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Application News

No. A615

Spectrophotometric Analysis

Difference in Quantifiable Concentration Ranges of UV-Vis Spectrophotometer and Fluorescence Spectrophotometer

Both the UV-Vis (ultraviolet-visible) spectrophotometer and the fluorescence spectrophotometer are used in quantitative evaluations. Quantitative analysis by the UV-Vis spectrophotometer measures absorbance, which is proportional to concentration, based on the Lambert-Beer law. The fluorescence spectrophotometer uses fluorescence intensity and enables quantitative evaluation of low concentrations because the fluorescence intensity is proportional to concentration.

In this experiment, we measured a rhodamine B solution with both a UV-Vis spectrophotometer and a fluorescence spectrophotometer. Rhodamine B is a fluorescent substance which is used in dyes for textiles and leather. This article introduces, based on the measurement results, the lower limits of quantitation and detection and the linearity of the calibration curves of the two instruments were compared.

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Absorbance Measurement of Rhodamine B Solution

A Shimadzu UV-2600i UV-Vis spectrophotometer was used in the absorbance measurement. Table 1 shows the measurement conditions. Rhodamine B in powder form was dissolved in distilled water, and standard solutions having concentrations of in the range of 0.003 to 5 µg/ml were prepared.

Fig. 1 shows the absorption spectra of the standard solutions of rhodamine B, and Fig. 2 and Fig. 3 show the calibration curves prepared from the 544 nm absorbance values. Fig. 2 was prepared for a high concentration region using 6 points in the range of 0.31 to 5 µg/ml and a blank sample (distilled water), and satisfactory linearity was obtained (square of correlation coefficient R^2 = 0.9999). However, in the low concentration region shown in Fig. 3, the effect of noise was relatively large, and linearity was low.

Table 1 Absorbance Measurement Conditions

Instrument	: UV-2600i UV-Vis spectrophotometer
Measurement value type	: Absorbance
Measured wavelength range	: 300 - 700 nm
Scan speed	: Medium
Sampling pitch	: 1.0 nm
Light source switching wavelength	: 340 nm
Slit width	: 1.0 nm

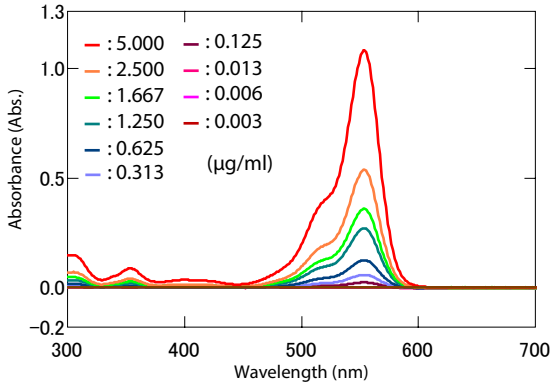

Fig. 1 Absorption Spectra (UV-2600i)

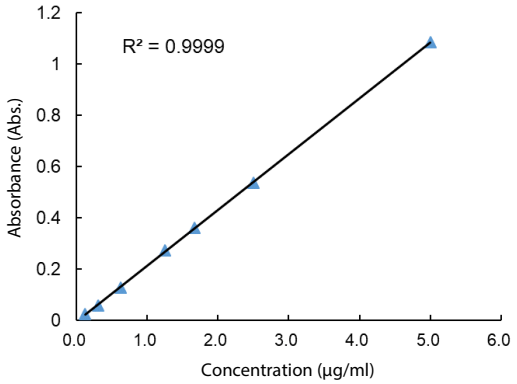

Fig. 2 Calibration Curve (UV-2600i)

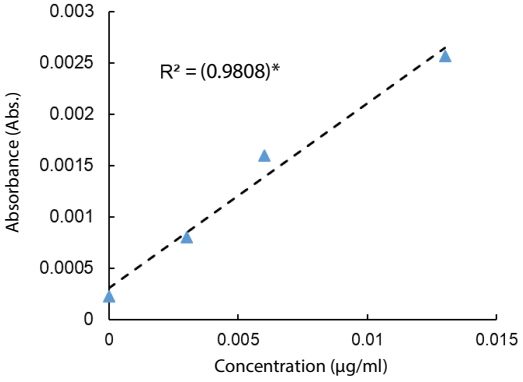

Fig. 3 Calibration Curve of Low Concentration Region (UV-2600i)

* Reference value, as this concentration range is lower than the lower limit of quantitation.

Fluorescence Intensity Measurement of Rhodamine B Solution

An RF-6000 fluorescence spectrophotometer was used in the fluorescence intensity measurement. Table 2 shows the measurement conditions.

Table 2 Fluorescence Intensity Measurement Conditions

Instrument	: RF-6000 fluorescence spectrophotometer
Spectrum type	: Fluorescence spectrum
Excitation wavelength	: 544 nm
Fluorescent wavelength range	: 540 - 700 nm
Scan speed	: 600 nm/min
Sampling pitch	: 1.0 nm
Bandwidth	: Ex. 5.0 nm, Em. 5.0 nm

Fig. 4 shows the fluorescence spectra of the standard solutions of rhodamine B. The shift of the peak top to the longer wavelength side as the concentration of the standard solutions increases is an effect due to reabsorption of fluorescence on the short wavelength side. Fig. 5 and Fig. 6 show the calibration curves prepared from the 577 nm fluorescence intensity values. In Fig. 5, the calibration curve is curved in the high concentration region of 0.125 µg/ml (0.025 Abs.) and above, whereas a calibration curve with good linearity was obtained in the low concentration region in Fig. 6.

