Toho University, Faculty of Science,
Department of Environmental Science
Master Thesis in 2011

Occurrence and Mechanism of Fluorescence Enhancement of Polycyclic Aromatic Ketones

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＜要旨＞

ナフトアントロン（NT）などの多環芳香族ケトンの蛍光は一般的に微弱である。しかし、その溶液を脱気し室温下で予め光照射しておく（予備照射）と、蛍光強度が約 100 倍増大し、スペクトル形も変化する。この蛍光増強現象は光励起された溶質分子と溶媒分子が会合体を形成し、その会合体が強い蛍光を発すると考えられている。しかし、その根拠は分光学データに限られているため、会合体形成の直接的な実験データは得られていない。本研究では、まずいくかの芳香族ケトンについて種々の条件下で蛍光測定を行い、蛍光増強現象を詳しく調べた。その結果、多くの実験データが会合体モデルで説明できることを確認した。次に、蛍光増強を誘起させた溶液を高速液体クロマトグラフィー（HPLC）で分析して会合体の存在を実証した。さらに、その会合体の質量を飛行時間型質量分析（TOF-MS）により決定した。

蛍光増強を起こす分子は、本来の蛍光が大変微弱な燐光性物質であった。つまり、蛍光増強は溶質分子の励起三重項状態を経由して起こる反応であると考えられる。また、一定強度以上の予備照射をしないと、蛍光増強は起こらなかった。さらに、溶媒中の水素を重水素化することで、蛍光増強は起こりにくくなった。このことより、蛍光増強は水素引き抜き反応に類似した反応であることが分かった。NT エタノール溶液の濃度を薄くすると、増強蛍光スペクトルはより短波長側にシフトし、その振動構造は明瞭になった。これらのことは会合体が周囲の溶媒分子にパッキングされていることを示唆している。

蛍光増強は 77K の NT エタノール溶液においても観測された。NT エタノール溶液の蛍光は室温ではブロードなスペクトルを示す。しかし、この NT エタノール溶液を 77K に冷却したまま予備照射をすることで蛍光強度が増大し、その試料を室温に戻したときの蛍光スペクトルには冷却時に現れた振動構造がそのまま残る。これらのことは、強い光照射で

NT と溶媒分子との会合体が形成され、それが低温で強く溶媒和し、その状態が室温下でも保持されることを示唆している。

逆相分配 HPLC を使用し、会合体の存在を明らかにした。蛍光増強を起こしていない NT メタノール溶液のクロマトグラムには保持時間 t = 4.5 min に NT 由来のピークが現れた。これに対して、蛍光増強を起こした溶液のクロマトグラムには新たなピークが保持時間 t = 2.3 および 6.7 min に現れた。予備照射時間増加とともに、すなわち蛍光増強が増大するにつれて、NT 由来のピークの強度は減少し、新たなピークのうち保持時間 6.7 min のピークの強度は増大した。また、このピークの保持時間は NT よりも長いので、会合体の極性は NT よりも小さいことが分かった。このことは会合体モデルとも合致する。

次に、TOF-MS により会合体の質量を測定した。蛍光増強を起こしていない NT メタノール溶液の質量スペクトルには NT 由来のピークが m/z = 255 に現れた。同溶液を脱気し予備照射をすることで蛍光増強を誘起させた溶液の質量スペクトルには新たな 2 つのピークが現れた。その 2 つのピークは m/z =239 と 368 に現れ、それぞれ 3 価イオン $[NT+H+2Na+13MeOH]^{3+}$ に、2 価イオン$[NT+2H+15MeOH]^{2+}$に帰属される。つまり、13 個または 15 個の溶媒分子は NT の周りに集まった会合体を考えることができる。以上より、蛍光増強を引き起こす会合体の分子数を初めて明らかにすることができた。

今まで分光学データより考察していた、会合体の存在とその質量を HPLC と TOF-MS による分析でより明確にすることができた。しかし、それらの分子が実際どういった構造を取っているかはまだ不明である。今後、分子軌道法計算を使用して妥当な構造を見つけ、さらなる蛍光増強現象の機構解明につなげていく。将来的には、この蛍光増強現象をディーゼル排ガス中の変異原性物質であるニトロ化多環芳香族ケトンの検出に役立てたい。

＜Abstract＞

The fluorescence intensity for degassed solution of polycyclic aromatic ketones, such as naphthanthrone (NT), is enhanced after pre-irradiation with strong light. This fluorescence enhancement has been considered to arise from association of the solute with the solvent. However, no chemical species formed by such association have experimentally been identified yet.

In the present study, we have studied the following subject: (1) spectroscopic studies on the fluorescence enhancement phenomena for several polycyclic aromatic ketones, (2) spectroscopic studies on the fluorescence enhancement of NT under various conditions, (3) analyses for chemical species causing the fluorescence enhancement in methanol solution of NT by high performance liquid chromatography (HPLC), and (4) identification of chemical species causing the fluorescence enhancement in methanol solution of NT by time of flight mass spectrometry (TOF-MS).

We have obtained following results: (1) The fluorescence enhancement is a general phenomenon for phosphorescent aromatic molecules and arises mainly from a reaction via the triplet state of the solute. (2) The fluorescence enhancement of NT depends strongly on the intensity of irradiation and there is a light-intensity threshold for the occurrence of the enhancement. Deuteration of solvents reduced the

fluorescence enhancement. The intensity, blue-shift, and shape of the enhanced fluorescence depended significantly on the concentration of solution. These findings led to modification of the former association mechanism of the fluorescence enhancement; the association proceeds in a solvent cage step-wisely. (3) The HPLC analysis for the solution that had exhibited the fluorescence enhancement revealed that a new peak appears at a retention time longer than that of the solute. The new peak was assigned to the association complex desired and the complex is less polar than the solute. (4) Mass spectra for the solution that had exhibited the fluorescence enhancement showed new peaks in addition to the NT peak and they were assigned to an association complex surrounding one NT molecule with thirteen and with fifteen solvent molecules. Thus the existence and chemical composition of the association complex, causing fluorescence enhancement, have been confirmed for the first time.

In future, the fluorescence enhancement may be applied to spectroscopic methods for the environmental analysis. A new analytic system using the fluorescence enhancement should be useful for detection of nitrated polycyclic aromatic ketones such as 3-nitro-benzanthrone, which are included in the exhaust gas and strongly mutagenic and carcinogenic.

＜Contents＞

Introduction

Aromatic ketones show several specific photochemical reactions such as hydrogen and electron abstraction, photocycloaddition, and photorearrangement [1]. This is because the compounds possess four lower-lying excited states, $^3n\pi^*$, $^1n\pi^*$, $^3\pi\pi^*$, and $^1\pi\pi^*$ in a simple-molecular-orbital denotation, and they interact with one another, causing the specific reactions. So far the photochemistry of small aromatic ketones such as benzophenone and acetonaphthone has been studied extensively and their various photochemical properties have been clarified and summarized [1]. In contrast, the photochemistry of polycyclic aromatic ketones, having more than two benzene rings, has barely been studied, because their synthesis and purification are difficult [2].

In 1997, nitrobenzoanthrones, nitrated ketones with four benzene rings, were reported to be strong mutagens, namely, chemical substances that damage or alter DNA in cells [3]. In particular 3-nitro-7*H*-benzo[*de*]anthracene-7-one, detected in diesel exhaust, showed the highest mutagenic activity among the known mutagenic mononitro-polycyclic aromatic compounds from environmental source. Since then nitrated polycyclic aromatic ketones (NPAKs) have received a considerable amount of attention because of their mutagenic activity and wide occurrence in the air; they are abundant in exhaust gas and particles from vehicle engines and in smoke and soot from burning wood. Accordingly it is important to measure the concentration of NPAKs in the environment and to provide the exposure information to the society. Fluorescence spectroscopy is a convenient and high-sensitive method to detect fluorescent compounds, and indeed has been widely used to detect polycyclic aromatic compounds. The fluorescence, however, of polycyclic aromatic ketones (PAKs) and NPAKs is generally so weak because their fluorescence is due to a forbidden transition, *i.e.*, an

nπ* transition [4]. Consequently fluorescence spectroscopy has not been applied to detect them, so far.

Recently it was found that the fluorescence of a degassed solution of naphthanthrone (NT), a ketone with five benzene rings, was enhanced about one hundredfold after preliminary irradiation of the solution with strong light for half one hour [5]. This fluorescence enhancement was considered to arise from association of NT with a solvent molecule, but its detailed mechanism remains unknown. From the viewpoint of environmental analysis, this phenomenon gives the promise of increasing detection efficiency of PAKs and NPAKs by fluorescence spectroscopy. To examine the applicability of fluorescence enhancement to environmental analysis, one needs detailed data on the phenomenon.

In the present study we firstly investigated the occurrence of the phenomenon for five polycyclic aromatic compounds except NT, 2-nitro-napthanthrone (2-NNT), benzanthrone (BA), 3-nitro-benzanthrone (3-NBA), anthracene, and perylene. The three ketones, 2-NNT, BA, and 3-NBA, exhibited the fluorescence enhancement, but, anthracene and perylene did not. We deduced that the fluorescence enhancement is a general phenomenon occurring in PAKs and NPAKs. Then we focused our attention to NT in alcohols and clarified the spectroscopic features of the fluorescence enhancement. We measured the effects of irradiation time, concentration, temperature, and deuteration of the solvents. The experimental data supported the association model for the fluorescence enhancement. Finally we tried to detect the chemical species causing the enhancement. We applied high performance liquid chromatography and time-of-flight mass spectrometry to an methanol solution of NT that had exhibited the fluorescence enhancement. The analyses of the data permitted us to identify the chemical species.

2. Experimental

2-1. Sample preparation

As solutes, we used four polycyclic aromatic ketones (PAKs), benzanthrone, naphthanthrone, 3-nitro-benzanthrone, and 2-nitro-naphthanthrone. Anthracene and perylene were also used for comparison. The PAKs were synthesized and purified in the Organic-Chemistry-II laboratory of Toho University. As solvents we used methanol, ethanol and their deuterated compounds, C_2H_5OD, CH_3OD, and CD_3OD. Non-deuterated solvents were purchased from Koso Chemical, Tokyo and the deuterated ones from Wako Pure Chemical Industries, Osaka. All solvents were used without further purification. The concentration of sample solutions were in the range from $1x10^{-6}$ to $1x10^{-4}$ M.

In the procedure of dissolving solutes into solvents, an ultrasonic cleaning device was utilized for quickening dissolution and degassing the solution. Such treatment has been widely used in HPLC to remove oxygen dissolved in the solution. We found that the treatment is very useful for preliminary degassing so that we could treat a large amount of solvents with the device.

Regular degassing of solutions was carried out as follows: (1) a solution was poured into a specially designed cell (see Figure 1), (2) the cell was connected to a glass vacuum line, and (3) the solution was degassed by the freeze-pump-thaw cycle method in the dark (see Figure 2). We took about one and half minutes to freeze the solution by use of liquid nitrogen and about half minutes to thaw the frozen solution with a blower. This cycle was repeated twice.

Fig. 1 *Specially designed cell for degas*

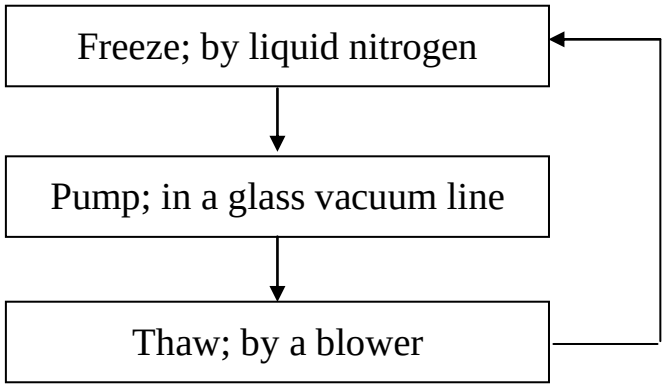


Fig. 2 *Flow chart of the degas cycle*

2-2. Measurements of enhanced fluorescence

Figure 3 shows the procedure for enhanced fluorescence measurements. A sample solution, prepared with the procedure described in the preceding section 2-1, was irradiated with intense light at wavelengths of the solutes, maximum absorption. Then fluorescence spectra were measured every 10 minutes. The irradiation and measurement processes were repeated.

