

UV

TALK LETTER

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UV Talk Letter

Introducing the QYM-01 Photoreaction Quantum Yield Evaluation System

For photoreaction research, including research into photocatalysts, it is important to evaluate which light wavelengths are absorbed and how a reaction progresses (excitation wavelengths), as well as how efficiently absorbed light is used in a reaction. In photochemical reactions, the quantum yield is an indicator that shows the efficiency of how absorbed light is used for a reaction. A chemical actinometer is the device typically used to measure quantum yield. However, chemical actinometers require a substantial amount of time and labor to be able to quantify the quantum yield. Shimadzu has developed the QYM-01 photoreaction quantum yield evaluation system, which is capable of calculating quantum yield through an easier procedure and with more accuracy compared to a chemical actinometer. Note there exists both a photoreaction quantum yield and a fluorescence quantum yield, and the QYM-01 measures the photoreaction quantum yield.

1. What is the Quantum Yield of a Photochemical Reaction?

The quantum yield is the ratio of the number of molecules that resulted in an actual chemical change to the number of photons absorbed in a photochemical reaction. (Reference: *Iwanami Dictionary of Physics and Chemistry*, 3rd Expanded Edition) In other words, if light is absorbed and causes a photochemical reaction, the quantum yield is the number of molecules

generated by the reaction or the number of substrate molecules "n" changed by a reaction over the number of photons absorbed "m" (n/m). For example, as shown in Fig. 1, if one substrate molecule reacts when three photons are absorbed, the quantum yield in this case would be $1/3$ (≈ 0.33).

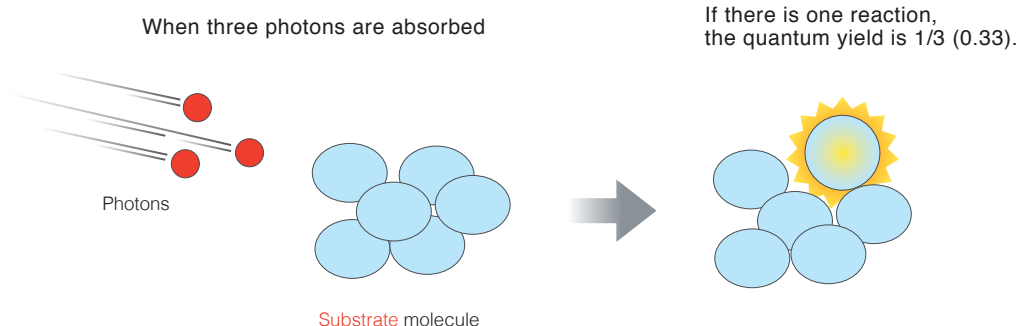


Fig. 1 Example Calculation of the Quantum Yield When a Target Molecule Absorbs Photons, Giving Rise to a Chemical Reaction

The quantum yield can also be measured based on the phenomenon of luminescence by fluorescence or luminescence by phosphorescence, where the fluorescence quantum yield is the ratio of the number of photons of excitation light absorbed to the number of photons emitted.

2. Conventional Method of Calculating the Number of Photons

A chemical actinometer can be used to measure the quantum yield of a photochemical reaction. The chemical actinometer irradiates with photoreaction-inducing excitation light a substance of known quantum yield, then the quantity of irradiation light is calculated based on the quantity of reaction products. A typical chemical actinometer uses iron oxalate and a reduction reaction where iron (III) is changed to iron (II) by the absorption of ultraviolet and visible (UV-VIS) light, and the quantity of irradiation light can be calculated based on the

quantitative value of iron (II) produced and quantum yield of iron oxalate. Experiments conducted with an iron oxalate chemical actinometer calculate the quantity of photoreaction-inducing excitation light, then subjects the actual sample solution to a photoreaction under the same irradiation conditions. This makes it possible to perform the photoreaction experiment in conditions that involve a known quantity of irradiation light. The iron oxalate chemical actinometer is a standard method but has the following weaknesses.

- (1) Iron oxalate reacts to sunlight and indoor lighting such as fluorescent light, so almost all procedural work from sample preparation up to performing the experiment must be performed in darkness, therefore requiring an experienced operator.
- (2) When using a chemical actinometer, the quantity of irradiation light must be ascertained experimentally each time there is a change in the irradiation conditions of the excitation light, such as wavelength or intensity.
- (3) Because a chemical actinometer is used to ascertain the quantity of irradiation light, obtaining the actual quantity of light absorbed by a sample solution requires the use of a UV-VIS spectrophotometer separately to measure absorbance.
- (4) A chemical actinometer is unable to correct for changes in absorbance of the sample solution during an ongoing photoreaction caused by reaction intermediates and other matters.
- (5) A chemical actinometer is unable to correct for changes in the light level of a light source that can occur when an experiment is performed over an extended period.
- (6) Actual excitation light has a wavelength range and is not of a single wavelength. A chemical actinometer is unable to correct for this wavelength distribution.

