ELSEVIER

Dyes and Pigments 42 (1999) 95–101

DYES and PIGMENTS

Fluorescence quenching by intermolecular $\pi-\pi$ interactions of 2,5-bis(*N,N*-dialkylamino)-3,6-dicyanopyrazines

Kazuko Shirai[a], Masaru Matsuoka[b,*], Koushi Fukunishi[a]

[a]*Department of Chemistry and Materials Technology, Kyoto Institute of Technology, Matsugasaki, Sakyo, Kyoto 606-0962, Japan*
[b]*Laboratory of Material Science, Kyoto Women's University, Imakumano, Higashiyama, Kyoto 605-8501, Japan*

Received 26 September 1998; accepted 3 December 1998

This paper is dedicated to Dr. Geoff Hallas on the occasion of his 65th birthday.

Abstract

The fluorescence properties of 2,5-bis(*N,N*-dialkylamino)-3,6-dicyanopyrazine dyes in the solid state were correlated with their molecular stacking behavior. The optimized molecular structures were evaluated by using MOPAC PM3 and AM1 methods and their stacking behaviors of the π-chromophoric system were simulated from their three dimensional molecular structures and ^1H NMR spectra. The steric requirements of the substituents in the parent chromophoric system were the main cause of the spectral changes in the absorption and fluorescence maxima and fluorescence intensity in the solid state. Intermolecular π–π interactions effectively quenched the solid state fluorescence and, consequently, the degree of fluorescence quenching can be used as an indicator of intermolecular π–π interactions of fluorescence dyes chromophores. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Aminopyrazine fluorescence dye; Fluorescence quenching; Solid state fluorescence; Fluorescence quantum yield; Molecular stacking; MOPAC AM1

1. Introduction

The color and constitution of dye chromophores at a molecular level have been quantitatively evaluated by semiempirical molecular orbital methods [1,2]. Solid state absorption spectra and the spectral shift from the solution could be correlated with intermolecular π–π interactions in crystals [3] and vapor deposited thin films [4]. Conformational analyses by the MOPAC PM3 method [5], and the aggregation and molecular stacking behaviors of pyrazine dyes have been studied with respect to their spectral changes in the solid state [6].

We are trying to correlate the solid state fluorescence properties such as fluorescence strength, spectral shift and fluorescence quenching with respect to their molecular stacking and intermolecular π–π interactions.

In this paper, the substituent effects of 2,5-bis(*N,N*-dialkylamino)-3,6-dicyanopyrazine fluorescence dyes on their solid state absorption and fluorescence spectra were evaluated systematically to correlate with their three dimensional molecular structures calculated by the MOPAC AM1 and PM3 methods [7,8]. In addition, fluorescence quenching by solvent polarity and intermolecular π–π interactions were studied in detail.

---

* Corresponding author. Tel.: +81-75-531-7175; fax: +81-75-531-7175; e-mail: GHA14151@nifty.ne.jp.

