Fluorometric Study of the Molecular States of 2,5-Diphenyloxazole in Ground Mixtures with γ-Cyclodextrin

**Keiji Yamamoto,[1,4] Toshio Oguchi,[1]
Etsuo Yonemochi,[1] Yoshisuke Matsumura,[1,2] and
Yoshinobu Nakai[1,3]**

Received April 8, 1993; accepted August 3, 1993

Molecular states of 2,5-diphenyloxazole (PPO) were investigated in ground mixtures with γ-cyclodextrin (γ-CD). Crystalline PPO gradually became amorphous upon grinding in the presence of γ-CD. Solid-state fluorescence spectra of the ground mixtures showed that the fluorescence emission peak due to PPO crystals was reduced in intensity with an increasing duration of grinding. A new fluorescence peak attributed to PPO excimer appeared at a longer wavelength. Results of the time-resolved fluorescence study indicated that the cogrinding caused an increase in the portion of PPO excimer in the γ-CD ground mixture. On the other hand, only a small portion of excimer was formed in ground mixtures with β-CD, which has a smaller cavity than γ-CD. These results suggest that cogrinding of PPO with γ-CD caused two PPO molecules to be included in the γ-CD cavity, contributing to excimer formation.

KEY WORDS: fluorescence; grinding; cyclodextrin; 2,5-diphenyloxazole; excimer; inclusion complex.

INTRODUCTION

The molecular states of a drug in solid dispersions are closely related to its pharmaceutical characteristics, e.g., stability, solubility, and bioavailability, in solid preparations. However, only a few spectroscopic methods are applicable to the evaluations of pharmaceutical dispersions. Previous analysis of the molecular states of drugs in solid dispersions by infrared spectroscopy (1,2) and photoacoustic spectroscopy (3) demonstrates the importance of the spectroscopic approach.

Fluorescence analysis has been used to evaluate the structure and dynamics of biomolecules and their aggregates (4) and to study molecular interactions such as the inclusion phenomena of cyclodextrin and guest compounds in solution (5–10). Solid-state fluorescence analysis has been used to study adsorbate on solid surfaces (11–19), dispersions in rigid matrices at low temperatures (20–22), solid dispersions in polymers (23), powdered solids (24), gels (25), and pharmaceutical formulations (26). These studies demonstrated that fluorescence can serve to investigate molecular state and photochemistry in solid systems.

Fluorescence studies of 2,5-diphenyloxazole (PPO) are of interest because the emitting excimer fluorescence has a long lifetime in concentrated solutions (27). Agbaria and Gill (28) prepared aggregates of the γ-cyclodextrin (γ-CD)/PPO system and observed that a suspension of the aggregates gave a marked excimer fluorescence, which they attributed to the formation of a PPO/γ-CD (2:1) inclusion complex. In the present paper, solid-state fluorescence measurements have been applied to γ-CD/PPO ground mixtures to elucidate the molecular behavior of PPO during the grinding process and the mechanism of inclusion complex formation by cogrinding with γ-CD.

EXPERIMENTAL

Materials

2,5-Diphenyloxazole (PPO; Nacalai Tesque) was special reagent grade. α-Cyclodextrin (α-CD; Nacalai Tesque), β-cyclodextrin (β-CD; Ando Kasei Co.), γ-cyclodextrin (γ-CD; Nacalai Tesque), and microcrystalline cellulose (MCC; Avicel PH-101, Asahi Chemical Ind. Co., Ltd.) were used after drying for 3 hr at 110°C in a vacuum.

Preparation of Ground Mixtures

Physical mixtures (2.0 g) of PPO and CDs were ground by means of a vibrational mill (Heiko Seisakusho TI-200) made of tungsten carbide. The mixing molar ratio of PPO to γ-CD was varied from 0.10 to 20.

