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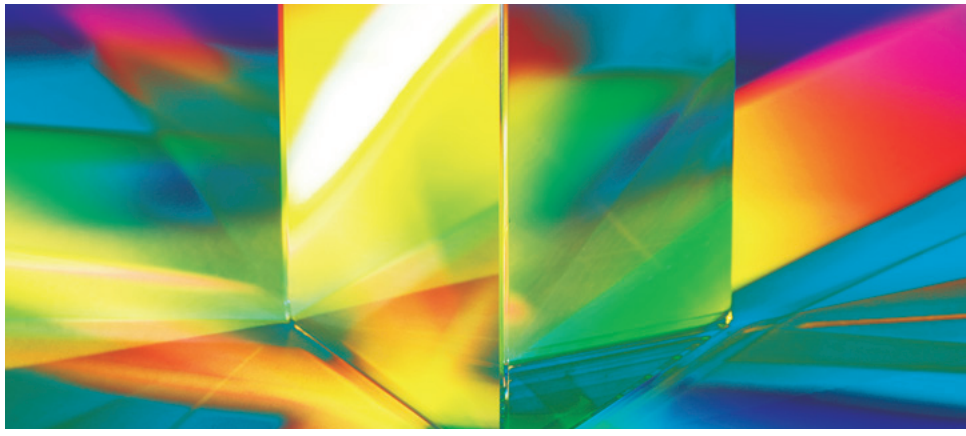
UV TALK LETTER

Vol. 18



UV-VIS Spectroscopy and Fluorescence Spectroscopy (Part 2 of 2)

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1. Introduction

In UV Talk Letter Vol. 17, we focused primarily on the principles and characteristics of fluorescence. Here, we describe the spectrofluorophotometers used in fluorescence spectroscopy, focusing on their configurations and the data they generate as compared to the data generated by UV-VIS spectrophotometers. Also described is how spectrofluorophotometers in particular can record multiple types of data, including excitation spectra, fluorescence spectra, and 3D spectra, and that data generated by each spectrofluorophotometer include a coefficient unique to that instrument, and recorded data must be corrected according to this coefficient.

2. About UV-VIS Spectrophotometers and Spectrofluorophotometers

• Relationship Between Spectra and the UV-VIS Spectrophotometer Configuration

First, we will review the structure of a UV-VIS spectrophotometer. Fig. 1 shows a simple schematic of Shimadzu's UV-2600 UV-VIS spectrophotometer. The four main components of a UV-VIS spectrophotometer are the light source, monochromator, sample compartment, and detector. The light sources include a deuterium (D_2) lamp for generating ultraviolet light and a halogen (WI) lamp for producing light in the visible to near-infrared region. A monochromator splits the UV light or visible light produced by either light source into monochromatic light. UV-VIS spectrophotometers with a dual-beam optical system have a sample compartment that can accommodate two cells: a sample cell for the sample light beam and a reference cell for the reference light beam. Light is passed through either the sample cell or reference cell, and each beam is alternatively detected by the detector.

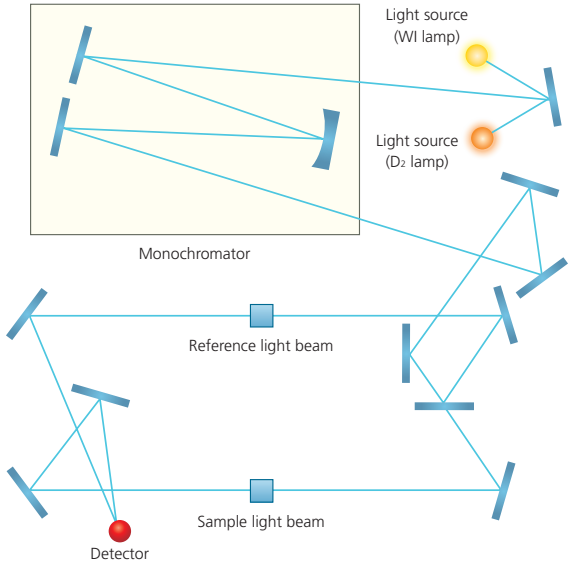

Fig. 1 Schematic of a UV-VIS Spectrophotometer

UV-VIS spectrophotometers generate spectra using the monochromator to change the wavelength of incident light and by recording the transmitted light that passes through the sample at each wavelength. A baseline measurement (solvent analysis) is also conducted before analyzing the sample. The ratio of light intensity recorded during baseline measurement to that recorded during sample measurement is calculated, thereby permitting accommodation for spectral emittance characteristics of the light source, wavelength characteristics of the monochromator, and wavelength sensitivity characteristics of the detector. This ratio and associated relationships are expressed as given in equation (1) below.

$$\frac{\text{(Intensity of measured light)}}{\text{(Intensity of baseline light)}} = \frac{[K_1\times\text{(Light source characteristics)}\times\text{(Monochromator characteristics)}\times(1 - \text{Sample absorption})\times\text{(Detector characteristics)}]}{[K_1\times\text{(Light source characteristics)}\times\text{(Monochromator characteristics)}\times(1 - \text{Solvent absorption})\times\text{(Detector characteristics)}]}$$
$$= \frac{(1 - \text{Sample absorption})}{(1 - \text{Solvent absorption})}$$
$$= (1 - \text{Sample absorption}) \quad (1)$$

Where K_1 is a constant.

