Ground and Excited State Dipole Moments of BADAN and ACRYLODAN Determined from Solvatochromic Shifts of Absorption and Fluorescence Spectra

A. Kawski, B. Kukliński, and P. Bojarski

Institute of Experimental Physics, University of Gdańsk, ul. Wita Stwosza 57, PL-80-952 Gdańsk, Poland

Reprint requests to Prof. A. K., ul. Gen. W. Sikorskiego 11, PL-84-200 Wejherowo, Poland

Z. Naturforsch. **56a,** 407–411 (2001); received March 16, 2001

The electric dipole moments in the ground μ_g and excited states μ_e of the fluorescent probes BADAN (6-bromoacetyl-2-dimethylamino-naphthalene) and ACRYLODAN (6-acrylolyl-2-dimethylamino-naphthalene) are determined from the solvatochromic shifts of their absorption and fluorescence spectra for two Onsager interaction radii (a = 4.2 and 4.6 Å). The obtained values of μ_g and μ_e for BADAN are comparable to those of PRODAN, while for ACRYLODAN they are distinctly greater.

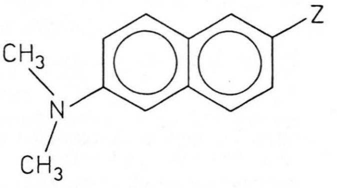
Key words: Solvatochromic Shifts; Dipole Moments in the Ground and Excited States; BADAN and ACRYLODAN-Fluorescent Probes.

1. Introduction

Recently, the interest in the properties of PRODAN-like fluorescent probes has grown because of the enormous difference in the dipole moments $\mu_e - \mu_g$ of PRODAN between the excited (e) and ground (g) state as a result of optical excitation to the lowest excited singlet state.

For PRODAN, the very high value of $\mu_e = 20$ D compared to that of $\mu_g = 2$ D, determined by Macgregor and Weber [1], is incorrect, as shown by Balter et al. [2]. Further work [3–5] demonstrated that the ratio μ_e/μ_g is approximately 3, similarly as for LAURDAN.

Several variants of the original fluorescent PRODAN probe, described by Weber and Farris [6], have later been prepared, including the thiol-reactive derivatives BADAN (6-bromoacetyl-2-dimenthylamino-naphthalene) and ACRYLODAN (6-acrylolyl-2-dimethylamino-naphthalene), which can be used to transfer the environment – sensitive properties of this fluorophore on bioconjugates. BADAN, PRODAN, and ACRYLODAN show fluorescence that is extremely sensitive to the solvent polarity [6–8].



MOLECULE :	PRODAN	BADAN	ACRYLODAN
Substituent Z :	$CH_3{-}CH_2{-}\underset{\underset{O}{\|}}{C}{-}$	$Br{-}CH_2{-}\underset{\underset{O}{\|}}{C}{-}$	$CH_2{=}CH{-}\underset{\underset{O}{\|}}{C}{-}$

Fig. 1. Structural formula of PRODAN, BADAN, and ACRYLODAN.

The present work reports on the effect of different substituents Z (given in Fig. 1) in -2-(dimethylamino)naphthalene on the dipole moment in the ground and excited state.

2. Basic Equations for the Analysis of Electric Dipole Moments in the Ground and Excited State

The following equations are based on quantum – mechanical perturbation theory [9, 10] of absorption ($\tilde{\nu}_A$) and fluorescence ($\tilde{\nu}_F$) band shifts (in wavenumbers) in solvents of permittivity ε and refractive index n, relative to the band positions of the molecule in the gaseous state. For a spherical molecule, if $\alpha/a^3 = 1/2$, where α is the polarizability and a the Onsager interaction radius of the solute, one has [11]

$$\Delta\tilde{\nu}_{A,F} = -\, m_{A,F}\left(\frac{2n^2+1}{n^2+2}\right)\cdot\left(\frac{\varepsilon-1}{\varepsilon+2}-\frac{n^2-1}{n^2+2}\right) - m_2\,\frac{3}{2}\,\frac{n^4-1}{(n^2+2)^2}\,. \qquad (1)$$

