

Application News

No. A560

Spectrophotometric Analysis

Observing Atypical Cases of Fluorescence

Absorbance spectra and fluorescence spectra make it possible to investigate the transitions in the state of electrons (ground state, excited state, etc.). There are many laws regarding the state of electrons such as Kasha's rule and the Franck-Condon principle, but more complex phenomena and exceptions are known to exist. In particular, regarding light emissions of organic substances, peak positions and shapes change significantly depending on the molecular structure and the effects of interactions with the surroundings. Points considered to be factors of influence include spatial structure, isomerization, solvent polarity, viscosity, and pH.

In Application News No. A533, we introduced the excimer emission of pyrene as an example of an emission transition where there are two different molecules. This article introduces measurements of a sample which exhibits differing fluorescence when in a polar solvent and in a nonpolar solvent, and a sample which starts emission from the second excited singlet state (S_2 state) both using the RF-6000 spectrofluorophotometer.

K. Sobue

Fluorescence in a Nonpolar Solvent and Polar Solvent

Samples for measurement were prepared by dissolving 10 mg of p-dimethylaminobenzonitrile (DMABN) and anthracene each separately in both a nonpolar solvent (cyclohexane) and a polar solvent (dichloromethane). The absorbance spectra of these samples were measured using the conditions listed in Table 1. As shown in Fig. 1, regarding the DMABN solutions, a peak is detected at 282 nm with the nonpolar solvent and at 294 nm with the polar solvent. For the anthracene solutions, peaks are detected at 339 nm, 357 nm, and 376 nm with the nonpolar solvent and at 341 nm, 359 nm, and 378 nm with the polar solvent. Generally, a high-polarity solvent shifts the absorption spectrum peak to longer wavelengths. Since the electric charge of excited molecules is greatly localized, the polarity is higher than when in the ground state. Therefore, solvents with a higher polarity have increased electrostatic interactions, thereby increasing stability (the energy becomes lower).

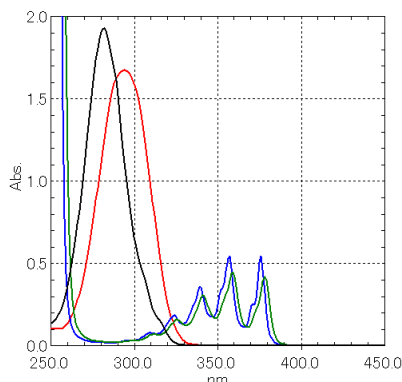


Fig. 1 Absorbance Spectra
Black: DMABN Solution (Solvent: cyclohexane)
Red: DMABN Solution (Solvent: dichloromethane)
Blue: Anthracene Solution (Solvent: cyclohexane)
Green: Anthracene Solution (Solvent: dichloromethane)

Table 1 Measurement Conditions

Instrument	: UV-2600
Wavelength Range	: 250 nm to 450 nm
Sampling Interval	: 1.0 nm
Scan Speed	: Medium speed
Bandwidth	: 1.0 nm
Light Source Changing Wavelength	: 323 nm

We next measured fluorescence spectra using the conditions listed in Table 2. Fig. 2 shows the fluorescence spectra of the DMABN solutions diluted by a factor of 100 and Fig. 3 shows the spectra data normalized on the peak values in Fig. 2. Fig. 4 shows the fluorescence spectra of the anthracene solutions diluted by a factor of 20.

Table 2 Measurement Conditions

Instrument	: RF-6000
Excitation Wavelength	: 282/294/339/341 nm
Measurement Wavelength Range	: 310 nm to 550 nm/ 350 nm to 550 nm
Data Interval	: 1.0 nm
Scan Speed	: 200 nm/min
Bandwidth	: Ex 5.0 nm/Em 5.0 nm

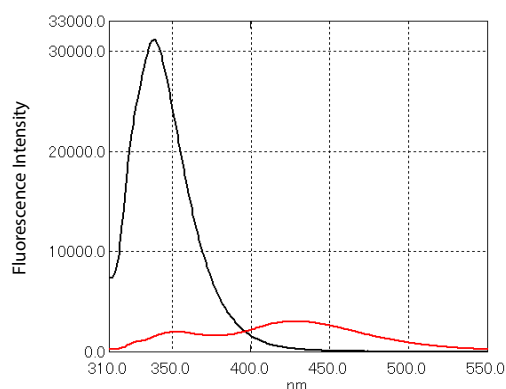


Fig. 2 Fluorescence Spectra
Black: DMABN Solution (Solvent: cyclohexane)
Red: DMABN Solution (Solvent: dichloromethane)