There are devices that can measure the quantity of irradiation light more simply than a chemical actinometer; these include a power meter or silicon actinometer. However, these devices share the same weaknesses as a chemical actinometer listed in (2) through (6). In addition, their measurements are affected by measurement position and angle.

3. QYM-01 Photoreaction Quantum Yield Evaluation System¹⁾

Fig. 2 is an external view of the QYM-01 photoreaction quantum yield evaluation system. The QYM-01 is capable of the accurate and easy measurement of the number of photons absorbed by a sample during irradiation of a sample solution with excitation light. The QYM-01 is also capable of monitoring the UV-VIS absorption spectrum of an ongoing photoreaction. The QYM-01 comes with a built-in spectrometer, which has been calibrated using a National Institute of Standards and Technology (NIST)-traceable actinometer of which absolute light quantity is managed. Therefore, calibration work using an actinometer is not necessary. Although the number of photons absorbed is

dependent on the quantity of irradiation light and absorbance of the sample, the quantity of irradiation light and sample absorbance are not necessarily constant during a photoreaction. The QYM-01 is capable of performing accurate measurements by accommodating changes in the quantity of irradiation light and absorbance through use of a correction function for changes in the light level of a light source and by monitoring the UV-VIS absorption spectra. The QYM-01 is the optimized system for evaluation of the internal quantum yield of homogeneous photocatalysts, and in situ measurements of photochemical reactions.



Fig. 2 External View of QYM-01 Photoreaction Quantum Yield Evaluation System
(shown together with the Asahi Spectra Co., Ltd. MAX-303 Xenon Light Source)

4. QYM-01 Instrument Configuration

Fig. 3 shows a schematic illustration of the QYM-01. The QYM-01 is comprised of a photoreaction excitation light source unit and a measurement unit, and both are controlled by the same software. Excitation light from the excitation light source passes through the optical fiber to irradiate a sample, then passes again through optical fiber into the spectroscopic detector, where the quantity of light at each wavelength is measured by a calibrated PDA detector. The wavelength of excitation light and the intensity of irradiation light can be changed by the bandpass filter inside the excitation light source and the continuously-variable ND filter, respectively. Furthermore, adding another ND filter (optional) inside the measurement unit makes it possible to lower the irradiation light intensity to 0.5 %.

The UV-VIS absorption spectra of the sample are measured in a direction perpendicular to the axis of the excitation light. The light source for spectra measurement (xenon flash lamp) illuminates for just 10 ms out of each set time period, irradiating the sample with white light. Light transmitted through the sample passes through optical fiber into the detector where the spectrum is detected and the UV-VIS absorption spectrum is obtained. The QYM-01 can perform automated measurements of UV-VIS absorption spectra at a maximum frequency of one-second intervals.

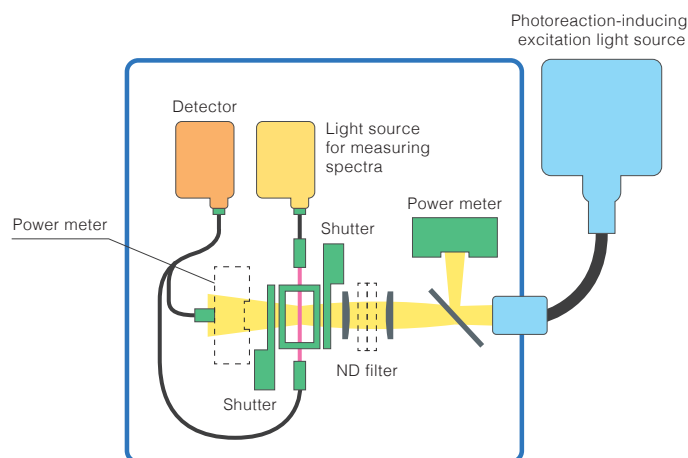


Fig. 3 Schematic Illustration of QYM-01

5. Example Measurement

5-1. Comparison of QYM-01 and Chemical Actinometer (Iron Oxalate) by Measuring Quantity of Irradiation Light

In this example, measurements of the absorbed light quantity between the QYM-01 and an iron oxalate chemical actinometer were compared. An aqueous potassium trioxalatoferate (III) sample was irradiated with photoreaction-inducing excitation light and the number of photons absorbed was measured using the QYM-01. The number of photons absorbed was also calculated from the quantity of iron (II) generated in solutions with different irradiation periods in accordance to the relevant section in the fifth edition of the Series of Experimental

Chemistry, published by the Chemical Society of Japan. Experimental results are shown as a plot of number of absorbed photons against irradiation time in Fig. 4 and Fig. 5, with the experimental conditions and the number of photons absorbed per second calculated for each of the experimental conditions shown in Table 1.