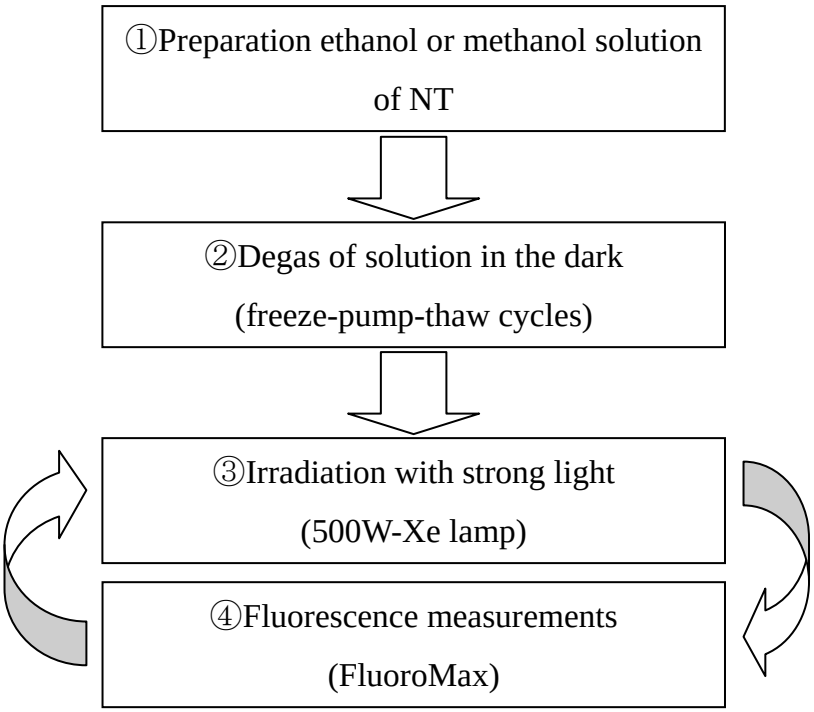


Fig. 3 *Flow chart for the measurement of enhanced fluorescence*

We used a fluorescence spectrometer, a FluoroMax spectrometer (Spex Industries, Edison, NJ, USA) for fluorescence and phosphorescence measurements. The conditions of fluorescence measurements are summarized in Table 1.

At 77K, we soaked a degassed solution in a cell in liquid nitrogen in a dewar with windows, and then performed the irradiation and fluorescence measurements

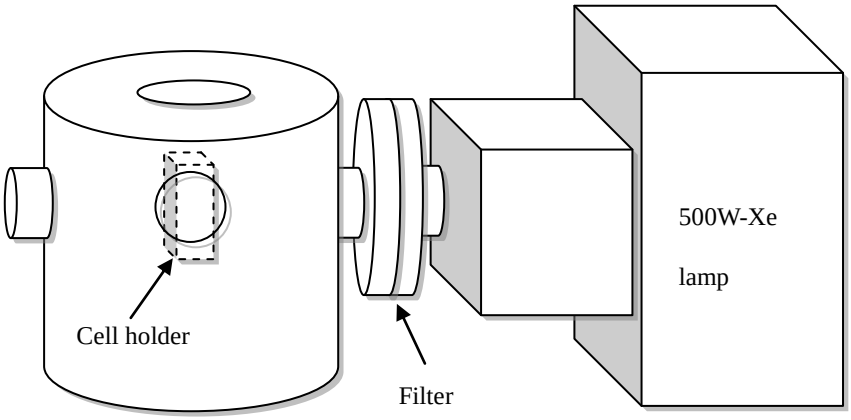
Table 1 Conditions of fluorescence measurement.

	BA	NT	3-NBA	2-NNT	Perylene	Anthracene
Excitation Wavelength	300nm	360nm	300nm	360nm	380nm	345nm
Wavelength region of fluorescence	320-590nm	370-630nm	320-590nm	370-630nm	400-600nm	365-515nm
Slit width	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm	0.5mm
Pre-irradiation wavelength	400nm	400nm	400nm	400nm	400nm	400nm

2-3. Preliminary irradiation

Two lamps were used for preliminary irradiation; the light-source 150W-Xe lamp of the FluoroMax fluorescence-spectrometer and a 500W-Xe lamp. In the use of the FluoroMax's light-source, the excitation spectrometer was positioned at the wavelength, where the absorption of the sample was maximized, and two slits on the excitation spectrometer were set to 7 mm, which corresponded to a band pass of 20 nm. To prevent scattered light from entering into the emission spectrometer, we adjusted two slits of it as narrowly as possible.

In the use of the 500W-Xe lamp (see Figure 4), an L-39 sharp-cut-filter was used. The filter prevents the samples from dissociating by light with shorter wavelengths.



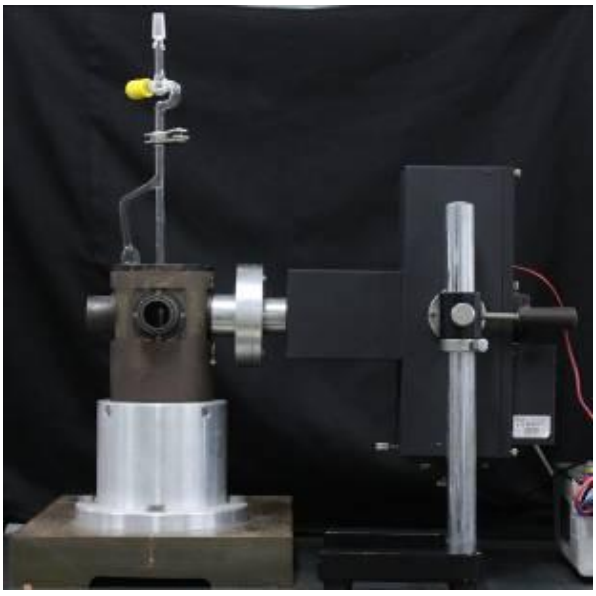


Fig. 4 *Schematic (upper) and picture (lower) of set up of the 500W-Xe lamp irradiation*

In fluorescence measurements, the slits of both excitation and emission spectrometers were adjusted so as that the band passes became 0.5 nm.

2-4 High performance liquid chromatography (HPLC) analysis

The analysis of chemical components of a solution was conducted on a high performance liquid chromatography (HPLC, Agilent 1200 series; Agilent Technologies, Santa Clera, United State, Figures 5 and 6) apparatus equipped with a photo-diode-array detector. Two solutions, before and after irradiation with strong light, were analyzed. After irradiation the solution exhibited the fluorescence enhancement. For separation a C18 column (ZORBAX Eclipse PAH; Agilent Technologies, Tokyo, Japan) was used and for a carrier solvent HPLC-grade methanol was used.

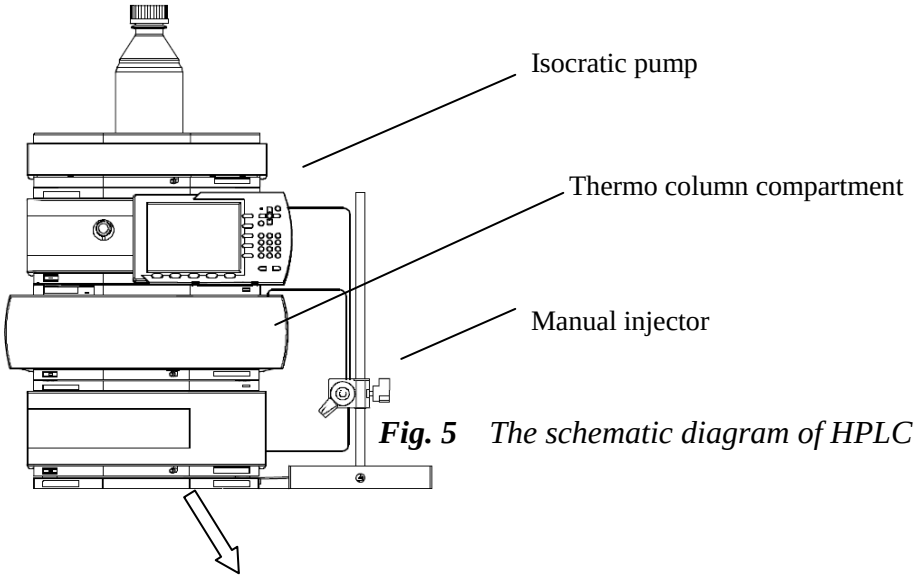


Fig. 5 *The schematic diagram of HPLC*

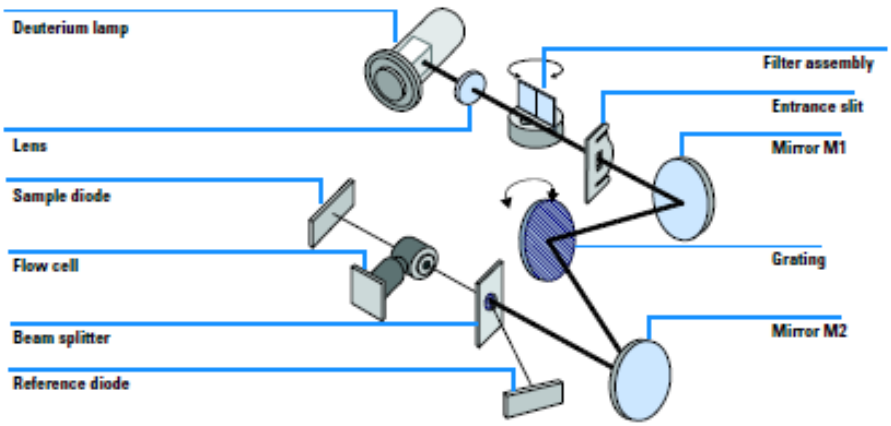


Fig. 6 *Optical Path of the Variable Wavelength Detector*

2-5 Time-of-flight mass spectrometric (TOF-MS) analysis

The analysis of chemical components of a solution was conducted on a time-of-flight mass spectrometer (JMS-T100LC Accu TOF; JEOL, Tokyo, Japan, Figure 7). The principle of TOF-MS is as follows. The kinetic energy of ion is written by

$$(\frac{1}{2})mv^2 = eV_a,$$

where *e* is elementary charge, Va is acceleration voltage, *m* is mass, *v* is velocity. If the flight distance is defined by L, the flight time is expressed by

$$t = \frac{L}{\sqrt{2eV_a}}\sqrt{m}.$$

Then, from the flight time t, one can determine mass of ion.

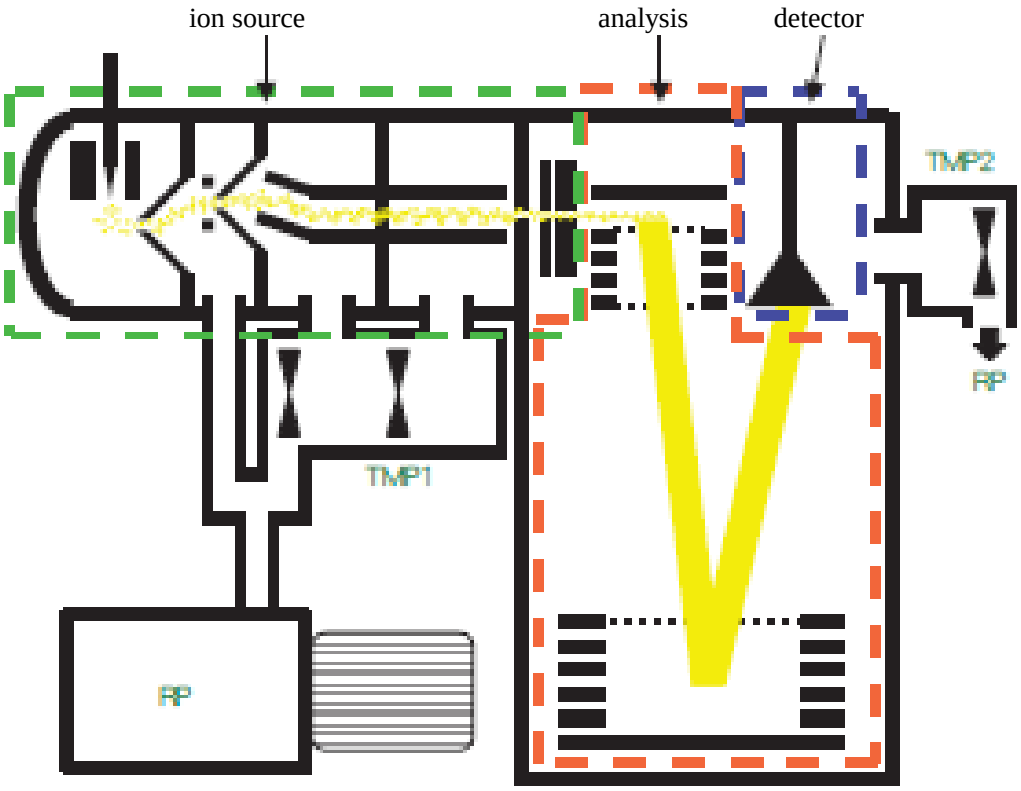


Fig. 7 *The schematic diagram of TOF-MS*

The conditions for the TOF-MS analysis are listed in Table 2.