0932-0784 / 01 / 0500-0407 $ 06.00 © Verlag der Zeitschrift für Naturforschung, Tübingen · www.znaturforsch.com

Hence, from (1) the following equations are obtained:

$$\tilde{\nu}_A - \tilde{\nu}_F = m_1 f(\varepsilon, n) + \text{const}, \tag{2}$$

$$\tilde{\nu}_A + \tilde{\nu}_F = -m_2 [f(\varepsilon, n) + 2g(n)] + \text{const}, \tag{3}$$

where

$$m_1 = \frac{2(\mu_e - \mu_g)^2}{hca^3}, \tag{4}$$

$$m_2 = \frac{2(\mu_e^2 - \mu_g^2)}{hca^3}, \tag{5}$$

$$m_A = \frac{2\mu_g(\mu_e - \mu_g)}{hca^3}, \tag{6}$$

$$m_F = \frac{2\mu_e(\mu_e - \mu_g)}{hca^3}. \tag{7}$$

The parameters of the solvent polarity are given by

$$f(\varepsilon, n) = \frac{2n^2+1}{n^2+2} \cdot \left(\frac{\varepsilon - 1}{\varepsilon + 2} - \frac{n^2-1}{n^2+2}\right), \tag{8}$$

$$g(n) = \frac{3}{2} \frac{n^4 - 1}{(n^2+2)^2}. \tag{9}$$

Assuming that the symmetry of the solute molecule remains unchanged on electronic transition and the ground and excited state dipole moments are parallel, together with (4) and (5) one obtains [9, 12]

$$\mu_g = \frac{m_2 - m_1}{2}\left(\frac{hca^3}{2m_1}\right)^{1/2}, \tag{10}$$

$$\mu_e = \frac{m_1 + m_2}{2}\left(\frac{hca^3}{2m_1}\right)^{1/2}. \tag{11}$$

Division of (11) by (10) gives

$$\frac{\mu_e}{\mu_g} = \frac{m_1 + m_2}{m_2 - m_1} = \frac{m_F}{m_A}, (m_2 > m_1), \tag{12}$$

where

$$m_A = \frac{m_2 - m_1}{2}, \tag{13}$$

$$m_F = \frac{m_1 + m_2}{2}. \tag{14}$$

As shown by Baba et al. [13], the dipole moments in the excited state in two solvents 1 and 2 which have the same refractive index n but different permittivities ε, can be determined. It follows from (1) that for the relative frequency shift between two different solvents 1 and 2 one has

$$\Delta\tilde{\nu}_{A,F} = \Delta\tilde{\nu}_{A,F}(2) - \Delta\tilde{\nu}_{A,F}(1) = -m_{A,F}\left(\frac{2n^2+1}{n^2+2}\right) \cdot \left(\frac{\varepsilon_2 - 1}{\varepsilon_2 + 2} - \frac{\varepsilon_1 - 1}{\varepsilon_1 + 2}\right). \tag{15}$$

In this case we obtain

$$\frac{\Delta\tilde{\nu}_F}{\Delta\tilde{\nu}_A} = \frac{m_F}{m_A} = \frac{\mu_e}{\mu_g}, \tag{16}$$

an equation which is similar to (12) for parallel μ_g and μ_e, although their directions may be not the same.

3. Experimental

3.1. Materials and Methods

BADAN and ACRYLODAN were purchased from Molecular Probes. The solvents were spectroscopically pure. Absorption and fluorescence spectra were measured with the apparatus described in [14, 15], upon excitation $\tilde{\nu}_{exc} = 25641$ cm^{-1} and 23810 cm^{-1} for BADAN and ACRYLODAN, respectively.