Based on these results, the measurements using the QYM-01 are similar to those using the iron oxalate chemical actinometer, with a difference between them of around 5 % to 6 %.

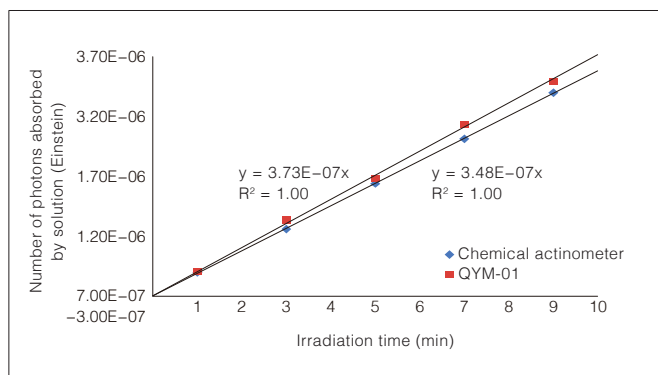


Fig. 4 Results of Measuring Quantity of Light at 365 nm

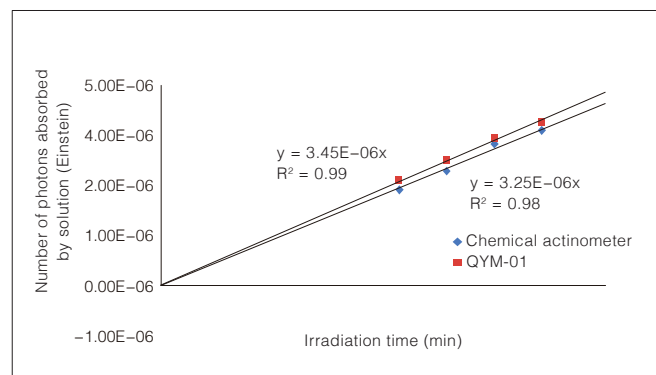


Fig. 5 Results of Measuring Quantity of Light at 480 nm

Table 1 Experimental Conditions and Results of Measuring Absorbed Photons

	Experiment 1	Experiment 2
Excitation Light Wavelength	365 nm (xenon lamp)	480 nm (xenon lamp)
Sample Concentration	6 mM	150 mM
Quantum Yield (values indicated in the fifth edition of the Series of Experimental Chemistry)	1.22	0.94
	Photons absorbed (Einstein/second)	
Chemical Actinometer	5.84×10^{-9}	5.40×10^{-8}
QYM-01	6.19×10^{-9}	5.78×10^{-8}

5-2. Measurement of Quantum Yield of CO₂ Reduction Reaction in Presence of Ru-Re Supermolecular Complex Photocatalyst

The quantum yield of a carbon dioxide reduction reaction in the presence of a Ru-Re supermolecular complex photocatalyst was measured. Absorbed photons were measured using the QYM-01, and the carbon monoxide generated by the reduction reaction was quantified using a gas chromatograph. The experimental conditions are shown in Table 2, and the number

of photons absorbed and quantity of carbon monoxide generated are shown in graphical form in Fig. 6. The slope in Fig. 6 represents the quantum yield. Based on the experimental conditions indicated below, the quantum yield for the carbon monoxide-generation reaction according to literature references is 0.15²⁾. Our experimental results gave a quantum yield of 0.16.

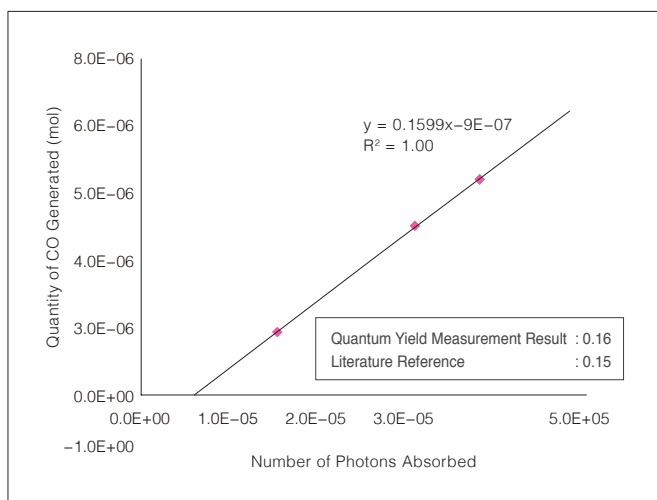


Fig. 6 Quantum Yield Measured for CO Generated in Presence of Ru-Re Supermolecular Complex Photocatalyst