TABLE 2 Conditions of the TOF-MS analysis

Concentration of sample		$1x10^{-5}$M	$1x10^{-6}$M
ion source [V]	needle	2000	2000
	ring lens	22	33
	orifice 1	101	105
	orifice 2	22	15
detection [V]	focus	-120	-120
	focusing lens	10	10
	quadrupole lens	20	20

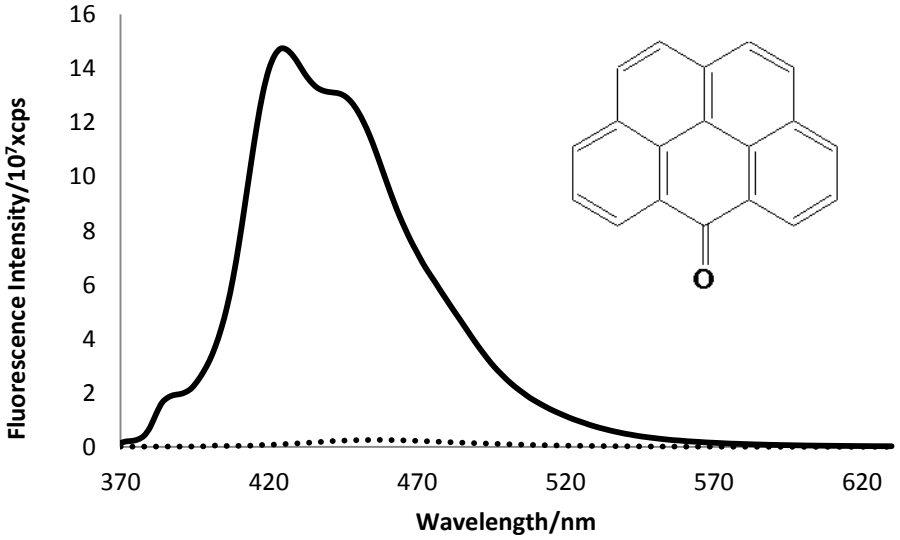
3. Spectroscopic study on fluorescence enhancement

3-1 Experimental Results

3-1-1 Fluorescence enhancement of polycyclic aromatic compounds

Figures 8 – 13 show fluorescence spectra of six aromatic compounds before (represented by dotted lines) and after (solid lines) strong light irradiation. It is clearly seen from the Figures 8-11 that NT, 2-NNT, BA, and 3-NBA exhibit the fluorescence enhancement, though the degree of enhancement largely depends on molecules. In contrast to this, perylene exhibits no changes in fluorescence on irradiation and anthracene shows no increase but reduction in the fluorescence intensity. The decrease in fluorescence of anthracene is due to the formation of anthracene dimers [7], which was confirmed by a strong absorption band newly appearing around 270 nm on irradiation (not shown in the figure).

Fig. 8 *Fluorescence and enhanced fluorescence spectrum of NT. Dotted and solid*



lines are before and after irradiation, respectively.

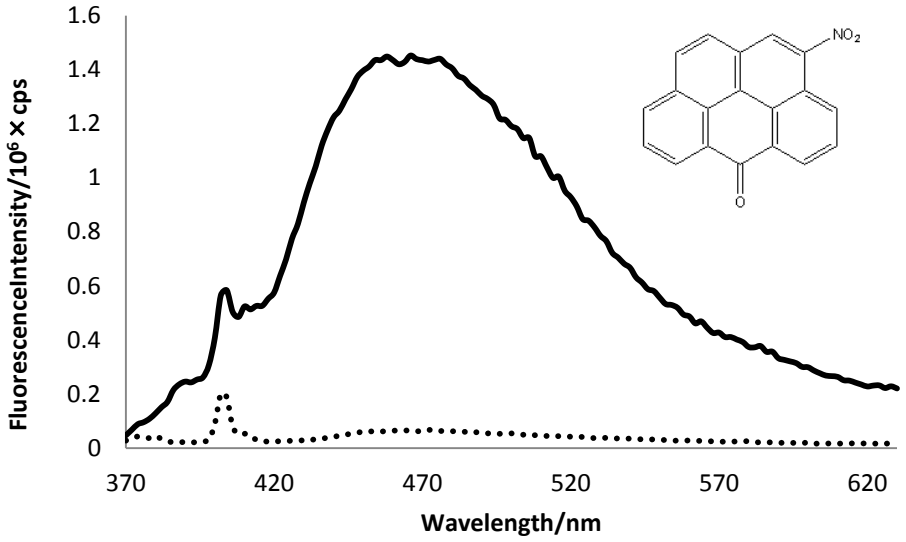


Fig. 9 *Fluorescence and enhanced fluorescence spectrum of 2-NNT. Dotted and solid lines are before and after irradiation, respectively*

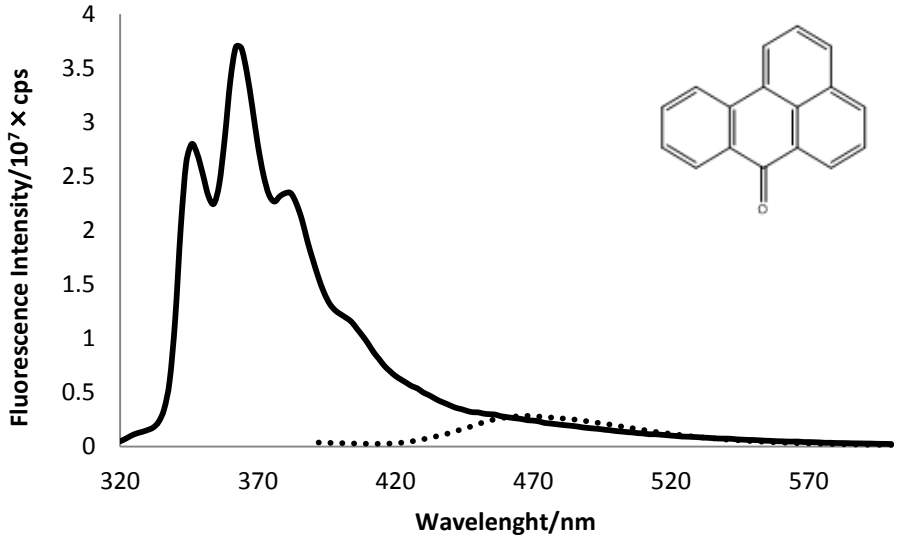


Fig.10 *Fluorescence and enhanced fluorescence spectrum of BA. Dotted and solid lines are before and after irradiation, respectively*

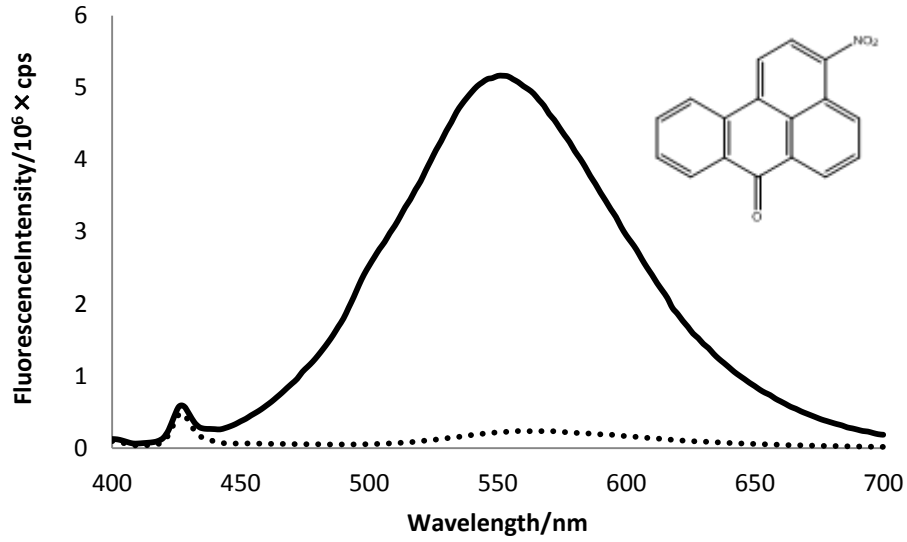


Fig. 11 *Fluorescence and enhanced fluorescence spectrum of 3-NBA. Dotted and solid lines are before and after irradiation, respectively*

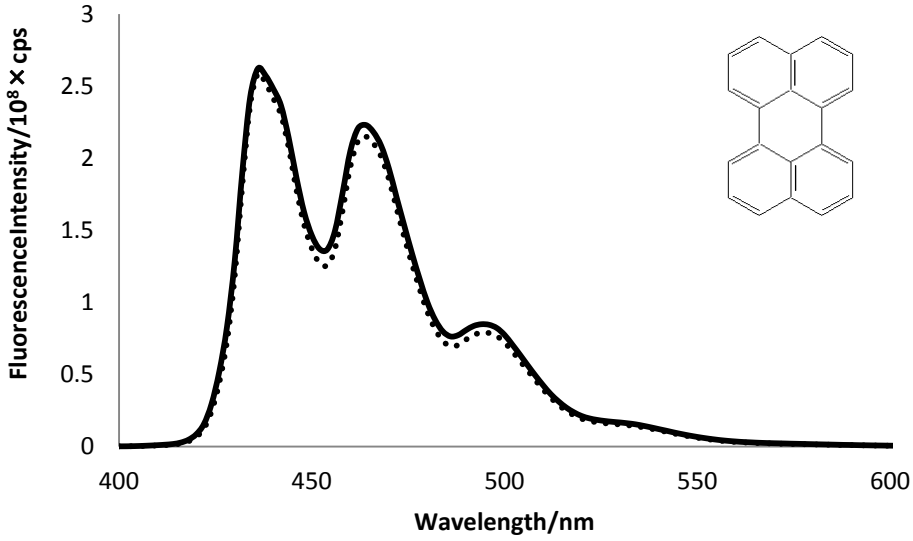


Fig. 12 *Fluorescence and enhanced fluorescence spectrum of perylene. Dotted and solid lines are before and after irradiation, respectively*

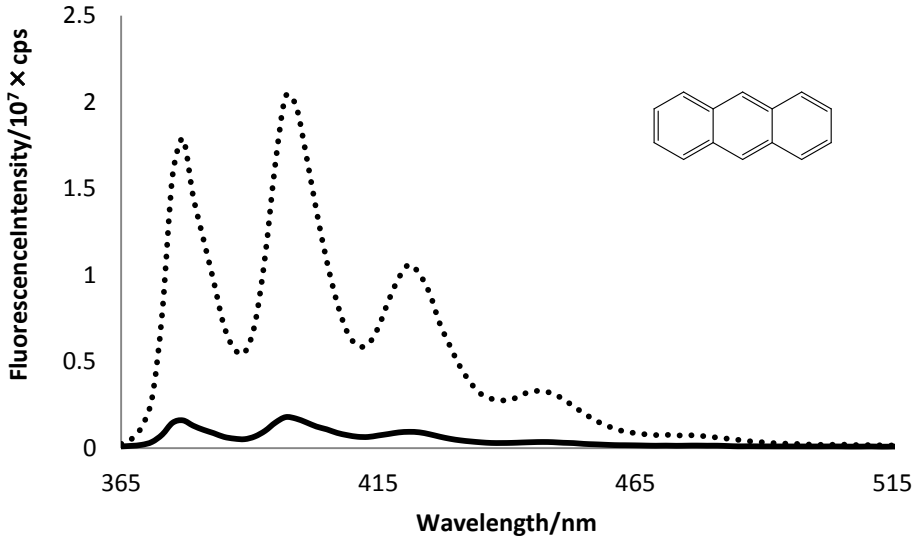


Fig. 13 *Fluorescence and enhanced fluorescence spectrum of anthracence. Dotted and solid lines are before and after irradiation, respectively*

Molecules exhibiting the fluorescence enhancement provide very weak fluorescence before irradiation and can be referred to as "non-fluorescent" molecules. On the other hand, the two molecules exhibiting no enhancement provide originally strong fluorescence; they are "fluorescent" molecules. In fact the fluorescence quantum yield of the "non-fluorescent" molecules is very small and ranges $10^{-5} – 10^{-2}$ [8]; and the yield of the two "fluorescent" molecules is 0.28 for anthracene and 0.98 for perylene [10]. The "non-fluorescent" molecules are phosphorescent and their phosphorescence intensity is comparable with their fluorescence one at 77 K [8, 9]. In these molecules, excited triplet states are considered highly populated through intersystem crossing from their excited singlet states produced on strong irradiation and they provide phosphorescence at liquid nitrogen temperature [11]. The triplet states also occur even at room temperature, though no phosphorescence is emitted. They are so long-lived that most of them are quenched by collisions with solvent molecules

before emission [11]. It is possible, however, that some of them react with solvent molecules to form new species such as association complex. Thus, the present finding that "non-fluorescent" molecules exhibiting the fluorescence enhancement confirms our previous report [9] that the association leading to the enhancement occurs from the triplet states.

