Molecular States of *p*-Dimethylaminobenzonitrile Coground with β-Cyclodextrin Investigated Using Solid-State Fluorescence Spectroscopy

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Changes in molecular states of *p*-dimethylaminobenzonitrile (DMABN) coground with β-cyclodextrin (β-CD) were examined using solid-state fluorescence measurements. Formation of a DMABN/β-CD inclusion complex by coprecipitation was confirmed by powder X-ray diffraction measurement. The powder X-ray diffraction pattern of the ground mixture was a halo pattern and differed from the pattern of the mixture prepared by coprecipitation. Solid-state fluorescence measurements revealed emission by DMABN crystals in a twisted intermolecular charge-transfer state at 473 nm. DMABN in the DMABN/β-CD coprecipitate had a fluorescence emission peak at 393 nm due to its planar structure. In contrast, DMABN in a DMABN/β-CD ground mixture had an emission peak at 473 nm due to its twisted structure. Grinding time-dependent structural changes in DMABN were evaluated using fluorescence lifetime and relative quantum yield measurements. Structural changes in DMABN in the DMABN/β-CD coprecipitate from a planar to a twisted structure were observed with grinding. DMABN, dispersed in microcrystalline cellulose (CC) molecules in a DMABN/CC ground mixture, had a fluorescence emission peak at 473 nm. However, the excitation spectrum of a DMABN/β-CD ground mixture differed from that of DMABN in CC. These results indicated that the molecular state of DMABN accommodated in the β-CD cavity differs between the coprecipitate and the ground mixture.

Key words   solid-state fluorescence; *p*-dimethylaminobenzonitrile; β-cyclodextrin; ground mixture; coprecipitate

Cyclodextrin (CD) molecules with hydrophobic cavities form inclusion complexes with hydrophobic guest molecules.[1] β-CD in particular can include a wide range of guest molecules, so β-CD has widely been utilized in formulations for medicines and food.[2,3] *p*-Dimethylaminobenzonitrile (DMABN) is known as a model probe for determining the mechanisms of inclusion in a solution.[4] DMABN molecules have been reported to adopt two different conformation, planar and twisted, in a solution. The two structures have been reported to lead to different fluorescence emission peaks, with DMABN molecules in a planar structure producing a fluorescence emission peak at a shorter wavelength, and DMABN molecules in a twisted structure in a twisted intramolecular charge-transfer (TICT) state producing a peak at a longer wavelength.[5,6] TICT state emission has been reported to be caused by an electron transfer of the dimethylamino group from a nitrogen atom to the cyano group, or to the entire benzonitrile group, because of the twist.[7] Since DMABN adopts different structures depending on the molecular environment, *e.g.* different CD cavity diameters, and DMABN's molecular state is reflected in the fluorescent spectrum, DMABN is an interesting research probe for intermolecular interactions with CDs.[8] As a probe, DMABN was coprecipitated or coground with β-CD in this study to analyze the solid-state fluorescence emission spectrum of the inclusion complexes, as well as the excitation spectrum, and fluorescence lifetime by time-resolved fluorometry, and to examine the changes in DMABN's molecular state.

Experimental

Materials   *p*-Dimethylaminobenzonitrile (DMABN) was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). β-Cyclodextrin (β-CD) was obtained from Mercian Co., Ltd., Japan. Crystalline cellulose (CC: PH-101) was obtained from Asahi Chemical Industrial Co., Ltd., Japan. β-CD and CC were used after they were dried under reduced pressure at 110 °C for 3 h. All other chemicals used were of reagent grade.

Preparation of a Physical Mixture and a Ground Mixture   A physical mixture of DMABN and β-CD (molar ratio of DMABN/β-CD=1/1) or CC (DMABN content 11.4%) was prepared by kneading in a mortar for 10 s. A ground mixture was prepared by grinding the physical mixture (2.0 g) using a vibrating mill (Heiko Seisakusho, TI200, Japan).

Preparation of Complexes by Coprecipitation   β-CD (2.0×10^{-3} mol) and DMABN (6.0×10^{-3} mol) were dissolved in distilled water and diethyl ether, respectively. The 2 solutions were mixed well. Each solution was slowly cooled to 4.0 °C to allow coprecipitation. The coprecipitate obtained in the aqueous phase was filtered out with filter paper and dried under reduced pressure at 120 °C for 6 h.