Table 2 Experimental Conditions for CO₂ Reduction Reaction in Presence of Ru-Re Supermolecular Complex Photocatalyst

Photocatalyst	Ru-Re (FPh) (Tokyo Chemical Industry Co., Ltd., Product No. R0100, used without purification)	
Reaction Conditions	Photocatalyst	Ru-Re (FPh) (0.3 mM)
	Donor reducing agent	BNAH (0.1 M)
	Solvent	DMF-triethanolamine (5:1 v/v solvent mixture)
	Solution volume	4 mL
	Irradiation light	480 nm xenon lamp
Operating Procedure	Reaction vessel	Four-sided quartz cuvette with branch (11 mL volume, 7 mL gas phase, 4 mL liquid phase)
	Using a 4-mL transfer pipette, the prepared solution is placed into the four-sided cuvette. After 30 minutes of bubbling with CO ₂ , the cuvette is sealed with a septum (three cuvettes are prepared) and irradiated with light for 1 hour, 2 hours, or 2.5 hours. A gas-tight syringe is then used to harvest 100 μ L from the gas phase of each cuvette, and these gas phase samples are used to quantify CO by gas chromatography (GC).	
References	Y. Tamaki, K. Watanabe, K. Koike, H. Inoue, T. Morimoto, O. Ishitani, <i>Faraday Discuss.</i> 2012, 155, 115.	

6. Summary

The QYM-01 photoreaction quantum yield evaluation system can be used to measure the number of absorbed photons more simply and more accurately than a chemical actinometer. The QYM-01 is also capable of monitoring the UV-VIS absorption spectrum of an ongoing photoreaction. The QYM-01 is the optimized system for evaluation of the internal quantum yield of homogeneous photocatalysts, and in situ measurements of photochemical reactions.

Acknowledgments

The two sets of experimental data used in the example measurement section were provided by the Ishitani/Maeda Laboratory, Department of Chemistry, Graduate School of Science and Engineering, Tokyo Institute of Technology. We are sincerely grateful for their help.

References

- 1) The QYM-01 photoreaction quantum yield evaluation system is a special order product developed in collaboration with the Ishitani/Maeda Laboratory, Department of Chemistry, Graduate School of Science and Engineering, Tokyo Institute of Technology.
- 2) Y. Tamaki, K. Watanabe, K. Koike, H. Inoue, T. Morimoto, O. Ishitani, *Faraday Discuss.* 2012, 155, 115.



Introducing a Simplified Quantitation Method for Highly Turbid Suspensions

There has been much research in recent years into the extraction of fuels and oils from micro algae. Examining the state of algae growth (quantitation of algal concentration) on a daily basis is an important part of micro algae research. The most commonly used method for quantifying algal concentration is a dry weight method using filter paper, but there is demand for a simpler method that does not consume as much time. The Shimadzu UV micro algae analysis system is capable of simple quantitation of micro algae suspensions dispersed in water using a spectrophotometer. This system includes both the reflection method (patent pending) to accommodate highly turbid suspensions and the transmission method to accommodate low turbidity suspensions. This section introduces an example of measuring a highly turbid suspension of Nannochloropsis micro algae using the reflection method. Highly turbid solutions are also commonly found in the food industry. The concentration of in-process beverage products during manufacturing operations, such as dilution and mixing, must be examined to confirm concentration consistency, and the Shimadzu UV micro algae analysis system will also be useful in these situations. Presented here are the results of an evaluation of the system when measuring the concentration (dilution ratio) of a fermented milk drink and a green vegetable drink.

1. Introducing a Simplified Quantitation Method for Highly Turbid Suspensions

1-1. Nannochloropsis Micro Algae

The Nannochloropsis micro algae sample (Higashimaru Co., Ltd., 10 billion cells/mL) was diluted with pure water to prepare measurement samples consisting of twelve sequential concentrations from 0 to 100 %. Fig. 1 shows a photograph of the prepared samples. These samples were divided into two groups, one to serve as standard samples for generating a calibration model, and the other to serve as calibration model validation samples. These are listed in Table 1 and Table 2, respectively.