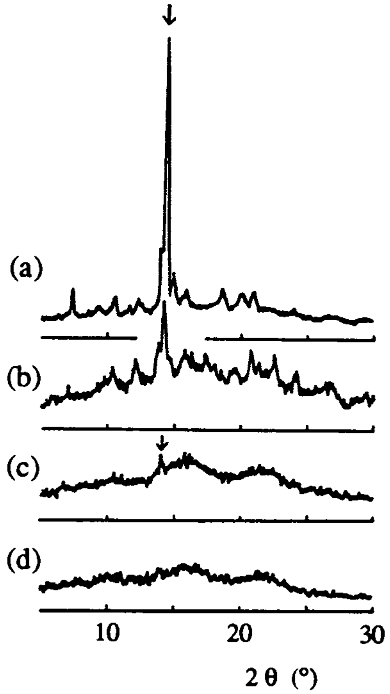

Fig. 1. Changes caused in the powder X-ray diffraction pattern of the γ-CD/PPO (1:2) system by grinding. (a) Intact; (b) 1 min of grinding; (c) 5 min of grinding; (d) 10 min of grinding.

---

[1] Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoicho, Inage-ku, Chiba 263, Japan.

[2] Yamanouchi Pharmaceutical Co., Ltd., 180 Ozumi, Yaizu-shi, Shizuoka 425, Japan.

[3] Tsumura & Co., 3586 Yoshiwara, Amimachi, Inashiki-gun, Ibaraki 300-11, Japan.

[4] To whom correspondence should be addressed.

0724-8741/94/0200-0331$07.00/0 © 1994 Plenum Publishing Corporation

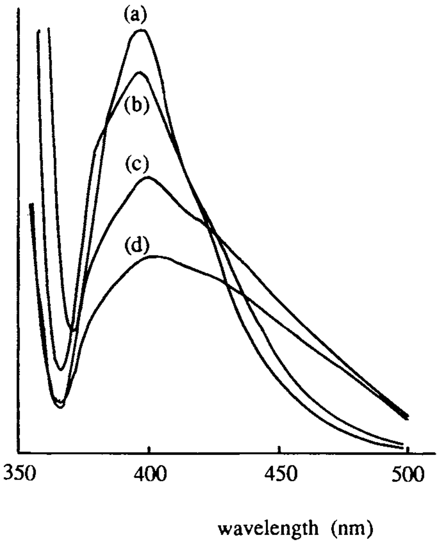

Fig. 2. Changes caused in the solid-state fluorescence spectrum of the γ-CD/PPO (1:2) system by grinding (λ_{ex} = 352 nm). (a) Intact; (b) 1 min of grinding; (c) 5 min of grinding; (d) 10 min of grinding.

Fluorescence Spectroscopy

An FP-770F spectrofluorometer (Japan Spectroscopic Co., Ltd.), equipped with an attachment for solid-sample measurement (FP-1060), was used for stationary measurement.

Determination of Fluorescence Lifetime

Measurements of fluorescence lifetimes and relative quantum yields were carried out using a single-photon counting apparatus, NAES-1100 (Horiba, Japan). Quartz demountable cells AB-20 (GL Sciences, Japan) were used for powdered samples.

Powder X-Ray Diffractometry

A Rigaku Denki 2027 diffractometer was used. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 30 kV; current, 5 mA; and scintillation counter.