3.2. Absorption and Fluorescence Spectra of BADAN and ACRYLODAN in Different Solvents

Figures 2 and 3 show selected spectra of BADAN and ACRYLODAN in different solvents, respectively. Among the solvents used only two have approximately the same refraction index: *n*-hexane $n = 1.37499$ ($g(n) = 0.2531$), ethyl acetate $n = 1.37257$ ($g(n) = 0.2517$), of legends of Figs. 4 and 5. The absorption peaks for BADAN are located in these two solvents at $\tilde{\nu}_A = 27151$ cm^{-1} and $\tilde{\nu}_A = 26427$ cm^{-1}, respectively, and the fluorescence peaks at $\tilde{\nu}_F = 24986$ cm^{-1} and $\tilde{\nu}_F = 22601$ cm^{-1}, respectively. Similarly, for ACRYLODAN we have: $\tilde{\nu}_A = 27502$ cm^{-1} and $\tilde{\nu}_A = 26343$ cm^{-1}, respectively, and $\tilde{\nu}_F = 24162$ cm^{-1} and $\tilde{\nu}_F = 19757$ cm^{-1}, respectively.

Figures 4–7 show the spectral shifts $\tilde{\nu}_A - \tilde{\nu}_F$ and $\tilde{\nu}_A + \tilde{\nu}_F$ of BADAN and ACRYLODAN in different solvents versus the solvent polarity functions $f(\varepsilon, n)$ and $f(\varepsilon, n) + 2g(n)$, respectively. A linear regression was carried out, and a fit to the data was obtained. The obtained slopes m_1 and m_2 of the fitted lines shown in Figs. 4–7 are given in Table 1.

If the dipole moments μ_g and μ_e are parallel it is possible, based on (12), to determine the ratio μ_e/μ_g for both molecules studied. For BADAN $\mu_e/\mu_g = 2.9$ was

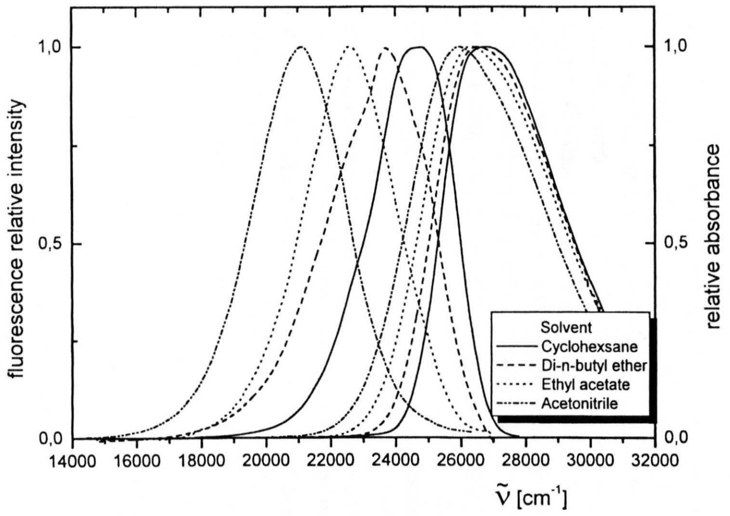


Fig. 2. Absorption and fluorescence spectra of BADAN in different solvents.