Fig.1 Measurement Samples of Nannochloropsis Micro Algae

Table 1 Standard Samples

Standard Sample	Concentration (%)
(1)	100
(2)	90
(3)	80
(5)	60
(6)	50
(7)	40
(9)	20
(10)	10
(12)	0 (pure water)

Table 2 Validation Samples

Validation Sample	Concentration (%)
(4)	70
(8)	30
(11)	5

1-2. Measurement Instrument

Analysis was performed using the Shimadzu UV micro algae analysis system consisting of the UV-2600 UV-VIS spectrophotometer combined with the integrating sphere attachment for algae analysis. The disposable screw-cap vials containing the samples were set in the integrating sphere attachment shown in Fig. 2, and the total reflectance of each

sample was measured. The use of disposable screw-cap vials for these measurements eliminated the need for manual operations such as the washing of vials, which also eliminated any concerns regarding contamination and carry-over due to insufficient washing.



Fig. 2 Integrating Sphere Attachment for Micro Algae Analysis

1-3. Measurement Results

Total reflectance was measured for the twelve samples shown in Table 1 and Table 2. Replacing the screw-cap vial for each sample, each sample of the same concentration was measured twice (12 samples \times 2 measurements = total of 24 data sets). These results are shown in Fig. 3, and the analytical conditions are shown in Table 3. The results indicated that the higher the concentration, the greater the reflectance, and the lower the

concentration, the lower the reflectance, demonstrating a correlation between the reflection spectrum and the concentration. The principle behind these measurement results is illustrated in Fig. 4. The greater the number of micro algae cells in the sample, the greater the proportion of light is reflected near the liquid surface, and so the greater the measured reflectance.

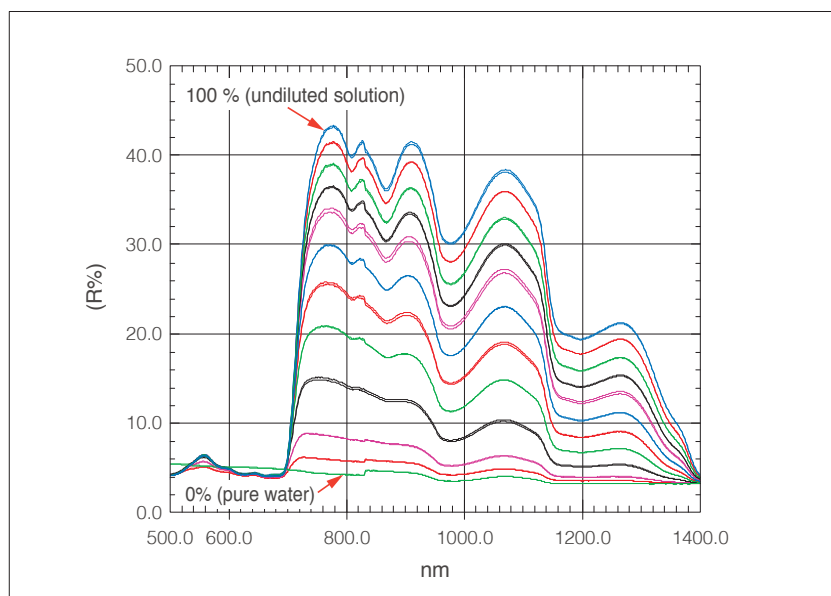


Fig. 3 Reflection Spectra of Micro Algae

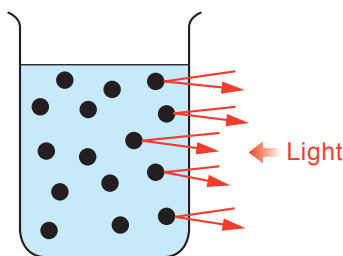


Fig. 4 Principle Behind Reflection Measurement

Table 3 Analytical Conditions

Instrument Used	UV-2600 UV-VIS spectrophotometer, Integrating sphere attachment for algae analysis
Measurement Wavelength Range	500 nm to 1400 nm
Scan Speed	High speed
Sampling Pitch	1.0 nm
Photometric Value	Reflectance
Slit Width	5 nm

