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Solvent and pH Effects on the Fluorescence of 7-(Dimethylamino)-2-Fluorenesulfonate

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Abstract A novel water-soluble solvatochromic molecule, 7-(dimethylamino)-2-fluorenesulfonate (2,7-DAFS), was prepared by a three-step reaction from 2-nitrofluorene in good overall yield. The pH and solvent effects on the UV-VIS absorption and fluorescence spectra of 2,7-DAFS have been studied. Protonation of the dimethylamino group switches the absorption from intramolecular charge-transfer (ICT) to $\pi \rightarrow \pi^*$ transition. The ground state pKa value of 2,7-DAFS was determined as 4.51. The fluorescence spectrum of the excited basic form, *(DAFS), shows a structureless single band with a large Stokes shift, whereas that of the acidic form, *($^+$HDAFS), exhibits a structured band with a small Stokes shift. The emission intensities of the basic and acidic forms versus pH/Ho plots show stretched sigmoidal curves and indicate that (1) the rate of deprotonation of *($^+$HDAFS) is comparable to the fluorescence decay of the species, and (2) the efficient proton-induced quenching of *(DAFS) fluorescence occurs. The pKa* was estimated as −1.7 from the fluorescence titration curve. The fluorescence maximum of *(DAFS) is blue-shifted as the polarity of solvent decreases. However, no clear dependency of the emission intensity and spectral half width, and thus fluorescence quantum yield, on the solvent polarity was revealed. It appears that the fluorescence sensitivity of 2,7-DAFS is 15∼25 times greater than the sensitivity of a widely utilized fluorescent probe, 5-(dimethylamino)-1-naphthalenesulfonate. This higher sensitivity, together with the ease of derivatization, would provide the fluorene-based fluorescent molecules significant advantages for a variety of applications.

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Introduction

Photoinduced intramolecular charge transfer (ICT) is one of the most frequently encountered processes in photochemistry [1, 2]. Molecules that can have ICT excited states usually exhibit a large dependence of their fluorescence properties on the environment, and have drawn a great deal of interest in their use as fluorescent probes, sensors, and components of light activated switches [1–4]. Aromatic amines, particularly those with a N,N-dimethylamine group, are typical ICT molecules, in which an electron is transferred from the amine group to the aromatic ring upon light absorption, producing an ICT excited state. Solvent effects on the fluorescence of aminonaphthalenes [5–9], aminobiphenyls [10–12], aminofluorenes [9, 12–19], and a variety of amine-substituted conjugated π-systems [1, 2, 20–27] have been studied. Protonation of the amine group results in loss of its electron-donating characteristics. Thus, the pH dependence of the absorption and fluorescence behavior of aromatic amines has been studied to investigate the protonation/deprotonation reactions of the compounds in the ground and excited states [7, 8, 10, 13–17, 20, 26, 28].

In order for a molecule to function as a highly sensitive fluorescent sensor or probe, it must contain a large molar absorptivity and fluorescence quantum yield (Φ_F), in addition to a sensitivity of the fluorescent properties on environment. Moderate solubility in aqueous media and a potentially high binding affinity for the target are desirable for applications with biological molecules. Also, a large spectral overlap between the absorption spectrum of the probe and the fluorescence spectrum of the host is desirable for efficient resonance energy transfer [27, 29–31]. (Dimethylamino)naphthalenes (DANs) and their derivatives are known to exhibit many of these desired properties [1–3, 5–9, 29–31], and they have been extensively used in the fluorescence investigation of the structure, solvation dynamics and unfolding of human serum albumin [29–31]. However, they usually have relatively small molar absorptivities (ε_{max} ~4,000 M^{-1} cm^{-1}) [8] in the near UV region. Aminobipyridines show large ε_{max} values (~20,000 M^{-1} cm^{-1}) [10], but their Φ_F values are quite small in polar solvent, presumably due to the formation of a twisted ICT state by rotation of the central C–C bond provoking efficient radiationless deactivation [1, 22]. Attaching aniline and phenyl groups to tetrahydropyrenes [21] and fluorene [22] restricts the rotation and results in higher Φ_F values.

The fluorescent properties of various aminofluorene compounds such as a 2-aminofluorene [14, 15], 2-amino-7-bromofluorene [16, 17], 2,7-diaminofluorene (2,7-AF) [9, 13], and 9-substituted-2-(dialkylamino)fluorenes [18, 19] have been studied. A large solvatochromic shift that is comparable to that of DANs has been observed [9, 13, 14]. This, together with the large ε_{max} and high Φ_F values [13–15], and high binding affinity of 2-(dimethylamino)fluorenes to β-amyloid [32], lead us to expect that novel sensitive fluorescent probes for biological and biomimetic systems can be derived from aminofluorenes. Here, we report the synthesis of a novel water-soluble aminofluorene derivative, 7-(dimethylamino)-2-fluorenesulfonate (2,7-DAFS), and the effects of pH and solvent on its absorption and fluorescence behavior. The spectral properties of 2,7-DAFS are compared with those of a prototypical naphthalene-based fluorescent probe, 5-(dimethylamino)-1-naphthalenesulfonate (1,5-DANS), and other aminofluorenes.