The characteristics of the fluorescence enhancement shown in Figures 8– 13 are summarized in Table 3; the degree of enhancement, *R*, is defined as $R=A/A_0$, where A_0 and *A* are the integrated spectral intensity before and after irradiation, respectively. The *R* values range from 6 to 53 for the "non-fluorescent" molecules, depending on characteristics of individual molecules. On the other hand, there is a large difference in the spectral shift; 2-NNT shows no shifts; 3-NBA only 10 nm; NT 60 nm; BA 100 nm. It is noticed that the nitration of NT and BA causes significant shifts, *i.e.*, 60 and 90 nm, respectively and that the addition of a benzene ring also causes 100 nm. Such large difference in spectral shifts may be considered to arise from the structural difference between association complexes, if the association model is true.

TABLE 3 The degree of fluorescence enhancement (*R*) (see text) and blue-shifts of enhanced spectra for the "non-fluorescent"molecules.

	Degree of enhancement, *R*	Blue shift /nm
NT	53	60
2-NNT	18	0
BA	6	100
3-NBA	17	10

3-1-2 Dependence of fluorescence enhancement on the irradiation intensity

Strong irradiation of a solution with 500W-Xe lamp enabled us to observe the spectral change of the solution in real time. The fluorescence from the solution changed of dark-green to light-blue in t_{ir}=5 min at room temperature. The feature of the color change resembles the aurora; once one point in the solvent changed to blue, the blue spot spread over the solvent just like convection, and finally the whole solvent became blue (see Figure 14).

Degassed ethanol solution of NT at $1x10^{-5}$M was irradiated by 400nm light, whose intensity was 5.0 mW, from the light source of SPEX FluoroMax. The degassed ethanol solution was irradiated by filtered light, whose intensity was 50 mW, from 500W-Xe lamp attached two L-39 sharp-cut-filters, and by filtered light, whose intensity was 100 mW, from 500W-Xe lamp attached one L-39 sharp-cut-filter. Figure 15 shows the fluorescence spectra under these conditions.

On irradiation with 5.0 mW-light, no change occurred in the spectrum even after 40 min. On irradiation with 50 mW-light, the fluorescence stayed almost constant for 20 min and then the fluorescence intensity increased, accompanied by a blue-shift at 40 nm. The degree of enhancement *R* value after 40 min irradiation was 63 for the solution irradiated with 50 W-light. On irradiation with 100 mW-light, the fluorescence intensity increased, accompanied by a blue-shift of 60 nm. The degree of enhancement *R* value after 20 min irradiation was 63 for the solution irradiated with 100 mW-light.

These findings show that the fluorescence enhancement depends strongly on the intensity of irradiation. When the irradiation intensity increases, the irradiation time until the fluorescence enhancement becomes shorter. Moreover, there seems to be a threshold for occurrence of the fluorescence enhancement.

It is also suggested that there is a latent period until the fluorescence enhancement occurs. Figure 16 shows a plot of the *R* value against the irradiation time,

t_{ir}, for the solution of NT. There can be seen clearly such a latent time in the plot; initially the R value remains constant, at t_{ir}~10min it gradually increases, and maximizes at t_{ir}=30 min.

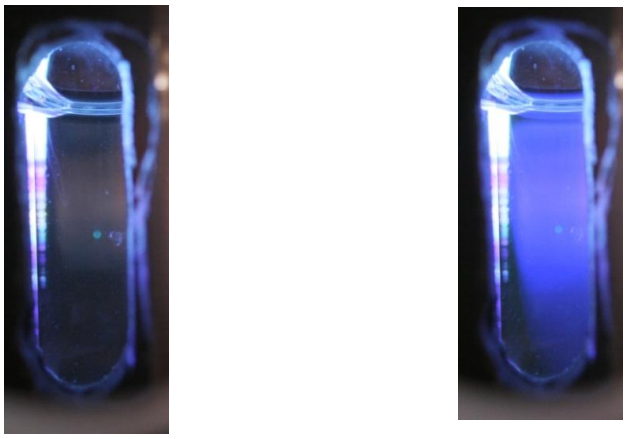


Fig. 14 *Change in fluorescence color of degassed ethanol solution of NT before (left) and after 5-minutes (right) irradiation, respectively*

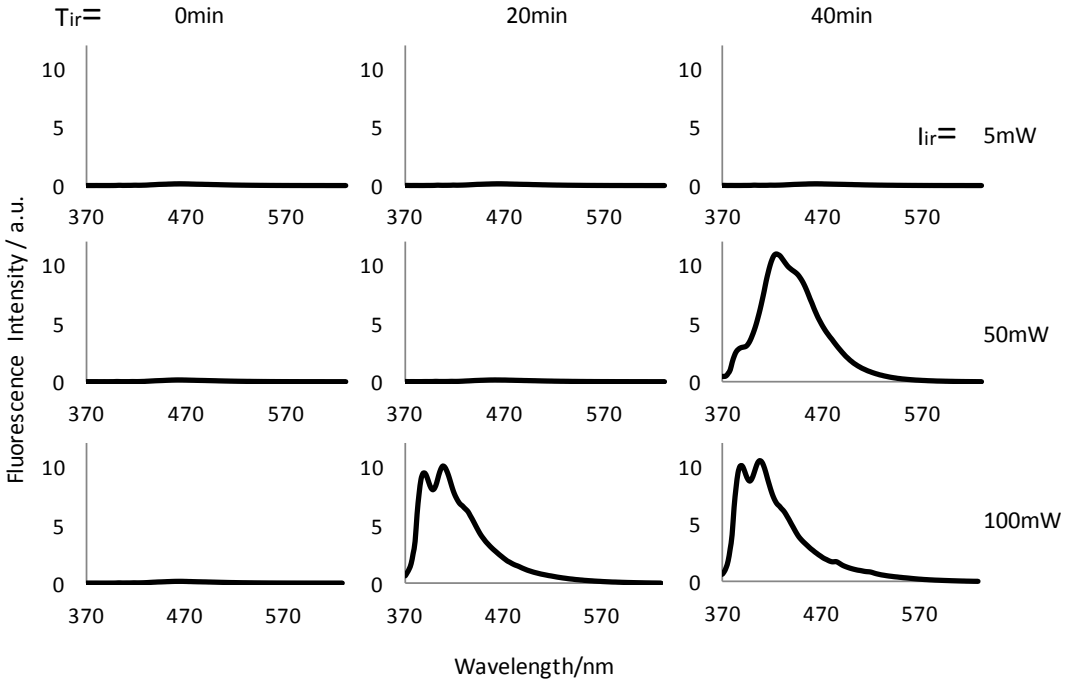


Fig. 15 *Fluorescence spectra of NT in degassed ethanol solution at 1x10^{-5}M as a function of light intensity and irradiation time*

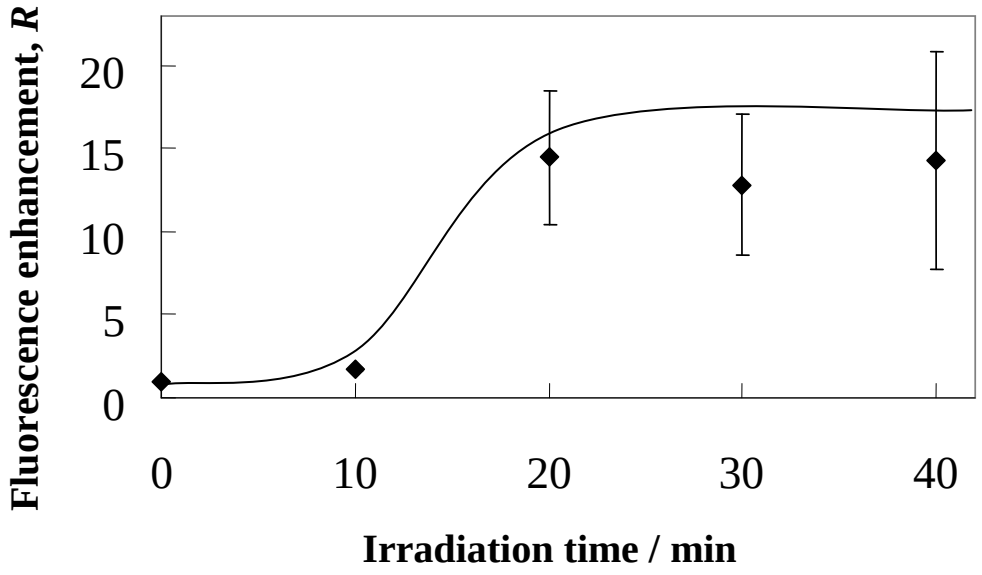


Fig. 16 *Plot of the degree of enhancement, R, against the irradiation time for a degassed ethanol solution of NT at 1x10^{-6}M*

As an additional experiment, we investigated the dependence of the fluorescence enhancement on the volume of solution. We prepared 10-5M ethanol solution of NT and carried out the degassing and pre-irradiation for 2 and 3 mL of the solution. Figure 17 shows the in fluorescence intensity as for the 2 and 3 mL volume of solution a function of irradiation time. This measurement was performed by use of the time base scan of the SPEX FluoroMax spectrometer; the irradiation and observed fluorescence wavelength was fixed at 400 nm and 440 nm, respectively, and data were taken every one second. As can be seen from Figure17, the latent period for the 3 mL solution (solid curve) becomes longer than that of the 2 mL solution (dotted curve) by 30 min. Furthermore, the enhanced-fluorescence intensity reduced gradually after maximigation.

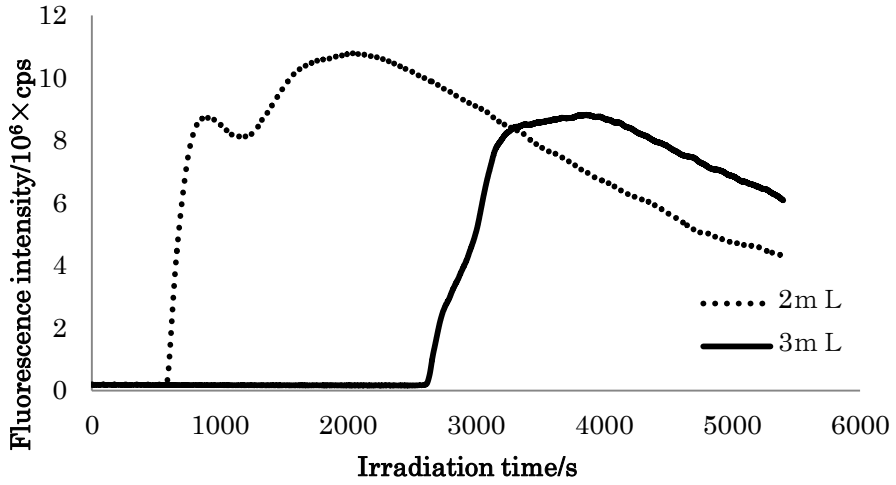


Fig. 17 *Fluorescence intensity for ethanol solution of NT at 1x10^{-5}M as function of irradiation time; the volume of solution was 2 (dotted curve) and 3 mL (solid curve).*

3-1-3 Effects of deuteration of solvent

Figure 18 shows the fluorescence spectra of NT in the solvent of CH_3OH, CH_3OD and CD_3OD before and after irradiation. The original spectra before pre-irradiation have the peaks at 470nm. After irradiation, the peak positions for all spectra were blue-shifted by the 40nm. The fluorescence enhancement is observed in every sample, but the enhancement becomes smaller in order of CH_3OH > CH_3OD > CD_3OD. The ratios of the *R* values for CH_3OD and CD_3OD to that for CH_3OH are 0.7 and 0.3, respectively. The latent period for the CH_3OH, CH_3OD and CD_3OD solution was 8, 9.5 and 10 min, respectively. Namely, when the number of deuterated hydrogen atoms of the solvent increases, the *R* value becomes smaller and the latent period becomes longer.