1-4. Results of Quantitative Analysis

The following presents an example of quantifying micro algae using measured reflection spectra. Quantitation was performed using the single regression method and the multivariate analysis multiple linear regression method, and the respective quantitative accuracies were then compared. A regression equation of the calibration curve model was obtained for each standard sample based on the samples of Table 1. For the multiple linear regression method, the regression equation is entered in the photometric measurement method window of the standard UVProbe software as shown in Fig. 5. With this setting, the predicted value calculated via the regression equation for each sample measured is output by the software. Table 4 shows the concentration results calculated from the reflectance of validation samples shown in Table 2. Comparing the results, it is clear that better results are obtained using the multiple linear regression method than by the single regression method. In

this way, measurement of unknown samples can easily be performed by simply entering the regression equation in the standard UVProbe software. In addition, the regression equation using the multiple linear regression method was determined using the "regression analysis" function in ExcelTM. RMSEP of Table 4 refers to the value defined in Fig. 6, and is an index that expresses the average difference between the predicted and true value. Thus, the smaller the RMSEP value, the better the prediction accuracy. Regarding the single regression method, quantitation was performed using the standard calibration function of the UVProbe software. Using the single regression method, calculation was performed based on the single wavelength data obtained at 910 nm. As for the multiple linear regression method, calculation was performed using four wavelength data sets: 870 nm, 910 nm, 980 nm, and 1070 nm.

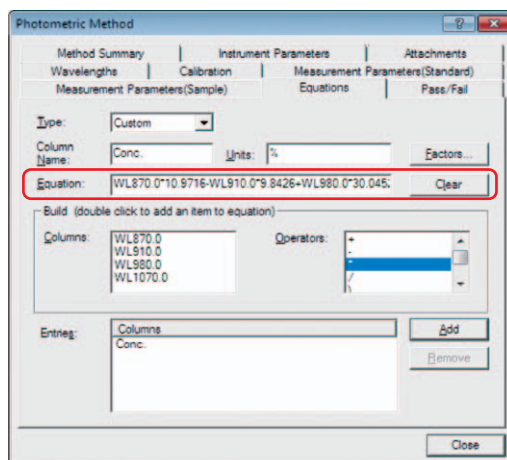


Fig. 5 Photometric Measurement Method Window of UVProbe Software

Table 4 Prediction Results Based on Concentration Calculated by Each Calibration Model for Validation Samples

Validation Sample	Actual Concentration (%)	Predicted Result by Single Regression Method (%)	Predicted Result by Multiple Linear Regression Method (%)
(4) - 1	70	72.9	69.2
(4) - 2	70	73.4	69.1
(8) - 1	30	32.4	28.3
(8) - 2	30	32.3	30.0
(11) - 1	5	1.4	5.3
(11) - 2	5	1.5	5.4
RMSEP		3.1	0.87

$$RMSEP = \sqrt{\frac{\sum_{i=1}^n (y'_i - y_i)^2}{N}}$$

y'_i is the predicted value, y_i is the actual value, and N is the number of validation samples.
* RMSEP: Root Mean Square Error of Prediction

Fig. 6 Equation that Defines RMSEP

2. Measurement of a Fermented Milk Drink

Measurements of the highly turbid suspension of a commercially available fermented milk drink were performed. As when performing measurements of micro algae, the fermented milk drink was diluted with pure water to prepare measurement samples consisting of twelve sequential concentrations from 0 to 100 %. These samples were divided into two groups as shown in Table 1 and Table 2, one to serve as standard samples for generating a calibration model, and the other to serve as calibration model validation samples. Fig. 7 shows a photograph of the samples, and Fig. 8 shows the reflection

spectra of the samples. A calibration model was generated with the standard samples by the single regression method and the multiple linear regression method, where the results of using the validation samples to validate each model are shown in Table 5. Better results were obtained using the multiple linear regression method. Using the single regression method, calculation was performed based on the single wavelength data obtained at 430 nm. As for the multiple linear regression method, calculation was performed using three wavelength data sets: 430 nm, 500 nm, and 700 nm.