Normally, a solvent that absorbs almost no UV-VIS region light is used so that solvent absorption is near zero. UV-VIS spectrophotometers display transmittance as equation (1) multiplied by 100, and absorbance as the base 10 logarithm of the inverse of equation (1). Also, since these results are not affected by instrument characteristics, they can be compared with measurements obtained using different UV-VIS spectrophotometers. Dual-beam instruments also eliminate the effect of light source fluctuations on recorded results by recording the ratio of sample light beam-to-reference light beam intensity. As for the differences between single- and dual-beam instruments, refer to UV Talk Letter Vol. 9.

• Relationship Between a Spectrum and the Spectrofluorophotometer Configuration

The configuration of a spectrofluorophotometer can be illustrated using the simple schematic of Shimadzu's RF-6000 spectrofluorophotometer shown in Fig. 2. The five main components of a spectrofluorophotometer are the light source, excitation monochromator, sample compartment, emission monochromator, and detector. Since the fluorescence intensity is proportional to the light source intensity, a high-intensity xenon (Xe) lamp is used as the light source, as later covered in detail. The sample is irradiated with monochromatic excitation light from the excitation monochromator, which diffracts light from the light source to produce monochromatic light. Spectrofluorophotometers are single-beam instruments that do not generate a reference light beam, therefore accommodating only a single cell in the sample compartment. The emission monochromator diffracts fluorescence emitted by the sample into light at different wavelengths. To reduce the effect of excitation light on the fluorescence spectrum being recorded, Shimadzu's RF-6000 is designed to detect fluorescence perpendicular to the direction of the excitation light. This design capitalizes on the phenomenon by which fluorescence produced by an irradiated cell is emitted in all directions. The cells used in spectrofluorophotometers and UV-VIS spectrophotometers are also different, as shown in Fig. 3. In spectrofluorophotometers, since fluorescence is recorded perpendicular to the direction of excitation light, a cell with four polished sides is required. UV-VIS spectrophotometers require a cell with just two polished sides to allow passage of incident light and transmitted light. In spectrofluorophotometers, fluorescence emitted by the sample is detected after it is diffracted by the emission monochromator.

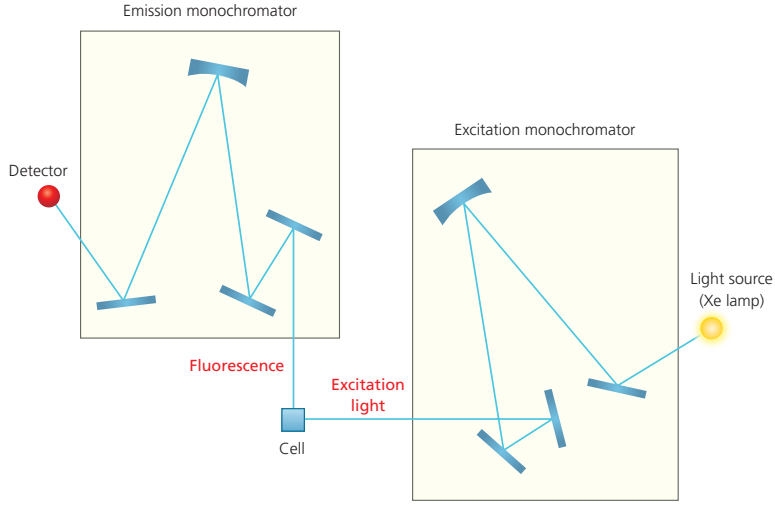

Fig. 2 Spectrofluorophotometer Structure

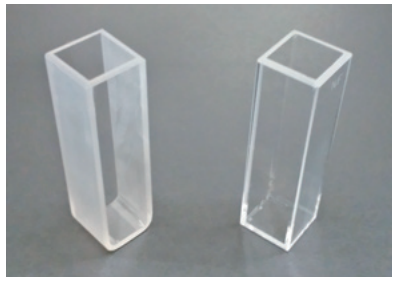

Fig. 3 Left: Cell with Two Polished Sides
Right: Cell with Four Polished Sides

Spectrofluorophotometers are primarily capable of recording two spectra: fluorescence spectra and excitation spectra. When recording fluorescence spectra, the emission monochromator scans a range of wavelengths of emitted fluorescence, all measured while the sample is irradiated with a fixed wavelength of excitation light (the excitation monochromator isolating a single wavelength of excitation light). When recording excitation spectra, fluorescence is observed at a single wavelength (the emission monochromator isolating one wavelength of fluorescence) while the excitation monochromator scans through a range of wavelengths of excitation light. The wavelength of excitation light that produces the greatest fluorescence from the sample is recorded. A 3D spectrum shows excitation wavelengths at which a sample emits light by recording the fluorescence spectrum at each excitation wavelength, and then changing the excitation wavelength by fixed amounts.

