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Spectrofluorophotometer RF-6000

Application News

Changes in Fluorescence Properties Due to Temperature—Using a Thermoelectric Single-Cell Constant-Temperature Holder—

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User Benefits

- Fluorescence properties can be confirmed quickly and easily while controlling the temperature with a thermoelectrically temperature-controlled single-cell holder.
- Thermoelectric control can control temperatures in a wider range of temperatures (0 to 100 °C) than thermostatic water control.

■ Introduction

The intensity and peak positions of fluorescence can vary depending on various external factors, such as temperature, solvent, and pH. Temperature is considered particularly important because it can affect the transition from an excited state to the ground state. Therefore, many experiments are conducted in low-temperature environments. (For an example of measuring data in a low-temperature state, refer to Application News No. A561.) Similarly, experiments are conducted in high-temperature states because fluorescent light measurements can also vary. (Peaks can shift or disappear.)

This article describes an example of using a thermoelectric single-cell constant-temperature holder*1 to measure temperature variations in the high-temperature direction.

■ Spectral Changes Due to Temperature of Lysozyme

Aromatic amino acids present in proteins (such as tryptophan, tyrosine, and phenylalanine) emit fluorescence. Tryptophan emits fluorescence with higher intensity than the other aromatic amino acids. In addition, because tryptophan is hydrophobic, three-dimensional structural decay caused by protein denaturing (unfolding) can expose tryptophan to solvents, which changes its fluorescence spectrum.[1)] One way to determine the correlation between the structure, physical properties, and function of proteins is by using fluorescence measurements.

In this example, an aqueous lysozyme solution was measured using the condition settings in Table 1. The temperature was varied from 25 to 90 °C. A stopper-sealed cell was used for measurements at increasing temperatures. A photograph of the system is shown in Fig. 1. Typical spectra are shown in Fig. 2. Fluorescence spectra normalized at peak intensity are shown in Fig. 3.

Table 1 Measurement Parameters

Instruments:	RF-6000
Optional Parts:	Thermoelectric single-cell constant-temperature holder*1 Constant-temperature water recirculation unit
Excitation (Ex) Wavelength:	281 nm
Fluorescence (Em) Wavelength Range:	290 to 500 nm
Data Interval:	1.0 nm
Scan Speed:	200 nm/min
Slit:	Ex 5.0 nm, Em 5.0 nm
Sensitivity:	Low

Fig. 1 RF-6000 Spectrofluorophotometer with Thermoelectric Single-Cell Constant-Temperature Holder

