JASCO Corporation was founded in 1958 to provide the scientific community with optical spectroscopy products.

In the mid-1950's a group of researchers in the Institute of Optics of what is now Tsukuba University needed an Infrared Spectrophotometer for their research. Since a commercially available instrument was not yet existing at the time, they undertook the challenge to develop their own.

The result was quite a success - a reliable instrument with excellent optical performance. As a second result, other research groups asked them to replicate the instrument for use within their laboratories.

With the introduction of HPLC in the mid-1970's JASCO's experience in highly sensitive and accurate optical systems led to the development of a series of chromatographic detection systems. Fixed and variable wavelength UV/Visible and Fluorescence detectors were introduced featuring excellent sensitivity and reliability in compact modules. In order to offer complete HPLC systems JASCO developed a variety of novel solvent delivery systems as well as other accessories such as column ovens, autosamplers, and PC based control and analysis software.

Today JASCO offers a wide variety of HPLC modules, accessories and analysis software. The new JASCO LC-4000 Liquid Chromatography series is designed to operate at pressures approaching 15,000 psi for either gradient or isocratic separations, providing researchers with a powerful tool when using the new generation of small particle columns. LC-4000 Series includes a versatile series of components offering unique flexibility to build systems for routine and specialized applications. LC-4000 features the widest choice of optical HPLC detector: UV, diode array, fluorescence, chemiluminescence, CD, chiral and refractive index detector.

Finally JASCO's modular Supercritical Fluid Chromatography and Supercritical Fluid Extraction platforms provide a low-cost, fast, green technology with reliable and worry-free performance for a wide variety of applications.

JASCO has a strong global presence, supplying customers in over 45 different countries.
**JASCO Europe** is in charge for marketing, sales, service and support for all Jasco products throughout **Europe, Middle East and Africa**.

With its service network, JASCO is ready to maintain the perfect reliability of customer’s instrumentation and minimize the laboratory down time.

- **Superior productivity**
- **Optimized analytical performance**
- **Lower cost of ownership**
- **Extended instrument life**

If your laboratory has specific Service and Support requirements, JASCO can help you with customized contract agreements. In addition, a full set of Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ) tests are available to verify the system proper installation, operation and performance, respectively.

**Get the most from your investment with JASCO Training Courses**

JASCO Training Courses ensure maximum skill development for the best value of your laboratory. Our team of highly-experienced specialists can help your staff to get the most from your instrument reducing your analysis run time and improve performance.

Build your knowledge with JASCO Training Courses:

- **Instrument and Software operation**
- **Troubleshooting**
- **Maintenance**
- **Calibration**
- **Applications and Methods developments**
- **Operating Techniques**
V-730 – V-730bio – V-750 – V-760
UV-Vis Spectrophotometers

With more than fifty years of experience in the design of spectrophotometers, JASCO offers a complete range of UV-Vis/NIR instruments. The **V-700 series** consists of six distinct models designed to meet a wide range of application requirements.

From an innovative optical layout to a simple comprehensive instrument control and data analysis software interface, the **V-700 series** does not compromise on accuracy, performance or reliability.

All spectrophotometers are controlled by **Spectra Manager™ II**, JASCO’s powerful cross-platform spectroscopy software package with USB communication.

FT/IR-4600 – FT/IR-4700
FT/IR Spectrometers

FT/IR-6600 – FT/IR-6700 – FT/IR-6800
FT/IR Spectrometers

IRT-5100 – IRT-5200
FT/IR Microscopes

IRT-7100 – IRT-7200
FT/IR Microscopes

JASCO is proud to release four innovative FT-IR Microscope, the **IRT-5000 and IRT-7000**, providing several new functions that drastically improve infrared micro-spectroscopy analysis.

Both microscope systems can be easily interfaced with either the FT/IR-4000 or FT/IR-6000 spectrometer, offering the most advanced microscopy and imaging systems available in the market today.

The microscope system automatically scans the specified points or area, rapidly collecting a full spectrum of each point without moving the sample stage.

IRT-7100 – IRT-7200
FT/IR Microscopes

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V-770 – V-780
UV-Vis/NIR Spectrophotometers

The **FT/IR-4000 and FT/IR-6000** models represent a broad range of instrumentation that redefine infrared spectroscopy as a powerful yet easy to use technique in a compact and reliable line of instruments with the highest signal-to-noise ratio.

All models are controlled by **Spectra Manager™ II**, JASCO’S powerful cross-platform spectroscopy software package with USB communication.

All models feature an auto-alignment function which maintains instrument optical alignment after beamsplitter changes or instrument movement.

The **FT/IR-4000 and FT/IR-6000** models represent a broad range of instrumentation that redefine infrared spectroscopy as a powerful yet easy to use technique in a compact and reliable line of instruments with the highest signal-to-noise ratio.

All models are controlled by **Spectra Manager™ II**, JASCO’S powerful cross-platform spectroscopy software package with USB communication.

All models feature an auto-alignment function which maintains instrument optical alignment after beamsplitter changes or instrument movement.
The performance expected on a micro-Raman spectrometer are fully provided with the JASCO NRS-5000/7000 series Raman systems, assuring consistent performance for rapid acquisition of high quality data with automated system control and minimal optical adjustments.

For application expansion, an automated multi-grating turret, 2 internally mounted detectors and a maximum of 8 lasers ranging from the UV through the NIR are capable of integration with the instrument system.

*Spectra Manager™ II* for the NRS-5000/7000 offers revolutionary features to simplify previously difficult measurement and analysis tasks, while adding various user-support tools such as autofluorescence-correction, wavenumber correction, intensity correction, and a novel user-advice function.

The system offers space-saving, automated switching laser light source and alignment adjustment to assist the analysis, NRS-4100 is easily used to quality control as well as research and development.

The micro-Raman NRS-4100 is equipped with measurement assist function that can be easily setup operation and a user advice function that automatically analyzes the spectrum and obtain a high-quality data even at the first time.

The automatic XYZ stage is equipped with a sample search function. Using a newly developed algorithm (patent pending) the microscope image, sample search function has used to set the measurement position automatically and gives you data from the location that is automatically registered with the click of a button measurement.

**NRS-4100**
*Laser Raman Spectrometer*

The MSV-5000 series is a microscopic spectrophotometer system providing transmittance/reflectance measurements of a microscopic sample area with a wide wavelength range from ultraviolet to near infrared.

**MSV-5100** Spectrophotometer is a dedicated UV-Vis microscope with a wavelength range of (200-900 nm).

**MSV-5200** Spectrophotometer includes a Peltier-cooled PbS detector and has a wavelength range of (200-2700 nm).

**MSV-5300** Spectrophotometer incorporates an InGaAs detector to obtain optimized NIR measurements and has a wavelength range of (200-1600 nm).

**MSV-5100 – MSV-5200 – MSV-5300**
*UV-Vis/NIR Microscopes*
Digital Polarimeter

The P-2000 is designed as a customizable polarimeter with various options for a range of applications and budgetary requirements.

Options such as polarizers, wavelength filters, lamps and photomultiplier detectors provide a wide range of analytical wavelengths from UV-Vis to NIR.

A newly redesigned intelligent remote module (iRM) with a color LCD touch screen conveniently guides the operator through routines from data acquisition to data processing. The obtained data can be automatically printed to USB printers, or saved to a compact flash memory card for further processing on a PC.

Circular Dichroism Spectropolarimeters

The latest effort in the JASCO commitment to lead the field of Circular Dichroism.

Unparalleled optical performance and optionally available measurement modes are combined in a manner to make the J-1000 Series Spectropolarimeter, a true "chiro-optical spectroscopy workbench", able to work up to 2,500 nm.

Instrument control and data processing are handled effortlessly by our JASCO's user friendly and innovative cross-platform software, Spectra Manager™ II.

Vibrational Circular Dichroism

The FVS-6000 not only allows you to easily obtain fingerprint VCD spectra, but also has several unique features such as a measurement range extension option of 4000-750 cm$^{-1}$.

Since the CD signals in the infrared region are one or more orders of magnitude lower than ECD signals in the UV-Vis region, high sensitivity and stability are required for a VCD spectrometer.

The FVS-6000 is the VCD spectrometer of choice for highly sensitive VCD measurements.

Spectrofluorometers

Designed with the latest technology, the FP-8000 Series spectrofluorometers incorporate the highest sensitivity, fastest spectral scanning capability and excellent analysis-oriented functionality offering integrated solutions for advanced materials research and biochemical analysis applications.

To meet the most stringent analysis demands, a variety of accessories are available for integration with a range of sophisticated control and analysis applications available in the user-friendly Spectra Manager™ II software to offer a flexible platform for any fluorescence and phosphorescence application.
JASCO’s new **RMP-500** Series has been developed to meet the needs of Material Science, Manufacturing and Biochemistry by combining the flexibility of a fiber optic probe with a portable Raman Microspectrometer.

The RMP-500 Series consists of three models, **RMP-510, RMP-520, RMP-530** ranging from small, portable units suitable for in-situ measurements to research-grade systems that will meet even the most difficult application requirements.

The **RMP-500** Series portable Raman spectrometer systems feature an integrated fiber optic probe with a small X-Y-Z stage, a compact laser, a high-throughput spectrograph and CCD detector.

JASCO is the first manufacturer to develop a powerful, cross-platform software package, **“Spectra Manager”**, for controlling a wide range of spectroscopic instrumentation. Spectra Manager program is a comprehensive package for capturing and processing data, eliminating the need to learn multiple software packages and offering the user a shallower learning curve.

Several types of measurement data files (UV-Vis/NIR, FT-IR, Fluorescence, etc.) can be viewed in a single window, and processed using a full range of data manipulation functions.