Data recorded by spectrofluorophotometers when measuring excitation/fluorescence spectra include the characteristics of the instrument. The excitation spectrum associated with the sample is multiplied by the spectral radiant characteristics of the light source and the wavelength characteristics of the excitation monochromator. However, since the emission monochromator is fixed at a specific wavelength, the wavelength characteristics of the emission monochromator and the wavelength sensitivity characteristics of the detector do not have an effect at each recorded wavelength of the excitation spectrum intensity. When an excitation spectrum that includes instrument characteristics is called an "Apparent excitation spectrum", it is expressed as shown in equation (2).

$$\text{(Apparent excitation spectrum} = K_2 \times \text{(Light source characteristics)} \times \text{(Excitation monochromator characteristics)} \times \text{(Sample excitation spectrum)} \quad (2)$$

Where K_2 is a constant.

Furthermore, the fluorescence spectrum of the sample is multiplied by the wavelength characteristics of the emission monochromator and the wavelength sensitivity characteristics of the detector. However, since the excitation monochromator is fixed at a specific wavelength, the spectral radiant characteristics of the light source and the wavelength characteristics of the excitation monochromator do not have an effect on each recorded wavelength of the fluorescence spectrum intensity. When the fluorescence spectrum that includes instrument characteristics is called an "Apparent fluorescence spectrum", it is expressed as shown in equation (3).

$$\text{(Apparent fluorescence spectrum} = K_3 \times \text{(Sample fluorescence spectrum)} \times \text{(Emission monochromator characteristics)} \times \text{(Detector characteristics)} \quad (3)$$

Where K_3 is a constant.

Since the "Apparent excitation spectrum" and the "Apparent fluorescence spectrum" include instrument characteristics, the spectrum shape of each will differ depending on the instrument model, and may also change within the same instrument due to the instrument changing over time. Consequently, instrument characteristics must be removed from excitation spectra and fluorescence spectra.

Without going into great detail, instrument characteristics as represented by [(Light source characteristics)×(Excitation monochromator characteristics)] in equation (2) and [(Emission monochromator characteristics)×(Detector characteristics)] in equation (3) can be calculated for each individual spectrofluorophotometer using a standard tungsten lamp of known intensity distribution and a light quantum meter (rhodamine B in ethylene glycol). After eliminating the instrument characteristics present in equation (2) and equation (3), the results are referred to as a corrected excitation spectrum and a corrected fluorescence spectrum, which are represented as shown in equations (4) and (5), respectively.

$$\text{(Corrected excitation spectrum)} = \frac{\text{(Apparent excitation spectrum)}}{\text{[(Light source characteristics)} \times \text{(Excitation monochromator characteristics)]}} = K_2 \times \text{(Sample excitation spectrum)} \quad (4)$$

$$\text{(Corrected fluorescence spectrum)} = \frac{\text{(Apparent fluorescence spectrum)}}{\text{[(Emission monochromator characteristics)} \times \text{(Detector characteristics)]}} = K_3 \times \text{(Sample fluorescence spectrum)} \quad (5)$$

The corrected excitation spectrum and corrected fluorescence spectrum are the excitation and fluorescence spectra of the sample with instrument characteristics eliminated, and they can be compared among different instrument models, without changing as the instrument ages.

3. Absorbance and Fluorescence Intensity

• Absorbance and Fluorescence Intensity at a Fixed Wavelength

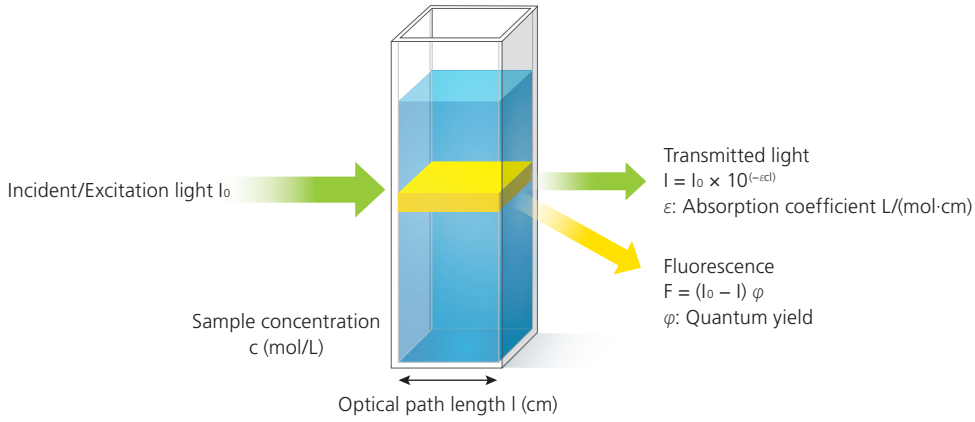

Fig. 4 Absorbance and Fluorescence Inside a Cell

As shown in Fig. 4, assuming sample concentration c (mol/L) and cell optical path length l (cm), the sample absorption coefficient at a given wavelength is ε (L/(mol·cm)). When incident light intensity is I_0 and intensity of transmitted light is I, absorbance A is given by equation (6) according to Lambert-Beer's law.