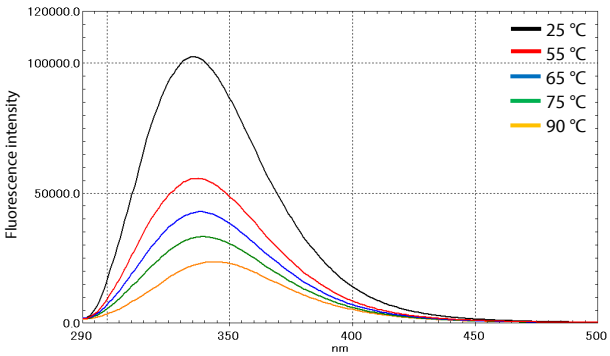

Fig. 2 Fluorescence Spectrum of Aqueous Lysozyme Solution

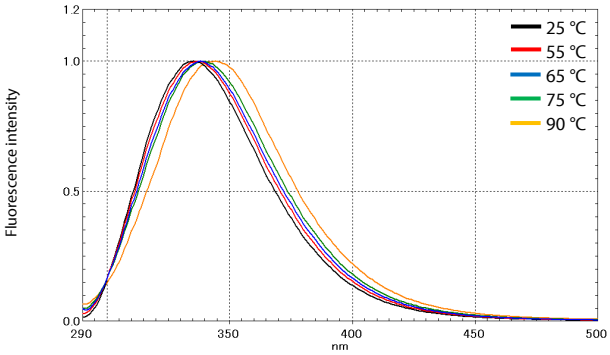

Fig. 3 Fluorescence Spectrum (Normalized) of Aqueous Lysozyme Solution

Fig. 2 shows how the fluorescence intensity decreased with increasing temperature. Presumably, the number of non-radiative transitions (deactivation of thermal energy) increased as photons returned from an excited state to the ground state. Fig. 3 shows how the peak position shifted farther in the long-wavelength direction (redshift) as the temperature increased. Presumably, that was due to the tryptophan in the lysozyme being disturbed by the external hydrophilic environment. Note, however, that the peak position remains the same at any temperature for the aqueous tryptophan solution alone (Fig. 4). Thus, changes in protein behavior (such as due to denaturation) can be inferred by measuring spectra while controlling the temperature.

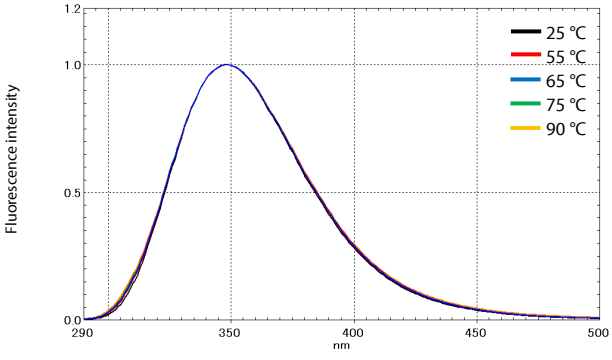

Fig. 4 Fluorescence Spectrum (Normalized) of Aqueous Tryptophan Solution

■ Spectral Changes Caused by Ink Temperature

Commercially marketed ballpoint pens with erasable ink are manufactured with a special process that causes the color of the ink to disappear when it is rubbed. This is due to a reaction caused by heat from friction.

3D spectra were measured from a commercially marketed fluorescent ballpoint pen (with a water-based orange color) by dissolving the ink in purified water to prepare a sample solution (an orange ink solution). This was measured as the temperature was varied according to the settings indicated in Table 2. Because it was a fluorescent pen, the results show fluorescence. The temperature was varied from 25 to 80 °C. A stopper-sealed cell was used for measurements with increasing temperatures. The appearance and 3D spectra of the sample at representative temperatures are shown in Figs. 5 and 6.

Table 2 Measurement Parameters

Instruments	RF-6000
Optional Parts:	Thermoelectric single-cell constant-temperature holder*1 Constant-temperature water recirculation unit
Excitation (Ex) Wavelength:	300 to 600 nm
Fluorescence (Em) Wavelength Range:	300 to 650 nm
Data Interval:	Ex 5.0 nm and Em 5.0 nm
Scan Speed:	30,000 nm/min
Slit:	Ex 5.0 nm, Em 5.0 nm
Sensitivity:	Low