Powder X-Ray Diffractometry   Powder X-ray diffraction patterns were measured using a Rigakudenki 2027 diffractometer (Japan). The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 30 kV; current, 15 mA; time constant, 0.5 s; scanning speed, 4 degree/min.

Thermal Analysis   Thermogravimetry-differential thermal analysis (TG-DTA) was carried out using a MAC Science TG-DTA 2000 (Japan) at a heating rate of 5 °C/min under a nitrogen gas flow.

Fluorescence Spectroscopy   An FP-770F fluorescence spectrometer (JASCO Co., Ltd., Japan) was used for stationary fluorescence spectroscopy. Powder samples were placed in a front-facing reflectance cell (FP-1060) for measurement.

Determination of the Fluorescence Lifetime and Time-Resolved Fluorescence Spectra   Measurement of fluorescence decay curves was carried out with a nanosecond time-resolved single-photon counter with a pulse width of 1.5 ns (Horiba NAES-770, Japan). The exciting pulse and emission response functions were measured simultaneously. The decay parameters (two components) were calculated by deconvolution of the excitation pulse profile using nonlinear least-squares fitting. The best fit for observed fluorescence decay curves was obtained by minimizing the value of χ^2.
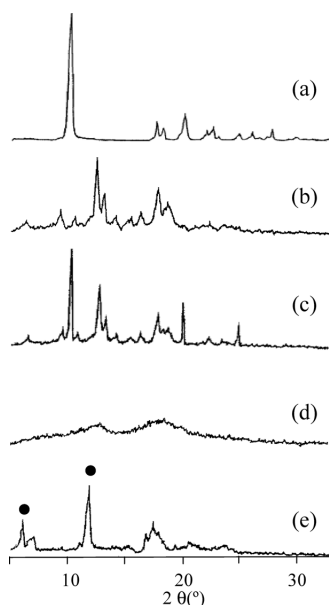
Fig. 1. Powder X-Ray Diffraction Patterns of DMABN/β-CD Systems during Grinding

(a) Unprocessed DMABN, (b) β-CD, (c) physical mixture, (d) ground mixture, and (e) coprecipitate.

Results and Discussion

Grinding and Inclusion Formation Figure 1 shows the powder X-ray diffraction patterns of the physical mixture (PM), ground mixture, and coprecipitate of β-CD and DMABN (DMABN/CD molar ratio=1/1). In the PM, the diffraction peaks due to the β-CD and DMABN crystals were observed, indicating that β-CD and DMABN existed in the crystalline state (Figs. 1a—c). The powder X-ray diffraction pattern of the DMABN/β-CD ground mixture (Fig. 1d) was a halo. The diffraction pattern of the coprecipitate had new diffraction peaks at $2\theta=5.6°$ and $11.4°$ (Fig. 1e) and differed from that of DMABN or β-CD crystals. The diffraction pattern of the coprecipitate was consistent with that of the $1:1$ inclusion complex with a cylindrical structure.[9] The current authors previously studied inclusion complexes as a result of freeze-drying β-CD and DMABN. The freeze-dried mixture of β-CD and DMABN was found to produce powder X-ray diffraction peaks at $2\theta=7.4°$ and $12.8°$, which are typical for β-CD inclusion complexes with a cylindrical structure.[10]

Guest molecules included in the CD cavity are known to sublimate at a higher temperature than the guest compound crystals.[11] TG measurements were conducted to investigate the sublimation behaviors of DMABN from a DMABN/β-CD ground mixture (DMABN content 11.4%: equivalent to a DMABN/β-CD molar ratio of 1/1). As shown in Fig. 2, PM (DMABN/β-CD molar ratio=1/1) started its weight loss due to the sublimation of DMABN at 80 °C, which is similar to the DMABN crystals (Fig. 2a). In contrast, the ground mixture (Fig. 2b) had 11.4% weight loss starting at 160 °C, indicating that DMABN sublimation was restrained through the co-grinding process. The ground mixture of DMABN and CC had powder X-ray diffraction with a halo pattern, like that of a β-CD ground mixture, but the ground mixture of DMABN and CC (DMABN content 11.4%) had weight loss starting at 80 °C (Fig. 2d). CC consists of glucose units but