The latest version, Spectra Manager II, includes four measurement programs, a spectra analysis program, an instrument validation program and the JASCO Canvas program as standard. It is possible to analyze data even during sample measurements.

**Spectra Manager CFR** provides features to support laboratories in compliance with 21 CFR Part 11.

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**VIR-100 – VIR-200 – VIR-300**

**Portable FT-IR Spectrometers**

The **VIR-100/200/300** series are compact, lightweight, flexible FT-IR systems.

The collimated entrance and exit ports make it an ideal instrument for a wide range of applications.

The standard instrument includes a hermetically sealed interferometer, DLATGS detector, high intensity source, KRS-5 windows and automatic alignment. Options can be added for increased sensitivity, optional spectral ranges including NIR, and battery operation.

For even greater flexibility, external connection optics allows the user to install up to three different attachments in one system, selecting the most appropriate application accessory by simply switching the PC controlled optical configuration.
JASCO has the largest range of optical detectors - from dual wavelength UV to diode array to unique chiral detectors. All the detector are designed to meet U-HPLC requirements, data acquisition rate of 100Hz.

**SFC-4000**

Supercritical Fluid Chromatography

The JASCO SFC/SFE 4000 integrated Analytical SFC system has been developed for all aspects of analytical SFC; including routine separation, method development and small scale preparation of samples at the mg scale.

With a simple intuitive software and robust engineering, the JASCO SFC system is a powerful tool for analytical separations.

Both HPLC and SFC/SFE systems are coupled with ChromNAV 2.0 data system to offer both HPLC and spectral data handling for most of the detectors even with the dual wavelength UV detector.

A newly added feature of ChromNAV 2.0 is the automatic e-mail notification on your smartphone/tablet, stay always updated on analysis status of your LC-4000. Full GLP compliance and 21 CFR part 11.
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Chromaticity and Turbidity Quantitative Measurement using UV/Vis Spectrophotometer

In this Application Note, the method for measurement of chromaticity and turbidity using UV/Vis spectrophotometer based on clean water test method will be described.

Chromaticity
Chromaticity measurement is to check the coloration degree of clean water and wastewater using humic acid. This test is applied by observing the absorption at 390 nm which shows the yellow color of humic acid by using UV/Vis spectrophotometer.

Measurement/analysis system
- V-630/650/660/670 UV/Vis spectrophotometer
- LSE-701 Long path cell holder
- VWWQ-789 Chromaticity/turbidity measurement program
- Rectangular cell 50 mm or 100 mm

Standard sample
Cobalt chloroplatinate which has a color similar to yellow-brown of humin is used as standard sample for chromaticity. 2.49 g of Potassium chloroplatinate and 2.02 g of cobalt chloride are dissolved in 200 mL of hydrochloric acid, and then purified water is added to make the solution of total volume 1 L. This solution is neat standard sample with chromaticity 1000 degree.

Test method
Measuring the absorbance of sample in cell of 50 mm or 100 mm pathlength at the wavelength of 390 nm.

Procedure
1. Standard solution is prepared from neat standard sample diluted by purified water. Blank sample is purified water filtrated using 0.2 μm membrane filter.
2. Chromaticity calibration curve is created from the measurement results of blank sample and standard solution prepared in 1.
3. Sample water is filtrated using membrane filter or centrifuged and the supernatant is used as sample.
4. The absorption of sample prepared in 3 at 390 nm is measured, and chromaticity is calculated from the results and calibration curve.

![Figure 1 Chromaticity calibration curve](image)

<table>
<thead>
<tr>
<th>Concentration [Chromaticity]</th>
<th>Absorbance</th>
<th>Quantitative Value [Chromaticity]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.000</td>
<td>-0.06</td>
</tr>
<tr>
<td>0.5</td>
<td>0.004</td>
<td>0.51</td>
</tr>
<tr>
<td>1</td>
<td>0.007</td>
<td>0.97</td>
</tr>
<tr>
<td>2</td>
<td>0.015</td>
<td>2.04</td>
</tr>
<tr>
<td>20</td>
<td>0.141</td>
<td>20.07</td>
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<tr>
<td>50</td>
<td>0.350</td>
<td>49.99</td>
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<tr>
<td>100</td>
<td>0.699</td>
<td>99.99</td>
</tr>
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</table>

Table 1 Chromaticity calibration curve
Chromaticity and Turbidity Quantitative Measurement using UV/Vis Spectrophotometer

In Standard solutions with chromaticity at 0, 0.5, 1, 2, 20, 50, 100 degree were measured using 50 mm light pathlength cell and these results are shown in the center column of Table 1. Measured absorbance values are input to the calibration curve and the calculated quantitative values of chromaticity are shown in the right column of Table 1. From the above results, the standard deviation (σ) between the obtained quantitative value and actual chromaticity is 0.04(6) degree, detection limit, 0.15 and quantitation limit, 0.46 degree. *1)

*1) Detection limit is calculated from 3.3σ and quantitative limit is calculated from 10σ.

Calibration curve information:  
\[ y = 0.0070x + 0.0004(5) \]  
\[ R^2 = 1.0000 \]

Turbidity

In turbidity measurement, the turbidity degree due to insoluble particles, microbe and organic substance in clean water and wastewater is tested. Scattering light at 660 nm is measured using UV/Vis spectrophotometer in transmission measurement method or integrating sphere photoelectric spectrophotometry.

Standard sample

Immixture polystyrene suspension is used as standard sample of turbidity. Mixture of 5 kinds of polystyrene particles shown in the Table 2 is stated as neat solution of turbidity at 100 degree, which is commercially available.

<table>
<thead>
<tr>
<th>Category</th>
<th>Normal diameter (um)</th>
<th>Mixture Ration (%)</th>
</tr>
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<tr>
<td>No. 6</td>
<td>0.5</td>
<td>6</td>
</tr>
<tr>
<td>No. 7</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>No. 8</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td>No. 9</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td>No. 10</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>

Table 2 Polystyrene standard particle of turbidity at 100

Measurement/analysis system

- V-630/650/660/670 UV/Vis spectrophotometer
- LSE-701 Long path cell holder
- Quantitative measurement program
- Rectangular cell 20 mm, 50mm and 100 mm

Procedure

1. Neat sample solution is diluted by purified water and prepared as standard solution. Purified water filtrated using 0.2 um membrane filter is used as blank sample.
2. Turbidity calibration curve is created from the measurement results of blank sample and standard solution prepared in 1.
3. The absorbance of sample water at 660 nm is measured, and turbidity is calculated from the results and calibration curve.
Chromaticity and Turbidity Quantitative Measurement using UV/Vis Spectrophotometer

Standard solutions with turbidity at 0, 5, 10, 50, 100 degree were measured using 20 mm light pathlength cell and the results are shown in Table 3. By the same method as chromaticity, the standard deviation is calculated as 0.36 degree, detection limit as 1.18 and quantitation limit as 3.6 degree.

Calibration curve information: \( y = 0.0050x + 0.0014 \) \( R^2 = 1.0000 \)

<table>
<thead>
<tr>
<th>Concentration [ Turbidity ]</th>
<th>Absorbance</th>
<th>Quantitative Value [ Turbidity ]</th>
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<tbody>
<tr>
<td>0</td>
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<tr>
<td>5</td>
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<td>10.07</td>
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<td>50</td>
<td>0.256</td>
<td>50.64</td>
</tr>
<tr>
<td>100</td>
<td>0.502</td>
<td>99.67</td>
</tr>
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</table>

Table 3 Turbidity calibration curve (transmission measurement method)

Integrating sphere photoelectric spectroscopy Measurement/analysis system

- V-650/660/670 UV/Vis spectrophotometer
- ISV-722/ISN-723 Integrating sphere unit
- VWWQ-789 Chromaticity/turbidity measurement program
- Rectangular cell 10 mm, 20 mm, 30 mm and 50 mm

Procedure

1. Standard solution is prepared from neat standard sample diluted by purified water. Blank sample is purified water filtrated using 0.2 um membrane filter.
2. Turbidity calibration curve is created from the measurement results of blank sample and standard solution prepared in Firstly, standard white plate is mounted in integrating sphere and total light transmittance (Tt) is measured, and then the plate is removed and diffuse transmittance (Td) is measured.
3. Tt and Td of sample water at 660 nm is measured, and turbidity is calculated from the results and calibration curve.

Concentration [ Turbidity ] | Td/Tt x 100 | Quantitative Value [ Turbidity ] |
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.004</td>
<td>-0.05</td>
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<tr>
<td>0.5</td>
<td>0.389</td>
<td>0.50</td>
</tr>
<tr>
<td>1</td>
<td>0.726</td>
<td>0.98</td>
</tr>
<tr>
<td>2</td>
<td>1.435</td>
<td>1.99</td>
</tr>
<tr>
<td>5</td>
<td>3.666</td>
<td>5.17</td>
</tr>
<tr>
<td>10</td>
<td>7.003</td>
<td>9.92</td>
</tr>
</tbody>
</table>

Table 3 Turbidity calibration curve (transmission measurement method)
Hexahydric chromium (Cr(VI)) is one of the regulated materials by RoHS directive. In this application note, the measurement example of two kinds of screws with chromate treatment is introduced.

1. Sample
Steel screws A: pan sems, spring washer + big washer, M3x6, 8 pieces, 5.7 g
Steel screws B: pan-head, M4x12, 19 pieces, 14.0 g

2. Measurement Procedures
Chromium was extracted by hot water and measured its quantity by chromogenic reaction of diphenylcarbazide method.

3. Chromium Extraction
Well-known extraction methods of chromium are the Hydrothermal Extraction (JIS H 8625), the Alkaline Decomposition (EPA 3060A), the Shaking Extraction (DIN53314), and the Volvo method. The Hydrothermal Extraction of JIS H 8625 is a widely-used easy method for extracting chromium treated to electrical and manufacturing products. The method was applied to the steel screws to extract chromium.