Experimental

Synthesis of 7-(dimethylamino)-2-fluorenesulfonic acid (2,7-DAFS)

7-Amino-2-fluorenesulfonic acid **2** was prepared from 2-nitrofluorene **1** by sulfonation with concentrated sulfuric acid followed by reduction of the nitro group with iron powder using the reported procedure [33]. To a mixture of **2** (1.84 g, 7.04 mmol) and paraformaldehyde (2.52 g, 84.0 mmol) in glacial acetic acid (65 ml) was added sodium cyanoborohydride (2.24 g, 35.0 mmol). After stirring at room temperature under nitrogen atmosphere for 18 h, the reaction mixture was poured into ice water (200 ml). The precipitate was filtered, washed with water, and put into hot water (150 ml) and then basified with 30% ammonia solution to obtain a clear solution. The solution was acidified to ca. pH 3 with 1 N HCl and the resulting precipitate was filtered, washed with water and then vacuum-dried to give 7-(dimethylamino)-2-fluorenesulfonic acid **3** (1.51 g, 74% yield).

3: mp>300°C; ^{1}H NMR (D$_2$O/DMSO-d$_6$/Na$_2$CO$_3$, 500 MHz): δ7.72 (s, 1 H), 7.65−7.61 (overlapped two d, 2 H), 7.58 (d, 1 H, J=7.5 Hz), 6.98 (s, 2 H), 6.78 (d, 1 H, J= 8.0 Hz), 3.80 (s, 2 H), 2.84 (s, 6 H); ^{13}C NMR (D$_2$O/ DMSO-d$_6$/Na$_2$CO$_3$, 125 MHz): δ152.15, 146.86, 144.85, 143.22, 142.29, 130.55, 125.63, 123.10, 122.24, 119.03, 113.54, 110.75, 41.65, 37.61. Anal. Calcd for C$_{15}$H$_{15}$NO$_3$S·H$_2$O: C, 58.61; H, 5.57; N, 4.56; S, 10.43. Found: C, 58.83; H, 5.68; N, 4.61; S, 10.38.

The ammonium salt **4** (84 mg, 96% yield) of compound **3** was obtained by adding two drops of 30% NH$_3$ solution to an aqueous suspension of **3** (83 mg in 25 ml H$_2$O) followed by evaporation of the solvent.

4: Anal: Calcd for C$_{15}$H$_{18}$N$_2$O$_3$S: C, 58.80; H, 5.92; N, 9.14; S, 10.47. Found: C, 58.49; H, 5.91; N, 8.93; S, 10.37.

The ammonium salt **4** was used in preparing the 2,7-DAFS solutions for spectroscopic measurements.

Other materials and methods

Other chemicals were of reagent grade quality and obtained from commercial sources. All solvents used for the spectroscopic measurements were spectroscopic grade and used without further purification. Stock solutions of 2,7-DAFS, 1,5-DANS, and 2,7-AF were prepared in methanol. To make solutions for spectral measurements, a predetermined amount of a stock solution was taken, methanol was evaporated off, and an appropriate solvent was added to make a desired concentration. Solutions of different pH were prepared by mixing basic (0.01 M sodium acetate+ 0.09 M NaCl) dye solution and acidic (0.01 M HCl+ 0.09 M NaCl) dye solution of the same concentration. Solutions of various Hammett's acidity function (H_o) were prepared by mixing the dye solutions of the same concentration in 65% H$_2$SO$_4$ and in water [34]. Absorption spectra were taken with an Agilent 8453 spectrophotometer. Fluorescence spectra were obtained with a Hitachi F4500 spectrofluorimeter. The emission slit-width was set at 2.5 nm. All spectra were taken at room temperature, 22± 2°C. For fluorescence spectral measurements of a given concentration of a fluorophore in different pH's or solvents,

the wavelength where absorbance varies little by the change was chosen as the excitation wavelength so that the difference in emission intensity indicates the difference in fluorescence quantum yield: for the cases where a small difference in absorption is inevitable, the emission intensities are corrected with the absorbance difference.

Results and discussion

The ammonium salt **4** of 2,7-DAFS was prepared starting from 2-nitrofluorene **1** as shown in Scheme 1. The compound **2** was prepared by sulfonation followed by the reduction of the nitro group to amine according to a reported procedure [33]. Reductive methylation [32] of **2** using paraformaldehyde and sodium cyanoborohydride gave **3** in 74% yield. Basification of **3** with concentrated NH$_3$ provided the ammonium salt **4**, which was used in preparing 2,7-DAFS solutions due to its better solubility in solvent media than the acid **3**.

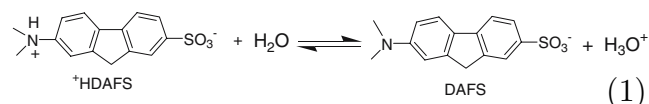### pH dependence of absorption spectra and pKa of 2,7-DAFS

The absorption spectra of 2,7-DAFS in aqueous solutions of pH 1.8~8 at constant ionic strength (0.1 M) were recorded (Fig. 1). The spectral characteristics are summarized in Table 1. At high pH, the spectrum above 260 nm shows a nearly structureless band with absorption maximum (λ_{max}) at 319 nm. The molar absorptivity (ε) at λ_{max} is 24,000 M^{-1} cm^{-1}. The λ_{max} is slightly red-shifted compared to those of 2-aminofluorene (315 nm) [14] and the monocationic form of 2,7-AF (317.5 nm) [13]. The ε_{max} value of 2,7-DAFS is about 3 times greater than that of 2,7-AF and 2-aminofluorene [13, 14].