PII: S0143-7208(99)00013-3

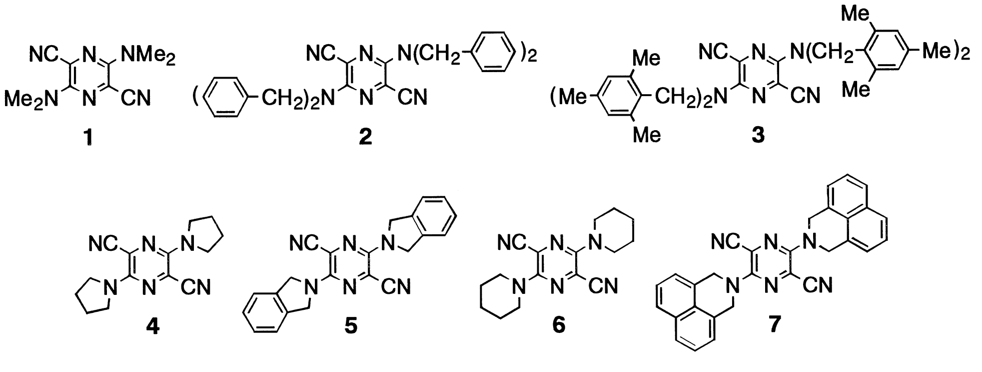
2. Results and discussion

A series of 2,5-bis(*N,N*-dialkylamino)-3,6-dicyanopyrazine dyes (**1**, **2**, **4**–**7**) have been previously synthesized by the alkylation of 2,5-diamino-3,6-dicyanopyrazine [9]. The sterically hindered dye **3** was also synthesized by a similar method [10]. The structures of these dyes are shown in Scheme 1. Each of their absorption and fluorescence spectra in chloroform, vapor deposited thin film and the powder state were determined by using the same equipment. Their spectral properties were compared with their chemical structures in each state. The difference in λ_{max} from the solid state to the solution is denoted as Δ_{λ}, and that in F_{max} is ΔF. The $\Delta\lambda$, ΔF and Stokes shift values were used to discuss the effect of substituents. The relative fluorescence quantum yields (Φ) of these dyes were determined in solution by using Rhodamine B as a standard at an excitation wavelength of 535 nm. All these experimental results were correlated in detail with respect to the nature of the substituents at the amino moieties of the parent dicyanopyrazine chromophore.

2.1. Substituent effects on the spectral properties of pyrazine dyes 1–7

The basic chromophoric system of 2,5-diamino-3,6-dicyanopyrazine has been evaluated by the Pariser–Parr–Pople molecular orbital (PPP MO) method. The first absorption band was characterized as having intramolecular charge-transfer character [9] with the amino groups acting as donors and the cyano groups as acceptors in the parent 2,5-diamino-3,6-dicyanopyrazine chromophore. The λ_{max} of dyes **1**–**7** shifted to longer wavelength in accordance with the electron donating strength of the *N,N*-dialkylamino groups. The λ_{max} and ε_{max} values of dyes **1**–**7** can be evaluated in detail by using dye **1** as a standard (Table 1). The λ_{max} of dye **1** was 502 nm in chloroform solution and the benzyl analogue **2** absorbed at 490 nm. The hypsochromic shift of 12 nm is due to the negative inductive effect of the phenyl groups, but loss of π-conjugation from the nitrogen lone pairs induced by the steric hindrance between the two benzyl groups on the amino moiety is also deduced from the decrease of ε value from 5040 for dye **1** to 4170 for dye **2**. The two benzyl groups on the amino moiety in dye **2** are oriented almost perpendicular to the pyrazine π-system due to the severe steric hindrance between the two benzyl groups. Even more severe steric hindrance in dye **3** gave rise to a further decrease of ε_{max} to 3250, whilst the increase in λ_{max} of 506 nm was attributed to electron-donating properties of the methyl groups. Free rotation of the benzyl groups in dyes **2** and **3** in solution at room temperature was confirmed by their ^{1}H NMR spectra; that is, the *ortho*-phenyl protons of dye **2** are observed as a doublet, and the *ortho*-methyl groups of dye **3** as a singlet (see Experimental section).

From these results, the benzyl groups of dyes **2** and **3** can rotate in solution, but are subject to severe steric hindrance in the solid state. Dye **4** absorbed at 526 nm and a bathochromic shift of 24 nm was observed. The pyrrolidine ring is



Scheme 1.

Table 1
Visible and fluorescence properties of dyes **1**–**7** in solution and in the solid states

	λ_{max} (nm)			F_{max} (nm)					
Compound no.	CHCl^3 (ε)	film[a]	$\Delta\lambda$[b] (nm)	CHCl$_3$[c] (strength)[h]	film[d]	powder[d] (strength)[i]	ΔF[e] (nm)	SS[f] (nm)	Φ[g]
1	502 (5040)	592	90	583 (940)	620	643 (6930)	37 (film) 60 (powder)	81	0.50
2	490 (4170)	495	5	574 (1330)	578	575 (10160)	4 (film) 1 (powder)	84	0.91
3	506 (3250)	510	4	573 (780)	571	580 (18430)	−2 (film) 7 (powder)	67	0.76
4	526 (5630)	461,^{s} 596	70	596 (840)	647	680 (610)	51 (film) 84 (powder)	70	0.33
5	514 (5600)	573	59	577 (1800)	593	644 (2230)	16 (film) 67 (powder)	60	0.65
6	492 (4000)	417, 566	74	597 (430)	594	633 (8230)	−3 (film) 36 (powder)	105	0.27
7	478 (3750)	480	2	573 (1100)	583	580 (2430)	10 (film) 7 (powder)	95	0.89