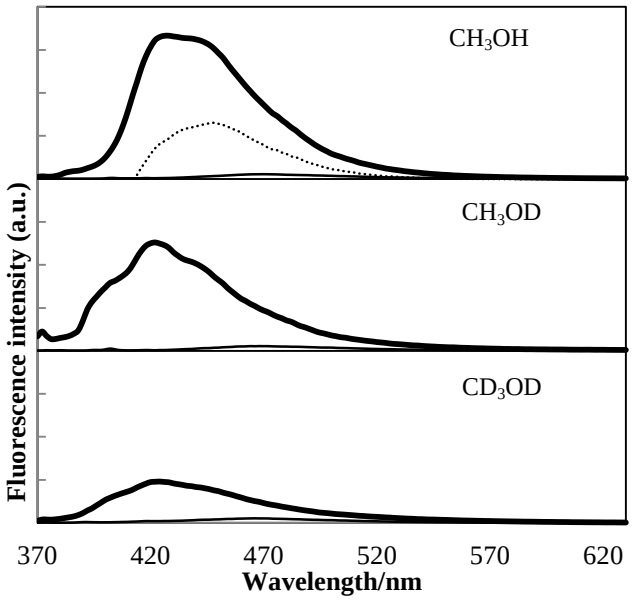


Fig. 18 *Fluorescence spectra of NT in methanol: CH_3OH, CH_3OD, CD_3OD , at $1x10^{-5}M$; thin curves denote original spectra and bold curves enhanced spectra. A 500W-Xe lamp with an L-39 sharp-cut-filter was used for preliminary irradiation. Excitation wavelength: 360nm; slit width for the measurements*

This isotope effect was also observed in the deuterated ethanol solvents. Figure 19 shows the fluorescence spectra of NT in the solvent of C_2H_5OH and C_2H_5OD. The fluorescence of both the solution is enhanced with a blue-shift of 40 nm, but the enhancement for the C2H5OD solution is smaller than that of the C2H5OH solution. The ratio of the *R* values for C_2H_5OD decreases to 0.28 compared with that for C_2H_5OH. The latent time for the C_2H_5OH and C_2H_5OD solution was 5 and 10 min, respectively.

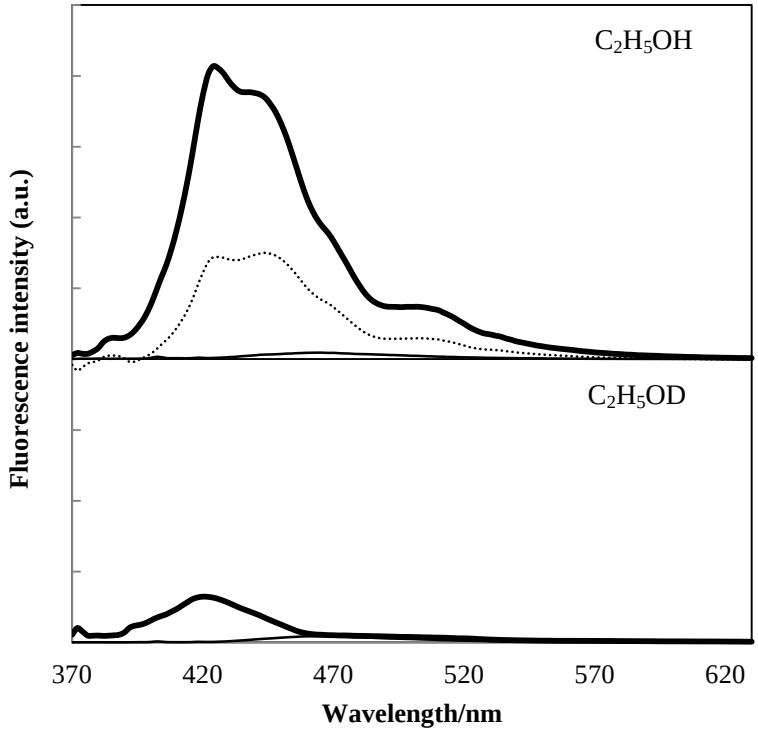


Fig. 19 *Fluorescence spectra of NT in ethanol: C_2H_5OH and C_2H_5OD, at 1x10^{-5}M; thin curves denote the original spectra and bold curves the enhanced spectra. A 500W-Xe lamp with an L-39 sharp-cut-filter was used for preliminary irradiation. Excitation wavelength: 360nm; slits width for the measurements: 3nm.*

By adding water to the ethanol solution, the enhancement also occurred but the peculiar enhanced fluorescence spectra appeared (see Figure 20). The enhanced spectrum have the sharp peaks which are blue-shifted, like those for the ethanol solution. An additional broad peak at 540 nm is observed due to the additional of water.

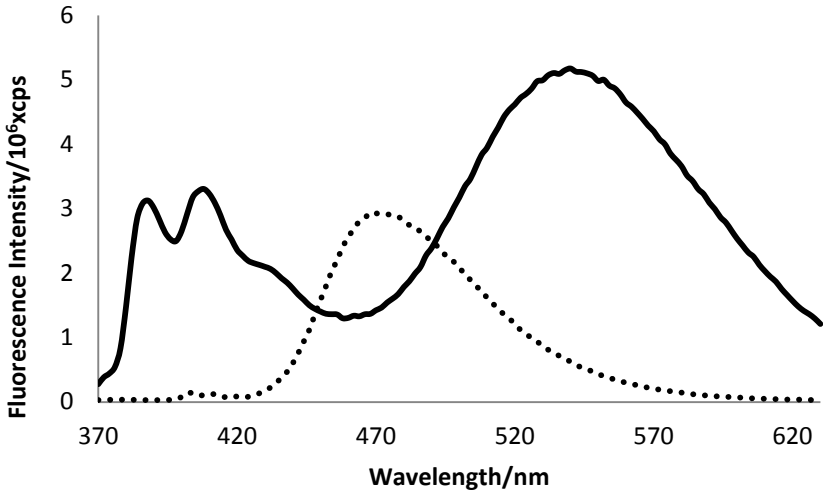


Fig. 20 *Fluorescence spectra of NT in ethanol and water. The dotted and solid lines were before and after irradiation, respectively. The solution was irradiated by a 500W-Xe lamp with an L-39 sharp-cut-filter; the light intensity was 100 mW.*

3-1-4 Molecular orbital calculations

In this section, we suggest that the association complex model by using the molecular orbital calculations. We used the software package, Scigress (Fujitsu), for molecular orbital calculations. The structure of NT and an association complex of NT with ethanol were optimized first by the MM2 molecular dynamics and then by the PM5 semi-empirical molecular orbital method. We calculated their absorption spectra using the ZINDO method for their optimized structures. The calculated results were compared with the experimental ones.

The calculated absorption spectrum of NT is shown in Figure 21 together with its optimized structure. The first absorption occurs at 440nm marked by a red arrow and its molar absorptivity is very small, showing that the absorption is due to the nπ* transition and optically forbidden. Fluorescence is the reversed process of the first absorption. Therefore it is predicted that the fluorescence of NT occurs at 440nm with very weak intensity.

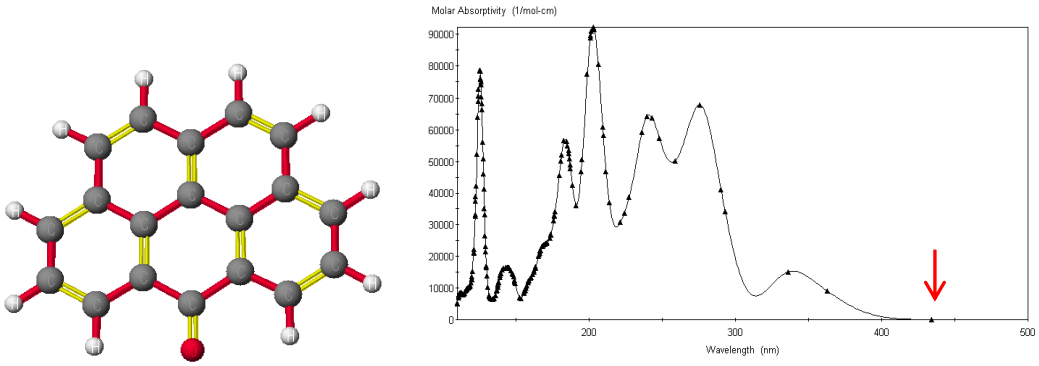


Fig. 21 *Structure of NT (left) and its calculated absorption spectrum (right).*

Figure 22 shows the optimized structure of an association complex between NT and ethanol and its calculated absorption spectrum is the complex a weak bond with of 1.3Å a bond-length is formed between them (oxygen molecule of NT and hydrogen molecule of ethanol). The first transition of the complex occurs at 360nm, marked by a red arrow, with a molar absorptivity of about $2x10^5\,M^{-1}cm^{-1}$. This strong absorption of the association complex indicates that the first transition has a character of an optical-allowed ππ* transition. Therefore, the fluorescence of the complex is expected to be blue-shifted by several tens nm and enhanced about one hundredfold compared with that of NT.

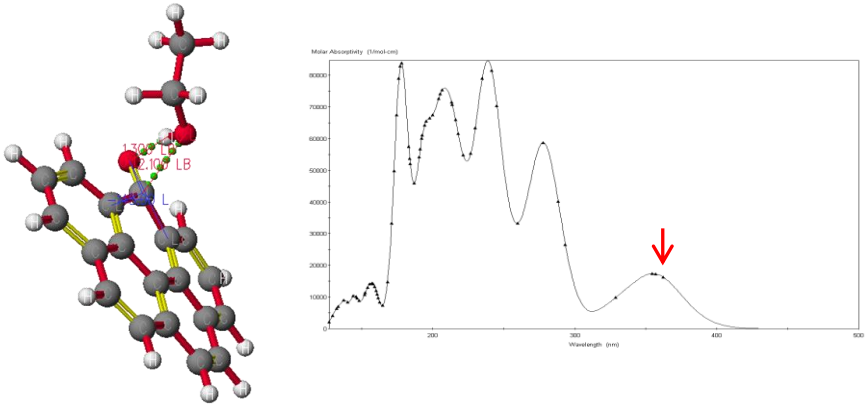


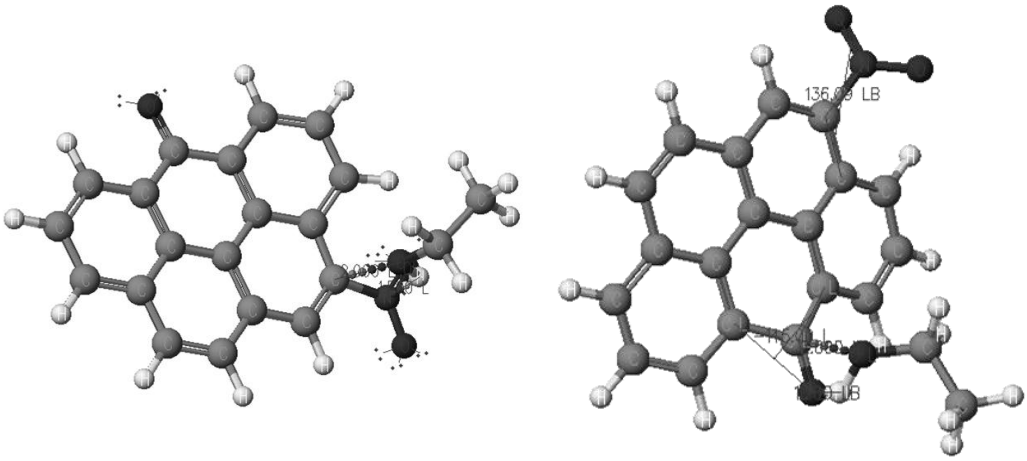
Fig. 22 *Optimized structure of an association complex of NT and ethanol (left) and its calculated absorption spectrum (right). The bond length is 1.3Å for O-H and 2.0Å*

for C-O.

Structure of an association complex of 2-nitronaphthanthrone (2-NNT) with ethanol

The ZINDO calculations for NT and 2-NNT and the association complexes of them provided their molar absorptivity and wavelength for the first transition, which are summarized in Table 4. 2-NNT associates with ethanol via its nitro group as shown in Figure 23 (a). This is because the first lowest excited state of 2-NNT is an $n_a\pi^*$ state, where n_a denotes the anti-symmetric nonbonding orbital localized on the O atoms of the nitro group [9, 12]. The association complex shown in Figure 23 (a) provides a red-shift of 23 nm and an increase in the molar absorptivity of 79 times as large as that for 2-NNT. These results are in rather poor agreement with the experimental ones: no spectral shift and an increase in the intensity of 18 times, see section 3-1-1. The smaller enhancement, however, is in part ascribed to the present experimental conditions, which were not optimized. It is noted that the red-shift is predicted for an isolated complex. In solution such a complex exists in a cage composed of a number of solvent molecules, which suppresses its motion even during photochemical processes [1]. As a result its fluorescence occurs practically from the Franck-Condon state and hence the fluorescence is blue-shifted [1]. Therefore the predicted red-shift can be compensated by a blue-shift owing to the solvation.

For comparison, the association of 2-NNT with ethanol via the carbonyl group (see Figure 23 (b)) provides a blue-shift of 36 nm and an increase in the molar absorptivity of 67 times as large as the molar absorptivity for 2-NNT. Since the solvation contributes to a larger blue-shift for the complex, this is not the case. Thus the calculated spectroscopic properties for the association complexes qualitatively explain the changes in the fluorescence spectra before and after irradiation.



(a) (b)

Fig. 23 *Association of 2-NNT with ethanol via the nitro group(a) and via the carbonyl group (b).*

TABLE 4 The molar absorptivity (in $M^{-1}cm^{-1}$) and wavelength for the first transition of NT and 2-NNT and their association complexes with ethanol; 2-NNT(nitro)-ethanol means an association complex of 2-NNT with ethanol via the nitro group.