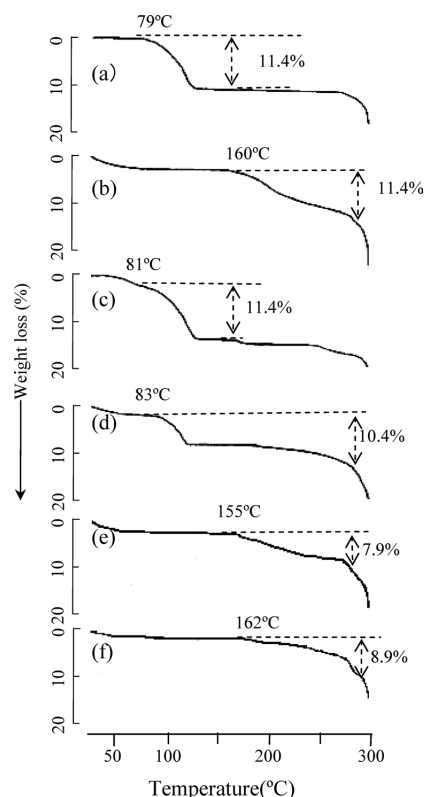Fig. 2. Thermogravimetry Curves of DMABN/β-CD and DMABN/CC Systems

(a) DMABN/β-CD PM, (b) sample (a) ground for 15 min, (c) DMABN/CC PM (DMABN content: 11.4%), (d) sample (c) ground for 15 min, (e) DMABN/β-CD coprecipitate, and (f) sample (e) ground for 15 min. Heating rate: 5 °C/min.

has no intramolecular cavity, preventing it from suppressing DMABN sublimation from the ground mixture with CC. Figures 2e and f show the TG curves of the coprecipitate and the ground samples. Both specimens started with weight loss at approximately 160 °C. Since sublimation onset temperature coincided with that of the DMABN/β-CD ground mixture, one could conclude that DMABN was included in the β-CD cavity as a result of grinding. This suggests that DMABN molecules were included in the β-CD cavity in the ground mixture.

Solid-State Fluorescence Emission Spectra In solution, DMABN is known to have an emission peak at 393 nm, derived from the "planar structure" in which the dimethylamino group and benzene ring are co-planar, and a peak at 473 nm, derived from the "twisted intramolecular charge transfer (TICT) state" in which bonding of the dimethylamino group and benzene ring is twisted 90°.[5] Figure 3 shows the fluorescence emission spectra of a DMABN/β-CD system. Solid-state fluorescence measurements revealed emission by DMABN in a TICT at 473 nm. As the grinding time increased, the ground mixtures had an increase in the peak intensity of TICT state emission at around 473 nm and decrease in the peak intensity of planar state emission at around 393 nm. This indicates that cogrinding of DMABN and β-CD produced DMABN molecules with more of a twisted than planar structure. In contrast, the coprecipitate had a fluorescence emission peak at 397 nm due to its planar structure, indicating that the molecular states of DMABN varied for specimens prepared by cogrinding and coprecipita-
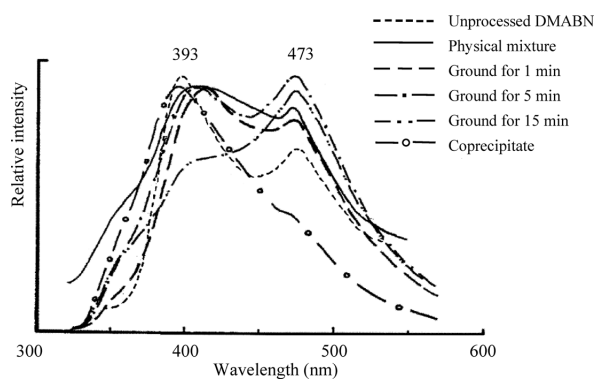
Fig. 3. Changes in Emission Spectra of DMABN/β-CD Systems during Grinding (λ_{ex}=290 nm)

Table 1. Changes in Fluorescence Lifetime (τ) and Relative Quantum Yield (Q) of DMABN in DMABN/β-CD Systems (λ_{ex}=313.5 nm, λ_{obs}=473 nm)

	Comp. **1** (LE)		Comp. **2** (TICT)		χ^2
	τ_1 (ns)	Q_1 (%)	τ_2 (ns)	Q_2 (%)	
DMABN crystals	0.27	69.2	5.52	30.8	1.26
Ground for 1 min	1.24	50.0	5.07	50.0	1.51
Ground for 5 min	1.42	46.9	4.9	53.1	1.40
Ground for 15 min	1.36	35.0	4.52	65.0	1.52
Coprecipitate	1.47	60.5	4.34	39.5	1.35

Molar ratio of DMABN/β-CD=1/1.

