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Application News

No. A533

Spectrophotometric Analysis

Monomer Emission and Excimer Emission of Pyrene Solution

Fluorescence is the phenomenon observed when a substance transitions from the electronically excited state to the ground state. However, association may occur between excited-state molecules and ground-state molecules, thereby causing an emission (fluorescence). This state is referred to as an excited complex. If it comprises identical molecules, it is referred to as an excited dimer (excimer). If it comprises two different molecules, it is referred to as an exciplex.[*1] Examples of excimer usage include excimer lasers and measurement of intermolecular distances.

This article introduces the measurement of monomer and excimer emissions of pyrene solution using the spectrofluorophotometer RF-6000.

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3D Measurement

Fig. 1 shows the exterior of the RF-6000. First, 3D spectrum measurement of pyrene solution (solvent: ethanol) was performed using the conditions in Table 1 in order to determine at what excitation wavelengths and at which wavelengths emission occurs. When the sample concentration is high, there are cases where the standard cell holder of the RF-6000, in which the detector is oriented 90° with respect to excitation light, may not be able to detect fluorescence. This is because the excitation light is only absorbed on the cell surface and fluorescence emission will occur only on the surface irradiated with excitation light. This time we used the solid sample holder and set the cell so that fluorescence emitted from the surface of the sample could be observed as shown in Fig. 2.

Fig. 3. shows the results of 3D spectrum measurement. The vertical axis of the 3D spectra indicates the excitation wavelength (Ex) and the horizontal axis indicates the fluorescence wavelength (Em). The fluorescence intensity for each Ex/Em is indicated using a color scale. With the low concentration sample (1.0×10^{-4} mol/L), the most intense fluorescence was observed in the Em 370 to 400 nm range at Ex 305 nm. The fluorescence in this region is monomeric (monomer emission). However with the high concentration sample (1.0×10^{-2} mol/L), the most intense fluorescence was observed in the Em 450 to 500 nm range at Ex 360 nm. The fluorescence in this region is excimeric (excimer emission).

Fig. 1 RF-6000 Exterior

Table 1 Measurement Conditions

Instrument used	:	RF-6000 and solid sample holder
Spectrum Type	:	3D spectrum
Measured Wavelength Range	:	Ex 280 to 400 nm, Em280 to 600 nm
Scanning Speed	:	6000 nm/min
Wavelength Interval	:	Ex 5.0 nm, Em 1.0 nm
Bandwidth	:	Ex 5.0 nm, Em 5.0 nm
Sensitivity	:	Low

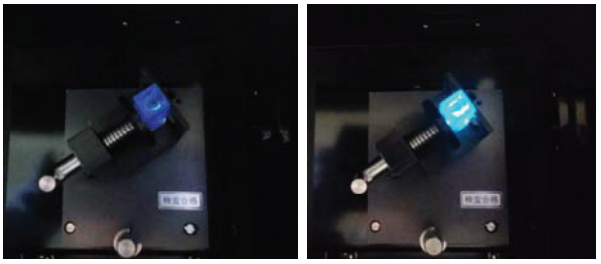

Fig. 2 Cell Set on the Solid Sample Holder
Left: Low Concentration (1.0×10^{-4} mol/L),
Right: High Concentration (1.0×10^{-2} mol/L)

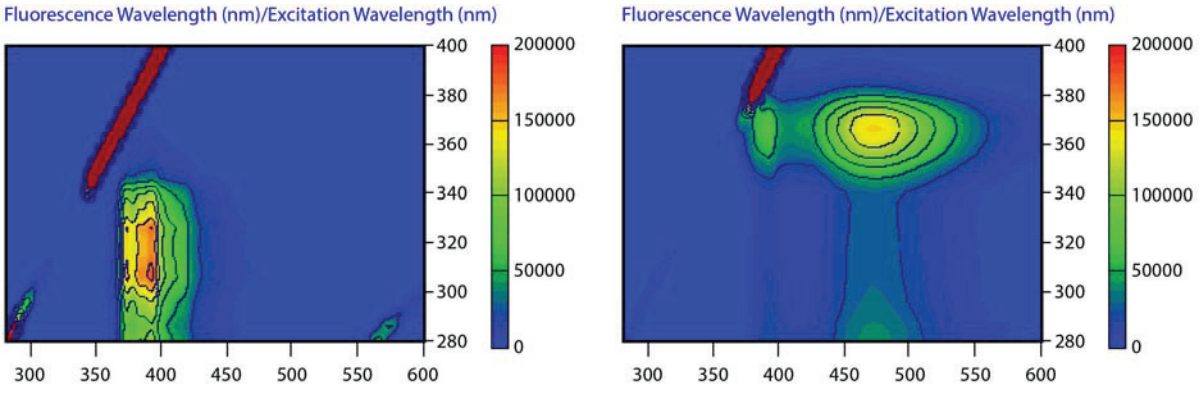

Fig. 3 3D spectra of Pyrene Solution Left: 1.0×10^{-4} mol/L, Right: 1.0×10^{-2} mol/L