[a] Vapor deposited thin film on glass, ^{s} = shoulder.
[b] $\Delta\lambda = \lambda_{max}$ (film)$-\lambda_{max}$ (CHCl$_3$).
[c] F_{max} (CHCl$_3$) excited at λ_{max} (CHCl$_3$) value.
[d] Solid state F_{max} excited at λ_{max} (film) value.
[e] $\Delta F = F_{max}$ (solid)$-F_{max}$ solution.
[f] Stokes shift.
[g] Fluorescence quantum yield.
[h] Measured in the same concentration of 1×10^{-5} mol/liter in chloroform.
[i] Measured in powder on the white plate.

almost planar but is out of the plane of the pyrazine π-system. The nitrogen atom in the pyrrolidine ring has sp^3-like hybridization by MOPAC AM1 and sp^2-like hybridization by PM3 methods (Fig. 1). The pyrrolidine nitrogen atom can be conjugated effectively to the pyrazine chromophore which results in a bathochromic shift relative to dye **1** and the increase of in ε_{max} to 5630. Dye **5** absorbed at 514 nm, a hypsochromic shift of 12 nm from dye **4** and again attributed to the negative inductive effect of the benzene rings. The ε value of 5600 for dye **5** is almost the same as the extinction coefficient for dye **4**. Dyes **4** and **5** have a planar π-system having almost planar substituents which are oriented in a chair-like array from the pyrazine ring.

Dye **6**, which contains a piperidine ring, absorbed at 492 nm and the ε value was 4000 which is considerably smaller than that for dye **4**. The piperidine ring has a non-planar chair-form and the π-conjugation through the whole molecule is not as effective as that for dye **4**. The hypsochromic shift and the decrease of ε values for dyes **6** and **7** were due to less π-conjugation of the piperidine nitrogen atoms with the pyrazine nucleus. The shapes of the molecules **6** and **7** are dumbbell-like which may prevent intermolecular π–π interactions. The planarity of π-conjugation systems and the space-filling molecular structure were deduced from the optimized molecular structures calculated by the MOPAC PM3 and AM1 methods and are shown in Fig. 1.

By way of contrast, the λ_{max} values in the solid state showed quite different behavior which can be evaluated by comparing the difference between λ_{max} in the solid state and that in solution ($\Delta\lambda$). The $\Delta\lambda$ value of dye **1** was 90 nm which indicated strong intermolecular π–π interactions in the solid state but $\Delta\lambda$ for dyes **2** and **3** was small (4–5 nm). There is little difference in λ_{max} between solution and the solid state, and no intermolecular π–π interactions are proposed for dyes **2** and **3**

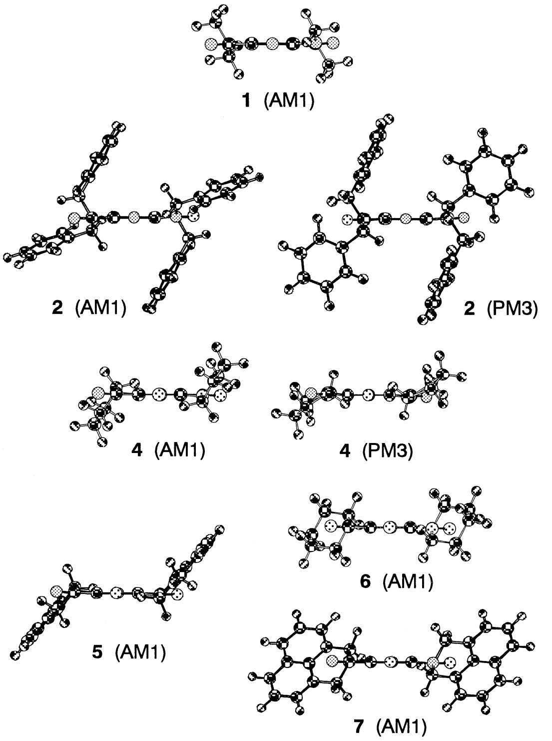


Fig. 1. Optimized molecular structures of dyes **1**–**7** by MOPAC AM1 and PM3 methods showing the side view from π-plane and the steric requirements of the substituents.

as a consequence of the steric requirements of the benzyl groups. Dyes **4**–**6** showed quite large Δλ values but dye **7** showed a Δλ value of only 2 nm. From these results, the Δλ value may be a good indicator of the presence of intermolecular π–π interactions which can be also evaluated by the optimized structures obtained by the MOPAC methods. The band splitting of λ_{max} in the film of dyes **4** and **6** has been discussed in a previous paper [11].

