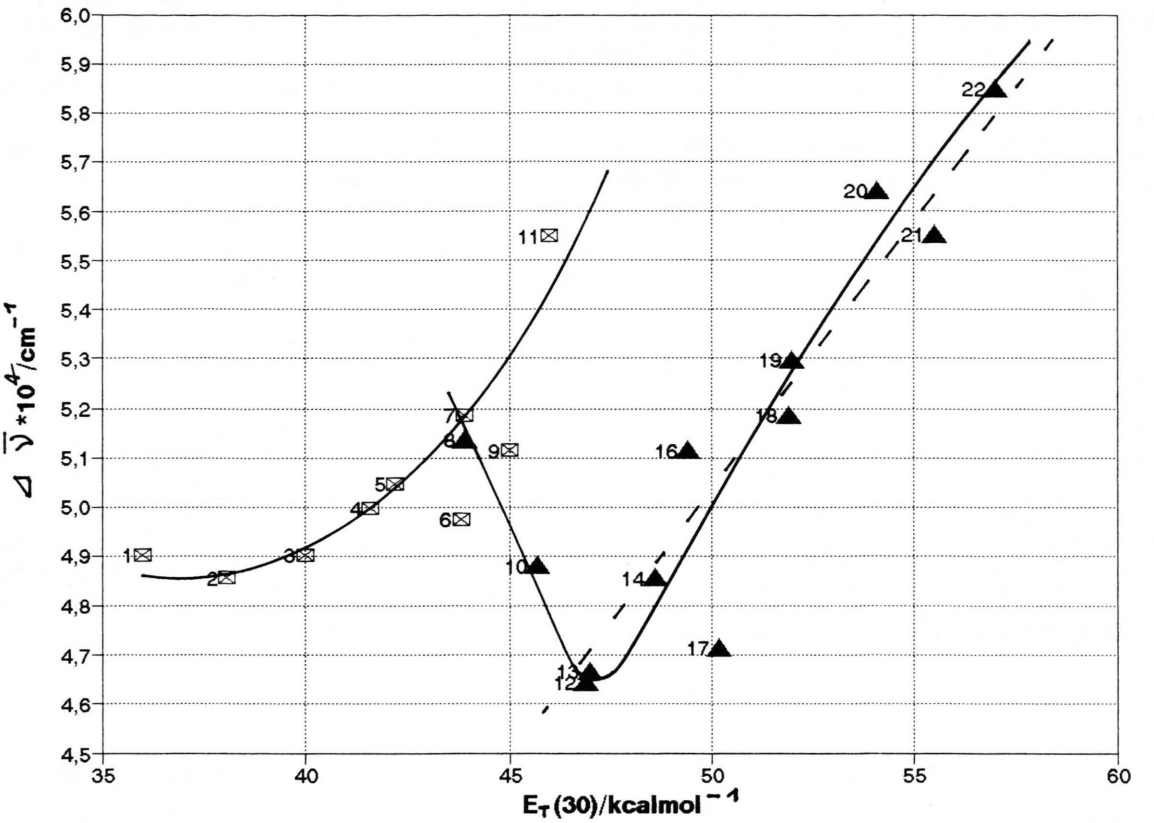


Table 1. Fluorescence properties of **1** in solvents of different polarity. $E_T(30)$ = polarity parameter of C. Reichardt, λ_{Exc}, λ_{Em} = excitation and emission wavelength, I = fluorescence intensity, $\Delta\bar{\nu}$ = Stokes' shift.