Fig. 7 Fermented Milk Drink

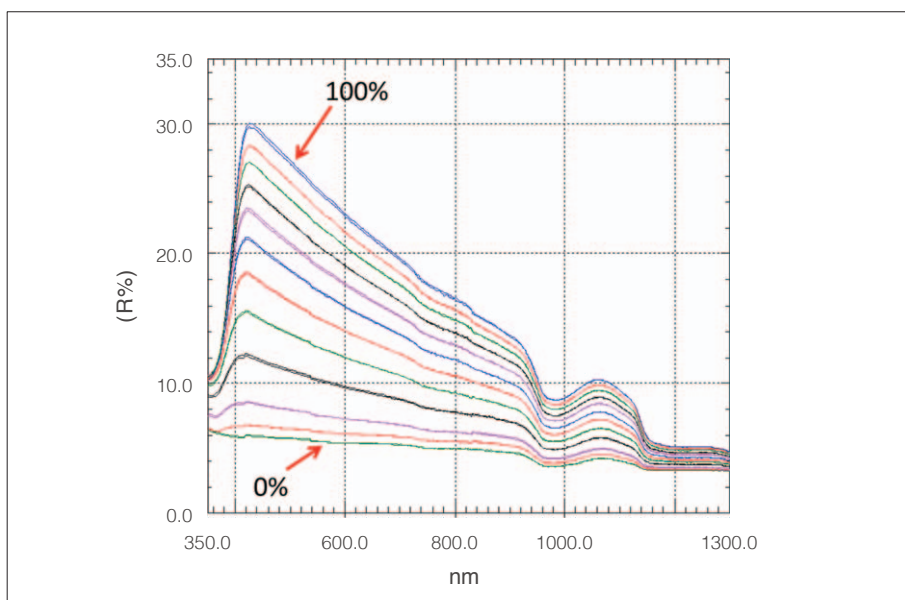


Fig. 8 Reflection Spectra of Fermented Milk Drink

Table 5 Prediction Results Based on Concentration Calculated by Each Calibration Model for Validation Samples (Fermented Milk Drink)

Validation Sample	Actual Concentration (%)	Predicted Result by Single Regression Method (%)	Predicted Result by Multiple Linear Regression Method (%)
(4) - 1	70	73.53	69.98
(4) - 2	70	73.93	68.60
(8) - 1	30	33.43	30.54
(8) - 2	30	33.91	30.20
(11) - 1	5	-1.74	5.20
(11) - 2	5	-1.69	5.20
RMSEP		4.92	0.63

3. Measurement of a Green Vegetable Drink

A commercially available green vegetable drink was used as the sample fluid. As when performing measurements of the micro algae and the fermented milk drink, the green vegetable drink was diluted with pure water to prepare measurement samples consisting of twelve sequential concentrations from 0 to 100 %. These samples were divided into two groups as shown in Table 1 and Table 2, one to serve as standard samples for generating a calibration model, and the other to serve as calibration model validation samples. Fig. 9 shows a photograph of the samples, and Fig. 10 shows the reflection spectra of the samples.

A calibration model was generated with the standard samples by the single regression method and the multiple linear regression method, where the results of using the validation samples to validate each model are shown in Table 6. Here also, better results were obtained using the multiple linear regression method. Using the single regression method, calculation was performed based on the single wavelength data obtained at 800 nm. As for the multiple linear regression method, calculation was performed using three wavelength data sets: 800 nm, 980 nm, and 1200 nm.

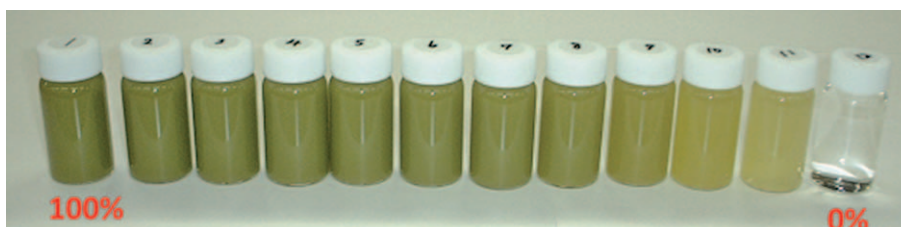


Fig. 9 Green Vegetable Drink

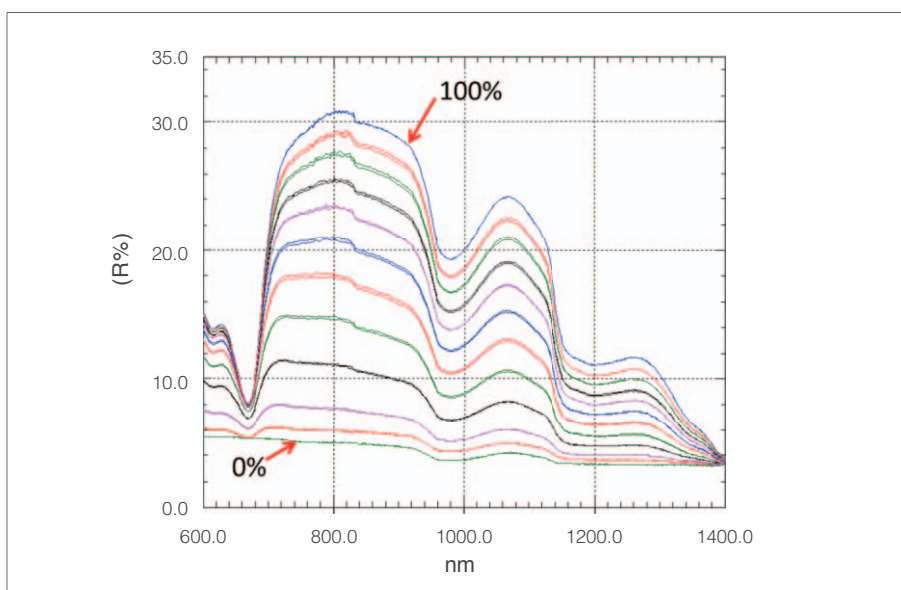


Fig. 10 Reflection Spectra of Green Vegetable Drink

Table 6 Prediction Results Based on Concentration Calculated by Each Calibration Model for Validation Samples (Green Vegetable Drink)