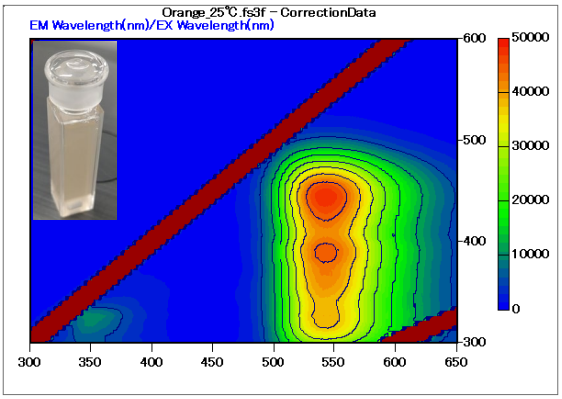

Fig. 5 Appearance and 3D Spectrum of Orange Ink Solution at 25 °C

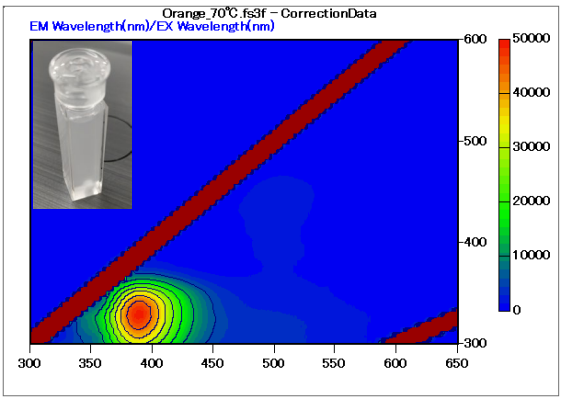

Fig. 6 Appearance and 3D Spectrum of Orange Ink Solution at 70 °C

The results show that fluorescence from the orange ink solution changed from appearing in the 500 to 600 nm region at room temperature (25 °C) to disappearing and appearing instead in the 360 to 430 nm region when the temperature reached 60 °C. To confirm the spectral changes in more detail, changes in the fluorescence spectra were measured using the parameter settings indicated in Table 3. The measurement results are shown in Figs. 8 and 9.

Table 3 Measurement Parameters

Excitation Wavelengths:	445 nm and 330 nm
Fluorescence (Em) Wavelength Range:	480 to 700 nm and 350 to 460 nm
Data Interval:	1.0 nm
Scan Speed:	200 nm/min
Slit:	Ex 5.0 nm, Em 5.0 nm
Sensitivity:	Low

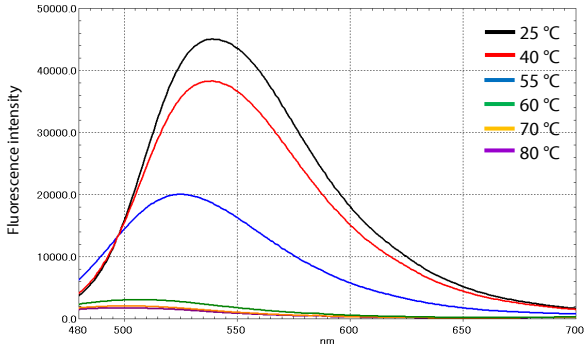

Fig. 7 Fluorescence Spectra of Orange Ink Solution at Ex 445 nm

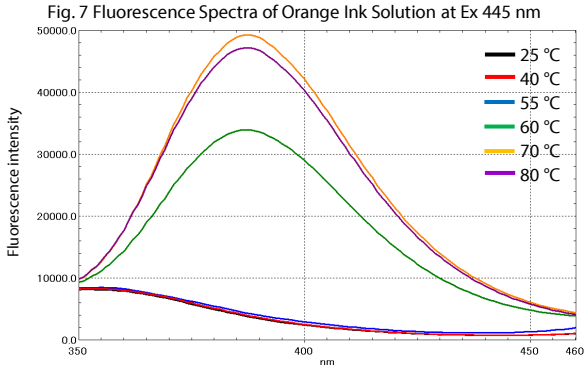

Fig. 8 Fluorescence Spectra of Orange Ink Solution at Ex 330 nm

The fluorescence spectrum for 445 nm excitation showed that fluorescence intensity decreased and peak wavelength shifted in the short-wavelength direction (blueshift) as temperature increased up to 55 °C, and almost all 500 to 600 nm fluorescence had disappeared by 60 °C. In contrast, with the fluorescence spectrum for 330 nm excitation, 360 to 430 nm fluorescence appeared at 60 °C and increased in intensity up to 70 °C. It then began decreasing after 80 °C. Presumably, this occurred due to chemical changes that were caused by temperature.

■ Conclusion

The fluorescence spectra of lysozyme and erasable fluorescent ballpoint pen ink were measured at varying temperatures. The spectra from the aqueous lysozyme solution showed a redshift in the peak wavelength above a certain temperature. The spectra from the erasable fluorescent ballpoint pen ink showed that the fluorescent color disappeared when the solution reached a certain temperature. It also showed that along with the disappearance of fluorescent color, additional fluorescence appeared in a separate wavelength region.

References

1) Tadashi Kamiyama, "Thermodynamics of Lysozyme in Binary Solutions of Water + DMSO," Netsu Sokutei, 36(5), 263-270, 2009.

Related Application News Articles

1. Light Emission Measurement at Low Temperature —Utilizing the Low-temperature Measurement Unit— Application News No. A561

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01-00909-EN First Edition: Jun. 2025

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