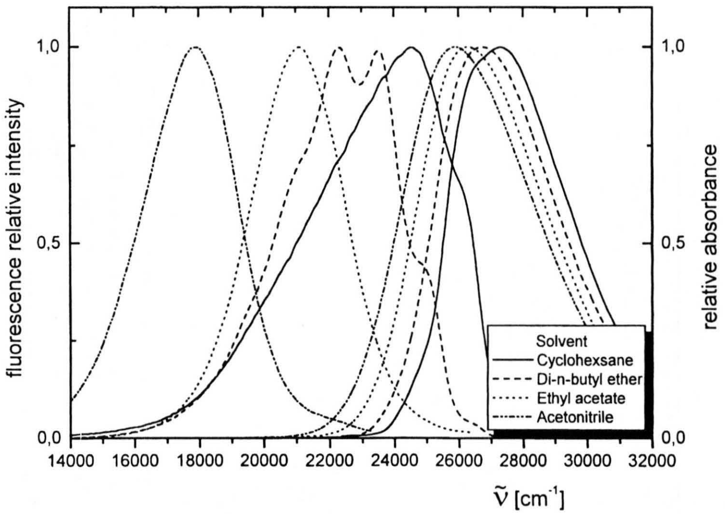


Fig. 3. Absorption and fluorescence spectra of ACRYLODAN in different solvents.

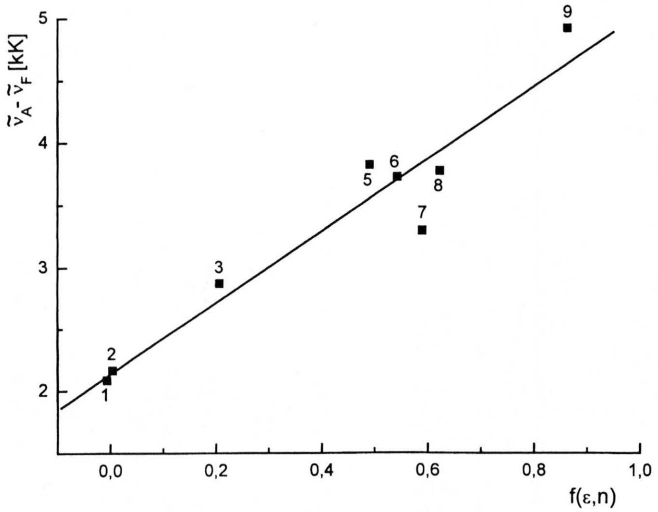


Fig. 4. Plot of $\tilde{\nu}_A - \tilde{\nu}_F$ versus $f(\varepsilon, n)$ for BADAN in different solvents: 1: cyclohexane, 2: *n*-hexane, 3: di-*n*-butyl ether, 5: ethyl acetate, 6: tetrahydrofuran, 7: 1,2-dichloromethane, 8: 1,2-dichloroethane, 9: acetonitrile.

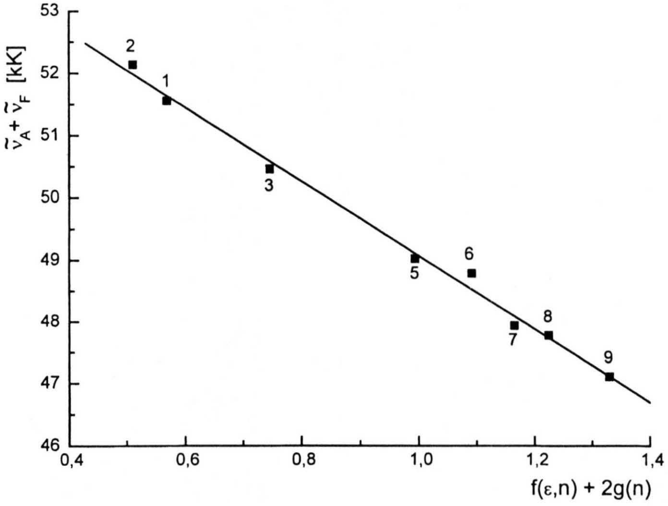


Fig. 5. Plot of $\tilde{\nu}_A + \tilde{\nu}_F$ versus $f(\varepsilon, n) + 2g(n)$ for BADAN in the same solvents as in Figure 4.