$$A = \log_{10}(I_0/I) = \varepsilon cl \quad (6)$$

Furthermore, transmittance T is shown by equation (7).

$$T\,(\%) = (I/I_0) \times 100 \quad (7)$$

Meanwhile, fluorescence intensity F, which arises due to the phenomenon of light emission after absorption of incident light inside the cell (I_0-I), is shown by:

$$F = (I_0 - I)\Phi = (1 - I/I_0)\Phi I_0 \quad (8)$$

Therefore, fluorescence intensity is proportional to incident light (light source intensity). In the above equation, Φ is called the quantum yield, and is the ratio of the number of photons of excitation light absorbed by the sample (N_{abs}) to the number of photons emitted as fluorescence (N_{em}), as shown in equation (9). Quantum yield is an important indicator of luminous efficiency. Note that equation (8) shows fluorescence intensity for a fixed wavelength, and therefore the monochromator and detector wavelength characteristics mentioned earlier are multiplied by the constants. However, in this representation, these constants can be omitted without issue.

$$\Phi = N_{em}/N_{abs} \quad (9)$$

When equation (6) is expressed as a natural logarithm,

$$(1/\log_e 10) \times \log_e(I_0/I) = \varepsilon cl \quad (10)$$

From equation (10),

$$I/I_0 = e^{-2.303\varepsilon cl} \quad (11)$$

From equations (8) and (11) we obtain,

$$F = (1 - e^{-2.303\varepsilon cl})\Phi I_0 \quad (12)$$

When the concentration is small enough, equation (12) can be expressed as,

$$F = 2.303\varepsilon cl\Phi I_0 = 2.303\,A\Phi I_0 \quad (13)$$

Therefore, as with absorbance, fluorescence intensity is also proportional to concentration.

As shown in equation (6), absorbance is calculated as the ratio of incident light intensity to transmitted light intensity. Consequently, the lower limit of detection of the detector determines its ability to distinguish a change in intensity between incident light and transmitted light at low sample concentrations. However, the phenomenon of fluorescence is measured directly as fluorescence, which is zero at its smallest level. This characteristic makes fluorescence spectroscopy more intensity sensitive than UV-VIS spectroscopy.

• Corrected Excitation Spectrum and Absorptance Spectrum

Taking apparent excitation spectrum Ex(λ), light source intensity L(λ), and excitation monochromator wavelength characteristics M(λ) (where λ represents wavelength), the apparent excitation spectrum is expressed as shown in equation (14).

$$\begin{aligned} Ex(\lambda) &= K_4 \times [L(\lambda) \times M(\lambda) - \alpha(\lambda) \times L(\lambda) \times M(\lambda)] \times \Phi(\lambda) \\ &= K_4 \times [1 - \alpha(\lambda)] \times L(\lambda) \times M(\lambda) \times \Phi(\lambda) \end{aligned} \quad (14)$$

Where K_4 is a constant, and $\alpha(\lambda)$ is the proportion of incident light that passes through the cell (1/100 of transmittance). From equation (14), the corrected excitation spectrum is expressed as given by equation (15).

$$\begin{aligned} (\text{Corrected excitation spectrum}) &= Ex(\lambda)/[M(\lambda) \times L(\lambda)] \\ &= K_4 \times [1 - \alpha(\lambda)] \times \Phi(\lambda) \end{aligned} \quad (15)$$

When quantum yield is fixed and not dependent on wavelength, it can be treated as a constant.

$$(\text{Corrected excitation spectrum}) = K_5 \times [1 - \alpha(\lambda)] \quad (16)$$

$$K_5 = K_4 \times \Phi(\lambda) \quad (17)$$

The corrected excitation spectrum from equation (16) matches the shape of the absorptance spectrum [1-$\alpha(\lambda)$] described using transmittance calculated with a UV-VIS spectrophotometer. Furthermore, if excitation spectrum intensity is linear, then concentration is sufficiently low, and according to the previous section equation (16) becomes,

$$\begin{aligned} (\text{Corrected excitation spectrum}) &= K_5 \times 2.303 \varepsilon(\lambda) cl \\ &= K_6 \times A(\lambda) \end{aligned} \quad (17)$$

$$K_6 = K_5 \times 2.303 \quad (18)$$

The shape of this spectrum matches that of the spectrum displayed for absorbance.