RESULTS AND DISCUSSION

A physical mixture of γ-CD and PPO at a molar ratio of 1:2 was ground with the vibrational mill. Figure 1 shows the changes in the powder X-ray diffraction pattern caused by grinding. Diffraction peaks due to PPO crystals, indicated by arrows in Fig. 1, decreased in intensity with increasing durations of grinding and, finally, disappeared after 10 min of grinding, indicating the amorphization of PPO crystals by cogrinding with γ-CD. Solid-state fluorescence measurements were carried out for the ground samples. Figure 2 shows the fluorescence emission spectra of the γ-CD:PPO (1:2) ground mixtures at an excitation wavelength of 352 nm. It was reported that excitation at 358 nm yielded nearly pure dimer fluorescence (28). In the solid sample, showing a broadened excitation spectrum, however, monomer species were considered also to be excited at 352 nm because no significant difference in the shape of the emission spectrum of PPO crystals was observed between the excitation wavelength of 313 nm and that of 352 nm. To detect changes in emission spectrum as a result of excimer formation, fluorescence measurements were carried out at an excitation wavelength of 352 nm. In the emission spectrum of the physical mixture, the fluorescence peak observed at 397 nm was regarded as being due to PPO crystals. Grinding with γ-CD reduced the intensity of this peak, accompanied by the appearance of a new fluorescence peak near 423 nm.

Fluorescence decay kinetics was also investigated for each sample. The excitation and observation wavelengths were 352 and 420 nm, respectively. As shown in Fig. 3, the fluorescence decay curves of PPO before and after the cogrinding with γ-CD were markedly different. The individual lifetimes of each component of PPO were determined by deconvolution with two or three exponentials. The values obtained of lifetimes and relative quantum yields are listed in Table I. The short-lifetime component, whose lifetime was about 1.0 nsec, was observed within a relative quantum yield of 10% for each sample. Since this component has too short a lifetime to be regarded as a fluorescence due to PPO molecules and its relative quantum yield increased with a decrease in PPO content (see Table II), this component was considered a result of spurious fluorescence e.g., stray, reflective, or scattered light (29). The third component in the ground sample had a long lifetime of more than 13 nsec, and the relative quantum yield of this component increased with the duration of grinding. Before grinding, the major compo-

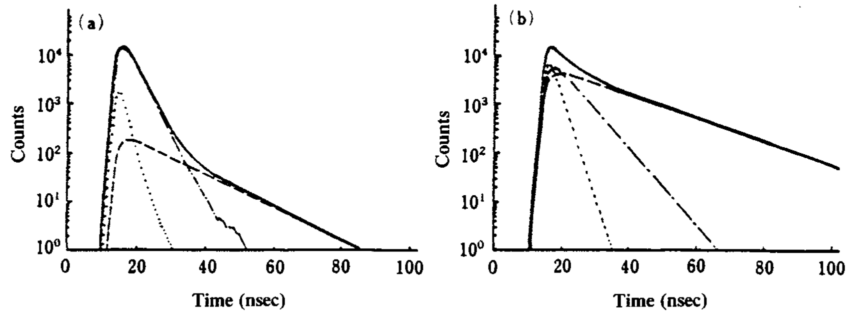

Fig. 3. Fluorescence decay curves of γ-CD/PPO (1:2) systems (λ_{ex} = 352 nm, λ_{obs} = 420 nm). (a) Physical mixture; (b) ground mixture (15 min of grinding). (——) Fluorescence decay curve; (······) component 1; (–·–·–) component 2; (– – – –) component 3.

Table I. Changes of Fluorescence Lifetime (τ) and Relative Quantum Yield (Q) of PPO by Cogrinding with γ-CD (Molar Ratio of γ-CD:PPO = 1:2): λ_{ex} = 352 nm, λ_{obs} = 420 nm

	τ_1 (nsec)	Q_1 (%)	τ_2 (nsec)	Q_2 (%)	τ_3 (nsec)	Q_3 (%)	χ^2[a]
Ground mixture							
0 min[b]	0.856	1.8	2.79	90.3	16.1	7.9	1.52
1 min	1.11	8.4	4.16	71.2	13.5	20.4	1.18
2 min	0.913	7.8	4.08	64.7	15.7	27.5	0.940
5 min	0.873	9.0	4.16	41.3	17.5	49.7	1.46
10 min	0.909	9.6	4.29	33.3	17.8	57.1	1.06
15 min	0.849	10.0	4.08	33.2	17.7	56.8	1.10
PPO crystals	0.291	5.4	2.97	94.6			1.23
Aggregate	0.408	2.5	5.59	18.5	19.4	79.0	1.25

[a] A parameter for judging the goodness of fit; an ideal fit will yield a χ^2 value of unity.