The fluorescence maxima (F_{max}) in solution and solid state, the difference between them (ΔF), the relative strength of fluorescence, and the Stokes shift (SS) are summarized in Table 1. Dyes **1**–**7** showed Stokes shift values of 60–105 nm indicating a significant energy loss from the first excited state to the emission state which is typically observed in diaminodicyanopyrazine chromophoric systems [11]. The F_{max} values of dyes **1**–**7** in solution changed from 573 to 597 nm depending on the substituents but the maximum difference of 24 nm was only half that of 48 nm in their λ_{max} values. The relative fluorescence strength of dyes **1**–**7** in solution were quite similar with dye **5** showing the strongest fluorescence (1800) and dye **6** the weakest (430).

On the other hand, the solid state fluorescence in vapor deposited thin film and in the powder state exhibited large changes in F_{max} values. The changes were evaluated by use of ΔF values. Dyes **1** and **4**, indicating effective intermolecular π–π interactions, showed large ΔF values in the thin films, but the sterically hindered dyes **2**, **3**, **6** and **7** showed small or negative ΔF values. In the powder state, larger ΔF values were obtained in dyes **1** (60 nm), **4** (84 nm) and **5** (67 nm) but dyes **2** (1 nm), **3** (9 nm), **6** (36 nm) and **7** (7 nm) showed smaller values. In contrast, the relative fluorescence strength of dyes **1** (6930), **2** (10 160) and **3** (18 430) were large, whilst dyes **4** and **5** gave rise to weak fluorescence indicating effective intermolecular π–π interactions in the powder state. Dye **6** (8230) showed relatively stronger fluorescence than that of dye **4** (610). From these results, fluorescence quenching was studied in detail from the viewpoint of solvent polarity and intermolecular π–π interactions.

2.2. Fluorescence quenching by solvent polarity

Effects of solvent polarity on the fluorescence properties of dye **1** are summarized in Table 2. Solvent polarity affects the fluorescence intensity; an increase in the polarity of the solvent decreases the fluorescence intensity. The singlet excited molecule is much more polar than in the ground state especially in the case of an intramolecular charge-transfer chromophoric system, and stronger electrostatic interactions between the excited dye molecule and polar solvent are proposed to account for decrease in the fluorescence strength. As indicated before, the λ_{max} and F_{max} of dye **1** produced a bathochromic shift from those in *n*-hexane with an increase in solvent polarity. The differences are indicated by δλ and δ*F* values, respectively. Non-polar benzene showed an anomalously large δλ value (14 nm) because of π–π interactions between dye **1** and solvent

Table 2
Effects of solvent polarity on the fluorescence quenching of dye **1**

Solvents	DM[a] (D)	λ_{max} (nm)	$\delta\lambda$ (nm)	F_{max} (nm)	δF_{max} (nm)	SS (nm)	FS[b]
n-Hexane	0	483	–	555	–	72	942
Benzene	0	497	14	579	24	82	573
Chloroform	1.02	502	19	583	28	81	597
Ethyl acetate	1.67	491	8	583	28	92	362
Ethanol	1.69	494	11	589	34	95	158
Acetone	2.85	493	10	588	33	95	247
DMA[c]		498	15	597	42	99	91
DMF[d]		500	17	599	44	99	84

[a] Dipole moment (Debye).
[b] Strength of fluorescence.
[c] *N,N*-Dimethylacetamide.
[d] *N,N*-Dimethylformamide.

molecule, and chloroform also showed a large $\delta\lambda$ value (19 nm) because of intermolecular hydrogen-bonding between the cyano groups of dye **1** and the acidic hydrogen in chloroform. The δF value increased with an increase of solvent polarity which is in accordance with the increase in the Stokes shift values. On the contrary, the strength of fluorescence in solution was the highest in *n*-hexane as a non-polar solvent and decreased with increasing solvent polarity. Some fluorescence quenching by the π–π interactions between dye **1** and benzene, and by intermolecular hydrogen-bonding between dye **1** and ethanol are proposed to account for the fluorescence strengths. From these solvents effects, it is apparent that fluorescence quenching increases with an increase in solvent polarity.