4. Conclusion

This article describes instrument configuration, absorbance, fluorescence intensity and spectrum correction for UV-VIS spectrophotometers and spectrofluorophotometers, while comparing the characteristics of each. A summary of the subject matter covered is shown in Table 1. UV-VIS spectroscopy and fluorescence spectroscopy both have distinguishing features that, when understood, allow for better utilization of both methods.

Table 1 Comparison of UV-VIS Spectroscopy and Fluorescence Spectroscopy

	Fluorescence Measurement	UV-VIS Absorbance Measurement
Instrument configuration	Light source → Excitation monochromator → Sample compartment → Emission monochromator → Detector	Light source → Monochromator → Sample compartment → Detector
Advantages	• High sensitivity as fluorescence (luminous phenomenon) starts from zero and is detected directly (three or more significant figures more sensitive than UV-VIS absorbance measurements*). • Selective due to substances having different excitation and fluorescence wavelengths.	• Any substance that exhibits absorbance in the UV-VIS region can be measured. • Baseline measurement allows for sample spectra to be recorded without including instrument characteristics. • Spectra and absorbances recorded on different UV-VIS spectrophotometers will basically match each other.
Disadvantages	• Substances that absorb light in the UV-VIS region may not necessarily fluoresce. • Excitation and fluorescence spectra include the instrument characteristics of the monochromators and detector.	• Detects the ratio of (difference between) incident light and transmitted light. Lower limit of detection is derived from whether the difference in intensity between incident and transmitted light is apparent. • Sensitivity is lower than fluorometric methods.
Other	• Corrected excitation spectra have the same shape as absorbance spectra. • The energy difference between vibrational energy levels of the excited state is reflected in excitation spectra, and the energy difference between vibrational energy levels of the ground state is reflected in fluorescence spectra.	• The energy difference between vibrational energy levels of the excited state is reflected in UV-VIS spectra.

*: The Japan Society for Analytical Chemistry, Hisanori Imura, Kazuya Kikuchi, Naoki Hirayama, Kotaro Morita, Hitoshi Watarai. Practical Analytical Technology Series, Instrument Analysis (Absorbance and Fluorescence Analysis), Kyoritsu Shuppan Co., Ltd.

Applications

Introduction of Examples of Chemiluminescence and Electroluminescence Analyses Using a Spectrofluorophotometer with an Automatic Spectral Correction Feature

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Some substances emit absorbed energy as light. This phenomenon is called luminescence, and is categorized based on the stimulating source of energy.
Photoluminescence is the name given to this phenomenon when light is the stimulus, chemiluminescence when a chemical reaction is the stimulus, electroluminescence when electrical energy is the stimulus, and bioluminescence when biological energy is the stimulus, as observed in fireflies.
This section describes the automatic spectral correction feature that automatically corrects readings for instrument-specific characteristics (instrument function), along with examples of electroluminescence (electrical energy stimulus) and chemiluminescence (chemical reaction stimulus) analyses.

1. Automatic Spectral Correction

The intensity and position of spectral peaks recorded from the same sample will differ when measured using different spectrofluorophotometers. This variation is caused by differences in the characteristics of the spectrofluorophotometer instrument itself, including differences in light source radiation characteristics, optical system spectral characteristics, and other component characteristics. Fig. 1 shows a corrected spectrum and an uncorrected spectrum in the same graph, indicating the differences in spectrum shape and peak positions. Shimadzu's RF-6000 spectrofluorophotometer simultaneously records corrected spectra without this instrument function as well as an uncorrected spectrum with this instrument function intact. Spectral correction permits the comparison of data that are generated using different instruments.

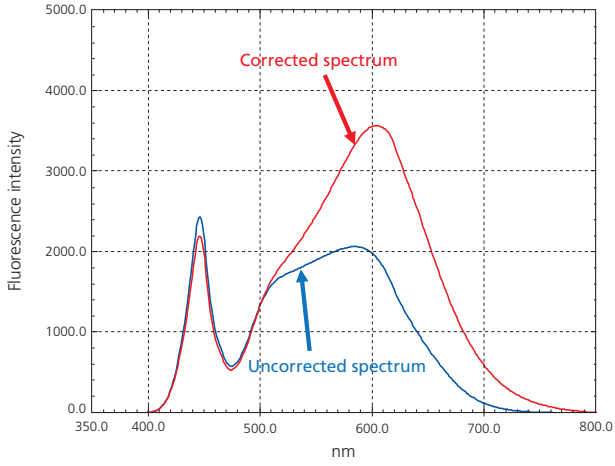

Fig. 1 Comparison of Corrected Spectrum and Uncorrected Spectrum

2. Measurement of LED Light Bulb Emission Spectra (Electroluminescence)

Light sources that emit visible light, such as LED light bulbs, generate unique emission spectra. The hue of light emitted by these light sources is determined by the intensity and specific wavelengths of the light they emit. Measurement of emission spectra therefore plays an important role in determining the type of light produced by a light source.