Lowering the pH of the solution causes the absorption band at λ_{max}=319 nm to diminish, while the one at λ_{max}= 269 nm grows with isosbestic points at 244 nm (ε= 7,600 M^{-1} cm^{-1}), 286 nm (ε=120,00 M^{-1} cm^{-1}), 290 nm (ε=16,000 M^{-1} cm^{-1}), and 300 nm (ε=19,000 M^{-1} cm^{-1}). The spectrum in acidic pH is highly structured with maxima at 269, 289, and 300 nm. The spectrum of the acidic form is quite similar to those of protonated aminofluorenes [13, 16,
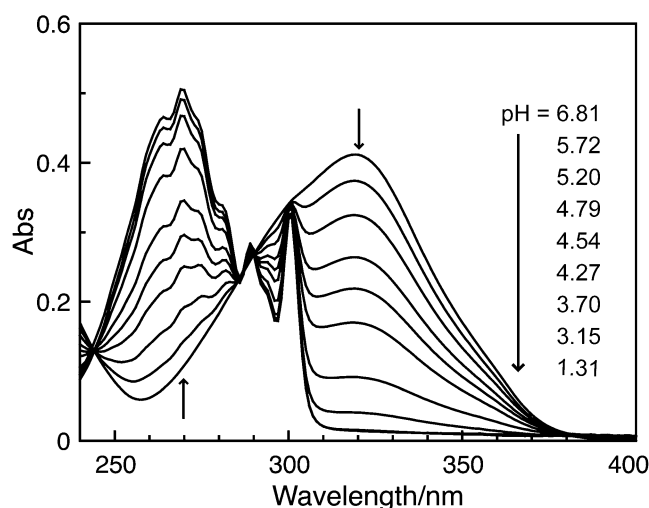
Fig. 1 Absorption spectra of 2,7-DAFS as a function of pH in water. [DAFS]=1.7×10^{-5} M. *Arrows* indicates the direction of the lower pH

17] and unsubstituted fluorene (in organic solvent) [18]. The pH-dependent absorption spectrum is attributed to the protonation/deprotonation equilibrium of the dimethylamino group of 2,7-DAFS (eq 1).

$$\text{}^+\text{HDAFS} + H_2O \rightleftharpoons \text{DAFS} + H_3O^+ \tag{1}$$

Analysis of the pH-dependent absorption spectra gave the pKa value of 2,7-DAFS as 4.51. This pKa value is similar to those of 2-aminofluorene (4.5) [15] and 2-amino-7-bromofluorene (4.35) [16, 17], but about one pH unit higher than the first pKa of 2,7-AF (3.6) [13].

We also performed a parallel study with 1,5-DANS and observed a similar pH dependence of the absorption spectra as 2,7-DAFS (spectra not shown). The λ_{max} of the basic form of 1,5-DANS is 314 nm with an ε_{max} value of 4,600 M^{-1} cm^{-1} which is about one-fifth of that of 2,7-DAFS: isosbestic points are observed at 262 nm (ε= 3,500 M^{-1} cm^{-1}) and 298 nm (ε=3,800 M^{-1} cm^{-1}). The pKa of 1,5-DANS was found to be 3.73.

The transition of the basic form of 2,7-DAFS, responsible for the absorption band at λ>260 nm shown in Fig. 1,

Scheme 1
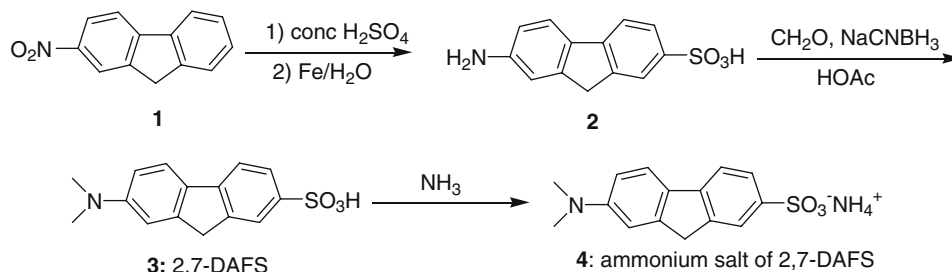
Table 1 Parameters of absorption and fluorescence spectra of 2,7-DAFS in various media