[b] Grinding time.

nent was found at a relative quantum yield of 90.3% and had a lifetime of 2.79 nsec, results consistent with those for PPO crystals. After sufficient grinding, two fluorescence components with lifetimes of 4 and 18 nsec were observed at relative quantum yields of about 33 and 57%, respectively. These distinct changes in fluorescence feature suggest that the molecular states of PPO were drastically converted by cogrinding with γ-CD.

For solutions containing low concentrations of PPO in cyclohexane, monomer fluorescent emission with a lifetime of 1.6 nsec was observed near 335 nm (30). At high concentrations of PPO, however, another fluorescence emission appeared at a longer wavelength, about 430 nm, and its lifetime of 15.2 nsec was considerably longer, indicating excimer formation of PPO in the concentrated solutions (30). Agbaria and Gill (28) have reported that γ-CD/PPO aggregates showed a typical excimer emission with a lifetime of 16 nsec at about 420 nm. They proposed the following structure model for the aggregates. In aqueous solutions, PPO formed a 2:1 (PPO:γ-CD) inclusion complex with γ-CD showing monomer emission at 370 nm, in which structure each of two PPO molecules would penetrate from the opposite ends of a γ-CD cavity. At high concentrations, these complexes would associate in the aqueous solutions to form aggregates in which the unit complexes were linked coaxially by the overlapping of PPO moieties.

In the fluorescence spectra for PPO/γ-CD dried aggregates, Agbaria and Gill observed strong dimer emission as well as PPO monomer emission (28). We investigated the fluorescence decay kinetics of the dried aggregates prepared according to their method. A fluorescent component which had quite a long lifetime (τ = 19.4 nsec) was found at a relative quantum yield of 79% as well as a minor component with a lifetime of 5.59 nsec. It was considered that the third component (τ > 13 nsec) in Table I corresponded to the excimer emission of PPO. The second component, regarded as being due to PPO crystals, had its lifetime elongated to approximately 4 nsec after grinding. Since no X-ray diffraction peak was observed in the 10-min ground mixture (Fig. 1), PPO molecules may, after sufficient grinding, exist in the molecular-dispersed state in γ-CD cavities or in the intermolecular hydrogen-bond network of γ-CD (1,2). The monomolecular species of PPO would provide the monomer emission with a lifetime of about 4 nsec and, together with PPO crystals, contribute to the second component.

Ground mixtures of PPO and γ-CD were prepared at various molar ratios (PPO/γ-CD). Powder X-ray diffractograms of the ground mixtures as a function of the PPO/γ-CD molar ratio are shown in Fig. 4. At a molar ratio of 20, where there was an excess amount of PPO, a diffraction peak due to PPO crystals was observed at a 2θ of 14.5°. At other molar ratios no diffraction peak due to PPO crystals was observed after 15 min of grinding, indicating that all the PPO molecules existed in an amorphous state. Fluorescence measurements were carried out in the solid state for these samples at an excitation wavelength of 352 nm (Fig. 5). The emission peak at 397 nm, observed at molar ratios of 20.0 and 2.0, gradually shifted toward 439 nm (dimer emission) with a decrease in PPO content to a molar ratio of 0.50 (PPO/γ-CD). With a further decrease in PPO content at a molar ratio of 0.10, the emission peak shifted back toward the initial shorter wavelength (monomer emission). These results were consistent with those obtained in the fluorescent decay kinetic study (Table II). The value of Q_3/Q_2, the ratio of the relative quantum yield of excimer to that of monomer, had a maximum value at a molar ratio of 0.50, suggesting that the

Table II. Fluorescence Lifetimes (τ) and Relative Quantum Yields (Q) of PPO in Ground Mixtures (15 min of Grinding) with γ-CD as a Function of Molar Ratio (PPO/γ-CD): λ_{ex} = 352 nm, λ_{obs} = 420 nm