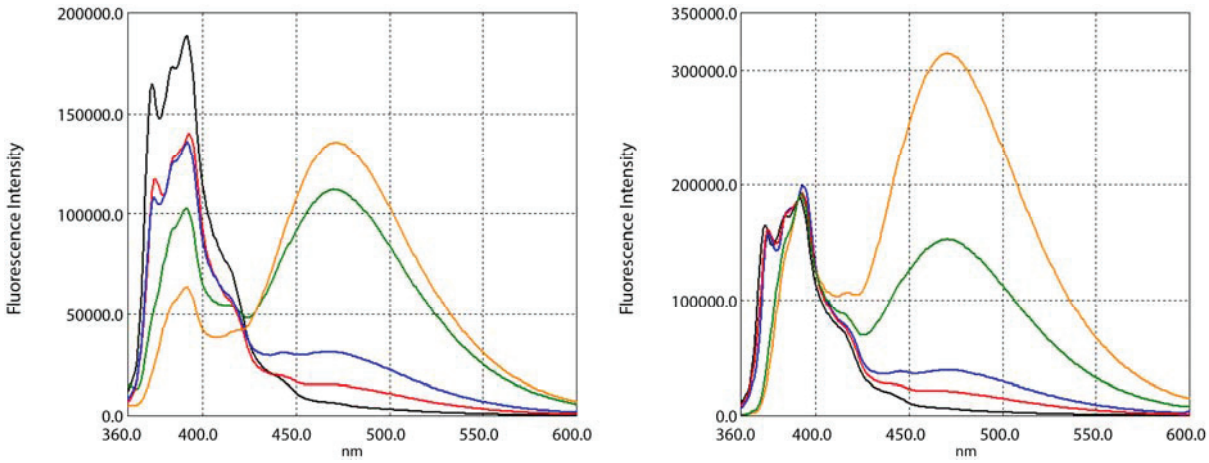

Fig. 4 Left: Fluorescence Spectra of Pyrene Solution
(all spectra measured at an excitation wavelength that induces the highest fluorescence intensity)
Black: 1.0×10^{-4} mol/L (Ex305 nm), Red: 5.0×10^{-4} mol/L (Ex305 nm), Blue: 1.0×10^{-3} mol/L (Ex345 nm),
Green: 5.0×10^{-3} mol/L (Ex360 nm), Orange: 1.0×10^{-2} mol/L (Ex360 nm)
Right: Fluorescence Spectra of Pyrene Solution (normalized to Ex 305 nm and Em 391 nm)

Monomer Emission and Excimer Emission

Fig. 4 shows the results of measuring monomer and excimer emissions for different concentrations of pyrene solution. The excitation wavelength that induced the highest fluorescence intensity in the 3D measurement was selected. Table 2 lists the measurement conditions. When the concentration of pyrene solution is low (1.0×10^{-4} mol/L), only monomer emission at 370 to 400 nm is observed. As concentration increases up to 1.0×10^{-3} mol/L, we start to observe excimer emission in the 470 nm range in addition to monomer emission, and as concentration reaches 5.0×10^{-3} mol/L and higher, excimer emission becomes more prominent compared to monomer emission. In the right graph of Fig.4 showing results that were normalized to the fluorescence intensity of 391 nm, we can also see the concentration dependence of excimer emission. As concentration of the pyrene solution increases, since interaction between molecules also increases, association occurs between pyrene molecules in the excited state and those in the ground state as shown in Fig. 5, and this is said to lead to intermolecular excimer emission. The measurement results obtained this reseach are considered to be due to this phenomenon.[*2] The formation of excimers stabilizes energy and can be observed on the long-wavelength side when checking their fluorescence. Also, when there are multiple pyrene groups in the same molecule, a phenomenon known as intramolecular excimer formation occurs between two pyrene groups within the same molecule. Intramolecular excimers are known to be observable at low concentrations compared to intermolecular excimers.[*2] Furthermore, the development of substances that exhibit new behaviors are underway through the creation of derivatives of organic substances such as pyrene. In fact, excimer emission can be observed at concentrations lower than pyrene in a pyrene derivative including four alkylamide chains. For details on this substance, refer to Application Note No. 38.

Table 2 Measurement Conditions

Instrument used	:	RF-6000 and solid sample holder
Spectrum Type	:	Fluorescence Spectrum
Excitation Wavelength	:	305/345/360 nm
Emission Wavelength Range	:	360 to 600 nm
Scanning Speed	:	600 nm/min
Wavelength Interval	:	1.0 nm
Bandwidth	:	Ex 5.0 nm, Em 5.0 nm
Sensitivity	:	Low

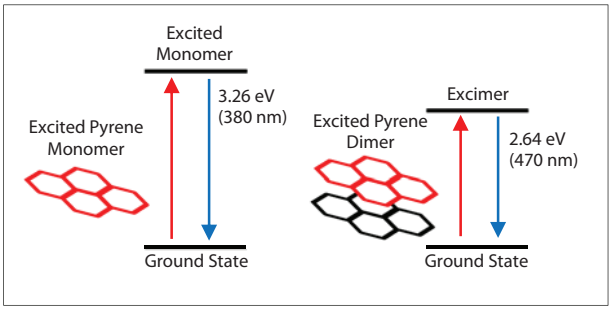

Fig. 5 Energy Level Diagrams and Association States

Summary

By measuring the 3D spectra of pyrene solutions with differing concentrations, we were able to rapidly perform scanning of optimal excitation wavelengths. We were also able to check the detailed behavior of monomer and excimer emissions of pyrene solution by measuring the fluorescence spectrum of solutions with differing concentrations.

References
*1 Nicholas J.Turro, V.Ramamurthy, J.C.Scaiano, Haruo Inoue/Osamu Ito (Japanese Translation): Principles of Molecular Photochemistry: An Introduction (Maruzen Publishing)
*2 Masatoshi Yamaguchi, Hitoshi Nohta, Hideyuki Yoshida, Kenichiro Todoroki: "Highly selective and sensitive derivatization method for biogenic substances, based on intramolecular excimer-forming fluorescence and its application to medical analysis," The CHEMICAL TIMES, Issue 211, No. 1 (2009)

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