Extraction solvent: Purified Water 25 mL
Extraction temperature: 80 degrees °C
Extraction time: 30 minutes °C

°C In the JIS H 8625, the extraction temperature is 100 degrees and the time is 5 minutes. The extraction here was performed by using thermostat bath at 80 degrees. The time for extraction was 30 minutes. Although this condition was determined by confirming the end of the Cr(VI) chromium extraction, the condition may change for the same kind of screws because the chromium coatings differ for each screw. Confirming the shape of spectrum, there were no contaminations of interference substances with the extraction condition.

4. Chromogenic Reaction of Cr(VI)
After hydrothermal extraction, screws were picked out from the sample vial. The extraction liquid was cooled to room temperature. Then, the chromogenic reagent of KYORITSU CHEMICAL-CHECK Lab (Figure 3) was added to the extraction liquid. The liquid was stirred for 1 minute and left to stand for 5 minutes.

Figure 1 Samples

Figure 2 Measurement procedures

Figure 3 Reagent set for water analyzer No.31- Cr (VI)
(KYORITSU CHEMICAL-CHECK Lab)
Quantitative Measurement of Chromium According to RoHS Directive and Hexahydric Chromium Treated to Screws

5. Quantitative Measurement
After leaving the sample for 5 minutes, turbidity was observed (Figure 4). The untouched sample and filtered sample were measured to compare their spectra.

Instrument
- JASCO V-630 spectrophotometer
- 10 mm rectangular quartz cell

Measurement Parameters
Mode: Abs
Measurement Range: 650 - 400 nm
Data Interval: 0.2 nm
UV/Vis bandwidth: 1.5 nm
Response: Medium
Scan Speed: 400 nm/min

6. Measurement Results
The spectra of untouched and filtered samples are illustrated in Figure 5. The comparison of two spectra shows higher baseline for the spectrum of untouched sample. This is caused by the dispersion by the interference substances. Three wavelengths quantitative analysis (peak wavelength: 542 nm, base wavelength: 635 and 402 nm) was considered to confirm the effects of the interference substances. Table 1 shows the results of the peak height calculations. Regardless of the pretreatment, almost same results can be obtained.

7. Quantitative Results
Three wavelengths quantitative analysis was examined on the peak height calculated with peak wavelength at 402 nm and base wavelengths at 635 and 542 nm. The calibration curve obtained by the diphenylcarbazide method in UV application data Vol.2 was applied to the quantitative analysis. Table 2 shows concentration of Cr(VI) for the sample A and sample B. The quantitative results in Table 2 show the two-sided 95% confidence interval. Both sample A and B have the confidence interval +/-0.005 mg/L against the quantitative values. With the method introduced here with JASCO spectrophotometer provides high precision measurement.

---

Table 1 Results of peak height calculation

<table>
<thead>
<tr>
<th></th>
<th>Sample A</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untouched</td>
<td>Filtered</td>
</tr>
<tr>
<td>One WL Absorbance at 542 nm</td>
<td>0.10393</td>
<td>0.09288</td>
</tr>
<tr>
<td>Three WL Peak height at 542 nm</td>
<td>0.08782</td>
<td>0.08765</td>
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Table 2 Quantitative Results of Cr(VI) (mg/L)

<table>
<thead>
<tr>
<th></th>
<th>Sample A</th>
<th>Sample B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untouched</td>
<td>Filtered</td>
</tr>
<tr>
<td>Quantitative Results</td>
<td>0.135 ±0.006</td>
<td>0.135 ±0.006</td>
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---
Superficially clean waters are analyzed in the field of not only inspection of drinking and environmental water, but quality control of glass. Of them, test of drinking water has water color analysis since it tells the concentration of humic materials that is precursor substance of trihalomethane.

The simplest method of color analysis defined in testing method of drinking water is visual check with color comparison tubes, however, results of the method depend on “observer’s aesthesia”. Here, JASCO introduces new method to analyze “Superficially clean water” with UV/Vis spectrophotometer and light path length 30 cm cell.

1. **System Configuration**
   - V-650/660/670 spectrophotometer
   - Extended sample compartment
   - 30 cm cylindrical cell

2. **Introduction of 30 cm Cell**
   JASCO extended sample compartment is dedicated for light path length 30 cm cylindrical cell.
   The 30 cm cell enables to measure samples with tiny absorbance precisely that cannot be measured by usual cell.
   An integrating sphere is mounted in the compartment to detect any size of light beam accurately. Easy measurement is now offered to you by just putting samples in the 30 cm cell and mounting it in the sample compartment.

3. **Samples**
   Ultra pure water, purified water, tap water, groundwater, and river water.

4. **Measurement Parameters**
   - Measurement Range: 850 - 220 nm
   - Data Pitch: 0.5 nm
   - UV/Vis Band Width: L5.0 nm
   - Response: Medium
   - Scan Speed: 400 nm/min

5. **Measurement and Analysis**
   Each sample was measured with 30 cm cell having air baseline. Ultra pure water indicated low absorbance at the all wavelength range (Figure 2). Since we regarded ultra pure water as almost clear, employed it to baseline to cancel the reflection or absorption of cell and obtained spectrum for each sample (Figure 3).

   The results obtained with 30 cm cell was analyzed with the [Color Diagnosis] program, and then plotted on the chromaticity diagram (Figure 4). Same samples were measured with 10 mm cell and same calculation was proceeded (Figure 5).

   Comparison between two chromaticity diagrams, colors that cannot be defined with 10 mm cell is clear with 30 cm cell. From the result, river water is yellower than the other waters and it is thought of as containing humic materials.

   Moreover, in the order of groundwater, tap water, and purified water, they come close to the light source that is white light.
With JASCO 30 cm cell, the color of “Superficially clear water” can be measured. It is available for the measurement of low concentration samples and in wide variety of water analysis fields. Extended sample compartment can be applied not only for liquid samples, but clear baculiform solid samples such as fibers.

From environmental analysis to quality control, JASCO extended sample compartment with 30 cm cell is useful in wide variety of fields.
Quantitative Determination of Chromium by Diphenylcarbazide Method

Chromium is widely used for metal materials such as iron and steel, chromium alloy, and refractory products. Plating, tanning, and pigments use the chromium as well. Chromium is very useful for our industrial society, however, against human body, chromium ions impinge as cell poison. For that reason, chromium requires careful handling and quantitative determination. The most widely used quantitative method is the diphenylcarbazide absorptiometrical method with spectrophotometer. The detection and determination limits of chromium were reviewed here with JASCO V-630 spectrophotometer. Calibration curves were created by using chromogenic kit of reagent manufacturers for easy quantitative determination.

1. Reagents
   • Reagent set for water analyzer No.31- Cr (VI) (KYORITSU CHEMICAL-CHECK Lab)
   • Special grade chemicals K2Cr2O7
   • Distilled water

2. Instrument
   • JASCO V-630 spectrophotometer
   • 10-mm rectangular cell: Quartz

3. Reagents preparation

3.1 Standard Solution
K2Cr2O7 of special grade chemicals was dried at 150°C for one hour and left in a desiccator, then 8.79 mg of the K2Cr2O7 were weighed out. In a 100-mL measuring flask, the K2Cr2O7 was dissolved in water and diluted to 100 mL. 80.4 mL of the solution was weighed out to the other 100-mL measuring flask and diluted to 100 mL. The diluted solution was a standard stock solution of 25.0 mg/L Cr (VI). The stock solution was diluted 100 times to prepare a standard solution of 0.25 mg/L.

3.2 Solutions for calibration curve
In accordance with Table 1, distilled water and standard stock solution were weighed out by pipettes and mixed to prepare 25 mL solutions.

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Stock Solution (µL)</th>
<th>DW (mL)</th>
<th>Concentration (mg/L)</th>
<th>Stock Solution (µL)</th>
<th>DW (mL)</th>
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<td>-</td>
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<td>-</td>
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</table>

*1 Solutions from 0.010 to 1.5 mg/L were prepared with the standard stock solution of 25.0 mg/L [Cr (VI)]. Solutions from 0.0010 to 0.0075 mg/L were prepared with standard solution of 0.25 mg/L.
Quantitative Determination of Chromium by Diphenylcarbazide Method

4. Chromogenic Reaction

Chromogenic reaction was processed at the room temperature (21°C). One package of the reagent set for water analyzer No.31- Cr (VI) was added to the calibration curve solutions right after opening it*2). The bottle of solution was agitated by hand for approximately 60 seconds*3). Dissolution of the reagent was confirmed *4).

4.1 Reaction Time

Cr (VI) solution of 25 mL with the concentration of 0.5 mg/L was prepared in order to determine the time from addition of the reagent to the measurement. The time course of the chromogenic reaction was observed. The reagent set for water analyzer No.31- Cr (VI) was added to the solution right after opening it and agitated for one minute, and then the time course of the absorption was measured at the wavelength of 540 nm. Here, 90 seconds were assumed as the time spent until measurement after addition of the reagent. The chromogenic reaction was completed 150 seconds after starting measurement (4 mins after addition of the reagent). The color became stable 210 seconds after starting measurement (5 mins after addition of the reagent).

4.2 Measurement

The Spectra Measurement Program was used for this experiment. The reagent was added to the solution and agitated for one minute, and then it was measured after leaving for 5 minutes. The light path of the reference side was empty. Baseline was measured with water in the cell used for sample measurement.