PPO/γ-CD	τ_1 (nsec)	Q_1 (%)	τ_2 (nsec)	Q_2 (%)	τ_3 (nsec)	Q_3 (%)	χ^2	Q_3/Q_2
0.10	0.807	26.5	2.96	48.9	17.3	24.6	1.37	0.50
0.20	0.637	22.2	3.26	47.5	16.3	30.3	1.36	0.64
0.50	1.07	11.5	4.92	27.5	18.4	61.0	1.17	2.2
2.0	0.849	10.0	4.08	33.2	17.7	56.8	1.10	1.7
20	1.81	18.7	3.34	69.1	13.3	12.2	1.04	0.18

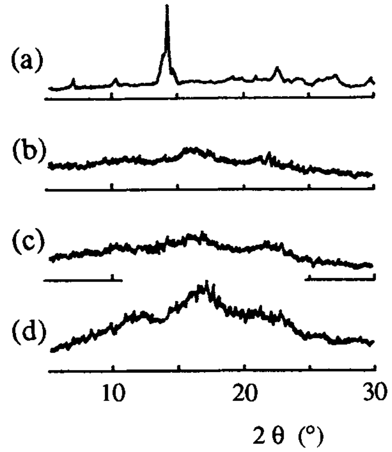

Fig. 4. Powder X-ray diffraction patterns of ground mixtures (15 min of grinding) in the γ-CD/PPO system at various molar ratios. Molar ratio of PPO/γ-CD: (a) 20; (b) 2.0; (c) 0.50; (d) 0.10.

excimer was formed extensively around this molar ratio. The most likely explanation for these results is as follows. In systems containing small amounts of PPO, PPO molecules are scattered monomolecularly in the dispersion, so that a monomer emission is predominant. An increase in PPO concentration allows two PPO molecules to be located within the γ-CD cavity, resulting in an increase in the PPO fraction of possible excimer formation. Further, in systems containing a large excess of PPO, most of the PPO molecules were in the crystalline state, showing intrinsically a monomer emission.

The cavity sizes of β- and γ-CD are 6.0–6.4 and 7.5–8.3 Å in diameter, respectively (31). β-CD usually formed a 1:1 inclusion complex with a variety of organic compounds because its cavity size was appropriate for the accommodation of one molecule. We investigated the fluorescence properties of a ground mixture of PPO with β-CD. The values for the lifetime and relative quantum yield of PPO in the β-CD ground mixture are listed in Table III. A long lifetime component (τ = 13.5 nsec) was observed at a relative quantum yield of 19.6% after 15 min of grinding, while the Q_3/Q_2 value (0.35) was significantly lower than that of the γ-CD-PPO system. A long lifetime component was also observed for the ground mixture of PPO with microcrystalline cellulose (MCC), which has no ability to form inclusion complexes (Table III). These results suggest that excimer formation was possible in the hydrogen-bond network formed among cellulose molecules. Since the cavity aperture of β-CD is too small to accommodate a pair of PPO molecules, the long lifetime fluorescence in the β-CD-PPO system should have arisen from the excimer formed in the intermolecular hydrogen-bond network of β-CD molecules. On the other hand, γ-CD has a cavity large enough to accommodate two PPO molecules. It seems reasonable to propose that the high Q_3/Q_2 value in the γ-CD/PPO systems was due to the inclusion formation of two PPO molecules in the γ-CD cavity by cogrinding, although the PPO excimer would also be formed, to some extent, outside of the γ-CD cavity in the same manner as in the β-CD system.