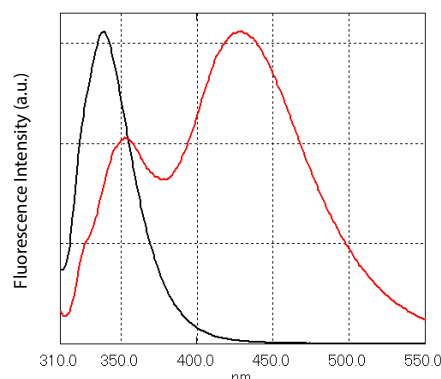


Fig. 3 Spectra Normalized on the Peak Values in Fig. 2

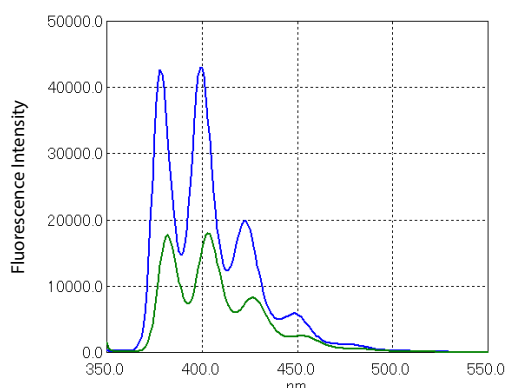


Fig. 4 Fluorescence Spectra
Blue: Anthracene Solution (Solvent: cyclohexane)
Green: Anthracene Solution (Solvent: dichloromethane)

The spectra of the anthracene solutions in Fig. 4 show a small shift between the nonpolar solvent and polar solvent, but the shape of the spectra are not greatly different. On the other hand, the spectra of the DMABN solutions in Fig. 2 greatly differ in shape, showing a fluorescence peak at about 340 nm with the nonpolar solvent and at about 350 nm and 430 nm with the polar solvent. This is because although DMABN has a planar structure before and after excitation in the nonpolar solvent, it stabilizes^{*1} due to rotations relating to C-N bonds after excitation in the polar solvent. Fluorescence is observed in this stabilized state.¹⁾ A planar structure is defined to be in a local excitation (LE) state, and a twisted structure is called a twisted intramolecular charge-transfer (TICT) state.

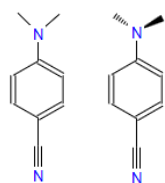


Fig. 5 Structure of DMABN
Left: Planar, Right: Twisted

*1 This is caused by charge transfers (CT) within a molecule from electron donor amino groups to electron acceptor cyan groups in the polar solvent.

Table 3 Measurement Conditions

Instrument	: UV-2600
Wavelength Range	: 300 nm to 750 nm
Sampling Interval	: 1.0 nm
Scan Speed	: Medium speed
Bandwidth	: 1.0 nm

Table 4 Measurement Conditions

Instrument	: RF-6000
Excitation Wavelength	: 339 nm
Measurement Wavelength Range	: 310 nm to 550 nm
Data Interval	: 1.0 nm
Scan Speed	: 200 nm/min
Bandwidth	: Ex/Em 5.0 nm
Sensitivity	: Low

Emission from the S₂ State

The absorbance spectrum of an azulene solution (solvent: ethanol) was measured using the conditions listed in Table 3 and then the fluorescence spectrum of the same solution diluted by a factor of 100 was measured using the conditions in Table 4.

The absorbance spectrum in Fig. 6 shows absorbance from the ground state (S₀) to the S₂ state in the ultraviolet range, and from the S₀ state to the first excited singlet (S₁) state in the visible range. Based on the emission position shown in the fluorescence spectrum in Fig. 7 and the results from Fig. 6, the emission can be expected to be fluorescence occurring due to a transition from the S₂ state to the S₀ state. Although organic compounds generally start emission from the lowest excited state according to Kasha's rule, we can see that azulene shows an emission transition that does not follow this rule.

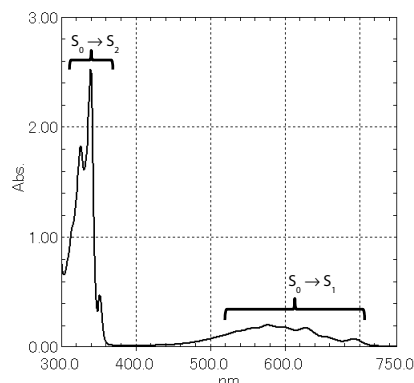


Fig. 6 Absorbance Spectrum of Azulene Solution (75 mg/L)

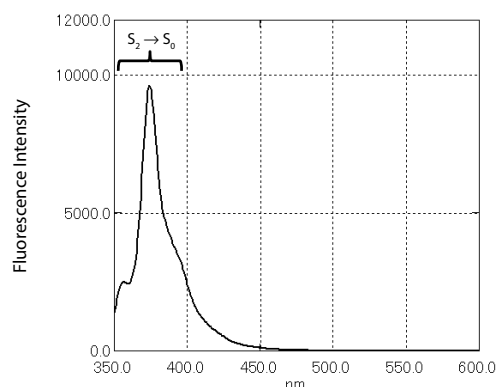


Fig. 7 Fluorescence Spectrum of Azulene Solution (0.75 mg/L)

Conclusion

We used the RF-6000 spectrofluorophotometer to measure samples which exhibit differing fluorescence when in a polar solvent and in a nonpolar solvent, as well as a sample which starts emission from the second excited singlet state. Since the transitions in the state of electrons can be understood in a complementary way using absorbance spectra and fluorescence spectra, the state of a substance can be investigated in greater detail.

Reference

- 1) Nicholas J. Turro et al. "Principles of Molecular Photochemistry - An Introduction", translation supervised by Haruo Inoue, Osamu Ito, Maruzen Publishing, 204-218 (2013) (in Japanese)