Photometric Mode: Abs
Measurement range: 650 - 400 nm
Data pitch: 0.2 nm
Band width (UV/Vis): 1.5 nm
Response: Medium
Scanning speed: 400 nm/min

*2) The solubility of the reagent set deteriorates with time after opening it, since the reagent absorbs moisture.
*3) Agitate the solution well, since the inadequate agitation impedes the smooth chromogenic reaction and has effect on the absorbance.
*4) Granular reagent remained even if the reagent dissolved perfectly in the solution. The remainder, however, was precipitated at the bottom of cell and did not have influence on the measurement.
Quantitative Determination of Chromium by Diphenylcarbazide Method

5. Results
The fluctuation of the bases occurred for Cr (VI) solution with the concentration between 0.01 mg/L and 0.001 mg/L. For that reason, the calibration curves were created with the peak height at 542 nm when the bases of wavelength were set at 402 nm and 635 nm. The determination limit of the calibration curve that was created with all concentration range was 0.09 mg/L. This shows that JASCO V-630 can determine the quantity of the chromium with the concentration range between 0.09 mg/L to 1.5 mg/L. Furthermore, when the calibration curve was created with the data of the Cr (VI) solution with the low concentration range between 0.01 mg/L and 0.001 mg/L, the determination limit was 0.0025 mg/L that proves higher sensitivity of the JASCO V-630.

<table>
<thead>
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<th>Concentration (mg/L)</th>
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<tr>
<td>-</td>
<td>-</td>
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<td>0.94904</td>
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Table 2 Concentration and Absorption
TCH-703 8-position Turret Micro Cell Holder

The TCH-703 eight-position turret micro-cell accessory is an attachment designed specifically for the V-630/630BIo instruments to provide a consistent measurement of eight samples with a minimum sample quantity of 4 μL.

It is a simple procedure to measure 8 samples as a maximum: pipette samples into the removable sample turret (Figure 1), set the turret cell into the holder (Figure 2), turn the knob to place a sample into the sample beam (Figure 3), and then press the start button. This attachment is very useful for the measurement of limited sample quantities for DNA and/or protein concentrations.

Figure 1

Figure 2

Figure 3

Measurement accuracy

Measurement accuracy of the 8 cells
To verify the measurement accuracy of the turret cells, analysis of DNA samples of raw bovine thymus was performed in the 8 cells at specific concentrations, measuring the quantity using the Warburg-Christian method. The CV’s for these measurements were 2% when the DNA concentration was 50 μg/mL, 11% for 10 μg/mL, and 10% for 5 μg/mL.

Reproducibility of the turret rotation
The same samples were measured while the turret was rotated to analyze the variation of the quantitative measurement in the same cell. The CV for these measurements was 0.3% when the DNA concentration was 50 μg/mL, 1.7% for a 10 μg/mL concentration, and 3.5% for 5 μg/mL (Table 1).

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<th></th>
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<th>C.V</th>
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<td>41.90</td>
<td>42.00</td>
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</table>

DNA 50μg/mL

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<th>3rd</th>
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<th>SD</th>
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<tr>
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<td>0.96</td>
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<td>1.09</td>
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</table>

DNA 10μg/mL

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<th>SD</th>
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</table>

DNA 5μg/mL
Detection limit and determination limit
The quantitative results outlined in Table 1 were used to create a calibration curve of the quantitative results vs. concentration (Figure 4). This calibration curve has a calculated detection limit of 0.38 μg/mL and a determination limit of 6.4 μg/mL. The definition of the detection limit used here is “the concentration calculated from the calibration curve when the maximum quantitative value of the 95% confidence interval is read at a calculated concentration of 0 μg/mL”. The definition of the determination limit is “the concentration calculated from the calibration curve using some specific quantitative results that has a coefficient of variation of 10%”.

Sample Infusion
A round-shaped gel loading tip is the most appropriate pipette tip to load samples into the turret cells. Using the gel loading tip prevents air bubbles in the cells.

Cleaning the turret cell
A round-shaped gel loading tip is used for both sample infusion and cleaning the cell with a cleaning solvent such as water. To remove protein samples, commercial detergents for instrument cuvettes can be used. If the turret cells are heavily contaminated, a concentrated nitric acid solution can also be used. JASCO offers a washing device, the μWash, which is designed to clean the cell easily using water, methanol, ethanol, and other solvents.

Washing accessory for micro cells such as 8-position Turret Micro Cell Holder
- Insert the needle tip into the cell. Pushing the buttons on the syringe several times cleans the cell using a ‘fill and drain’ action.
- The Wash device can wash the cell with a small quantity of a washing solution.
- The MW-2000 is applicable to the sample volume less than 100 μL.

Specifications

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<tr>
<td>Maximum injection and suction</td>
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</tr>
<tr>
<td>Cleaning solvent</td>
<td>Water, Methanol, Ethanol and other volatile solvents</td>
</tr>
</tbody>
</table>

Figure 4 Calibration curve of the quantitative results vs. concentration

\[
Y = A \times E + B \\
A = 0.824697 \pm 0.010735 \\
B = 0.236037 \pm 0.311315 \\
\text{Correlation coefficient} = 0.998451 \\
\text{Standard error} = 1.12974 \\
\text{Detection limit} = 0.37749 \\
\text{Determination limit} = 6.44095 \]
Quantitative Determination of Proteins

Generally, the concentration of proteins is measured using UV-Vis spectrophotometers. The JASCO V-630 Bio is a UV/Vis spectrophotometer designed for biochemical analysis. The V-630 Bio is equipped with 6 quantitative calibration curves based on UV absorption spectrophotometry including the Lowry, Biuret, BCA, Bradford, and WST methods.

Table 1 shows the features of the six different quantitation methods. As outlined, the preferred method can be selected by reviewing the sample and range of the quantitation and existence of any possible contaminants.

Five of the analysis methods are quantitative methods that utilize a chromogenic reaction. Reagent manufacturers produce chromogenic kits for BCA, Bradford or WST with an instruction manual explaining the measurement procedures\(^1\). On the other hand, the chromogenic reagents for the Lowry and Biuret methods need to be prepared by the user. The measurement procedures for the Lowry and Biuret methods differ depending on each document. For that reason, this application data explains how to create the calibration curves for the Lowry, Biuret and UV absorption analysis methods. Fluctuations of the values depending on the type of proteins and the differences of the concentration ranges calculated as a result of the cell volume\(^2\) were also investigated.
### Quantitative Determination of Proteins

<table>
<thead>
<tr>
<th>Method</th>
<th>Principle</th>
<th>Concentration range</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV Abs</td>
<td>The absorption maximum at 280 nm corresponds to the response of the tyrosine and tryptophan and is used for the analysis method.</td>
<td>50 to 2000 µg/mL (*BSA)</td>
<td>Simple method. Sample can be used after measurement.</td>
<td>The absorbance differs for each protein. Proteins such as collagen and gelatin that do not have absorption at 280 nm cannot be measured. Contamination by nucleic acids with absorption in the UV region obscures the measurement.</td>
</tr>
<tr>
<td>Biuret</td>
<td>Protein solutions turn purple after the polypeptide chain chelates with a copper ion. An alkaline solution of Biuret reagent including copper sulfate and Rochelle salt is added to a protein solution. Uses the absorption maximum at 540 nm to determine the quantity.</td>
<td>150 to 9000 µg/mL (BSA)</td>
<td>Simple procedure. The chromogenic rate is constant for most proteins.</td>
<td>Low sensitivity. Sample with a low protein concentration cannot be measured. The chromogenic reaction is influenced by high concentrations of triaminomethane, amino acids, and ammonium ion.</td>
</tr>
<tr>
<td>Lowry</td>
<td>Add an alkaline copper solution to a protein solution. Tyrosine, tryptophan, and cisteine of proteins reduce molybdemen acid and phosphotungstic acid of a phenol reagent, turning the solution blue. Uses the absorption maximum at 750 nm to determine the quantity.</td>
<td>5 to 200 µg/mL (BSA)</td>
<td>High sensitivity. Widely used.</td>
<td>Complicated procedure with a long preparation. Since the chromogenic reaction occurs by a reduction reaction, contamination of the reduction material interferes with the quantitative determination. The chromogenic rate differs for each protein.</td>
</tr>
<tr>
<td>BCA</td>
<td>The BCA method combines the Biuret method and Bicinchoninic Acid (BCA). BCA has high sensitivity and selectivity for copper ions. When a copper ion that is formed by the reduction action of protein reacts with 2 molecules of the BCA, the solution turns purple. Uses the absorption maximum at 560 nm to determine the quantity.</td>
<td>20 to 2000 µg/mL (BSA)</td>
<td>Simple procedure. High sensitivity with wide Concentration range.</td>
<td>Thiol, phospholipid, and ammonium sulfate interferes with the measurement.</td>
</tr>
<tr>
<td>Bradford</td>
<td>The absorption maximum of a protein shifts from 465 nm to 600 nm when the protein binds to the Coomassie Brilliant Blue G250, one of triphenylmethane blue pigment. Uses the absorption maximum at 600 nm to determine the quantity.</td>
<td>10 to 2000 µg/mL (BSA)</td>
<td>Very simple operation. Hardly influenced by the blocking materials</td>
<td>The chromogenic rate differs for each protein. Contamination by a surfactant can interfere with the chromogenic reaction.</td>
</tr>
<tr>
<td>WST</td>
<td>Reduce WST-8 with proteins with a high pH, turning the sample blue. Uses the absorption maximum at 650 nm to determine the quantity.</td>
<td>50 to 5000 µg/mL (BSA)</td>
<td>Simple operation. Hardly influenced by a surfactant.</td>
<td>The chromogenic rate differs for each protein.</td>
</tr>
</tbody>
</table>
1. UV Absorption Method
Figure 4 illustrates the absorption spectrum of Human Serum Albumin (HSA). Simple UV Absorption spectrophotometry can determine the quantity of proteins in the sample by using the maximum absorption at 280 nm.