The cavity size of γ-CD is large enough to accommodate a pair of PPO molecules from consideration of CPK molecular modeling (28). In aqueous solution, however, the inclusion complex of PPO and γ-CD (2:1) showed only monomer fluorescence, while the aggregate of the complex showed pure excimer fluorescence. Agbaria and Gill speculated as follows (28). Two PPO molecules would partly penetrate the

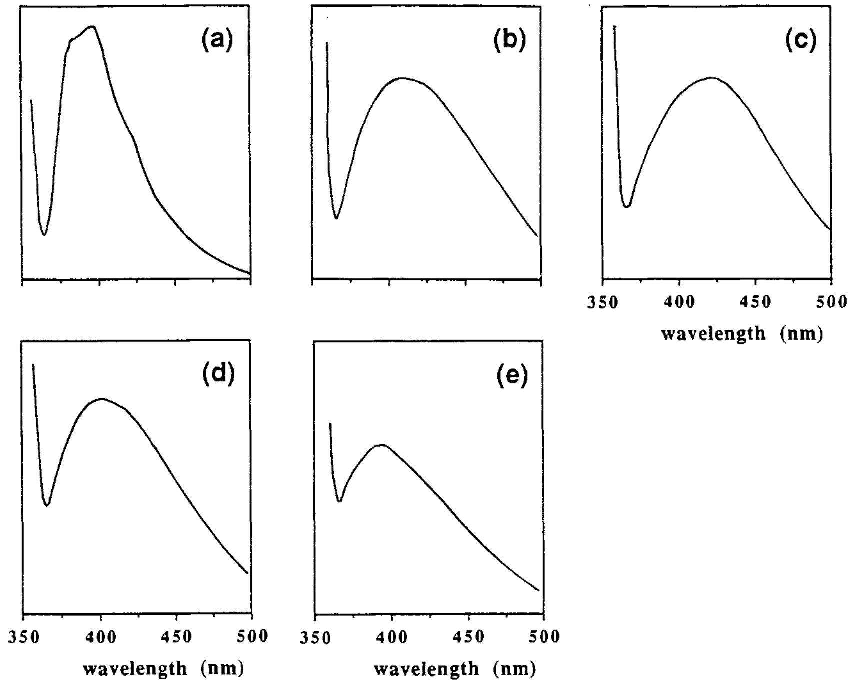

Fig. 5. Solid-state fluorescence spectra of ground mixtures (15 min of grinding) in the γ-CD/PPO system at various molar ratios (λ_{ex} = 352 nm). Molar ratio of PPO/γ-CD: (a) 20; (b) 2.0; (c) 0.50; (d) 0.20; (e) 0.10.

Table III. Fluorescence Lifetimes (τ) and Relative Quantum Yields (Q) of PPO in Ground Mixtures (15 min of Grinding) with β-CD and MCC: λ_{ex} = 352 nm, λ_{obs} = 420 nm

	τ_1 (nsec)	Q_1 (%)	τ_2 (nsec)	Q_2 (%)	τ_3 (nsec)	Q_3 (%)	χ^2
β-CD/PPO (1:1)	0.803	23.6	3.73	56.8	13.5	19.6	1.13
MCC/PPO (5% w/w)	1.14	41.2	4.51	37.1	15.2	21.7	0.969

opposite site of the CD tunnel, namely, only a phenyl ring of PPO would penetrate a CD cavity, while the oxazole ring would stay in the water phase because of its hydrophilicity. When the complexes associated to form the aggregate at a high concentration, PPO pairs would be compelled to overlap by accommodation between adjacent γ-CD molecules.

In the ground mixture of PPO and γ-CD, some portion of PPO molecules existed as the overlapping pair since excimer fluorescence of PPO was observed. It might be reasonable to consider the following three possibilities. (i) The γ-CD cavity participates in the overlapping of PPO. (ii) In the solid dispersion, providing an environment distinct from the aqueous solution, hydration does not affect the stability of the inclusion complex. (iii) The complex cannot establish the highly ordered structure of the aggregate of PPO and γ-CD, because the complexes in the ground mixture were disordered and amorphized by the mechanochemical process. It can be concluded that the inclusion of two PPO molecules in the γ-CD cavity took place during the process of cogrinding. Thus, an overlapping pair of PPO molecules may be included in a γ-CD cavity, resulting in the emission of the excimer fluorescence with a long lifetime.