Fig. 4. Effect of Grinding on Emission Spectra of DMABN/β-CD and DMABN/CC Systems (λ_{ex}=290 nm)

(a) Unprocessed DMABN, (b) sample (a) after grinding for 15 min, (c) DMABN/β-CD PM, (d) sample (c) after grinding for 15 min, (e) coprecipitate, (f) sample (e) after grinding for 15 min, (g) physical mixture with CC, and (h) sample (g) after grinding for 15 min.

tion. DMABN is reported to adopt a planar structure in β-CD cavities when in a solution.[12] This indicates that DMABN in the coprecipitate with β-CD might have a structure similar to that of the DMABN inclusion complex in solution.

Table 1 shows the fluorescence lifetimes and relative quantum yields of the DMABN/β-CD coprecipitate and mixtures ground for 1 min, 5 min, and 15 min. Specimens were found to have short-lived components with a fluorescence lifetime of about 1 ns and long-lived components with a lifetime of about 5 ns. Focusing on changes in the fluorescence lifetimes of components and the relative quantum yield due to grinding indicated that the quantum yield of Comp. **2**, which had long-lived components, gradually increased with grinding. This finding indicates that short-lived components are due to the planar structure of DMABN while long-lived components are due to the twisted structure of DMABN. Prolongation of grinding time increased in the relative quantum yield of long-lived components due to the twisted structure. Relative quantum yield of long-lived components was *ca.* 65% in the mixture ground for 15 min. This indicated that cogrinding of DMABN and β-CD increased the proportion of molecules with a twisted structure. The planar structure of the coprecipitate led to a relative quantum yield of short-lived components that reached 60%, indicating that DMABN in the ground mixture has a different molecular state than DMABN in the inclusion complex.

In order to examine the changes in the molecular state of DMABN during the co-grinding process, the coprecipitate was ground for 15 min and then its fluorescence emission spectrum was compared to that of the ground mixture (Fig. 4). TICT emissions of DMABN and DMABN after grinding
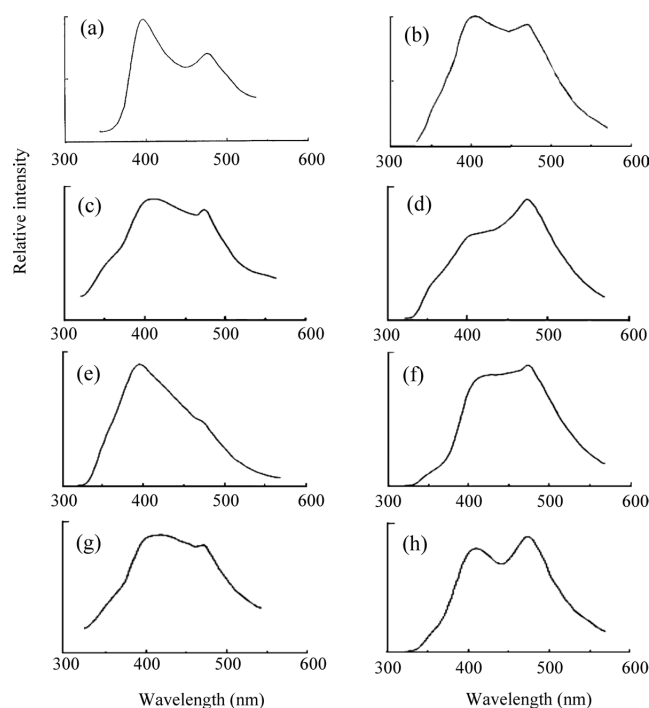
for 15 min were noted at 473 nm. While the coprecipitate had an emission peak at 393 nm, due to the planar structure of DMABN (Fig. 4e), grinding caused the position of the peak to vary to 473 nm due to the twisted structure of DMABN inside β-CD cavities (Fig. 4f). These results coincided with those for the emission peaks of the fluorescence emission spectrum of the ground mixture (Fig. 4d). The inclusion complex obtained by coprecipitation was in a crystalline state, while the inclusion complex obtained by cogrinding was in an amorphous state where CD molecules were expected to be randomly oriented. The difference in the crystalline state of CD is, therefore, presumed to have greatly affected the conformational state of the included DMABN. Figure 4f shows the emission spectrum of the ground mixture of CC and DMABN. The CC ground mixture had emission peaks at around 400 nm and around 473 nm.