1.1 Samples
Bovine Serum Albumin (BSA): 0.02, 0.025, 0.05, 0.1, 0.2, 0.25, 0.4, 0.5, 1, (1.5, 2) mg/mL
Hen Egg Lysozyme: 0.02, 0.025, 0.05, 0.1, 0.2, 0.25, (0.4, 0.5) mg/mL
Chymotrypsin from bovine pancreas: 0, 0.1, 0.2, 0.25, 0.4, 0.5 mg/mL
Bracketed values are for concentrations used with the 10 mm rectangular cell. The other values are concentrations used with the 10 mm rectangular cell and micro cell.

1.2 Measurement Procedures
Measure the absorbance of the protein solution using the wavelength of 280 nm.

Figure 4 Absorption spectrum of HSA

<table>
<thead>
<tr>
<th>Cell</th>
<th>Concentration range</th>
<th>Calibration curve formula</th>
<th>Correlation function</th>
<th>Standard error</th>
<th>Detection limit</th>
<th>Determination limit</th>
</tr>
</thead>
</table>
| BSA                      | 10 mm rectangular cell (quartz) | to 2 mg/mL | Y = AX + B
A = 0.6652 ± 0.0079
B = -0.0130 ± 0.0064 | 0.9994 | 0.0219 | 0.0097 mg/mL | 0.0479 mg/mL |
|                         | Micro cell          | to 1 mg/mL | Y = AX + B
A = 0.6713 ± 0.0043
B = -0.0016 ± 0.0008 | 0.9999 | 0.002 | 0.0012 mg/mL | 0.0218 mg/mL |
| HEL                      | 10 mm rectangular cell (quartz) | to 0.5 mg/mL | Y = AX + B
A = 0.6474 ± 0.0459
B = -0.0150 ± 0.0109 | 0.9991 | 0.0076 | 0.0041 mg/mL | 0.0680 mg/mL |
|                         | Micro cell          | to 0.25 mg/mL | Y = AX + B
A = 2.7499 ± 0.0429
B = -0.0060 ± 0.0055 | 0.9995 | 0.0031 | 0.0020 mg/mL | 0.0096 mg/mL |
| α-Chymotrypsin           | 10 mm rectangular cell (quartz) | to 0.5 mg/mL | Y = AX + B
A = 1.904 ± 0.0237
B = -0.0055 ± 0.0070 | 0.9997 | 0.0042 | 0.0037 mg/mL | 0.0615 mg/mL |
|                         | Micro cell          | to 0.5 mg/mL | Y = AX + B
A = 2.1279 ± 0.0655
B = -0.0202 ± 0.0193 | 0.9983 | 0.0104 | 0.0091 mg/mL | 0.1263 mg/mL |

Table 2 Results for the UV absorption method
2. Biuret Method
Protein solutions turn purple with the absorption maximum of 540 nm when Biuret reagent is added (Figure 5).
The time course of the chromogenic reaction was measured at 540 nm absorption. The absorbance became stable approximately after 60 minutes (Figure 6). Figure 7 demonstrates the spectra of the HSA aqueous solutions that were measured after 60 minutes. Biuret method determines the quantity by using the absorption maximum at 540 nm after reacting with the sample for 60 minutes.

2.1 Chromogenic Reagent
Biuret reagent: Add 60 mL of 10% NaOH to 100 mL of aqueous solution to dissolve 0.3 g CuSO4 and 1.2 g Rochelle salt, and then add water to 200 mL. (Standard reagent can be saved in a polyethylene bottle.)

2.2 Samples
Bovine Serum Albumin (BSA): 0, 0.25, 0.5, 1, 5, 9 mg/mL (10 mm rectangular cell (quartz))
0, 1, 3, 5, 9 mg/mL (Micro cell)
Hen Egg Lysozyme: 0, 1, 3, 5, 9 mg/mL (10 mm rectangular cell (quartz), Micro cell)
Chymotrypsin from bovine pancreas: 0, 1, 3, 5, 9 mg/mL (10 mm rectangular cell (quartz), Micro cell)

2.3 Measurement Procedures
Add Biuret reagent (2.0 mg) to 500 μL of protein aqueous solution and mix them well. Then react the solution for 60 minutes. Measure the absorbance at the wavelength of 540 nm.
### Quantitative Determination of Proteins

<table>
<thead>
<tr>
<th>Cell</th>
<th>Concentration range</th>
<th>Calibration curve formula</th>
<th>Correlation function</th>
<th>Standard error</th>
<th>Detection limit</th>
<th>Determination limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA 10 mm rectangular cell (quartz)</td>
<td>to 9 mg/mL</td>
<td>$Y = AX^2 + BX + C$</td>
<td>1</td>
<td>0.0109</td>
<td>0.008 mg/mL</td>
<td>0.1483 mg/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$A = -0.0005 \pm 4.3444 \times 10^{-3}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$B = 0.0548 \pm 0.0004$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Micro cell</td>
<td>to 9 mg/mL</td>
<td>0.9998</td>
<td>0.0775</td>
<td>0.0696 mg/mL</td>
<td>1.0127 mg/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$Y = AX^2 + BX + C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$A = -0.0016 \pm 0.0003$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$B = 0.0655 \pm 0.0027$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$C = 0.0450 \pm 0.0045$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lysozyme 10 mm rectangular cell (quartz)</td>
<td>to 0.9 mg/mL</td>
<td>$Y = AX^2 + BX + C$</td>
<td>1</td>
<td>0.0144</td>
<td>0.0133 mg/mL</td>
<td>0.2420 mg/mL</td>
</tr>
<tr>
<td></td>
<td>Micro cell</td>
<td>to 9 mg/mL</td>
<td>0.9999</td>
<td>0.0786</td>
<td>0.0640 mg/mL</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$Y = AX^2 + BX + C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$A = -0.0053 \pm 0.0005$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$B = 0.0983 \pm 0.0048$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$C = 0.0476 \pm 0.0063$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Chymotrypsin 10 mm rectangular cell (quartz)</td>
<td>to 9 mg/mL</td>
<td>$Y = AX^2 + BX + C$</td>
<td>0.9996</td>
<td>0.0958</td>
<td>0.0934 mg/mL</td>
<td>1.2987 mg/mL</td>
</tr>
<tr>
<td></td>
<td>Micro cell</td>
<td>to 9 mg/mL</td>
<td>0.9998</td>
<td>0.0697</td>
<td>0.0695 mg/mL</td>
<td>0.9987 mg/mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$Y = AX^2 + BX + C$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$A = -0.0010 \pm 0.0003$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$B = 0.0614 \pm 0.0025$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$C = 0.0343 \pm 0.0043$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Results for the Biuret method

#### 3. Lowry method

Protein solutions turn blue with an absorption maximum of 750 nm when Alkline copper solution and Phenol reagent are added to the protein solution (Figure 8). The time course of the chromogenic reaction was measured at 750 nm absorption. The absorbance became stable approximately after 60 minutes (Figure 9). Figure 10 illustrates the spectra of the HSA aqueous solutions that were measured after 60 minutes.

Lowry method determines the quantity by using the absorption maximum of 750 nm after reaction of the sample for 60 minutes.

**Figure 8**
Color change after adding Phenol reagent

**Figure 9**
The time course trace of the chromogenic reaction
Quantitative Determination of Proteins

3.1 Reagents
Alkaline copper solution: Mix 50 mL of 2% Na2CO3 solution*3) with 1 mL of 0.5% CuSO4 solution*4) (can only be used for immediate analysis).

*3) 2% Na2CO3 solution: dissolve anhydrous sodium carbonate (2 g) and caustic soda (0.4 g) in 100 mL water.

*4) 0.5% CuSO4 solution: dissolve copper (II) sulfate pentahydrate (50 mg) and potassium sodium tartrate tetrahydrate (0.1 g) in 10 mL of water.

Phenol reagent: Dilute commercial solution (2N phenol chemical reagent of Kanto Chemical) to 1N.

3.2 Samples
Same concentrations are used both for the 10 mm rectangular cell and the micro cell of 10 mm Bovine Serum Albumin (BSA): 0, 2, 20, 50, 100, 200 µg/mL
Hen Egg Lysozyme: 0, 1, 5, 10, 20, 50, 100, 200 µg/mL
α-Chymotrypsin from bovine pancreas: 0, 2, 20, 50, 100, 200 µg/mL

3.3 Measurement Procedures
Add 2.5 mg of alkaline copper solution to 500 µL of protein solution. Allow to react for 10 minutes after mixing well. Then, add phenol reagent. Rapidly mix and allow the solution to react for 60 minutes. Measure the absorbance at 750 nm.