ACKNOWLEDGMENTS

The authors would like to thank Ms. Mayumi Narita for her assistance. The authors are grateful to Dr. Lim Lee Yong for her helpful advice in writing the English manuscript. This research was supported in part by Grant-in-Aid for Scientific Research (C) No. 03807141 from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. Y. Nakai. Molecular behavior of medicinals in ground mixtures with microcrystalline cellulose. *Drug Dev. Ind. Pharm.* 12:1017–1039 (1986).
2. T. Oguchi, K. Terada, K. Yamamoto, and Y. Nakai. Freeze-drying of drug-additive binary systems. I. Effects of freezing condition on the crystallinity. *Chem. Pharm. Bull.* 37:1881–1885 (1989).
3. Y. Nakai, K. Yamamoto, K. Terada, and M. Sakai. Application of photoacoustic spectroscopy for quantitative analysis of drugs in the pharmaceutical preparations. *Yakugaku Zasshi* 108:1107–1109 (1988).
4. R. F. Steiner. Dynamic fluorescence methods and their applications to biochemistry. *Protein Nucleic Acid Enzyme* 37:1415–1432 (1992).
5. J. Nishijo, M. Yasuda, M. Nagai, and M. Sugiura. Inclusion mode of 8-anilino-1-naphthalenesulfonate (ANS) in the ANS-β-cyclodextrin complex. *Bull. Chem. Soc. Jpn.* 65:2869–2871 (1992).
6. T. Soujanya, T. S. R. Krishna, and A. Samanta. The nature of 4-aminophthalimide-cyclodextrin inclusion complexes. *J. Phys. Chem.* 96:8544–8548 (1992).
7. A. Ueno, S. Minato, and T. Osa. Host-guest sensors of 6^A, 6^B-, 6^A, 6^C-, 6^A, 6^D-, and 6^A, 6^E-bis(2-naphthylsulfonyl)-γ-cyclodextrins for detecting organic compounds by fluorescence enhancement. *Anal. Chem.* 64:1154–1157 (1992).
8. A. Nakamura, K. Saitoh, and F. Toda. Fluctuation in structure of inclusion complexes of cyclodextrins with fluorescent probes. *Chem. Phys. Lett.* 187:110–115 (1991).
9. J. B. Zung, A. M. de la Pena, T. T. Ndou, and I. M. Warner. Influence of alcohol addition on the γ-CD:pyrene complex. *J. Phys. Chem.* 95:6701–6706 (1991).
10. F. V. Bright, G. C. Catena, and J. Huang. Evidence for lifetime distributions in cyclodextrin inclusion complexes. *J. Am. Chem. Soc.* 112:1343–1346 (1990).
11. V. Ramamurphy, D. F. Eaton, and J. V. Casper. Photochemical and photophysical studies of organic molecules included within zeolites. *Acc. Chem. Res.* 25:299–307 (1992).
12. L. F. V. Ferreira, M. R. Freixo, and A. R. Garcia. Photochemistry on surface: Fluorescence emission quantum yield evaluation of dyes adsorbed on microcrystalline cellulose. *J. Chem. Soc. Faraday Trans. I* 88:15–22 (1992).
13. T. Fujii, A. Ishii, H. Satozono, S. Suzuki, M. Che, and M. Anpo. Photophysics on solid surfaces. Time-resolved fluorescence spectra of pyrene adsorbed on calcinated vycor glass. *Bull. Chem. Soc. Jpn.* 63:2475–2480 (1990).
14. X. Liu, K.-K. Iu, and J. K. Thomas. Photophysical properties of pyrene in zeolites. *J. Phys. Chem.* 93:4120–4128 (1989).
15. T. Fujii, E. Shimizu, and S. Suzuki. Photophysics at solid surfaces. Evidence of dimer formation and polarization of monomer and excimer fluorescences of pyrene in the adsorbed state on silica-gel surfaces. *J. Chem. Soc. Faraday Trans. I* 84:4387–4395 (1988).
16. G. J. Burrell and R. J. Hurtubise. Extended luminescence calibration curves for studying surface interactions in solid-surface luminescence analysis. *Anal. Chem.* 59:965–970 (1987).
17. S. M. Ramasamy, V. P. Senthilnathan, and R. J. Hurtubise. Determination of room-temperature fluorescence and phosphorescence quantum yields for compounds adsorbed on solid surfaces. *Anal. Chem.* 58:612–616 (1986).
18. S. L. Suib and A. Kostapapas. Intermolecular pyrene excimer formation in zeolites. Decay parameters and ground-state association. *J. Am. Chem. Soc.* 106:7705–7710 (1984).
19. R. K. Bauer, R. Borenstein, P. de Mayo, K. Okada, M. Rafalska, W. R. Ware, and K. C. Wu. Surface photochemistry. Translational motion of organic molecules adsorbed on silica gel and its consequence. *J. Am. Chem. Soc.* 104:4635–4644 (1982).
20. A. K. Mishra, M. Sato, H. Hiratsuka, and H. Shizuka. Dual emission from ion pairs produced by excited-state proton transfer in the naphthol-amine system at 77 K. Steric orientation of contact and separated ion pair. *J. Chem. Soc. Faraday Trans.* 87:1311–1319 (1991).
21. A. Matsuyama and H. Baba. Fluorescence and phosphorescence spectra of α-naphthol in relation to hydrogen bonding and proton transfer. *Bull. Chem. Soc. Jpn.* 44:1162–1164 (1971).
22. J. Ferguson. Excited dimer (excimer) luminescence from aromatic molecules in crystalline cyclohexane. *J. Chem. Phys.* 43:306–307 (1965).
23. P. Avis and G. Porter. Effect of concentration on the absorption and fluorescence spectra of pyrene in a solid solution of poly(methyl methacrylate). *J. Chem. Soc. Faraday Trans. 2* 70:1057–1065 (1974).
24. M. S. Wrighton, D. S. Ginley, and D. L. Morse. A technique for the determination of absolute emission quantum yields of powdered samples. *J. Phys. Chem.* 78:2229–2233 (1974).
25. T. Fujii, T. Mabuchi, H. Kitamura, O. Kawauchi, N. Negishi, and M. Anpo. Fluorescence spectra of 1-naphthol during the