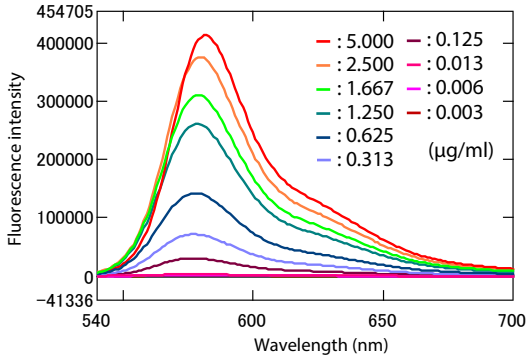

Fig. 4 Fluorescence Spectrum (RF-6000)

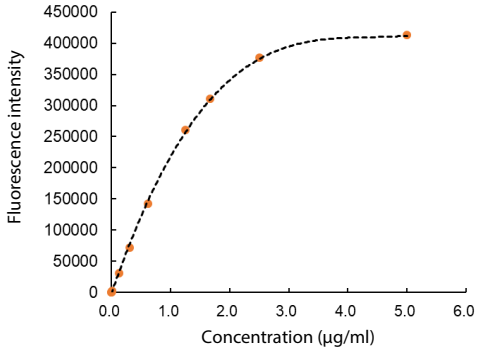

Fig. 5 Calibration Curve (RF-6000)

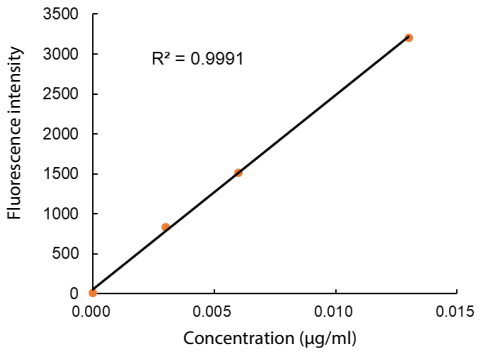

Fig. 6 Calibration Curve for Low Concentration Region (RF-6000)

Comparison of Sensitivity by Lower Limits of Detection/Quantitation

As in Application News No. A606, the lower limit of quantitation (10σ) and lower limit of detection (3σ) were calculated by using the standard deviation σ obtained by 10 measurements of the calibration curve and blank, applying the calibration curve with the highest linearity.

Table 3 shows the lower limit of quantitation and lower limit of detection of the UV-2600i and the FR-6000. The ratio of the lower limits of quantitation calculated in this experiment indicated that the sensitivity of the RF-6000 was more than 400 times higher than that of the UV-2600i. Moreover, a comparison of the calibration curves for the lower concentration region in Fig. 3 and Fig. 6 also showed that deviation of the calibration curve was suppressed in Fig. 6 (RF-6000). Unlike the absorbance method, which detects the irradiated light that is not absorbed by the sample, low noise level and high sensitivity can be obtained with the fluorescence method because fluorescence is detected against a zero baseline.

Table 3 Lower Limit of Quantitation and Lower Limit of Detection

UV-2600i	Lower limit of quantitation	1.9×10^{-2} µg/ml
	Lower limit of detection	5.6×10^{-3} µg/ml
RF-6000	Lower limit of quantitation	4.3×10^{-5} µg/ml
	Lower limit of detection	1.3×10^{-5} µg/ml

Comparison of Linearity in Each Concentration Range

Table 4 shows the relationship between the concentration range and the square of the correlation coefficient R^2 in the calibration curves of the UV-2600i and RF-6000. With the UV-2600i, the points below the lower limit of quantitation other than the blank were excluded.

The RF-6000 can obtain a calculation curve with good linearity even in the region (0 to 0.013 µg/ml) below the lower limit of quantitation for the UV-2600i.

On the other hand, when the calibration curve was prepared using the standard solutions with higher concentrations, a calibration curve with low linearity was obtained with the RF-6000. Thus, because extinction occurs in the high concentration region, the measurement values may be lower than the assumed fluorescence intensity.

Table 4 Relationship of Concentration Range and R^2 of Calibration Curves

Range (µg/ml)	R^2 (UV-2600i)	R^2 (RF-6000)
0 - 5.000	0.9999	0.8091
0 - 1.667	0.9990	0.9924
0 - 0.013	(0.9808)*	0.9991

* Reference value, as the concentration range is below the lower limit of quantitation.

Conclusion

The results of quantitative evaluations using a UV-2600i UV-Vis spectrophotometer and an RF-6000 fluorescence spectrophotometer were compared. With the RF-6000, quantitative evaluation was possible in the low concentration region below the lower limit of quantitation of the UV-2600i. On the other hand, it was found that quantitation in the high concentration region, where extinction occurs with the RF-6000, was possible with the UV-2600i.

As demonstrated here, quantitative evaluation is possible in various concentration ranges by appropriate use of the UV-Vis spectrophotometer and fluorescence spectrophotometer.

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