	Molar absorptivity / $M^{-1}cm^{-1}$	Wavelength of the first transition /nm
NT	224	434
NT-ethanol	2.0×10^4	370
2-NNT	233	483
2-NNT(nitro)-ethanol	1.8×10^4	506
2-NNT(carbonyl)-ethanol	1.6×10^4	447

3-1-5 Concentration dependence

Fig. 24 shows the fluorescence and enhanced fluorescence spectra of NT in methanol solution at the concentration range from $1x10^{-4}$ to 10^{-6} M. All original spectra have a peak at 470nm independent of concentration. The peak position of enhanced fluorescence spectra after irradiation is 408nm for 10^{-4}M, 448nm for 10^{-5}M and 428nm for 10^{-6}M. Therefore the blue-shift of the enhanced fluorescence is significantly influenced by the change of the concentration; 20nm for 10^{-4} M, 40nm for 10^{-5} M, and 60nm for 10^{-6} M. The intensity also depends largely on the concentration. In particular, the enhancement is very small at 10^{-6} M. The *R* value is 1.7 for 10^{-6} M, 47 for 10^{-5}M, and 43 for 10^{-4} M, with t_{ir}=10 min. Moreover, all the enhanced fluorescence spectra in Figure 24 show well defined vibrational structure in spite of room-temperature, and the structure becomes clearer with decreasing concentration. These findings are peculiar from a spectroscopic point of view and will be discussed in the Discussion.

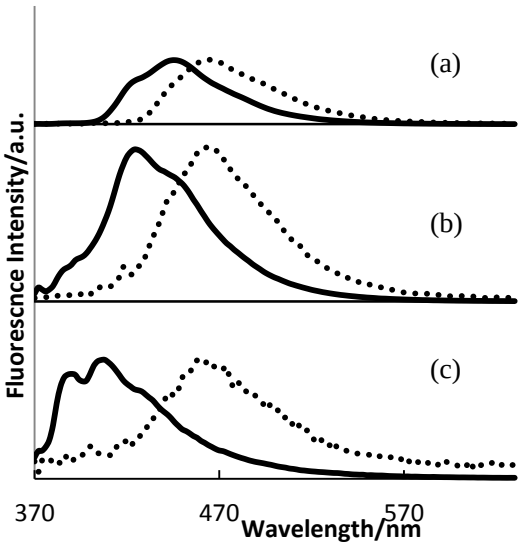


Fig.24 *Fluorescence and enhanced fluorescence spectra for NT in ethanol at the concentration (a) 10^{-4}, (b) 10^{-5}, and (c) 10^{-6} M. The broken curves represent the original spectra of NT before irradiation; they are enlarged so as that their peak is coincident with that of the enhanced spectra.*

3-1-6 Temperature dependence of the fluorescence enhancement

1. The fluorescence enhancement at liquid nitrogen temperature (77K)

The fluorescence enhancement was also observed at liquid nitrogen temperature (77 K) as shown in Figure 25. The enhanced fluorescence spectra provide the vibrational structure more clearly compared with those at room temperature. When the solution, in which the fluorescence enhancement had been induced at 77K, was heated up to room temperature, its enhanced fluorescence spectrum was red-shifted by about 10nm with a decrease in the intensity and a less clear vibrational structure. The spectral shape is almost identical with that of the enhanced fluorescence induced at room temperature, see section 3-1-1. This means that the chemical species causing the enhancement at 77 K is identical with that causing the enhancement at room temperature.

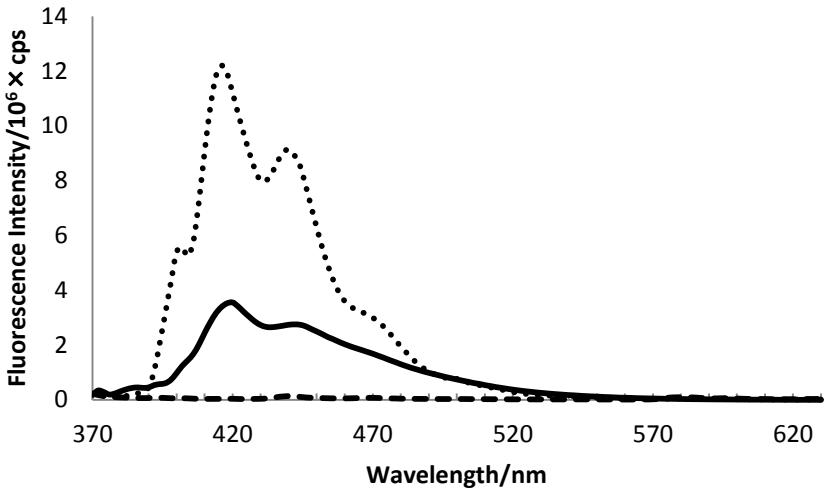


Fig. 25 *Fluorescence spectra of NT in ethanol at 77 K: before (broken line) and after (dotted line) pre-irradiation; after heating the solution from 77 K up to room temperature (solid line).*

The intensity of pre-irradiation also significantly affected the fluorescence enhancement at 77K as well as at room temperature. Pre-irradiation of 10 min only weakly induced the enhancement with R = 1.3, but that of 30 min much more with R = 9.3 in the same conditions. These aspects at 77 K were reflected in the spectra heated up to room temperature after enhancement at 77K as shown in Figure 26. The fluorescence spectrum via enhanced fluorescence spectrum with R = 1.3 at 77K is similar to the original spectrum at room temperature, but that with R = 9.3 exhibits a clear vibrational structure. In the vibrational structure the peak height at a shorter wavelength of 420 nm is almost the same as that at a longer wavelength of 440 nm.

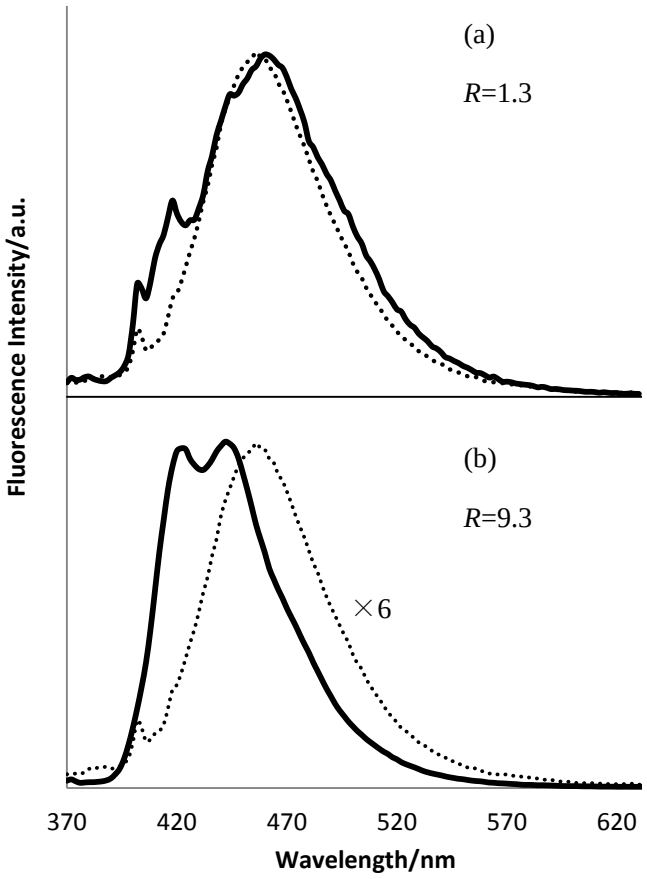


Fig. 26 *Fluorescence spectra (dotted line) and fluorescence spectra after enhanced at 77 K (solid line) of ethanol solution of NT at room temperature*

The concentration dependence for the fluorescence enhancement at 77K is similar to that of at room temperature. The blue-shift becomes larger and the vibrational structure more clearly with a decrease in the concentration. Moreover, such aspects remain in the spectra even when the solution is heated up to room temperature, as shown in Figure 27.

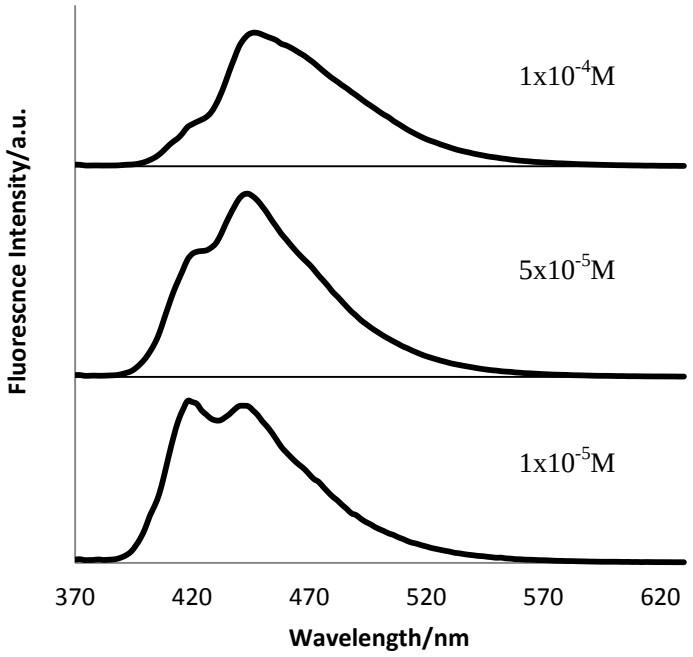


Fig. 27 *Concentration dependence of the fluorescence spectra; the solution were heated up to room temperature after inducing fluorescence enhancement at 77K.*

According to the association complex model, the formation of the complex should hardly occur at 77K, because the motion of solute and solvent molecules is suppressed at low temperature. Contrary to this speculation, the fluorescence enhancement occurs even at 77 K and its features are almost the same as those at room temperature. The findings indicate that in the cooling process solute and solvent molecules are attracting each other to form a structure similar to that of the association complex.

The fluorescence enhancement at 100 and 200K

Figures 28 and 29 show the enhanced fluorescence spectra of an ethanol solution of NT at 100 and 200K, respectively. Pre-irradiation was performed with 400 nm light from the FluoroMax light source; the light intensity was 5 mW and irradiation time was 20 min. Although the pre-irradiation was rather weak, the degree of enhancement *R* is 20 at 100K and 1.2 at 200K. Namely, the fluorescence enhancement occurs more easily at lower temperatures, indicating that the formation of association complex is promoted by cooling.

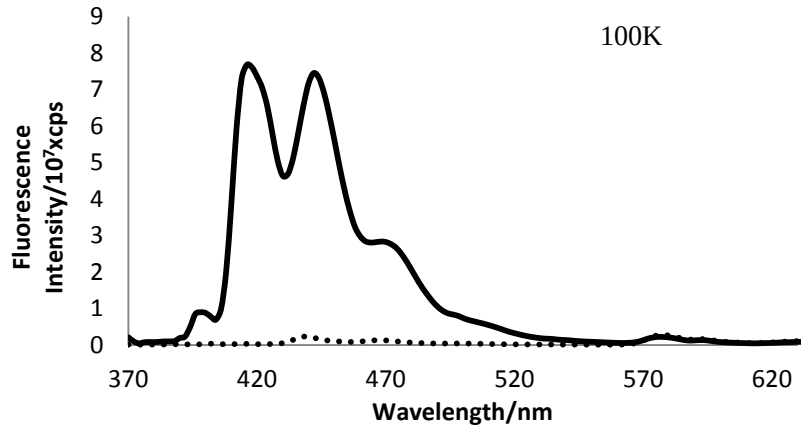


Fig. 28 *The original (dotted line) and enhanced (solid line) fluorescence spectra of ethanol solution of NT at 100K.*

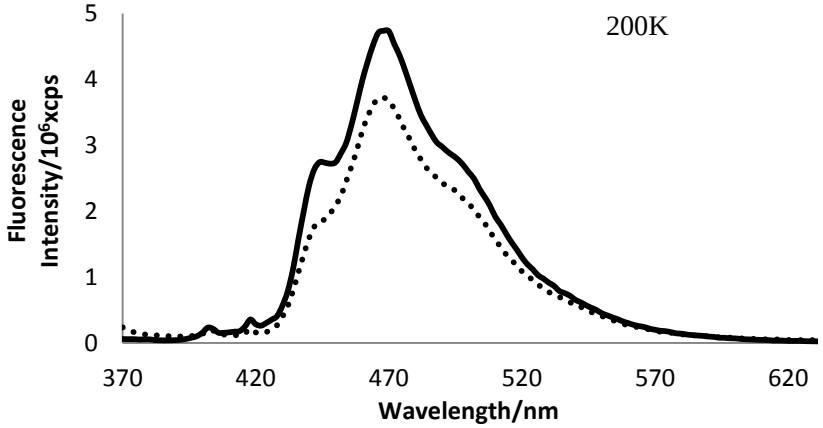


Fig. 29 *The original (dotted line) and enhanced (solid line)fluorescence of ethanol solution of spectra at 200K.*

3-2 Discussion

3-2-1 Occurrence of fluorescence enhancement

As described in section 3-1-1, “non-fluorescent” compounds, i.e., phosphorescent compounds (2-NNT, BA and 3-NBA) show fluorescence enhancement (Figures 6, 7 and 8), but “fluorescent” compounds (perylene and anthracene) did not show fluorescence enhancement (Figures 9and 10). Thus the fluorescence enhancement phenomenon is considered to be common for the phosphorescent aromatic compounds. This suggests that fluorescence enhancement occurs mainly via excited-triplet state. This is consistent with the fact that degassing of a sample solution is critical in the phenomenon: oxygen, s strong quenches of triplet state, is removed from the solution thorough the procedure.