Shimadzu's RF-6000 spectrofluorophotometer includes a feature that automatically eliminates the instrument function (automatic spectral correction function), allowing the RF-6000 to record accurate emission spectra that are unaffected by instrument function characteristics.

Measurements conducted using commercially available LED light bulbs are shown below. Fig. 2 shows photographs of a warm, white color LED light bulb and a daylight white color LED light bulb. Fluorescence spectra recorded from each light bulb (four colors from the warm white, and four colors from daylight white) are shown on the same graph in Fig. 3 and Fig. 4. Fig. 3 shows the highest intensity visible light emitted from the warm white color LED bulb occurs in the green band (500 to 600 nm) and the red band (600 to 700 nm), and the light in these two bands combines to produce the yellowish hue emitted by the LED bulb. Fig. 4 shows that for the daylight white color LED bulb, the area under the graph is approximately equal across blue (400 to 500 nm), green, and red bands of light, and light from these bands mixes to produce the hueless white of the LED bulb. For further details, refer to Shimadzu Application News A497 Emission Spectra of LED Light Bulbs.

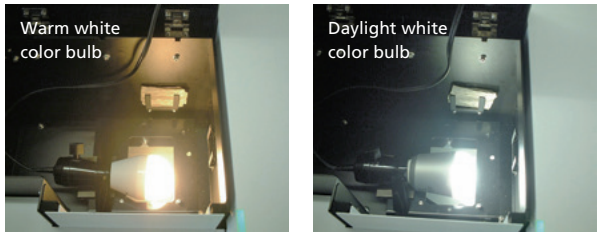

Fig. 2 LED Light Bulbs

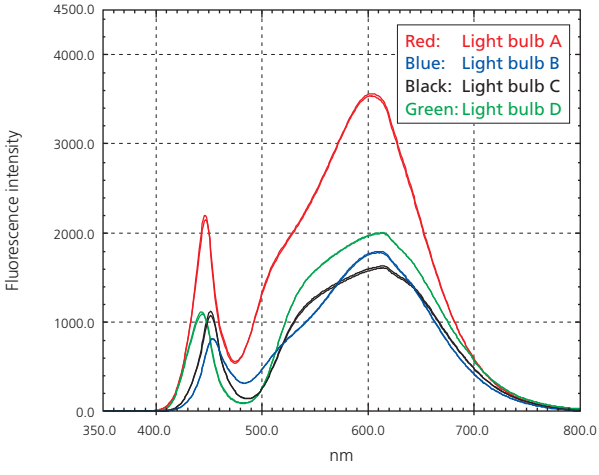

Fig. 3 Emission Spectra of Warm White Color Bulb

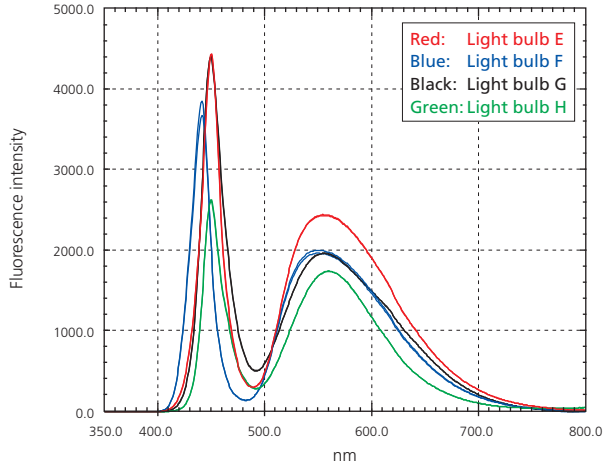

Fig. 4 Emission Spectra of Daylight White Color Bulb

3. Peroxyoxalate Luminescent Process and Spectral Measurement of Glow Stick Fluorescent Pigment (Chemiluminescence)

Peroxyoxalate chemiluminescence is caused by a chemical reaction between an oxalate ester and hydrogen peroxide within a fluorescent dye solution. This luminescence is characterized by high luminous efficiency and long duration of luminosity. The luminescent process is shown in Fig. 5.