Fluorescence excitation spectra at observed wavelengths of 393 nm and 473 nm were measured for the DMABN/β-CD physical mixture, the DMABN/β-CD mixture ground for 15 min, the ground coprecipitate, and the DMABN/CC ground mixture (Figs. 5, 6). The excitation spectrum changed at both of the observed wavelengths as a result of the grinding process. The ground precipitate had a spectrum similar to that of the ground mixture, indicating that DMABN molecules in ground coprecipitates and ground mixtures had more of a twisted than planar structure inside β-CD cavities. The fluorescence excitation spectrum of the DMABN/CC ground mixture differed from that of the DMABN/β-CD mixture.

The molecular state of DMABN molecules included in β-CD cavities differs from that of DMABN in a monomolecular dispersion or in a nanocrystalline state in the hydrogen-
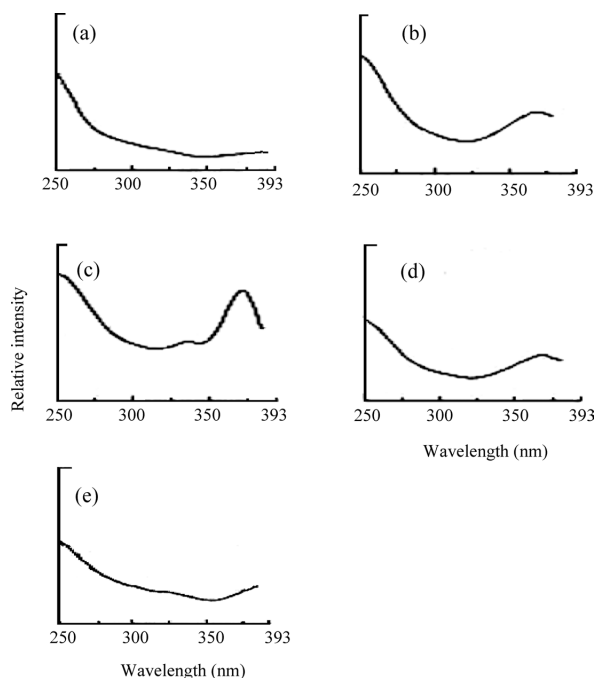
Fig. 5. Changes in Excitation Spectra of DMABN after Grinding for 15 min (λ_{obs}=393 nm)

(a) DMABN/β-CD PM, (b) sample (a) after grinding for 15 min, (c) DMABN/β-CD coprecipitate, (d) sample (c) after grinding for 15 min, and (e) DMABN/CC GM.
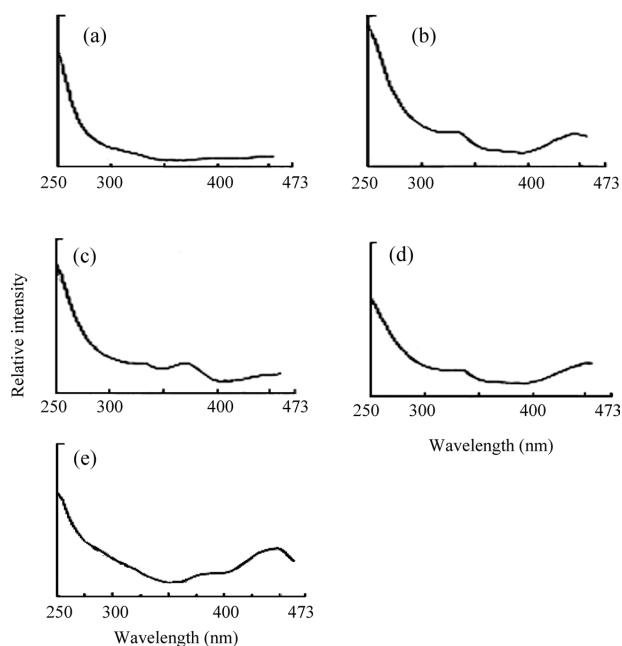