3-1-2 Process of association of NT with solvent molecules

In the $n\pi^*$ state of a ketone an electron is transferred from the O atom to a π^* orbital so that the bond dipole moment is opposite to that in the ground state [11, 12]. This is considered to be true for NT. Then the oxygen atom of NT* is electron-deficient and becomes reactive in reductive reaction [11]. As a consequence, NT* attracts a nearest hydrogen atom of solvent to form a photochemical species.

As described in the section 3-1-2, there is a latent period for the fluorescence enhancement and the period depends strongly on the light intensity of irradiation (Figure 15). These findings suggest that the latent period is the time that NT requires to form a photochemical species. Since such interaction lasts only during the survival of the triplet state of NT, NT*, it is much more effective than the singlet state because of longevity of the former. Moreover, the latent period becomes longer with increasing volume of the solution in a cell. This result indicates that whether the NT* is easy for the reactor which reacts with NT* to encounter, or not.

As will be described in detail in the section 3-1-4, a NT molecule is closely surrounded with many solvent molecules and its movement is so restricted that it is hardly possible to form the photochemical species by one excitation of NT. If one NT molecule is excited more than one time, it has much interaction-time so that it can change its position step-wisely.

3-2-3 Interpretation of the isotope effects

In general, hydrogen abstraction for alcohols occurs more preferentially at their alkyl group than at the hydroxyl group [1]; hence deuteration of the former leads to an effective decrease in the abstraction. In the fluorescence enhancement, however, deuteration of the hydroxyl group gives rise to a decrease of about 70% for ethanol and of about 30% for methanol; the 30% decrease is slightly smaller than the decrease (about 40%) due to of the alkyl group. Accordingly the solvent-deuteration effect for the fluorescence enhancement differs from the usual hydrogen abstraction where deuteration of the alkyl group dominates. The difference in the deuteration effect reinforces our assumption that the fluorescence enhancement arises from "association of NT with the solvent", not from hydrogen abstraction of NT from the solvent, though their initial steps resemble each other [5]. Furthermore the experimental findings indicate that there are two kinds of association complex; an NT molecule interacts strongly with solvent's hydroxyl group in one complex (referred to as complex I) and with solvent's alkyl group in the other complex (referred to as complex II). The enhanced spectra in CH_3OD and C_2H_5OD are considered to originate from complex II because the contribution of the hydroxyl interaction is decreased. On the other hand, the spectra of complex I are obtained by the subtraction of enhanced spectra in CH_3OD and C_2H_5OD from those in CH_3OH and C_2H_5OH, respectively, which are shown by

dotted lines in Figures 18 and 19. The spectra of complex I are red-shifted compared with those of complex II. As preliminary experiment we added H_2O into a C_2H_5OH solution of NT and investigated the fluorescence enhancement. Addition of H_2O suppressed the enhancement in the shorter wavelength, but provided a new broad band at 540nm in the spectrum (Figure 20). Such a longer-wavelength band is considered to be consistent with the spectra of complex I, because the hydroxyl interaction largely contributes.

Next we discuss the mechanism of the solvent-deuteration effects that causes reduction of the fluorescence enhancement by 30 – 70%. According to the association model for the fluorescence enhancement, the reduction is ascribed to a kinetic isotope effect: the rate for association of NT with the deuterated solvents is smaller than that with the normal solvents. Such kinetic isotope effects have been observed in the hydrogen abstraction and the mechanism has also been discussed [1, 4]; therefore we can discuss our findings on the analogy of the hydrogen abstraction. Figure 30 shows an energy profile for the course of the association via the hydrogen atom of solvent's hydroxyl group. In the initial state, the vibrational energy of the O-D stretch lies below that of the O-H stretch by the difference in their zero-point energies (ZPE's). On the other hand, in the transition state, the difference in the ZPE between the O-H and O-D bonds can be neglected. This is because the vibrational-energies are lowered owing to an interaction with massive NT, which substantially reduces the difference between their ZPE's [13]. Thus the activation energy for the association through hydrogen becomes smaller than that through deuterium. The same explanation can be applied for the association via C-H or C-D bonds of the solvents. Accordingly associative complexes containing deuterium are slowly produced, resulting in a decrease in the fluorescence enhancement.

From the above discussion, the fluorescence enhancement is considered to occur through the association between solute and solvent molecules. In fact, such an association complex can be generated by use of molecular orbital calculations, and its calculated spectra are consistent with the experimental ones.

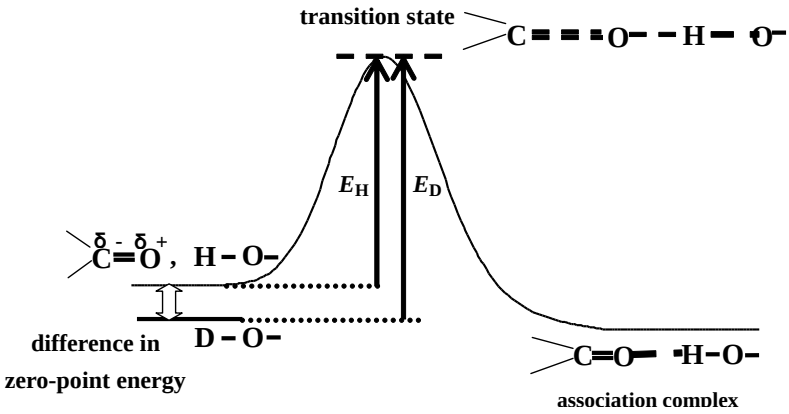


Fig. 30 *Energy profile for association of NT with methanol through the O-H and O-D bond of methanol. The zero-point energy of the O-D vibration lies below that of the O-H vibration, leading to higher activation energy of association through the O-D bond.*

3-2-4 Photochemical reaction in a solvent cage

As can be seen from Figure 24, the spectra of fluorescence enhanced on irradiation show fairly defined vibrational structure in the concentration range 10^{-6}-10^{-4} M even though at room temperature and the structure becomes clearer with decreasing concentration. This indicates that the polar solvent behaves as a non-polar medium for the fluorescent species and such non-polar character becomes stronger at lower concentrations [4, 11]. The original fluorescence spectra of NT provide practically one broad band at 470 nm and no vibrational structure, showing NT is in a polar solvent. We will discuss the spectral differences in the enhanced fluorescence at different concentrations, taking into account the association model.

On the basis of the established notion of solvation [4, 10, 11], NT is in a solvent cage and the solvent molecules are oriented with restricted motion, because both the solute (NT) and solvent (alcohols) are polar. The triplet state of NT, NT*, produced by irradiation interacts with a nearest solvent molecule constituting the cage to form an associative complex. Since the association occurs between the oxygen atom of NT's carbonyl group and a hydrogen atom of the alcohol, as described above, the n π *-character of NT is substantially weakened in the complex; semi-empirical molecular orbital calculations predict that the associative complex is strongly fluorescent [5]. In addition the complex becomes held more rigidly to the cage through the associated solvent molecule. On irradiation, the complex in the solvated ground state is excited to the Franck-Condon state, the geometry of which is identical to that in the ground state, and relaxes to the solvated excited state that is energetically most stable (see Figure 31). If the solvation is very strong, the movement of the complex in the solvent cage is suppressed so largely that the relaxation from the Franck-Condon state is prevented [4]. In this case, the fluorescence occurs practically from the Franck-Condon state and therefore it is blue-shifted. The finding that the enhanced spectra are shifted to the blue with decreasing concentration indicates (Figure 24) that at lower concentrations the solvation becomes stronger and the NT's motions are confined. Accordingly we speculate that more solvent molecules cluster around one complex at lower concentrations and contribute more strongly to "freeze" their motions. Furthermore, such frozen molecules should provide the spectra with more defined vibrational structure [4]. This is the case, as shown in the spectrum at 10^{-6} M (Figure 21). It is also expected that the restriction of molecular motions at low concentration inhibits the association processes. In fact the degree of the fluorescence enhancement reduces with decreasing concentration as can be seen in Figure 24.

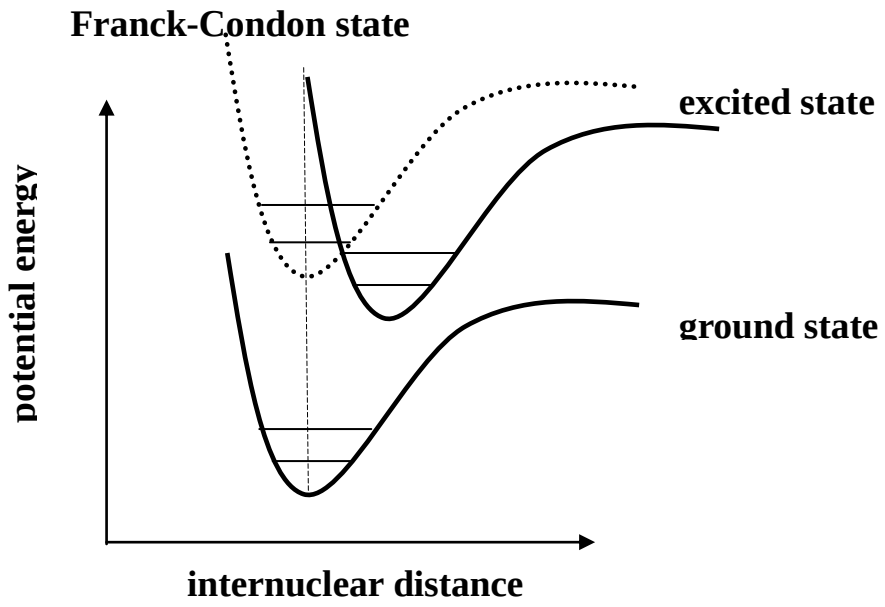


Fig. 31 *Potential energy curves of the ground, Franck-Condon, and excited states of an association complex.*

4. Identification of chemical species leading to fluorescence enhancement

4-1 Analysis of chemical species by thin layer chromatography (TLC)

To identify the chemical species which cause the fluorescence enhancement in ethanol solution of NT, we have tried to separate it by thin-layer-chromatography (TLC). Figure 32 shows a chromatogram obtained; in the measurement, cyclohexane was used as a developer of the TLC. An extended spot for a sample before irradiation is shown on the left side of the plate and for those a sample after irradiation sample on the right side. As can be seen, the before irradiation sample exhibits only one spot, but the after irradiation sample two spots. This finding suggested that the latter contains a new chemical species produced by irradiation.

Fig. 32 *TLC chromatogram of samples before (left) and after (right) irradiation.*

4-2 Analysis of chemical species by high performance liquid chromatography (HPLC)

We have analyzed the chemical species by HPLC. Figure 33 shows HPLC chromatograms of a methanol solution of NT before and after irradiation sample. In the chromatogram for the before irradiation sample, only one peak appears at a retention time, t_R, of 4.5 min, and it is reduced to about 1/2 by irradiation in the chromatogram for the after irradiation sample. This peak corresponds to NT. In the chromatogram for the after irradiation sample that exhibited the fluorescence enhancement, new peaks appear at t_R = 2.5 and 6.5 min. These new peaks are naturally assigned to new chemical species produced by the irradiation, which was the association complex. The intensity of the new peak at 6.5 min increases as the irradiation time increases. This means that the new peak is assigned to the chemical species desired, i.e., the association complex. The intensity of the NT peak at t_R = 4.5 min corresponding to NT decreases as the irradiation time increases. The t_R of the association complex is longer than that of NT. As a C18 reverse phase HPLC column was used in this measurement, the retention

time of a stronger polarization sample is shorter. Then the polarization of the chemical species observed at t_R = 6.5min is weaker than that of NT (t_R = 4.5 min). Then, from this finding we can say that the polarization of the association complex of NT is thought to be weakened by a solvent cage surrounded by methanol molecules.