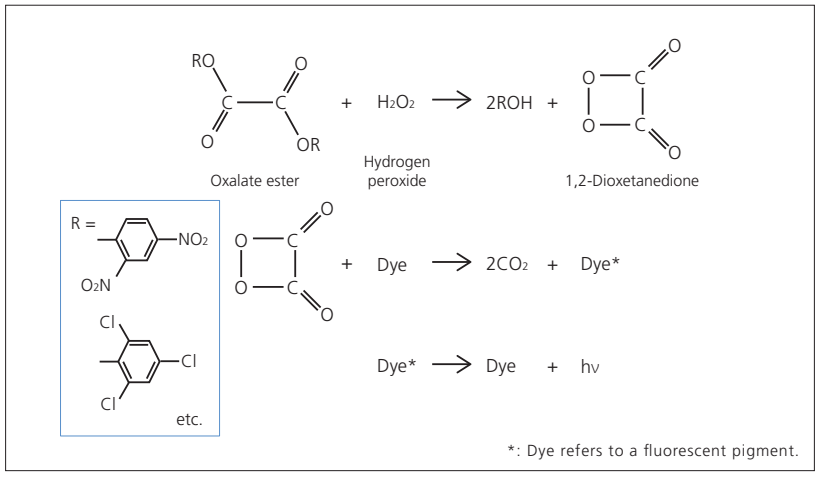

Fig. 5 Luminescent Process of Peroxyoxalate Chemiluminescence

As shown in Fig. 5, the oxalate ester is oxidized by hydrogen peroxide to produce ROH and 1,2-dioxetanedione.
1,2-Dioxetanedione is a high-energy reaction intermediate that exchanges electrons with the fluorescent pigment as it breaks down to carbon dioxide. When the electrons return to the fluorescent pigment, they enter the lowest unoccupied molecular orbital, elevating the fluorescent pigment to an excited state. The fluorescent pigment then releases light (hν) on returning to its ground state. The wavelength of the light released differs depending on the fluorescent pigment.

Three commercially marketed glow sticks are shown in Fig. 6. In these glow sticks, oxalate ester and fluorescent pigment solution are placed in a sealed thin-walled glass container fixed within a polyethylene tube that itself contains hydrogen peroxide solution and added catalyst. Bending the polyethylene tube breaks the glass container, causing the solutions to mix and the glow stick to fluoresce. Polycyclic aromatics are commonly used as the fluorescent pigments in glow sticks, and the color of light emitted by glow sticks varies depending on the wavelength of light emitted when the fluorescent pigment returns to its ground state from an excited state.

Fig. 6 Glow Sticks

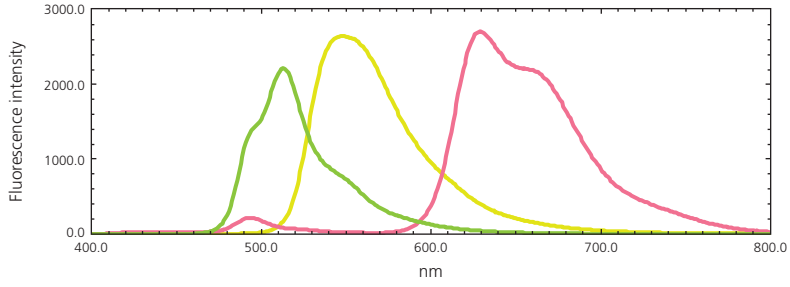

Fig. 7 Fluorescence Spectra of Glow Sticks

Fig. 7 shows emission spectra for yellow-green, yellow, and red glow sticks. The color used to represent each spectrum on the graph corresponds to the color of the glow stick.

4. Summary

This article introduced examples of electroluminescence and chemiluminescence analysis. The spectra presented in this article were subjected to automatic spectral correction, and can therefore be considered as true spectra with the effects of instrument function eliminated.

Q&A

Q What is the difference between quantum yield and quantum efficiency?

A Quantum yield is the name given to the number of photons of light emitted as fluorescence by a fluorescent substance relative to the number of photons of excitation light absorbed by the substance. There are two methods used for calculating quantum yield: the relative method and the absolute method. Quantum yields calculated by these methods are referred to as relative quantum yield (or quantum yield) and absolute quantum yield (or quantum efficiency), respectively.

Measuring quantum yield by the relative method relies on the use of a fluorescent substance of known quantum yield as a standard sample for calculating the quantum yield of an unknown sample. The formula used is similar to that shown in equation (1).

$$\phi_{un} = \phi_{st} \times \left(\frac{F_{un}}{F_{st}}\right) \times \left(\frac{Abs_{st}}{Abs_{un}}\right) \times \left(\frac{n_{un}^2}{n_{st}^2}\right) \times \left(\frac{D_{un}}{D_{st}}\right) \qquad (1)$$

Here, φ is quantum yield, *Abs* is absorbance at the excitation wavelength, *F* is area under the graph of the corrected fluorescence spectrum, *n* is solvent refractive index, and *D* is the dilution ratio used in sample dilution for a fluorescence spectrum measurement. In formula (1), *st* refers to the standard sample and *un* refers to the unknown sample. When the relative method is used, a standard sample of known quantum yield must first be chosen. Then, in addition to using a spectrofluorophotometer to measure the fluorescence spectrum of the standard and unknown samples, a UV-VIS spectrophotometer is required to measure absorbance at the excitation wavelength. As the recording of fluorescence spectra using a high-concentration sample can result in concentration quenching, diluted samples are used. As a rough guide for sample dilution, absorbance no greater than 0.05 Abs at the excitation wavelength is recommended. The absorbance spectra of uranine in ethanol and rhodamine B in ethanol are shown in Fig. 1, and the fluorescence spectra are shown in Fig. 2. The spectra show that when using the relative method, calculation of quantum yield requires the fluorescence spectra of two samples. For further details, refer to Application News No. A504.