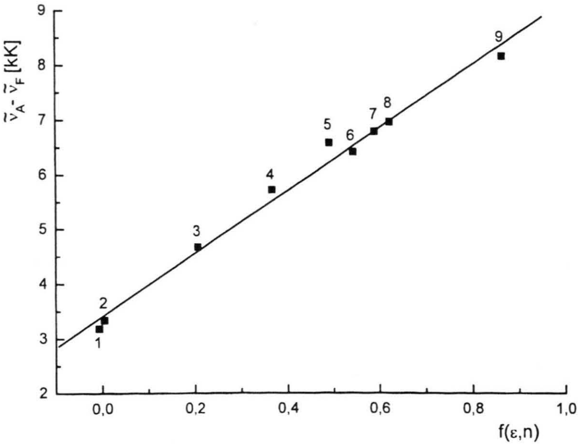


Fig. 6. Plot of $\tilde{\nu}_A - \tilde{\nu}_F$ versus $f(\varepsilon, n)$ for ACRYLODAN in different solvents: 1: cyclohexane, 2: *n*-hexane, 3: di-*n*-butyl ether, 4: diethylether, 5: ethyl acetate, 6: tetrahydrofuran, 7: 1,2-dichloromethane, 8: 1,2-dichloroethane, 9: acetonitrile.

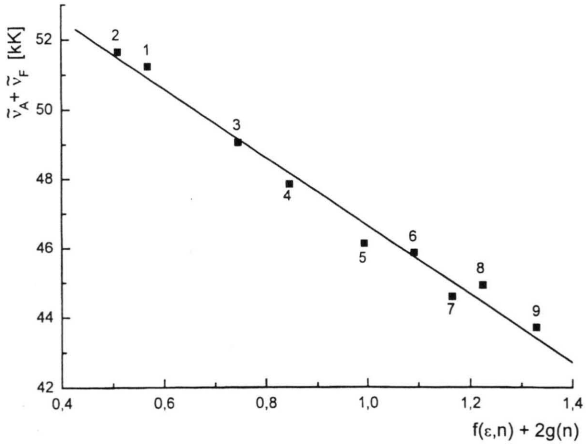


Fig. 7. Plot of $\tilde{\nu}_A + \tilde{\nu}_F$ versus $f(\varepsilon, n) + 2g(n)$ for ACRYLODAN in the same solvents as in Fig. 6.

Table 1. Dipole moments of PRODAN, BADAN, and ACRYLODAN determined from solvatochromic shifts of absorption and fluorescence spectra. The conversion factor for the dipole moment: $[\mu]_{SI}/\mathrm{Cm} = 3.33564 \times 10^{-30} [\mu]_{cgs}/\mathrm{D}$, where D is the symbol of Debye and $1\mathrm{D} = 10^{-18}$ esu cm.

Molecule	Onsager radius a [Å]	μ_g	μ_e	$\Delta\mu$	μ_e/μ_g	m_1	m_2
		in Debye [D]				[cm^{-1}]	
PRODAN	4.2	2.14	6.43	4.29	3.00	2450	4900
	4.6	2.46	7.37	4.91			
BADAN	4.2	2.44	7.05	4.61	2.9	2895	5950
	4.6	2.79	8.08	5.29			
ACRYLODAN	4.2	2.36	8.97	6.61	3.8	5750	9860
	4.6	2.66	10.12	7.46			

obtained, which is close to μ_e/μ_g determined previously for PRODAN [3, 4] (cf. Table 1). However, for ACRYLODAN the higher value $\mu_e/\mu_g = 3.8$ was obtained.

In the case of BADAN, based on the relative shifts of absorption $\Delta\tilde{\nu}_A = 724\ \mathrm{cm}^{-1}$ and fluorescence bands $\Delta\tilde{\nu}_F = 2385\ \mathrm{cm}^{-1}$ according to (16) we obtain $\Delta\tilde{\nu}_F/\Delta\tilde{\nu}_A = \mu_e/\mu_g = 3.29$. However, for ACRYLODAN $\Delta\tilde{\nu}_F/\Delta\tilde{\nu}_A = \mu_e/\mu_g = 3.8$ is obtained. In the latter case the values of μ_e/μ_g determined in two different ways (from (12) and (16)) are equal. For BADAN, however, two distinctly different values for μ_e/μ_g are obtained, based on (12) $\mu_e/\mu_g = 2.9$ and (16) $\mu_e/\mu_g = 3.29$.