Medium			Absorption		Fluorescence		
	Dielectric constant	$E_T(30)$ (kcal/mol)	$\lambda_{max,abs}$ (nm)	log ε_{max}	$\lambda_{max,em}$ (nm)	$I_{F,max}$ [a]	$\Delta\nu_{1/2}$ (cm^{-1})
2-C$_3$H$_7$OH	18.30	48.6	314	4.41	376	1.13	2,830
p-Dioxane	2.21	36.0	319[b]	4.44[b]	375	1.15[c]	2,680[c]
EtOH	24.55	51.9	318	4.40	385	1.12	2,970
CH$_3$OH	32.7	55.5	321	4.39	397	1.11	3,080
CH$_3$CN	36.7	46.0	314	4.42	383	1.06	2,710
H$_2$O (pH 7)	78.4	63.1	319	4.38	432	1.00	2,850
H$_2$O (pH 1.3)			300	4.24	432	0.56	
			289	4.22	317	0.31	
			269	4.47	303	0.32	
H$_2$O (H_o −5)			300	4.27	317	1.04	
			289	4.21	304	1.06	
			270	4.42			

[a] Normalized to light absorbed by dividing the measured intensities by (1–10^{-abs}).
[b] In 96% p-dioxane-4% water.
[c] From data in 98% p-dioxane-2% water ($\lambda_{max,em}$=378 nm).

is attributed to the intramolecular charge transfer (ICT) transition that arises from mixing of the $\pi \rightarrow \pi^*$ transition of the aromatic ring with the lone pair transition from the dimethylamino group. The protonated dimethylamino group in the acidic form does not have the electron lone pair for the ICT transition, and thus the lowest energy transition becomes a $\pi \rightarrow \pi^*$ transition of the aromatic ring. This is supported by the similarity of the absorption spectra of the protonated aminofluorenes with that of fluorene itself [18]. No appreciable spectral change is observed by further acidification of the solution of 2,7-DAFS from pH 1.3 to H_o −5. This implies that the protonation of the sulfonate group does not affect the electronic energies of the aromatic ring significantly.

### pH dependence of fluorescence spectra and pKa* of 2,7-DAFS

Figure 2 shows the fluorescence spectra of 2,7-DAFS taken in aqueous solutions of various pH. When pH ≫pKa, a structureless band with an emission maximum at 432 nm is obtained. The Stokes shift ($\Delta\nu_{ss}$) is 8,200 cm^{-1}. The $\Delta\nu_{ss}$ value is smaller than that of 1,5-DANS (λ_{fl}=509 nm), 12,200 cm^{-1}, but similar to the value of 8,900 cm^{-1} [35] of a widely used fluorescent probe, 6-propionyl-2-(dimethyla-mino)naphthalene (Prodan) [29–31]. The large Stokes shift is typical of fluorescence from an ICT excited state, since the ICT excitation accompanies a large increase in the dipole moment and the solvent reorientation around the charge separated ICT excited state lowers the excited state energy. Lowering the pH of the solution of 2,7-DAFS causes the emission band around 432 nm to decrease and a new structured emission band around 310 nm to grow. The

new emission band is assigned to the fluorescence from the excited acidic form of the fluorophore, *($^+$HDAFS). The variations of the fluorescence intensities at 432 nm and 312 nm and also the absorbance at 320 nm with H_o/pH are compared in Fig. 3.

In addition to the ground state protonation/deprotonation equilibrium which determines the ratio of the initially produced excited state acid *($^+$HDAFS) and base *(DAFS), various dynamic processes in the excited states contribute to the steady-state concentrations, i.e. fluorescence intensities, of the excited species (Scheme 2). These are protonation/deprotonation reactions, non-radiative and radiative decays, and proton-induced quenching reactions of
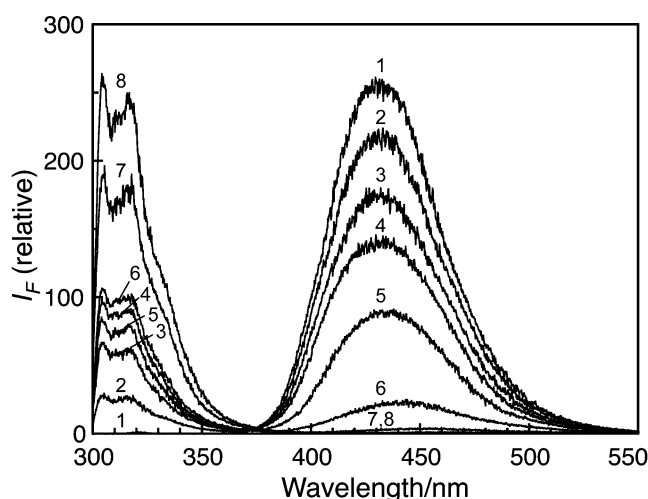
Fig. 2 Fluorescence spectra of 3.0×10^{-6} M 2,7-DAFS as a function of pH/H_o in aqueous media. The excitation wavelength was 290 nm, an isosbestic point in the absorption spectra. The pH/H_o values are **1**. 7.20; **2**. 5.44; **3**. 4.45; **4**. 2.38; **5**. 0.13; **6**. −0.84; **7**. −2.28; **8**. −3.32
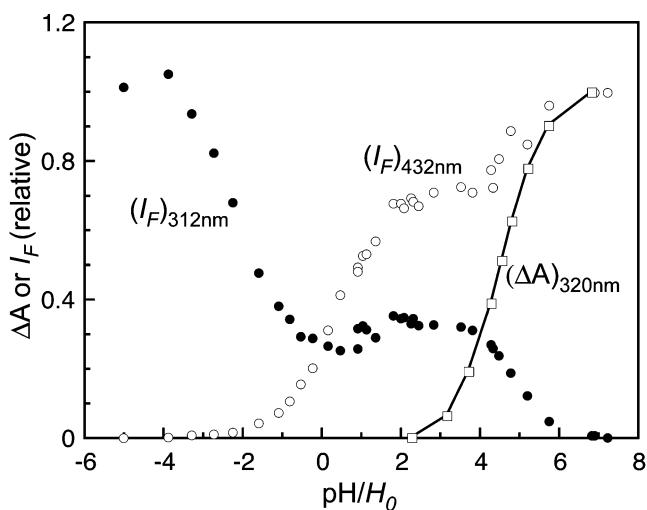
Fig. 3 Variation of relative absorption change at 320 nm (*open square*), and fluorescence intensities at 432 (*open circle*) and 312 nm (*filled circle*) of 2,7-DAFS in aqueous media with pH/H_o. Emission intensities are normalized to the intensity at 432 nm of pH=7.2 solution. λ_{ex}=290 nm