<table>
<thead>
<tr>
<th>Cell</th>
<th>Concentration range</th>
<th>Calibration curve formula</th>
<th>Correlation function</th>
<th>Standard error</th>
<th>Detection limit</th>
<th>Determination limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mm rectangular cell (quartz) to 200 µg/mL</td>
<td>Y = AX² + BX + C A = 4.3663 x 10⁻⁷ ± 5.5049 x 10⁻⁷ B = 0.0041 ± 0.0001 C = 0.0250 ± 0.0034</td>
<td>0.9999</td>
<td>1.2336</td>
<td>0.8385 µg/mL</td>
<td>3.9441 µg/mL</td>
<td></td>
</tr>
<tr>
<td>Micro cell to 200 µg/mL</td>
<td>Y = AX² + BX + C A = -4.0578 x 10⁻⁶ ± 1.3689 x 10⁻⁶ B = 0.0041 ± 0.0003 C = 0.0150 ± 0.0097</td>
<td>0.9994</td>
<td>2.5325</td>
<td>2.3903 µg/mL</td>
<td>10.1765 µg/mL</td>
<td></td>
</tr>
<tr>
<td>10 mm rectangular cell (quartz) to 200 µg/mL</td>
<td>Y = AX² + BX + C A = -5.6033 x 10⁻⁶ ± 7.2903 x 10⁻⁷ B = 0.0049 ± 0.0001 C = 0.0293 ± 0.0037</td>
<td>0.9998</td>
<td>1.3861</td>
<td>0.7598 µg/mL</td>
<td>3.5722 µg/mL</td>
<td></td>
</tr>
<tr>
<td>Micro cell to 200 µg/mL</td>
<td>Y = AX² + BX + C A = -4.8873 x 10⁻⁶ ± 8.2675 x 10⁻⁷ B = 0.0047 ± 0.0002 C = 0.00076 ± 0.0042</td>
<td>0.9997</td>
<td>1.6514</td>
<td>0.8911 µg/mL</td>
<td>4.1471 µg/mL</td>
<td></td>
</tr>
<tr>
<td>10 mm rectangular cell (quartz) to 200 µg/mL</td>
<td>Y = AX² + BX + C A = -1.0948 x 10⁻⁷ ± 4.3250 x 10⁻⁷ B = 0.0061 ± 8.8164 x 10⁻³ C = 0.0112 ± 0.0027</td>
<td>1</td>
<td>0.685</td>
<td>0.4371 µg/mL</td>
<td>7.6764 µg/mL</td>
<td></td>
</tr>
<tr>
<td>Micro cell to 200 µg/mL</td>
<td>Y = AX² + BX + C A = -8.8298 x 10⁻⁷ ± 8.8527 x 10⁻⁷ B = 0.0054 ± 0.0002 C = 0.0167 ± 0.0055</td>
<td>0.9999</td>
<td>1.3341</td>
<td>1.0214 µg/mL</td>
<td>16.2006 µg/mL</td>
<td></td>
</tr>
</tbody>
</table>
Melting measurement is one of remarkable measurement methods in Biotechnology field such as DNA melting and thermal denaturation of Proteins. For those measurements, 10mm rectangular type cell has been generally used, which requires large volume of sample for each measurement. By using JASCO’s water-cooled Peltier thermostatted cell holder PAC-743/743R with micro 8-position cell it is possible to measure sample with volume as small as 10 µL *, however, for measurement of sample with much lower volume, it has been wished for a long time to have an accessory enabling such requirement.

*Refer to DNA melting measurement #3 in using of PAC-743/743R No.UV-0017-E.

As one of solutions, JASCO introduces an application “DNA melting by One Drop measurement method” using JASCO V-630 BIO UV/VIS Spectrophotometer with capillary jacket for melting measurement, capillary and adaptor.

**Capillary jacket for melting measurement**

Capillary is disposal type quartz glass cell, whose optical pathlength is 0.5 mm and minimum sample volume is 3 µL. After sampling by a capillary phenomenon, both edges of capillary are lapped by seal, which helps to avoid volatilization of sample. Such capillary is inserted into capillary jacket for melting measurement to be mounted to 6 channel cell block of Peltier cell holder or to PAC-743/ PAC 743R Water-cooled Peltier thermostatted cell holder. This capillary jacket has temperature sensor insertion port and temperature measurement in this port helps to measure accurate actual temperature of sample.

**Measurement System**
- V-630 BIO UV/VIS Spectrophotometer
- ETCS-761 Water-cooled Peltier thermostatted cell holder with stirrer
- OPS-515 Temperature sensor assy
- MCB-100 Mini water circulation bath
- Capillary jacket for melting measurement, adaptor, capillary, seal material

**Measurement Program**
Temperature ramping / DNA melting program (Standard software of V-630 Bio)

**Sample**
Poly (dA-dT)-Poly(dA-dT) pH7 phosphoric acid buffer

**Measurement Condition**
- Start setting: 3 seconds in the set temperature +/- 0.10 degrees C
- Data acquisition interval: 0.1 degrees C
- Temperature gradient: degrees C / min
- Response: Fast
- Measurement wavelength: 260 nm
DNA melting by One Drop measurement using capillary jacket

Result

Fig. 3 shows the result of measurement of Poly (dA-dT)-Poly(dA-dT). The green spectrum is the measurement result data by using 10 mm rectangle type cell and blue one is the result data by using capillary cell. Temperature is measured by the temperature sensor inside of capillary jacket.

As the result of calculation based on above data for melting temperature, the similar results have been obtained for both measurement, such as 63.8 degrees C in 10 mm rectangular type cell and 63.9 degrees C in capillary cell respectively. It obviously proves that the measurement using small volume capillary is as reliable as measurement by general method using 10 mm cell.
For the melting measurement of DNA samples, a temperature sensor can be inserted into the sample cells and the actual temperatures of samples plotted on the horizontal axis in order to increase the accuracy of the temperature readings for the melting experiment. This measurement technique is easy to be applied for 10-mm rectangular cells with a larger sample volume. However, for cells with a small sample volume such as the 8-position micro cell (100 µl), a temperature sensor blocks the instrument optical path. It is then difficult to obtain both absorbance and temperature of a sample simultaneously.

Here, a DNA measurement example using the 8-position micro cell with a temperature sensor is outlined. By using one of the 8-position micro cells as a temperature monitor (Figure 1), the horizontal axis of the temperature course data can be plotted with actual temperatures obtained by the sensor. This increases the temperature accuracy of measurements with the 8-position micro cell.

**Measurement System**
- PAC-743 water-cooled Peltier cell changer
- 8-position micro cell block
- 8-position micro cell
- Silicon cap
- Cap pressure fixture
- Sensor in cell

**Measurement Program**
VWTP-780 Temperature Gradient Measurement Program

**Sample**
Poly (dA-dT)-Poly(dA-dT) pH7 KH2PO4-NaOH buffer solution (20 µg/mL)

**Measurement Parameters**
- Number of cells: 7
- Temperature control sensor: holder
- Temperature monitor sensor: cell 8
- Start condition: Keep within +/- 0.01°C of the target temperature for 3 seconds
- Data interval: 1°C (20-50°C), 0.1°C (50-70°C), 1°C (70-100°C)
- Ramp rate: 2°C/min
- Response: Fast
- Measurement wavelength: 260 nm
- Reverse temperature measurement: ON

*1) Cell 8 was used only for temperature monitor. Absorbance was not measured.
*2) A silicon cap with a hole illustrated in Figure 2 was used for the temperature monitor cell. Silicon caps without holes were used for cells 1 to 7.
DNA Melting Measurement with the PAC-743/743R Water-cooled Peltier Cell Changer Measurement with a Temperature Sensor in Cell

Results
Measurement results are illustrated in Figure 3. The horizontal axis of the graph was plotted versus the values of the temperature monitor sensor in cell 8. The sample measurement required a total of 5 hours; 2.5 hours for the temperature increase data and 2.5 hours for the reverse temperature course. Table 1 indicates the melting temperature calculated from the temperature course data outlined in Figure 3. These results demonstrate melting temperatures for the various cells from 61.8 to 62.2°C (Ave. of 62.0°C), with a standard deviation of 0.13°C, and a coefficient of variance of 0.20%.

The same measurement was performed while obtaining sample temperatures using the standard temperature sensor (the holder sensor) within the PAC-743/743R accessory. Table 2 records the melting temperatures calculated from the temperature data using the holder sensor. Measurement results from this experiment provide a melting point varying from 63.0 to 63.3°C that is around one degree higher than the temperature obtained when using the sensor in the sample cell. These results indicate that the temperature of the holder was around one degree higher than the actual temperature of the sample. On the other hand, the standard deviation and coefficient of variance are the same for both measurements. Considering these results, the holder sensor offers sufficient capability to measure a reproducible melting temperature for all sample cells. However, to obtain the absolute value for sample melts, a cell temperature sensor is highly recommended.

<table>
<thead>
<tr>
<th>Melting g Temperature (sensor in cell)</th>
<th>Rising</th>
<th>Falling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell 1</td>
<td>62.0</td>
<td>61.9</td>
</tr>
<tr>
<td>Cell 2</td>
<td>62.0</td>
<td>62.1</td>
</tr>
<tr>
<td>Cell 3</td>
<td>61.9</td>
<td>62.1</td>
</tr>
<tr>
<td>Cell 4</td>
<td>61.9</td>
<td>62.0</td>
</tr>
<tr>
<td>Cell 5</td>
<td>61.8</td>
<td>61.9</td>
</tr>
<tr>
<td>Cell 6</td>
<td>62.0</td>
<td>61.9</td>
</tr>
<tr>
<td>Cell 7</td>
<td>62.2</td>
<td>62.3</td>
</tr>
<tr>
<td>Average</td>
<td>62.0</td>
<td>62.0</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>C.V.</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Table 1

<table>
<thead>
<tr>
<th>Melting g Temperature (holder sensor)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell 1</td>
</tr>
<tr>
<td>Cell 2</td>
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<tr>
<td>Cell 3</td>
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<tr>
<td>Cell 4</td>
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<tr>
<td>Cell 5</td>
</tr>
<tr>
<td>Cell 6</td>
</tr>
<tr>
<td>Cell 7</td>
</tr>
<tr>
<td>Average</td>
</tr>
<tr>
<td>Std. Dev.</td>
</tr>
<tr>
<td>C.V.</td>
</tr>
</tbody>
</table>

Table 2

Figure 3 Temperature Course Data
When performing a DNA Melting measurement, most of the samples are only available in extremely small amounts. Due to a limited amount of sample, it is essential that the amount used for measurement/analysis purposes be as little as possible. However, when sampling a small amount in a high temperature range, volatilization of the sample occurs, frequently complicating the analysis process. Additionally, in order to increase the accuracy of the temperature readings of the samples, one of the 8-Position Micro Cell Block cells is utilized for the dedicated temperature monitoring so that the temperature in a cell can be used in the melting data.