sol-gel process of a mixed aluminum-silicon alkoxide (Si:Al = 94:6). *Bull. Chem. Soc. Jpn.* 65:720–727 (1992).

26. A. Villari, N. Micali, M. Fresta, and G. Puglisi. Simultaneous spectrophotometric determination in solid phase of aspirin and its impurity salicylic acid in pharmaceutical formulations. *J. Pharm. Sci.* 81:895–898 (1992).
27. I. B. Berlman. Transient dimer formation by 2,5-diphenyloxazole. *J. Chem. Phys.* 34:1083–1084 (1961).
28. R. A. Agbaria and D. Gill. Extended 2,5-diphenyloxazole-γ-cyclodextrin aggregates emitting 2,5-diphenyloxazole excimer fluorescence. *J. Phys. Chem.* 92:1052–1055 (1988).
29. K. Kinoshita and K. Mihashi. *Photofluorometry, Application for Biological Science,* Gakkai Shuppan Center, Tokyo, 1983.
30. J. Yguerabide and M. Burton. Luminescence decay times: Concentration effects. *J. Chem. Phys.* 37:1757–1774 (1962).
31. W. Saenger. Cyclodextrin inclusion compounds in research and industry. *Angew. Chem. Int. Ed. Engl.* 19:344–362 (1980).