*(DAFS) and *($^+$HDAFS). The quantitative analysis of the fluorescence titration curves shown in Fig. 3 considering all of these processes is very complicated and beyond the scope of this work. Instead, qualitative explanations for the curves are attempted. The levelling off of the 432 nm emission band at pH>6.8 indicates that *(DAFS) is the exclusive excited species above pH 6.8. Similarly, it appears that *($^+$HDAFS) is the predominant excited species present at H_o<−4. Fortuitously, the intensity at 432 nm from *(DAFS) at the high pH end is virtually the same as that at 312 nm from *($^+$HDAFS) at the low pH/H_o end. By lowering pH from 7 to 4, the fluorescence from *(DAFS) decreases while that from *($^+$HDAFS) increases. The sum of intensities of the two species remains nearly constant, suggesting that the decrease in the steady-state concentra-

tion of *(DAFS) by lowering the pH is compensated by the increase in the concentration of *($^+$HDAFS). The behavior in this pH region reflects the ground state equilibrium between $^+$HDAFS and DAFS. However, the decrease in the fluorescence intensity of *(DAFS) continues to occur at the lower pH region compared to the absorbance change, and a substantial *(DAFS) fluorescence is observed below pH 2 where no significant amount of DAFS is present. This indicates that *($^+$HDAFS) is a stronger acid than the ground state counterpart, $^+$HDAFS. This is a common trend among aromatic amines [8, 10, 13, 14, 17], and consistent with the ICT character of the emitting state of *(DAFS).

In the pH region of 4∼1.8, the intensities from both species, *(DAFS) and *($^+$HDAFS), depend little on pH. As the pH is lower than the pKa, most of 2,7-DAFS is present as the protonated form, $^+$HDAFS. Therefore, the excited state base *(DAFS) would be formed mostly by the deprotonation reaction of the initially produced excited state acid *($^+$HDAFS) with water molecules. The independence of the emission intensities on pH in this pH region indicates that the rates of dynamic processes involving protons, proton-induced quenching reactions and the protonation of *(DAFS) to produce *($^+$HDAFS), are slower than the rates of the deprotonation reaction of *($^+$HDAFS) and fluorescence decays. The presence of about 70% of *(DAFS) fluorescence, compared to that at high pH, suggests that the rate of deprotonation of *($^+$HDAFS) is about twice of the rate of fluorescence decay of the species, i.e. $k_{+1}[H_2O] \approx 2(k'_{nr}+k'_f)$. A similar behavior has been observed with 3-aminobiphenyl [10].

Further acidification of the solution to pH 0 results in a large decrease in *(DAFS) fluorescence. This can be attributed to proton-induced quenching, that has been observed with aromatic amine and hydroxy compounds [8, 10, 13, 15, 28, 36]. The decrease of the *($^+$HDAFS) fluorescence by lowering the pH is much less significant

Scheme 2
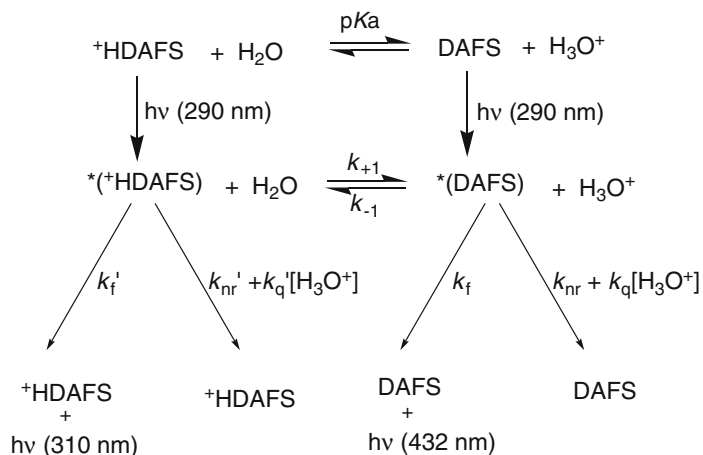
than that of *(DAFS). This implies that the proton-induced quenching of *($^{+}$HDAFS) fluorescence is much less efficient than that of *(DAFS), i.e. $k'_{q} << k_{q}$.