**Measurement Systems**
- PAC-743 Water-cooled Peltier Cell Changer
- 1 mm 8-position micro cell block
- 1 mm 8-position micro cell
- Silicon Cap (attached to 1mm 8-position micro cell block)
- Cap fixture (attached to 1mm 8-position micro cell block)

**Measurement program**
VWTP-780 [Temperature Ramping Measurement /Melting Analysis] Program

**Sample**
Poly (dA-dT)
Poly (dA-dT) pH7KH2PO4-NaOH buffer solution (200 μg/mL)

**Measurement Parameters**
Start condition: Keep within +/-0.1°C of the temperature setting for 3 seconds
Data interval: 1°C (20 - 50°C), 0.1°C (50 - 70°C), 1°C (70 - 100°C)
Temperature gradient: 2°C / min
Response: Fast
Wavelength for measurement: 260 nm

**Holder sensor**
- Number of cells: 8
- Temperature control sensor: holder
- Temperature monitor sensor: holder

**Internal Cell Sensor**
- Number of cells: 7
- Temperature control sensor: holder
- Temperature monitor sensor: cell (8)

**Results**
The melting curves from the results of sample measurements with all eight micro cell using the holder sensor are plotted as shown in Figure 2. The time required for the measurement was totally 2.5 hours and the changes of samples volume during the measurement process are shown in Figure 3. During the measurement, Nujol was placed on top of the sample cells to prevent the sample from adhering to silicon caps. After completion of the measurement of samples, the solution levels for each of the 8 cells were higher than the upper limit of cells (indicated using a red dotted line), and the decrease in sample volume were almost not observed by the human eye. In short, by using the silicon cap and cap fixture, volatilization of samples can be prevented.
DNA Melting Measurements (3) with the PAC-743/743R Water-cooled Peltier Cell Changer “1 mm 10 μL 8-Position Micro Cell Block”

In order to enhance the accuracy of the temperature, one of the eight cells (referred to as “cell 8”) was used exclusively to monitor the sample temperature. Figure 4 shows a result of melting curves using the temperature readings from internal cell sensor. These temperature values were plotted in the horizontal axis in Figure 4 using data collected from internal cell sensor in cell 8. No evaporation was observed in this case.

The results of the melting points, calculated from the melting curves data in Figure 2 and 4, are shown in Table 1 and 2 below. The results using the holder sensor are shown in Table 1 ranging melting temperature between 66.0°C ~ 66.2°C (average 66.1°C) with standard deviation of 0.08°C and coefficient of variance of 0.13%. On the other hands, the results using internal cell sensor are shown in Table 2 ranging melting temperatures between 63.6°C ~ 63.8°C (average 63.7°C) with standard deviation of 0.08°C and coefficient of variance of 0.12%. This indicates that the temperature using the holder sensor was approximately 3.5°C higher than using the internal cell sensor. From these data, it can be concluded that the actual temperature of the holder was 3.5°C higher than the temperature of the original sample in the cell, while both the standard deviation and coefficient of variance had no major differences in their result. In conclusion, to compare the melting temperatures relatively between each sample, the holder sensor is believed to be sufficient, while the internal cell sensor in the sample cell is ideal for measuring the absolute value of melting temperatures.

<table>
<thead>
<tr>
<th>Melting Temperature (Holder Sensor)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell 1</td>
<td>66.1</td>
</tr>
<tr>
<td>Cell 2</td>
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</tr>
<tr>
<td>Cell 3</td>
<td>66.0</td>
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<tr>
<td>Cell 4</td>
<td>66.1</td>
</tr>
<tr>
<td>Cell 5</td>
<td>66.1</td>
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<tr>
<td>Cell 6</td>
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</tr>
<tr>
<td>Cell 7</td>
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<tr>
<td>Cell 8</td>
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<tr>
<td>Average</td>
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</tr>
<tr>
<td>Std. Dev.</td>
<td>0.08</td>
</tr>
<tr>
<td>C.V.</td>
<td>0.13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Melting Temperature (Internal Cell Sensor)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
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<td>63.6</td>
</tr>
<tr>
<td>Cell 2</td>
<td>63.6</td>
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<tr>
<td>Cell 5</td>
<td>63.7</td>
</tr>
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</tr>
<tr>
<td>Average</td>
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</tr>
<tr>
<td>Std. Dev.</td>
<td>0.08</td>
</tr>
<tr>
<td>C.V.</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Table 1

Table 2
The Model SAH-769 One-Drop: A Dedicated Accessory for Extremely Small Amounts of Protein and Nucleic Acid Samples

JASCO introduces a new measurement method for an extremely small amount of DNA samples, or any other sample, by using the model V-630BIO spectrophotometer with the model SAH-769 One-Drop accessory. The SAH-769 measures 5 or 0.6 μL of sample dropped on the disk cell with a 1- or 0.2-mm optical path, respectively. The precise optical path is secured by covering the liquid sample with the cover glass integrated with the unit. The cell and cover glass can be washed by simply wiping them clean with laboratory wipers. The simple method for the measurement of proteins and nucleic acids allows users to measure large numbers of samples promptly. The shorter optical path length configuration allows measurement of high concentration samples without further dilution.

The V-630BIO, the main unit of the system, utilizes a monochromator with a diffraction grating and a double-beam optical system to ensure high stability for extremely reliable measurements. The V-630BIO can be operated by the intelligent Remote Module (iRM) color touch panel control module or utilizing the cross-platform Spectra Manager software designed for the Windows operating system. In either case, the software includes standard programs for life science analyses. The [Protein/Nucleic Acid Measurement] program measures the sample absorbance at 260 and 280 nm to calculate the protein and nucleic acid ratio. The [Temperature Control Measurement] program with optional Peltier thermostatted cell holders can be utilized for the DNA melting analysis experiment.

System Configuration
• V-630BIO Spectrophotometer for life science field
• SAH-769 One-Drop
• Dedicated disk cell 1-mm optical path with 5 μL of sample volume
• (Optional disk cell 0.2-mm optical path with 0.6 μL of sample volume)

Measurement Procedure

1) Drop sample on the cell
2) Close the cover glass and the lid of sample compartment
3) Start sample measurement
4) Cleaning the cell

less than 20 seconds
The Model SAH-769 One-Drop: A Dedicated Accessory for Extremely Small Amounts of Protein and Nucleic Acid Samples

Precision of Quantitative Analysis
Solutions of Calf Thymus DNA (KH2PO4 / NaOH buffer at pH7) at several concentrations were measured by using cells with 1-mm and 0.2-mm optical paths. The spectra (collected with identical instrument parameters) using each cell are illustrated in Figures 1 and 2. The graphs 1 and 2 illustrate the calibration curves created using the absorbance maxima at 260 nm. Both calibration graphs demonstrate good linearity.

Table 1 Sample Conc. and Abs [OP: 1mm]

<table>
<thead>
<tr>
<th>Legend</th>
<th>Conc. [ng/µL]</th>
<th>Abs</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0.0228</td>
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<tr>
<td></td>
<td>26</td>
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<td></td>
<td>52</td>
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<td></td>
<td>250</td>
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<td>520</td>
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<td>780</td>
<td>1.3443</td>
</tr>
<tr>
<td></td>
<td>1040</td>
<td>1.8137</td>
</tr>
</tbody>
</table>

\[ y = 0.0017x - 0.0032 \]
Correlation coefficient 1.0000
Standard deviation 3.09

Figure 1
Absorbance spectra of DNA solution
[optical path: 1 mm]

Measurement Parameters
Data interval: 0.5 nm
Measurement range: 220 to 315 nm
Band width: 1.5 nm
Response: Medium
Scan Speed: 200 nm/min

Graph 2 Calibration Curve [Optical path : 1 mm]
The Model SAH-769 One-Drop: A Dedicated Accessory for Extremely Small Amounts of Protein and Nucleic Acid Samples

**Figure 2**
Absorbance spectra of DNA solution [optical path: 0.2 mm]

**Graph 3** Calibration Curve [Optical path: 0.2 mm]

**Contamination of Samples**
The disk cell and cover glass were wiped clean after measuring the absorbance of a 5-μL high concentration DNA sample. Then, a 5-μL solvent was measured to evaluate sample cross-contamination of the disk cell. The results indicated in Table 3 indicate the wiping is enough to wash the sample from the cell.

**Table 3** Carry over of DNA sample

\[
y = 0.0002x + 0.0029
\]
Correlation coefficient 0.999880002
Standard deviation 18 4930641
After the acquisition of the spectrum, it is possible to perform data manipulation, calculating the temperature of melting from collected experiment. This can be done in two ways:

- A selected routine within Spectra Analysis allows to make spectra interpolation in order to extrapolate Tm (Figure 4).
- First derivative of the curve can be performed and then Tm can be easily determined by evaluating the maxima of previously obtained curve (Figure 5).

**Comments**

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<td>End</td>
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**Measurement Information**

<table>
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<tr>
<td>Response</td>
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<tr>
<td>Monitor wavelength</td>
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<tr>
<td>Correction</td>
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Melting Experiments with V-600 Spectrophotometers Series

V-600 Spectrophotometers Series provide a powerful tool for performing temperature dependent experiments, when main instrument is coupled with any of the newly developed single or multiple position Peltier thermostatted cell holders listed below (see Figure 1):

- EHCS-760 air cooled single position Peltier cell holder
- ETCS-761 water cooled single position Peltier cell holder
- ETCR-762 water cooled single position Peltier cell holder with thermostatted reference side
- PSC-763 air cooled 6 x 1 multiple position Peltier cell holder
- PAC-743 water cooled 6 x 1 or 8 x 1 multiple position Peltier cell holder
- PAC-743R water cooled 6 x 1 or 8 x 1 multiple position Peltier cell holder with thermostatted reference side
- PWC-718 water cooled 8 x 8 multiple position Peltier cell holder

Spectroscopy Suite Spectra Manager 2 standard functionality can be extended by adding several optional routines to software main part. In particular, Temperature Programming measurement option allows for the monitoring of variations in the absorbance of a sample at fixed wavelength when its temperature is gradually changed. The Software Parameters Setting (Figure 2a,b,c) allows to setup completely automated temperature dependent experiments, by performing thermal gradients having complex profiles. It is possible to automatically collect absorbance variation over an extended temperature interval by selecting the most proper data pitch according to the sample transmittance variation in the selected region: for example in regions where variation is very low, data pitch may be wider, while this has to be narrower in regions of high and rapid variation.