2.3. Fluorescence quenching by intermolecular π–π interactions

It is generally known that a decrease in fluorescence intensity or fluorescence quenching in solution is observed along with an increase of dye concentration and/or the formation of an excimer. Intermolecular interactions of a singlet excited dye molecule with a ground state dye molecule give rise to the energy-transfer to quench the fluorescence. Solid state fluorescence was determined in vapor deposited thin film and powder states in which dye molecules formed aggregates or stacked strongly, and strong intermolecular interactions between dye molecules are proposed. The interactions can be evaluated in terms of the ΔF value, and the strength of solid state fluorescence can be correlated with the optimized molecular structures.

The solid state fluorescence properties of dyes **1**–**7** in the thin film and powder states are summarized in Table 1. The thickness of the vapor deposited thin film could not be easily controlled and the strengths of fluorescence in the thin films therefore could not be compared. In the powder state, the conditions such as particle size and the sample amounts for measurement could be controlled, and fluorescence strength can be compared with their structures. In the series of dyes **1**–**3**, dye **3** has the strongest fluorescence followed by dye **2** and then dye **1** in powder state, while in chloroform their strengths were in the order **2**, **1** and **3**. The fluorescence quantum yields (Φ) of these dyes are in the order of dyes **2** (0.91), **3** (0.76) and **1** (0.50).

From these results, it was found that fluorescence quenching in the solid state occurred in the order of dyes **1**, **2** and **3** by the possibility or the increase of intermolecular π–π interactions. In a series of dyes **4**–**7**, the sterically hindered six membered dyes **6** and **7** having a dumbbell structure have stronger fluorescence in the powder state than the corresponding dyes **4** and **5**. Again the reverse was observed in solution. The Φ values of these dyes are in the order of dyes **7**, **5**, **4** and **6**. As mentioned before, fluorescence quenching will be induced by intermolecular π–π interactions of the chromophore, and sterically hindered dye molecules (evaluated by the MOPAC method) have stronger solid state fluorescence than less hindered ones.

Relative fluorescence quantum yield of dyes **1**–**7** were determined at low concentration for fluorescence measurement to avoid fluorescence quenching in higher concentration. Rhodamine B has a high quantum yield ($\Phi = 0.97$ in ethanol) and its absorption ranges overlapped with those of dyes **1**–**7** and was selected as a standard. Pyrazine dyes **1**–**7** have relatively high fluorescence quantum yields of 0.27–0.91 and critical substituent effects are observed among them. Tetrabenzyl derivatives **2** and **3** and the annelated dyes **5** and **7** showed larger Φ values than the dyes **1**, **4** and **6**. It is generally known that a planar and rigid π-system increases the Φ value and a flexible molecule loses the absorbed energy in the excited state and thus the Φ value decreases. It is therefore reasonable that dyes **4** and **6** have smaller Φ values than the corresponding dyes **5** and **7**, respectively. In the case of dyes **1**–**3**, dyes **2** and **3** have much more rigid substituents, but dye **1** has more freedom for molecular motions through the molecule. The Stokes shift of dye **4** is larger than that of dye **5**, and that of dye **6** is larger than that of dye **7**. It was found that the larger the Stokes shift value is, the smaller the Φ value is.

We conclude that strong intermolecular π–π interactions effectively quench the fluorescence in the solid state, and sterically hindered dyes such as **2** and **3** having no intermolecular π–π interactions showed strong solid state fluorescence. Thus, these pyrazine dyes can be applied as functional fluorescence materials in various application fields.

3. Experimental

3.1. Materials and equipment

Dyes **1**, **2**, **4**–**7** were synthesized and characterized as in a previous paper [7]. Dye **3** was supplied by Nippon Soda Co Ltd (mp 222–224°C). The purity of dye **3** was checked by TLC and the structure confirmed by ^{1}H NMR. The preparations of vapor deposited thin films were performed using the Nippon Shinku. Kikou VSP-060 apparatus. Visible spectra were recorded on a HITACHI 220A spectrophotometer. Fluorescence spectra in solution and thin film were recorded on a HITACHI F-4500 fluorescence spectrophotometer, and those in the powder state were measured on a Hamamatsu Photonic Multi-channel Analyzer PMA-11 using a Jasco SM-3 type monochromator as a light source. ^{1}H NMR spectra of dyes **2** and **3** were recorded on an FT-NMR QE 300 MHz Shimadzu spectrometer.