	Solvents	$E_T(30)$ kcal/mol	λ_{Exc} nm	λ_{Em} nm	I arb.units	$\Delta\bar{\nu}$ cm^{-1}
1	Dioxan	30.0	378	464	1000	4.90
2	Ethylacetate	38,1	376	460	950	4.86
3	Methylacetate	40.0	374	458	820	4.90
4	Triacetin	41.6	376	463	862	5.00
5	Acetone	42.2	372	458	733	5.05
6	DMF	43.8	373	458	1200	4.98
7	Acetanhydride	43.9	374	464	672	5.19
8	tert.-Butanol	43.9	376	466	600	5.14
9	DMSO	45.0	375	464	1047	5.11
10	3-Pentanol	45.7	377	462	863	4.88
11	Acetonitrile	36.0	369	464	534	5.55
12	Cyclohexanol	46.9	379	460	1127	4.65
13	3-Methylbutanol	47.0	378	459	950	4.67
14	Isopropanol	48.6	376	460	659	4.86
15	1-Pentanol	49.1	377	462	770	4.88
16	Ethylacetoacetat	49.4	375	464	634	5.11
17	1-Butanol	50.2	378	460	687	4.72
18	Ethanol	51.9	374	464	609	5.19
19	Methylacetamide	52.0	375	468	550	5.30
20	Methylformamide	54.1	374	474	222	5.64
21	Methanol	55.5	374	472	269	5.55
22	Glycerol	57.0	376	482	87	5.85

Fig. 3. Plot of the Stokes' shift of **1** versus the polarity parameter $E_T(30)$ of different solvents. For numbers see Table 1. ☒ = aprotic solvents, ▲ = protic solvents.



3. Results and Discussion

There exist protic and aprotic solvents over a wide range of polarities. For both groups, we have chosen solvents covering the whole polarity scale. Table 1 lists these media together with their polarity $E_T(30)$ and the corresponding excitation and emission maximum of **1** dissolved therein.

Figure 2 shows the fluorescence intensity of **1** versus $E_T(30)$ of the used solvents and Fig. 3 the corresponding Stokes' shift.

The protic solvents show a distinct maximum of the fluorescence intensity at $E_T(30) = 46.0$ kcal/mol and a corresponding minimum of the Stockes' shift. Looking for the fluorescence intensities, dimethylformamide (DMF) and dimethylsulfoxide (DMSO) are not near the curve of the other aprotic solvents but seem to fit more to the protic-solvent curve. But whereas DMF and DMSO show "protic" behaviour as to the intensities, their Stokes' shifts are close to the aprotic line. As

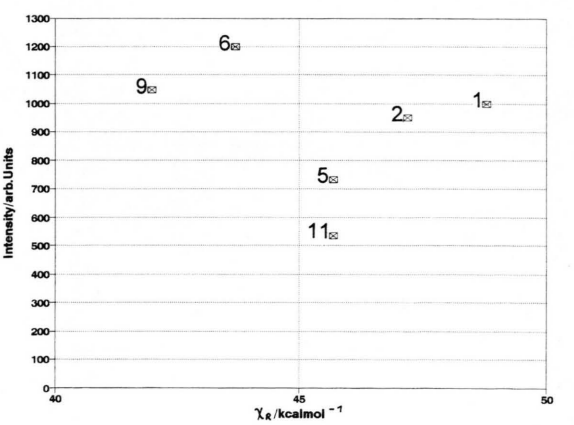


Fig. 4. Plot of fluorescence intensity of **1** versus the polarity parameter χ_R of different solvents. For numbers see Table 1.

these two solvents are very polarizable, the correlation of their fluorescence intensities with the solvent polarity χ_R of Brooker, which is more sensitive to polarisation than to orientation effects [10, 11], was tested.

However, Figure 4 shows that the correlation does not become better.

Besides these two solvents, the fluorescence properties of **1** in tert.-butanol are quite different from what was expected. This solvent seems to behave as an aprotic one.

Neglecting theses three exceptions, one finds linear correlations for the remaining measurements of the fluorescence intensity with $r = 0.9635$ for the aprotic solvents without DMF and DMSO, and $r = 0.9448$ for the protic ones without tert.-butanol (Fig. 2, dashed lines). In case of the Stokes' shifts, the aprotic solvents do not yield a linear plot. The calculation of the linear correlation of the protic solvents (excepting tert.-butanol) results in $r = 0.9188$ (Fig. 3, dashed line).

Although no clear linear correlation of the fluorescence intensity or Stokes' shift of **1** in neither the protic nor the aprotic solvents is found, it seems obvious from the different plots that the solvatochromic fluorescence of **1** is mainly influenced by the protic character of the solvent.

Presently, no interpretation can be given for the fact that the possible linear correlation lines for the intensity meet for $I \rightarrow 0$ in a common polarity value of $E_T(30) = 58.4$ kcal/mol. A substance specific value of **1** might be proposed for that.

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