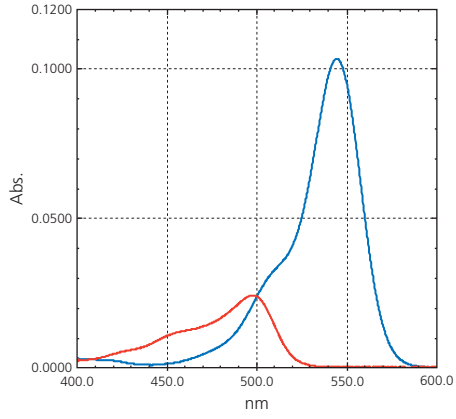

Fig. 1 Absorbance Spectra of Uranine in Ethanol (Red) and Rhodamine B in Ethanol (Blue)

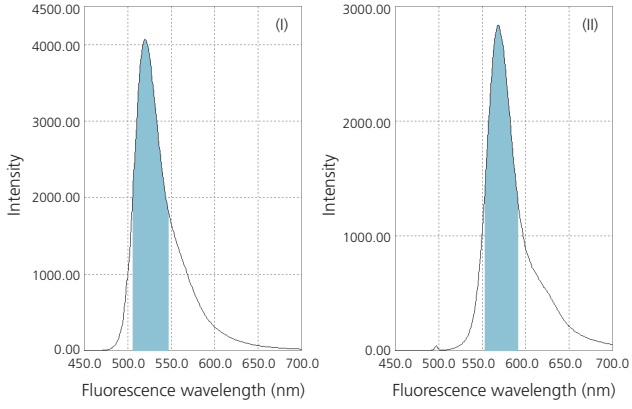

Fig. 2 Fluorescence Spectra of Uranine in Ethanol (I) and Rhodamine B in Ethanol (II)

Measurement by the absolute method requires the use of an integrating sphere to calculate the number of photons absorbed and emitted by an unknown sample based on the area of scattered excitation light and fluorescence under the graph. These numbers are used to calculate the quantum yield. The formula used is similar to that shown in equation (2).

$$\phi = \left(\frac{S_s E_m - S_b E_m}{S_b E_x - S_s E_x} \right) \qquad (2)$$

Here, S_sE_m and S_bE_m refer to the surface area under the graph of the corrected fluorescent spectrum recorded for the sample and blank solution (solvent only), and S_sE_x and S_bE_x refer to the area under the graph of scattered excitation light for the sample and blank solution, respectively. The procedure used involves first recording the fluorescence spectrum of a blank solution to measure the fluorescence and excitation light scattered by the solvent. Next, the fluorescence spectrum of the sample is recorded to measure the excitation light scattered by the sample and fluorescence emitted by the sample. The denominator of equation (2) corresponds to the amount of excitation light absorbed by the sample, and the numerator corresponds to the amount of fluorescence emitted by the sample. These values are used to calculate the quantum efficiency. Fig. 3 shows the fluorescence spectra of a quinine sulfate solution (solvent: 1.0 N sulfuric acid) and the solvent. These graphs indicate that when calculating quantum yield by the absolute method, in addition to the fluorescence spectrum of the sample and solvent, measurement of the scattered excitation light is also required. For further details, refer to Application News No. A496.

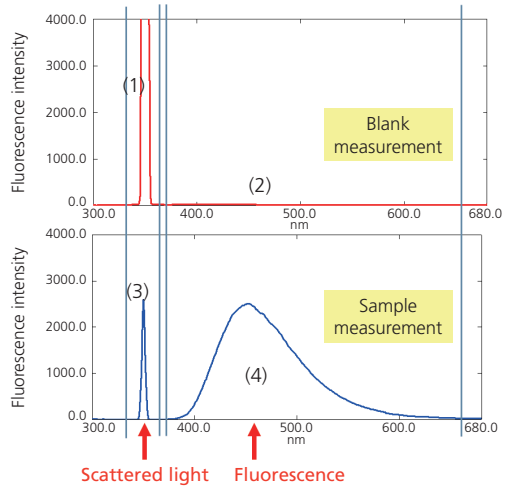

Fig. 3 Absolute Method Procedure (1) S_bE_x (2) S_bE_m (3) S_sE_x (4) S_sE_m

Table 1 compares the respective advantages and disadvantages of using quantum yield and quantum efficiency, respectively, for analysis.

Table 1 Comparison of Quantum Yield and Quantum Efficiency

	Quantum Yield (Relative Quantum Yield)	Quantum Efficiency (Absolute Quantum Yield)
Advantages	• Simple comparison of fluorescence spectra. • Inexpensive, requiring only a cell with four polished sides.	• Standard sample is not required. • Absorbance measurement is not required.
Disadvantages	• Requires a standard sample of known quantum yield. • Requires absorbance measurement of both standard and unknown samples. • After measuring absorbance, sample dilution may be necessary.	• Integrating sphere is expensive. • Labor-intensive management of integrating sphere deterioration and the need to conduct spectral correction.

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