3.2. Determination of fluorescence quantum yield

The absolute fluorescence quantum yield of Rhodamine B excited at 535 nm is quoted as $\Phi = 0.97$ [12]. The fluorescence spectra of Rhodamine B was measured using our equipment and its Φ value excited at 535 nm was determined as $\Phi = 0.97$. The fluorescence quantum yields of our samples excited at 535 nm were determined using Rhodamine B as a standard by the method described [12]. The concentration for fluorescence measurements was 3×10^{-5} mol/liter for pyrazine dyes and 3×10^{-7} mol/liter for Rhodamine B, because the ε values of pyrazine dyes are several thousand but that of Rhodamine B is several hundred thousand. The fluorescence quenching by increased dye concentration was determined as described in the literature [12].

3.3. Solid state fluorescence measurement

Fluorescence strengths in the solid state were determined by using monochromator (±10 nm wavelength range) for excitation, and the emitted fluorescence was quantitatively determined using a multi-channel analyzer. The fluorescence strengths given in the Table 1 are relative values in the small wavelength ranges 480–596 nm for excitation and 575–680 nm for fluorescence.

3.4. ^{1}H NMR of dyes **2** and **3**

^{1}H NMR of **2** and **3** in chloroform-d were recorded at room temperature. The structures of **2** and **3** are confirmed from the following results. **2**: δ 4.68 (8 H, s, CH_2), 7.22 (8 H, dd, $J = 7.5$ and 2 Hz, ortho-H), 7.31 (12 H, t, $J = 7.5$ Hz, m- and p-H). **3**: δ 1.88 (24 H, s, o-Me), 2.23 (12 H, s, p-Me), 4.39 (8 H, s, CH_2), 6.75 (8 H, s, phenyl). There are no differences in either the chemical shifts of the two ortho-phenyl protons in **2** or the two *ortho*-methyl protons in **3**.

References

[1] Griffiths J. Colour and constitution of organic molecules. London: Academic Press, 1976.

[2] Fabian J, Hartmann H. Light absorption of organic colorants. Berlin: Springer–Verlag, 1980.

[3] Mizuguchi J, Rihs G, Karfunkel HR. Solid-state spectra of titanylphthalocyanine as viewed from molecular distortion. Journal of Physical Chemistry 1995;99:16217–27.

[4] Kim JH, Matsuoka M, Fukunishi K. Syntheses and solid state absorption spectra of aminonaphthoquinone dyes. Dyes and Pigments 1996;31:263–72.

[5] Shiozaki H, Oshida A, Hou DF, Matsuoka M. Conformational analysis of aminovinylpyrazines by semiempirical molecular orbital calculations. Dyes and Pigments 1997;33:33–9.

[6] Kim JH, Shin SR, Matsuoka M, Fukunishi K. Self-assembling of aminopyrazine fluorescent dyes and their solid state spectra. Dyes and Pigments 1998;39:341–57.

[7] Stewart JJP. MOPAC ver. 6, CPE no. 455.

[8] Hirano T. Revised MOPAC ver. 6.01, JCPE newsletter 1991;2:26.

[9] Shirai K, Yanagisawa A, Takahashi H, Fukunishi K, Matsuoka M. Syntheses and fluorescent properties of 2,5-diamino-3,6-dicyanopyrazine dyes. Dyes and Pigments 1998;39:49–68.

[10] Dye **3** was supplied from Nippon Soda Co Ltd.

[11] Shirai K, Jaung JY, Matsuoka M, Fukunishi K. Structure and spectral properties of dicyanopyrazine fluorescence dyes. Journal of the Society of Photographic Science and Technology of Japan 1998;61:261–7.

[12] Nishikawa Y, Hiraki K. Analysis methods for fluorescence and phosphorescence. Tokyo: Kyoritsu, 1984 (in Japanese).