Fig. 6. Changes in Excitation Spectra of DMABN after Grinding for 15 min (λ_{obs}=473 nm)

(a) DMABN/β-CD PM, (b) sample (a) after grinding for 15 min, (c) coprecipitate, (d) sample (c) after grinding for 15 min, and (e) DMABN/CC GM.

bonding network of CC. Powder X-ray diffraction revealed that the inclusion complex obtained by coprecipitation was in a crystalline state with regular arrangement of CD. In contrast, the inclusion complex obtained by co-grinding was in a

non-crystalline state in which CD molecules were randomly oriented. Results in terms of the quantum yield during fluorescence emission also indicate that most DMABN molecules in a ground mixture have a twisted structure in β-CD cavities. In a ground mixture, the molecular state of DMABN molecules is disrupted, so most DMABN molecules assume a TICT state. TICT emissions are known to be significantly affected by the polarity in the cavity.[13—15] The observation of enhanced TICT emissions indicates that in the complex the DMABN molecule does not remain totally inside the hydrophobic cavity. Therefore, the molecule might be located partially inside the cavity but also project outside, where the molecule is in a polar environment. The DMABN molecule is completely included in the complex prepared by coprecipitation while the guest molecule is located in a hydrophobic environment. In the co-grinding complex, however, the DMABN is barely included in the complex, where it is deposited around the hydrophilic hydroxyl groups of β-CD, while the guest molecule is in many different inclusion modes. This indicates that enhancement of the nonpolar and TICT emissions of DMABN in the presence of β-CD are due to different sets of molecules. The molecular state of DMABN in a complex could be significantly affected by host CD molecules in a crystalline state.

In conclusion, cogrinding of DMABN and β-CD produced an inclusion complex. DMABN molecules had a planar structure when a DMABN/β-CD inclusion complex was prepared by coprecipitation. In contrast, they mainly had a twisted structure in an inclusion complex that was coground, indicating that different methods of preparation lead to different conformational states of DMABN in CD cavities.

References

1) Frömming K.-H., Szejtli J., "Cyclodextrins in Pharmacy," Kluwer Academic Publishers, Dordrecht, 1994.
2) Hashizaki K., Kageyama T., Inoue M., Taguchi H., Ueda H., Saito Y., *Chem. Pharm. Bull.*, **55**, 1620—1625 (2007).
3) Inoue M., Hashizaki K., Taguchi H., Saito Y., *Chem. Pharm. Bull.*, **56**, 668—671 (2008).
4) Hara K., Rettig W., *J. Phys. Chem.*, **96**, 8307—8309 (1992).
5) Salgado F. P., Herbich J., Kunst A. G. M., Rettschnick R. P. H., *J. Phys. Chem.*, **103**, 3184—3192 (1999).
6) Grabowski Z. R., Rotkiewicz K., Rettig W., *Chem. Rev.*, **103**, 3899—4032 (2003).
7) Leinhos U., Kuhnle W., Zachariasse K. A., *J. Phys. Chem.*, **95**, 2013—2021 (1991).
8) Santra S., Zhang P., Tan W. H., *J. Phys. Chem.*, **104**, 12021—12028 (2000).
9) Ohmura M., Kawahara Y., Okude K., Hasegawa Y., Hayashida M., Kurimoto R., Kawaguchi A., *Polymer*, **45**, 6967—6975 (2004).
10) Inoue Y., Ii N., Yonemochi E., Oguchi T., Higashi K., Moribe K., Yamamoto K., *Yakugaku Zasshi*, **129**, 253—262 (2009).
11) Kawashima H., Yonemochi E., Oguchi T., Yamamoto K., *J. Chem. Soc., Faraday Trans.*, **90**, 3117—3119 (1994).
12) Monti S., Bortolus P., Manoli F., Marconi G., Grabner G., Köhler G., Mayer B., Boszczyk W., Rotkiewicz K., *Photochem. Photobiol. Sci.*, **2**, 203—211 (2003).
13) Nag A., Bhattacharyya K., *Chem. Phys. Lett.*, **151**, 474—476 (1988).
14) Nag A., Dutta R., Chattopadhyay N., Bhattacharyya K., *Chem. Phys. Lett.*, **157**, 83—86 (1989).
15) Nag A., Kundu T., Bhattacharyya K., *Chem. Phys. Lett.*, **160**, 257—260 (1989).