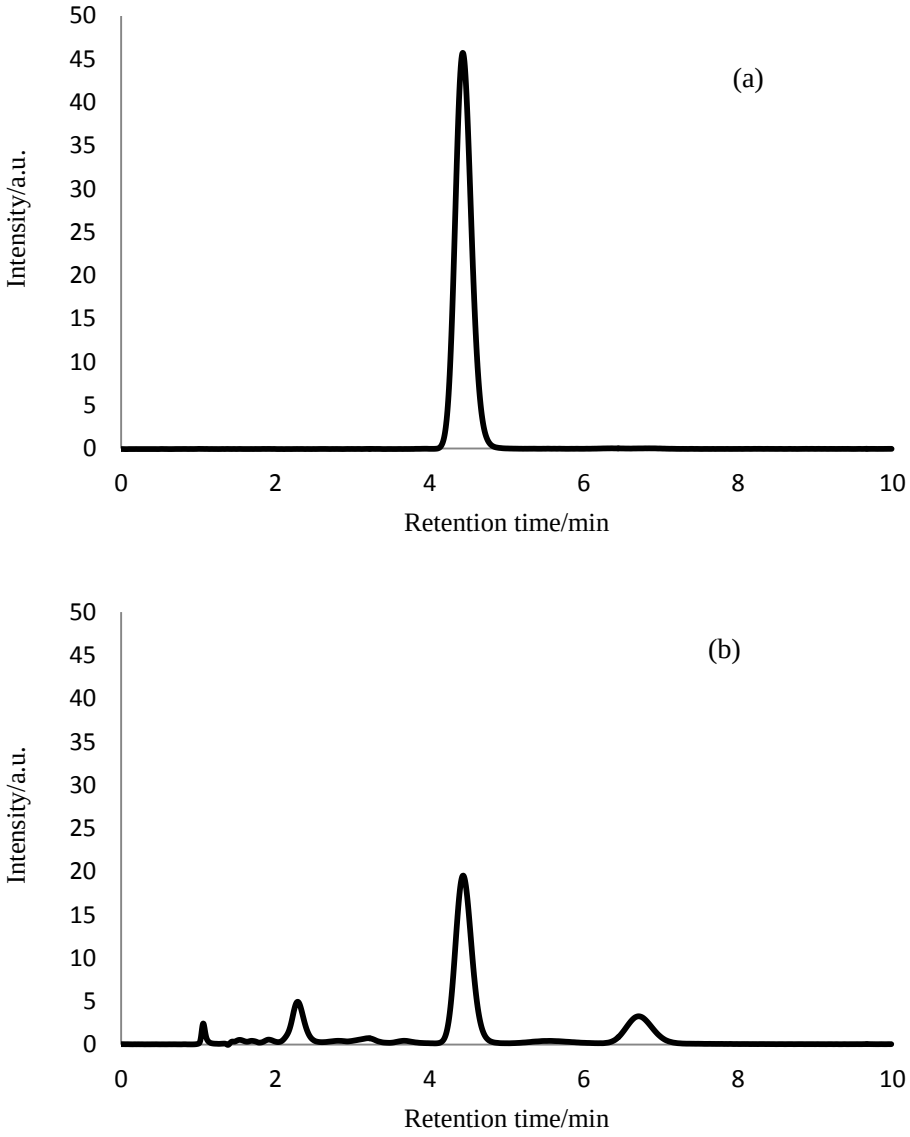


Fig. 33 *The HPLC chromatograms of NT methanol solution of before and after irradiation. Dotted and solid lines are the chromatograms of before (a) and after (b) irradiation sample.*

4-3 Analyses of chemical species by time of flight / mass-spectrometry (TOF-MS)

We have tried to detect the association complex by TOF-MS. Samples used were methanol solutions of NT at the concentration $1x10^{-4}$, $1x10^{-5}$, and $1x10^{-6}$ M, which had exhibited the fluorescence enhancement by irradiation. The irradiation time was set for 10, 20, and 30 min so as to accomplish the enhancement with different degrees; for comparison solutions without irradiation were also prepared. In the TOF-MS measurement, we introduced the sample solution directly into the ionization chamber using a syringe and allowed sample molecules to be ionized by the electrospray method. The mass of ions produced was selected through their times of flight.

The TOF-MS spectra are shown in Figures 34 (a), (b), (c). The spectrum of the sample without irradiation shows a peak at *m*/*z*=255 (Figure 34 (a)), which is assigned to NT. The spectra for the irradiated samples at higher concentrations of $1x10^{-4}$ and $1x10^{-5}$ M show a new peak at *m*/*z*=239 (Figure 34 (b)). We assigned this peak to $[NT+H+2Na+13MeOH]^{3+}$, by taking it into account that the ionization by electrospray may often produce ions with multiple charged ions owing to the addition of protons and sodium ions. Therefore the ion with m/z=239 is ascribed to the association complex + methanol molecules; namely the complex formed at higher concentrations is composed of one NT and 13 MeOH molecules. Figure 34 (c) shows a TOF-MS spectrum for the sample at a lower concentration of $1x10^{-6}$ M. An additional peak appears at *m*/*z*=368 in the spectrum. We assigned this peak to $[NT+2H+15MeOH]^{2+}$, which is the association complex + methanol molecules that consists of one NT and 15 MeOH molecules.

The height of the three peaks varied with a change in the irradiation time of the solution. With increasing irradiation-time, the peak height at *m*/*z*=255 decreased and those at *m*/*z*=239 and 368 increased, as shown in Figure 34 (c). On the other hand, the number of NT becomes smaller and that of association complexes greater with

increasing irradiation-time, because the fluorescence enhancement proceeds. Therefore the assignment described above is consistent with the change in the TOF-MS spectra as a function of irradiation time. Furthermore, the assignment indicates that the association complex formed at lower concentrations contains more solvent molecules than that formed at higher concentrations. From the concentration dependence of the fluorescence enhancement, we assumed the formation of a solvent cage. If this is the case, NT interacts strongly with one methanol solvent to make the association complex and then the complex is surrounded by twelve or fourteen methanol molecules to make the cage. The present results of TOF-MS measurements suggest that the size of the cage tends to enlarge as the concentration of the solution decreases, leading to formation of a more strengthen cage. This is also consistent with the concentration dependence, described in section 3-2-4.

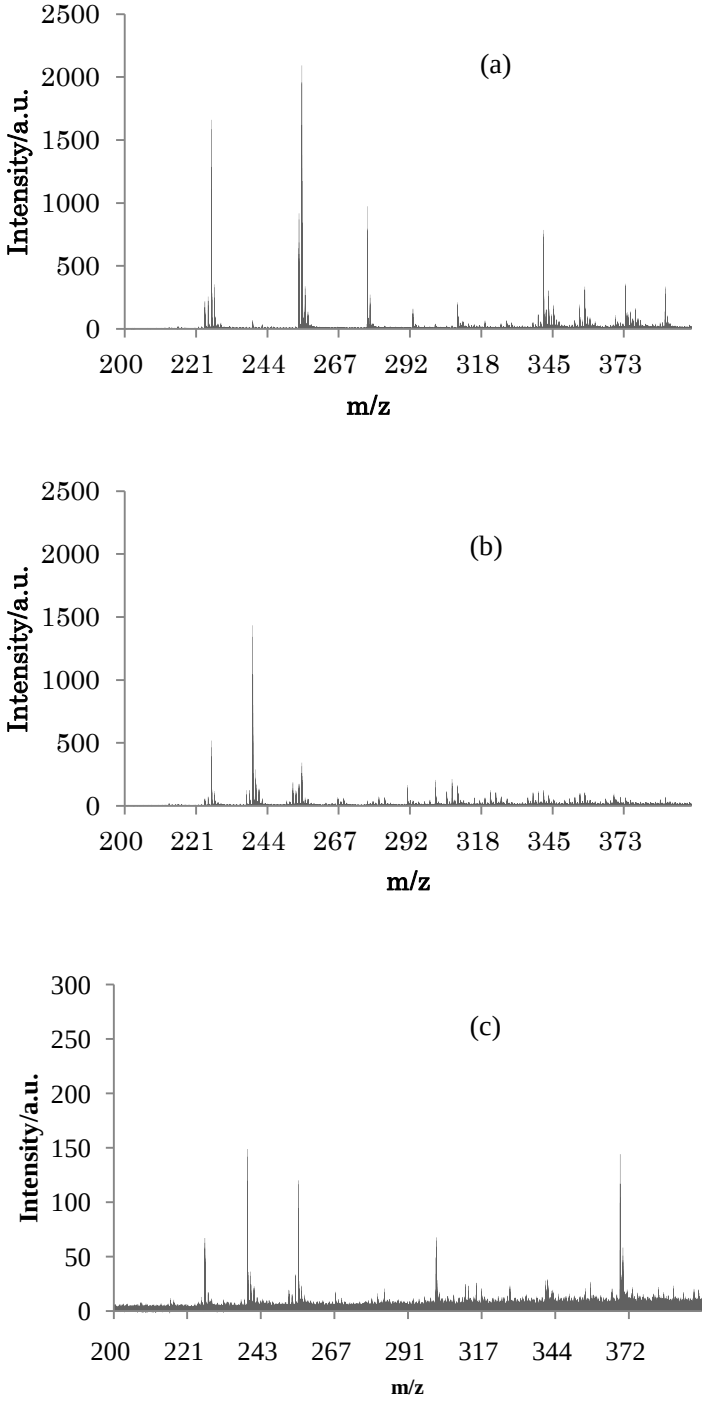


Fig. 34 *The mass spectra of sample for methanol solution of NT (a) before enhancement, 10^{-5}M, (b) after enhancement, 10^{-5}M and (c) after enhancement 10^{-6}M.*

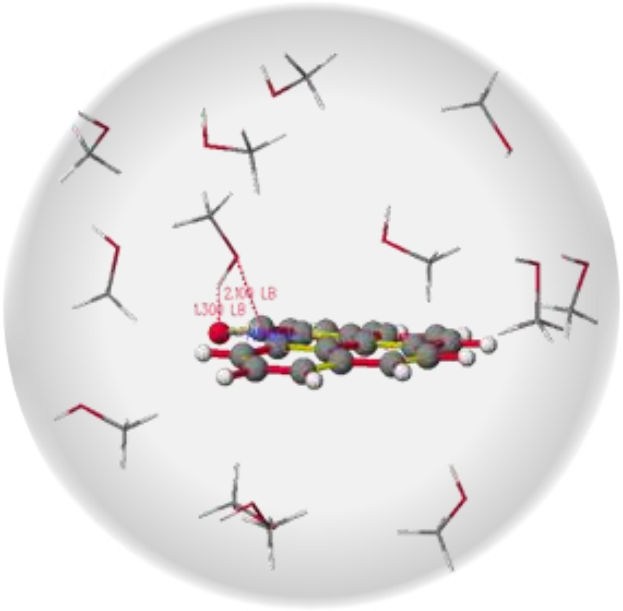


Fig. 35 *The model of a solvent cage surrounded the association complex between NT and solvent (methanol) molecules. The solvent cage is composed of thirteen methanol molecules*.

Conclusion

The fluorescence enhancement, reported only for naphthanthrone (NT) so far, has also been observed in benzanthrone, 2-nitronaphthanthrone, and 3-nitrobenzanthrone. The molecules that exhibit the enhancement are non-fluorescent, or phosphorescent in other words. Namely the fluorescence enhancement is a general phenomenon occurring in phosphorescent molecules and arises mainly from a reaction via their triplet state. For NT, we have investigated the effects of irradiation intensity, deuteration of the solvents, and concentration of the solution. The enhancement occurs more rapidly with stronger irradiation, though there was a latent period of several minutes. The enhancement is reduced to 30 − 70 % by deuteration of the solvents. The spectra of enhanced fluorescence showed more resolved vibrational structure and larger blue-shift as the concentration decreases.

The HPLC chromatography of a methanol solution of NT after the enhancement provides a peak at a retention time longer than that of NT. Namely the chemical species for the longer retention time peak has less polarizability than that of NT. We concluded that the peak was due to the chemical species of NT and methanol, i.e., the association complex.

TOF-MS spectra for the solution exhibited the fluorescence enhancement provided new peaks belonging to $[NT+2H+15MeOH]^{2+}$ and $[NT+H+2Na+13MeOH]^{3+}$. Then the chemical species are identified to association complexes surrounding one NT molecule with thirteen and fifteen solvent molecules. Thus, the mass and composition of the association complexes, causing fluorescence enhancement, have been confirmed for the first time.

3-Nitrobenzanthrone (3-NBA) is known as a potent mutagen. 3-NBA is not detectable by the fluorescence spectroscopy, because it is hardly fluorescent. The fluorescence enchantment of polycyclic aromatic ketones, studied intensively by the present work, could be applied for a practical use of spectroscopic analysis for mutagenic aromatic compounds such as 3-NBA.

Acknowledgement

I am grateful to Prof. Ohshima of the department of environmental science for his supervising my research and encouraging me all the time. I appreciate all the members of Ohshima laboratory for their friendship.

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