Below H_{o}/pH≈0, the fluorescence intensity from *($^{+}$HDAFS) increases as the acidity of the medium increases and levels off near H_{o}=−4. In this region, the fluorescence emission maximum of the remaining *(DAFS) is also observed at about 450 nm, red-shifted from the normal 432 nm. The shift seems to reflect the protonation of the sulfonate group. The higher *($^{+}$HDAFS) fluorescence intensity in more acidic medium reflects the higher steady-state concentration of the fluorophore. Both the increased protonation rate of *(DAFS) due to the acidity increase and the slower deprotonation rate of *($^{+}$HDAFS) as a result of the lower concentration of the proton acceptor and the higher viscosity of the medium may contribute to the high concentration of *($^{+}$HDAFS).

For an excited state acid, whose conjugate base undergoes proton-induced fluorescence quenching, the determination of the correct pKa* requires complicated dynamic analysis [28]. Instead of this, many researchers have taken the midpoint of the fluorescence titration curve of the excited state acid as the pKa* of the acid [8, 10, 13, 15]. Applying this to the present case, the pKa* of 2,7-DAFS is obtained as −1.7. This is close to the corresponding value of 2-aminofluorene, −1.6 [14], but about one H_{o}/pH unit higher than the first pKa* of 2,7-AF [13].
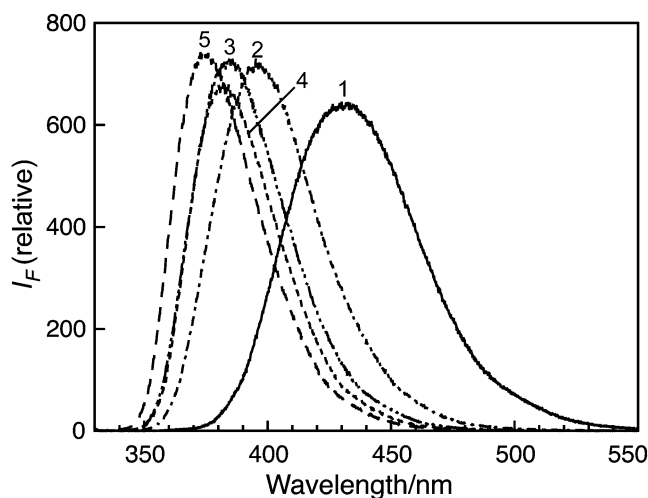
Solvent effects on the absorption and fluorescence spectra of 2,7-DAFS

In Figs. 4 and 5, the absorption and fluorescence spectra of the basic form of 2,7-DAFS in different solvent media are
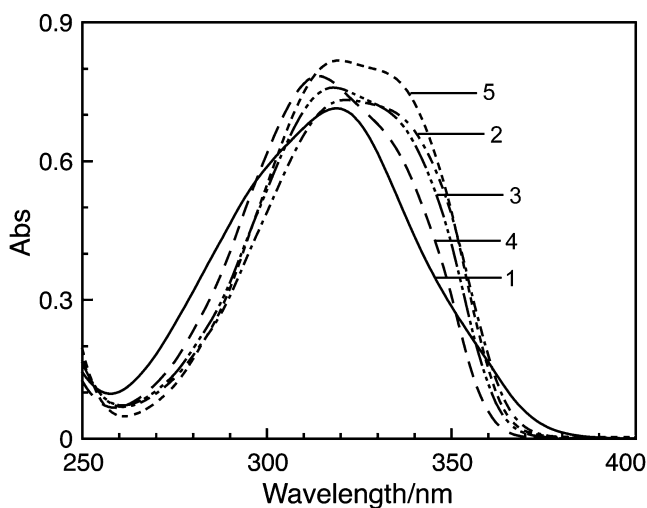
shown: poor solubility of 2,7-DAFS in highly non-polar solvents such as THF and CHCl$_3$ prevented us to take the spectra in these solvents media; the addition of a small amount of water was required to increase the solubility in p-dioxane for the spectral measurements. The absorption maximum ($\lambda_{max,abs}$) and log ε, and fluorescence maximum ($\lambda_{max,em}$) and relative intensities are summarized in Table 1. Though the absorption spectra in organic media are slightly red-shifted from that in aqueous media, no systematic dependence of the shift on solvent parameters could be deduced. Interestingly, it was found that the shape of the absorption spectra of 2,7-DAFS in the near UV region depends on the solvent medium (Fig. 4). A close examination of the absorption band in the 260–380 nm region reveals that it is a sum of bands from at least two transitions. The spectra in various H$_2$O/CH$_3$OH or H$_2$O/p-dioxane mixtures showed isosbestic-like points: similar behavior was also observed with 1,5-DANS. These imply that the transition energies do not depend significantly on the macroscopic dielectric constant or E_{T}(30) scale of the medium, while the relative contributions of the transitions vary with the nature of the solvent. A plausible explanation for this is the presence of both hydrogen-bonded and non-hydrogen-bonded chromophore molecules in solution. However, we cannot rule out, at this point, the possibility of a change in the Franck–Condon excited state (e.g. the vibration level of the excited state) as a result of the change in solvent.