4. Ground and Excited State Dipole Moments of BADAN and ACRYLODAN

According to (10) and (11) the dipole moments μ_g and μ_e depend not only on the parameters m_1 and m_2, but also on the Onsager interaction radius a. For BADAN and ACRYLODAN one can assume the same values of a as for PRODAN (see Fig. 1). Assuming $a = 4.2$ Å, determined from crystallographic data [6], and $a = 4.6$ Å, based on the calculation of the spherical cave volume being the sum of all atomic volumes [16], we can calculate μ_g and μ_e for the values of m_1 and m_2 given in Table 1. The results are also given in Table 1.

The values of μ_g and μ_e obtained for BADAN differ slightly from those for PRODAN. Comparison of the structural formulae (Fig. 1) leads to the conclusion that the compounds BADAN and PRODAN differ only in the substituents Br and CH_3, respectively. However, for ACRYLODAN much higher values of the dipole moments μ_g and μ_e as well as $\mu_e/\mu_g = 3.8$ were obtained.

5. Conclusions

The obtained values of μ_g and μ_e for BADAN (see Table 1) are comparable to those of PRODAN, but for ACRYLODAN they are distinctly greater. The ratio

μ_e/μ_g determined by the method of Baba et al. [13] for only two solvents with different permittivities ε and identical refractive indexes n is completely accidental. Besides, great difficulties are encountered to find solvents which could satisfy this condition. The most reliable values of dipole moments are obtained based on measurements of absorption and fluorescence band shifts performed in many different solvents with various ε and n. For a known value of μ_g determined experimentally based on dielectric studies it is possible for parallel μ_g and μ_e to obtain μ_e from (12) without knowing the Onsager radius a.

[1] R. B. Macgregor and G. Weber, Nature (London) **319,** 70 (1986).
[2] A. Balter, W. Nowak, W. Pawełkiewicz, and A. Kowalczyk, Chem. Phys. Letters **143,** 565 (1988).
[3] A. Kawski, Z. Naturforsch. **54a,** 379 (1999).
[4] A. Kawski, B. Kukliński, and P. Bojarski, Z. Naturforsch. **55a,** 550 (2000).
[5] A. Kawski, B. Kukliński, P. Bojarski, and H. Diehl, Z. Naturforsch. **55a,** 817 (2000).
[6] G. Weber and F. J. Farris, Biochem. **18,** 3075 (1979).
[7] F. G. Prendergast, M. Meyer, G. L. Carlson, S. Jida, and J. D. Potter, J. Biol. Chemistry, **258,** 7541 (1983).
[8] T. Hiratsuka, J. Biol. Chem., **274,** 29156 (1999).
[9] L. Bilot and A. Kawski, Z. Naturforsch. **17a,** 621 (1962); **18a,** 10 and 256 (1963).
[10] A. Kawski, Acta Phys. Polon. **29,** 507 (1966).
[11] A. Kawski, Acta Phys. Polon. **25,** 285 (1964).
[12] A. Kawski, Naturwissensch. **51,** 82 (1964).
[13] H. Baba, L. Goodman, and P. C. Valenti, J. Amer. Chem. Soc., **88,** 5410 (1966).
[14] A. Kawski, G. Piszczek, B. Kukliński, and T. Nowosielski, Z. Naturforsch. **49a,** 824 (1994).
[15] A. Kawski, G. Piszczek, and B. Kukliński, Z. Naturforsch. **50a,** 949 (1995).
[16] W. Nowak, P. Adamczak, A. Balter, and A. Syguła, J. Mol. Struct. THEOCHEM **139,** 13 (1986).