Application Note

FP-0003

Measuring fluorescence anisotropy spectrum of Rhodamine B

Measuring fluorescence anisotropy is performed using polarizers on both excitation(Ex) side and emission(Em) side. It is known that Rhodamine B has wavelength dispersion of fluorescence anisotropy depending on the excitation wavelength. Therefore the Rhodamine B was measured to obtain wavelength dispersion of fluorescence anisotropy and degree of polarization.

[Measurement principle]

With Ex side polarizer in the vertical(V) position, each excitation spectrum is measured under Em side polarizer in the vertical(V) and horizontal(H) positions. These fluorescence intensities of the excitation spectra are defined as I_V and I_H.

In order to correct the difference of sensitivity for polarization on Em side detector, the spectra are measured with Ex side polarizer in the H and Em side polarizer in the V and H positions, and the ratio is multiplied by I_H to obtain I_H(corrected). Then calculated fluorescence anisotropy(r) is obtained using the following equation.

$$r = \{ I_V - I_H(\text{corrected})\} / \{I_V + 2\, I_H(\text{corrected})\}$$

[Measurement condition]

Instrument:	FP-6500
Polarizer:	FDP-203 polarizer
Measurement mode:	Ex spectrum
Ex side bandwidth:	3 nm
Em side bandwidth:	10 nm
Response:	0.5 sec
Sensitivity:	Medium
Measurement wavelength range:	350-580 nm
Data acquisition interval:	0.2 nm
Em wavelength:	625.0 nm
Wavelength scan speed:	200 nm/min

[Measurement procedure]

1. Set Ex side polarizer in the V position and set Em side polarizer also in the V position. Using FP-6500 spectrofluorometer, measure Ex spectrum of ethylene glycol solution (0.588 mg/mL). Then the fluorescence intensity of the spectrum is defined as I_V.
2. Set Ex side polarizer in the H position and set Em side polarizer in the V position, and measure spectrum in the same way like item 1.
3. Set Ex side polarizer in the H position and set Em side polarizer in the H position, and measure spectrum in the same way like item 1.
4. Set Ex side polarizer in the V position and set Em side polarizer in the H position, and measure spectrum in the same way like item 1.
5. Calculate the ratio of the above item 2.and 3. results, and then approximate average value is determined to be $\text{ratio}_{avg} = 3.0$.
6. Multiply the spectrum of the above item 4. with the $\text{ratio}_{avg} = 3.0$, to determine this fluorescence intensity as I_H(corrected).
7. Calculate the fluorescence anisotropy(r) using I_V and I_H(corrected).

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[Measurement result]

The absorption band that has peak wavelength around 560 nm shows large constant fluorescence anisotropy(r). On the other hand, r around 430nm, 360 nm show negative peaks, and shows small positive peak around 380 nm. These fluorescence anisotropy variation suggests that different electronic transition bands are overlapped.

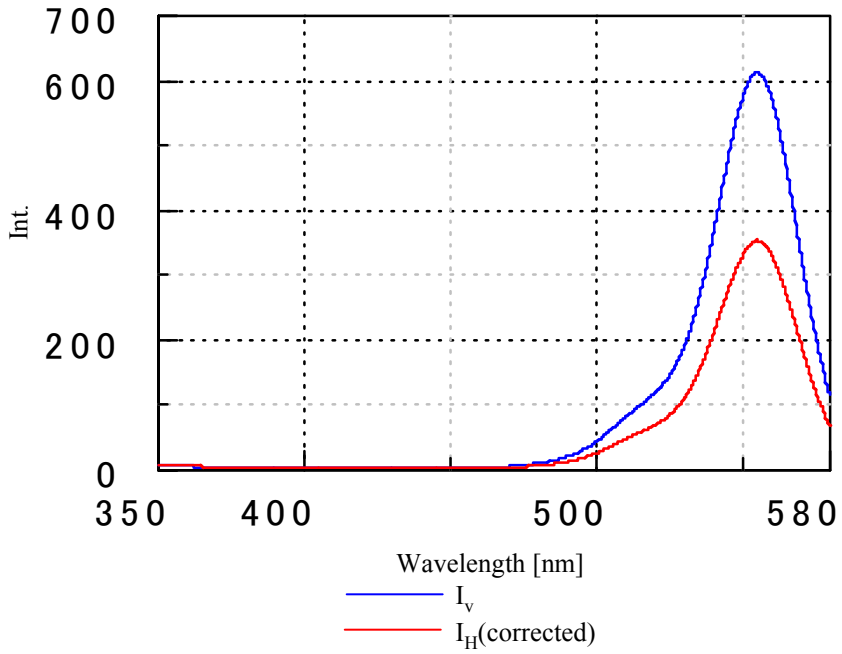

Fig. 1 Ex spectra of I_V and I_H(corrected)

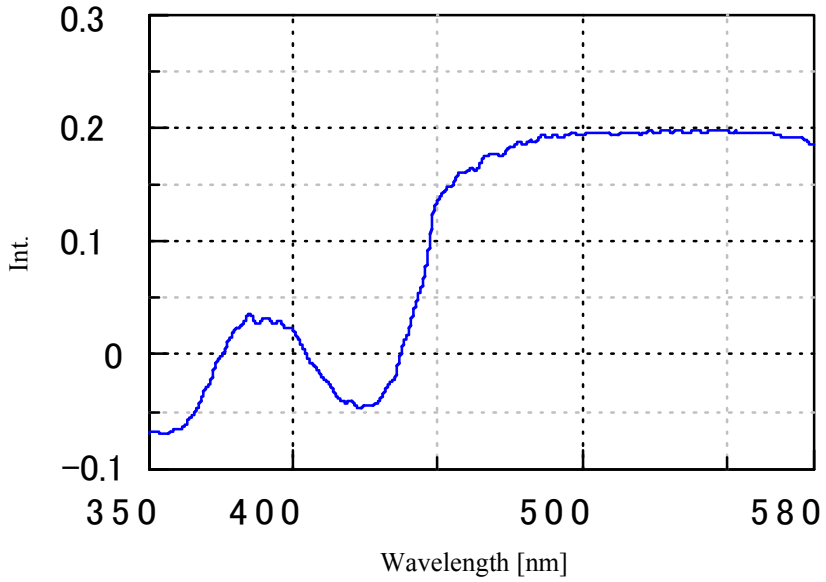

Fig. 2 Ex spectrum of fluorescence anisotropy

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