In all solvent media studied, the fluorescence spectrum of the basic form of 2,7-DAFS shows a single band and does not indicate any hints of the dual fluorescence that has

Fig. 4 Absorption spectra of 3.0×10^{-5} M 2,7-DAFS in various solvent media. 1. H$_2$O (pH 7); 2. CH$_3$OH; 3. C$_2$H$_5$OH; 4. CH$_3$CN; 5. p-dioxane (with 4 % H$_2$O). The spectrum in 2-C$_3$H$_7$OH was little different from that in CH$_3$CN (4) and is omitted for clarity

Fig. 5 Fluorescence spectra of 3.0×10^{-6} M 2,7-DAFS in various solvent media. 1. H$_2$O (pH 7); 2. CH$_3$OH; 3. C$_2$H$_5$OH; 4. CH$_3$CN; 5. 2-C$_3$H$_7$OH. The fluorescence intensities are normalized to light absorption by dividing the measured intensities by (1–10^{-abs}). The spectrum in p-dioxane (with 2 % H$_2$O), which appears at ∼2 nm longer wavelength than that in 2-C$_3$H$_7$OH (5), is not shown for clarity. λ_{ex}=325 nm

been often observed with dimethylamine-substituted aromatic compounds such as 4-(dimethylamino)benzonitrile [1, 37, 38] and 4-(dimethylamino)-1-naphthalenesulfonate [7]. The excitation spectra (not shown) resemble the absorption spectra in the corresponding solvent media. This indicates a rapid relaxation to a lowest ICT state before the fluorescence emission, though the excitation may occur to different Franck–Condon excited states or to species of different hydrogen-bonding character.

The fluorescence emission maximum of 2,7-DAFS is blue-shifted as the polarity of the solvent decreases (Table 1). The shift is correlated better with $E_T(30)$ than the dielectric constant. This is in agreement with the general trend for fluorescence emission from an excited ICT state [1, 3, 5] and with the observation made with aminofluorenes [9, 14, 15]. The Stokes shift ($\Delta\nu_{ss}$) of 2,7-DAFS in water (8,200 cm^{-1}) is considerably larger than those of the monocationic form of 2,7-AF (7,100 cm^{-1}) [13] and 2-aminofluorene (5,200 cm^{-1}) [14]. This implies that methyl substitution on the amine group results in a larger increase in dipole moment upon excitation, i.e. facilitation of internal charge transfer from the amine group to the aromatic ring.

Unlike the fluorescence emission maximum, the fluorescence intensity and full-width-half-maximum ($\Delta\nu_{1/2}$) do not show any clear dependence on the polarity of the solvent. This is similar to the behavior of 1,5-DANS [5], but differs from those of (arylamino)naphthalenesulfonates [4] and dimethylaminostyryl derivatives [20, 27] which exhibit a large enhancement in fluorescence intensity with decreasing solvent polarity.

We also recorded the fluorescence spectrum of 2,7-DAFS in water–methanol and water-p-dioxane binary mixtures. The fluorescence intensity and emission maximum ($\lambda_{max,em}$) are shown in Fig. 6 as a function of methanol or p-dioxane content in the mixed solvent: the data for 1,5-DANS in water–methanol are also shown. In agreement with the results in pure solvents, the emission maximum of 2,7-DAFS is blue-shifted as the content of organic component in the solvent increases showing a virtually linear relationship between $\lambda_{max,em}$ and v/v% of solvent composition. The fluorescence intensities of 2,7-DAFS and 1,5-DANS are highest in 30% water-70% methanol. However, the dependence of emission intensity on solvent composition is much smaller for 2,7-DAFS than 1,5-DANS. Similar conclusions can be drawn for the behavior in water–dioxane mixtures by comparison of the 2,7-DAFS data in Fig. 6 and 1,5-DANS data in ref 5. The bell shape of the emission intensity (or fluorescence quantum yield) versus solvent composition plots was attributed to the presence of two closely spaced excited states of different polarity [5, 6] and solvent dependent coupling of the states [37, 38]. If this is the case, the weaker solvent dependency of the emission intensity of 2,7-DAFS compared to those of 1-(dimethylamino)naphthalenes might reflect the larger energy gap between the two states of the compound. This can explain the absence of the dual fluorescence in the compound.