Validation Sample	Actual Concentration (%)	Predicted Result by Single Regression Method (%)	Predicted Result by Multiple Linear Regression Method (%)
(4) - 1	70	72.91	69.51
(4) - 2	70	73.69	70.37
(8) - 1	30	32.69	27.95
(8) - 2	30	33.27	28.80
(11) - 1	5	0.40	5.23
(11) - 2	5	0.21	5.01
RMSEP		3.75	1.01

4. Summary

A system that enables simple measurement of the concentration (dilution ratio) of highly turbid suspensions has been developed. Good results were obtained when performing quantitative measurements of *Nannochloropsis* micro algae using the reflection method. Similarly good results were also obtained with the highly turbid suspensions of a fermented milk drink and a green vegetable drink.

Quantifying highly turbid fluid samples in their unprocessed state is generally difficult, but feasible and simple to quantify them when using this system. This system will allow for quick and easy control of fluid concentration for a variety of highly turbid suspensions in micro algae research and in the food industry.

1) Excel is a registered trademark or trademark of Microsoft Corporation.



What absorbances can a UV-VIS spectrophotometer measure?



UV-VIS spectrophotometers can measure the amount of light absorbed by a sample in terms of absorbance. However, the range of absorbances that can be measured differs depending on the spectrophotometer used for measurement. The measurable range of absorbance of a given model depends largely on the stray light level in the instrument. (Refer to *UV Talk Letter* Vol. 2 for more information on stray light.)

At absorbances of one or less, absorbance can be measured accurately regardless of which spectrophotometer is used. However, when absorbance exceeds two, the accuracy of measurements depends greatly on the spectrophotometer. We performed absorbance measurements at a wavelength of 525 nm using a number of concentrations (differing absorbances) of a potassium permanganate solution in water (0 to 325 mg/L). We plotted true absorbance on the x-axis and apparent absorbance (measured result) on the y-axis. When a data point lies on the black line this indicates the measured absorbance was the same as the true absorbance. Fig. 1 shows the results of absorbance measurements using the UV-1280, and Fig. 2 shows the results of absorbance measurements using the UV-2700. For the UV-1280 spectrophotometer, the measured absorbance is obviously lower than the true absorbance at values above two. Meanwhile, with the UV-2700 spectrophotometer, the absorbance measurements remain accurate even above three.

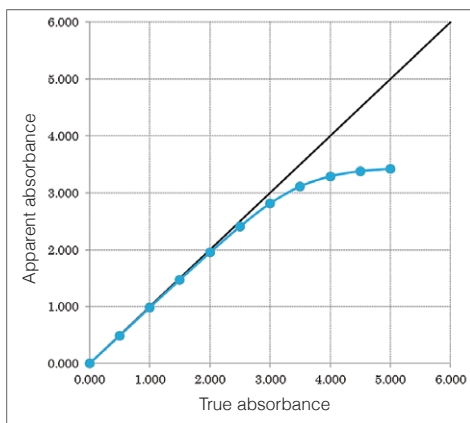


Fig. 1 UV-1280 Absorbance Measurements

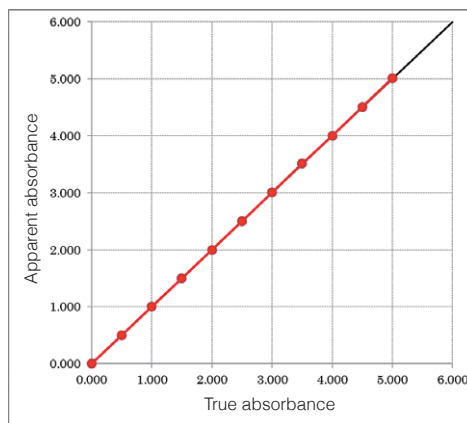


Fig. 2 UV-2700 Absorbance Measurements

Although also affected by measurement wavelength, stray light, and other factors, as an approximate guide, spectrophotometers with a single monochromator give accurate measurements up to an absorbance of around two, and spectrophotometers with a double monochromator give accurate measurements up to an absorbance of around five.