Fig. 6 Effect of organic solvent content (v/v %) on the fluorescence emission maxima (*Left*) and intensities (*Right*) of 2,7-DAFS in the water-p-dioxane (*open circle*) and in the water–methanol (*filled circle*) mixtures, and of 1,5-DANS in the water–methanol mixtures (*open triangle*)
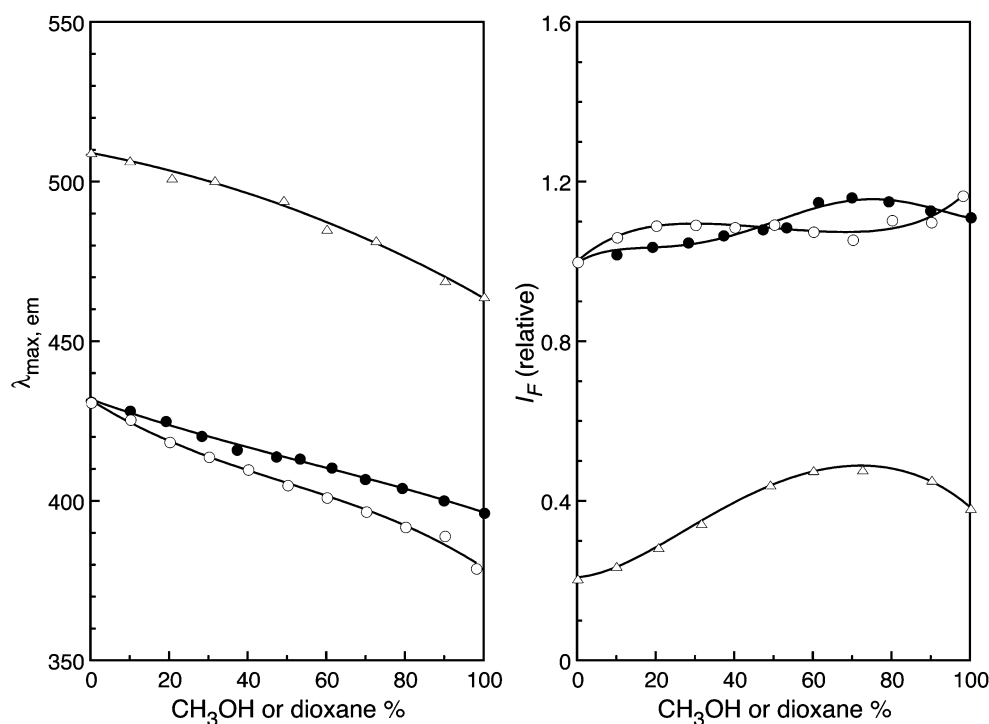
Finally we would like to comment on the sensitivity of 2,7-DAFS and its derivatives, in comparison with the widely used (dimethylamino)naphthalene compounds such as 1,5-DANS. The molar absorptivity of 2,7-DAFS is 5 times greater than 1,5-DANS, though the wavelength region of the absorption bands of the two compounds is similar. When solutions with the same absorbance were irradiated, 2,7-DAFS gave 3∼5 times stronger fluorescence than 1,5-DANS (Fig. 6). This implies that 2,7-DAFS is about 15∼25 times more sensitive a fluorescent molecule than 1,5-DANS. The stronger fluorescence intensity of 2,7-DAFS indicates a high Φ_F value. To investigate the Φ_F value of 2,7-DAFS, we compared the fluorescence behavior of 2,7-DAFS and 2,7-AF in methanol. The fluorescence maximum (388 nm) and $\Delta\nu_{1/2}$ (2,730 cm^{-1}) of 2,7-AF are very similar to the corresponding values of 2,7-DAFS (Table 1). However, the fluorescence intensity of 2,7-AF was about one-third of that of 2,7-DAFS, when solutions of the same absorbance were excited. This implies that the Φ_F value of 2,7-DAFS is about three times larger than that of 2,7-AF. As the reported Φ_F value of 2,7-AF in methanol is 0.25 [13], the Φ_F value of 2,7-DAFS in methanol is estimated as approximately 0.7. This is close to the value of 2-aminofluorene in organic solvents [14]. Considering the little dependence of the fluorescence intensity and $\Delta\nu_{1/2}$ on solvent, similar Φ_F values are expected in other solvents. This agrees with the trend of 2,7-AF [13] and 2-aminofluorene [14] in organic solvents: these compounds show considerably lower Φ_F values in water.

In addition to desirable spectral characteristics such as high absorptivity, high Φ_F values, and a large solvatochromic shift, 2,7-DAFS can be easily modified for various applications. The potential modification sites are the amine group (substitution of the dimethyl groups to other functional groups), the hydrogen atoms at the 9-position (to fluoren-9-yl derivatives), and the sulfonate group (e.g. to sulfonyl chloride and then derivatization to appropriate compounds). These modifications to 2,7-DAFS may provide various fluorene-based highly sensitive fluorescent sensors, switches, probes, and labeling agents.

Conclusions

A novel water-soluble and highly fluorescent solvatochromic molecule 2,7-DAFS was synthesized. The pH and solvent effects on the absorption and fluorescence spectra of 2,7-DAFS have shown clearly that the excitation and fluorescence emission of the basic form are to and from an ICT excited state, respectively, whereas those of the acid form are associated with a $\pi \rightarrow \pi^*$ transition of the aromatic ring. The rate of deprotonation of the excited acid *($^+$HDAFS) is comparable to the rate of fluorescence decay

of the species. The fluorescence of the excited base * (DAFS) is efficiently quenched by the proton-induced quenching reaction. The fluorescence emission maximum of *(DAFS) is blue-shifted as the polarity of its environment decreases. However, the emission intensity and Φ_F depend little on the solvent polarity. The solvatochromic shift of 2,7-DAFS is similar to that of (dimethylamino) naphthalene compounds, but the fluorescence sensitivity of 2,7-DAFS is 15 ∼25 times greater than DANS due to its high molar absorptivity and Φ_F. 2,7-DAFS and other related fluorene-based ICT molecules are promising as fluorescent sensors and probes for biological targets, and studies in this direction are underway.

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