At the same time even temperature change speed can be selected to be different from one temperature interval to the other.

For example, it is possible to setup an experiment like the following one:

- change temperature from 20°C to 60°C at a rate of 5°/min and collect one sample data each 2°
- increase temperature from 60°C to 80°C at a rate of 1°/min and collect one sample data each 0.5°
- change temperature from 80°C to 95°C at a rate of 2°/min and collect one sample data each 1°

![Figure 1 V-630 with PSC-763](image1)

![Figure 2a Temperature Parameters setting](image2)
Measurement of the reduction reaction of 2,6-dichloroindophenol (DCIP) using the absorption stopped-flow system

Introduction
Using the absorption stopped-flow measurement system, consisting of FS-110 fast scan spectrophotometer and SFS-852 stopped-flow system, two to four kinds of liquid sample can be mixed quickly and the changes in absorption spectra can be measured at intervals of 5 msec. This system allows measurement of rapid enzymatic, catalytic and oxidation-reduction reactions. This application note illustrates an example of determining the reaction rate by using the absorption stopped-flow system for the reduction of DCIP, whose color in aqueous solution is changed from blue to colorless as a result of reaction with L-ascorbic acid.

Measurement and analysis system
• Absorption stopped-flow measurement system
• FS-110 fast scan spectrophotometer
• SFS-852 stopped-flow system
• Stopped-flow measurement program
• Reaction rate calculation program

Samples
• 20 mmol/L L-ascorbic acid aqueous solution (Dissolve the L-ascorbic acid with NaOH/Na2HPO4 aqueous solution and make it to a constant volume. Then, adjust the pH to 7.6.)
• 1 mmol/L DCIP aqueous solution

Measurement conditions
Spectrophotometer
Optical pathlength: 2 mm
Wavelength range: 300 to 800 nm
Data interval: 1 nm
Measurement interval: 0.010 sec
Measurement time: 0 to 3 sec

Stopped-flow system
Time of solution sending: 10 msec
Mixing ratio: 1:1
Volume of solution sending: 50 μL
The measurement is started when the syringe is stopped.

Results
Figure 2 shows the 3D spectra of the sample. When the reaction is started, the spectrum indicates an absorption maximum at approximately 600 nm and the sample exhibits a blue color. Then, the absorbance in the visible wavelength range changes to approximately zero within 1 sec after starting the measurement, and the sample turns colorless. Figure 3 shows the time course data for absorbance at the absorption maximum (604 nm) and the curve fitted to the data between 0.03 and 2.00 sec for the reaction range, assuming the reaction to be a primary reaction. The fitted curve is in excellent agreement with the measurement results. A reaction rate of 4.3 sec-1 was calculated.

Figure 1 Absorption stopped-flow system

Figure 2 3D spectra of reduction of DCIP

Figure 3 Time-course measurement results and fitted curve for sample absorbance at 604 nm

Curve fitting analysis parameters
Number of reaction steps: 1
Reaction range: 0.03 to 2.00 sec

Curve fitting analysis result
Reaction rate calculation equation: \( Y(t) = 0.615066 \times \exp(-t/0.230571) \)
Baseline equation: \( Y(t) = 0.0105329 \)
Time constant: 0.230967 sec
Rate constant: 4.32963 sec-1
Half-life period: 0.160094 sec
Transmission measurement of Volvox by using MSV-5000 series

Introduction
The MSV-5000 series microscopic spectrophotometer is capable to analyze micro sample/area by both transmission/reflection measurement in the region from UV to Near IR, which can be applied to characterization of micro sized sample/area and also impurity analysis. Recently, this type of technology is getting very popular in bioscience field such as the analysis of localized constituents in living cells. Volvox which has localized cellular density due to its internal daughter colonies, was measured to obtain absorption spectra and fixed-wavelength mapping.

System configuration
• MSV-5100 UV/Vis Microscopic Spectrophotometer
• MAXY-501 Automatic XYZ Stage

Sample
Water containing Volvox was dropped onto a microscope slide glass and dried. (Figure 1)

Measurement conditions
Spectral bandwidth (UV/Vis): 5.0 nm
Scanning speed: 1000 nm/min
Response: Quick
Data pitch: 1 nm
Cassegrain objective: 16 times
Aperture: 50 mmφ

Spectrum measurement
One of the daughter colonies inside of mother colony is measured to obtain absorption spectrum.

Results
Measured absorption spectrum is shown in Figure 2. Chlorophyll a and chlorophyll b are well known as major chlorophylls included inland plants and green alga \(^1,2\) and those absorption spectra are shown in Figure 3.\(^2\) This published data on the literature is measured under acetone solvent condition, and the peak positions of those chlorophylls are slightly different depending on the solvent used, but it’s only approx. 2-7 nm difference in wavelength. Comparing the spectra of chlorophylls with Volvox spectrum, it is assumed that chlorophyll a and b are included in Volvox.

---

**Figure 1** Observation Image of dried Volvox

**Figure 2** Absorption spectrum of Volvox

**Figure 3** Absorption spectra of chlorophylls\(^2\) (with Acetone solvent)
Transmission measurement of Volvox by using MSV-5000 series

Fixed Wavelength Mapping Measurement
Fixed wavelength mapping measurement was executed at 672 nm since the peak was observed at this 672 nm by the absorption spectrum measurement. Mapping measurement at specific fixed wavelength makes it possible to generate high speed mapping data.

Measurement conditions
Measurement Mode: Lattice measurement
Measurement wavelength: 672 nm
Response: Fast
Spectral Bandwidth: 2 nm
Cassegrain objective: 16 times
Aperture: 30 μmφ
Measurement interval: 30 μm

Results
Observation image and its color-coded diagram by mapping measurement are shown as below and it is confirmed that the area with higher cellular density in observation image is exactly in good agreement with the area of higher absorbance in color-coded diagram.

Reference Literatures
2) Biochimica et Biophysica Acta (BBA) - Bioenergetics. 2011, 1807, 968-976.
The increasing awareness of the risks of skin cancer with sun exposure requires that sunscreen products be appropriately tested and labeled. The numerous possible sunscreen formulations demands that a rigorous analysis method be available.

Introduction
Sunscreens work by either reflecting or absorbing the ultraviolet (UV) radiation before it reaches the skin. The UV-A and UV-B region (400 - 290 nm) is the spectral region that must be blocked for effectiveness of the sunscreen. Whether organic or inorganic, the active ingredient that shields the skin from the sun must be present in sufficient quantity and uniformity to ensure skin protection. The traditional method for sunscreen analysis is based on a quantitative analysis of a diluted sample. A series of standards based on different concentrations of the active ingredient are measured and a quantitative method developed based on Beer's Law, \( A = abc \). Where, \( A \) = the absorbance value of an analyte band; \( a \) = the absorptivity coefficient of the analyte band - a constant; \( b \) = the pathlength - generally considered a constant; and \( c \) = analyte concentration.

Results and Discussion
Spectra of sunscreen formulations were collected over the spectral range 400 - 290 nm with a 0.5 nm data point resolution on a JASCO V-530 UV-Vis spectrophotometer. A fixed slit width of 2 nm was used with a 'Fast' detector speed and a scan speed of 400 nm/min. Sunscreen formulations were spread onto a 20 cm² piece of the 3M Transpore™ tape, the sunscreen covered substrate placed into the sample compartment and several spectra collected from different areas. Representative spectra of two different sunscreen formulations are presented as Figure 1.

<table>
<thead>
<tr>
<th>Wavelength [nm]</th>
<th>Monochromator adjustment factor</th>
<th>Measured transmittance</th>
<th>Date [mm/dd]</th>
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<td>295</td>
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<td>400</td>
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</tr>
</tbody>
</table>

An average sunscreen spectrum is calculated from the multiple sample spectra and the average submitted to a Windows Excel macro. The Excel macro calculates the SPF rating and other relevant data for the sunscreen sample (Figure 2) which is printed as a single page report. The results can be used during formula development or for quality control.

Conclusions
The Diffey Robson in vitro SPF calculation is a simple, rapid and universal analysis method for the determination of SPF values of sunscreens using either inorganic and organic screening components. The versatility of the JASCO V-530 spectrophotometer can accomplish the SPF calculation in addition to providing a full suite of UV-Vis analysis capabilities.

In recent years, solar reflective material attracts attention as one of remarkable countermeasure technique against heat island phenomenon and as sustainable household building material. These cutting edge sustainable materials have been evaluated by an independent organization as Environmental Technology Verification Project under Ministry of the Environment and its evaluation criterion is in process to be studied and to be established as standard regulation in JIS*. There is solar reflective paint material as sustainable material which has been approved by JIS (Japanese Industrial Standard) regulation. As shown in Fig.1, solar light has significant energy in near IR region. This solar reflective paint material has specific capability to reflect NIR region light of solar with higher efficiency than the other general paint materials. This capability reduces thermal energy on surface to penetrate into buildings, helping to improve an efficiency of air conditioner and contributes to saving energy.

In this Application Note, some example of evaluation about solar reflective paint material based on JIS K5602 is explained.

**System configuration**
V-670 UV/VIS/NIR Spectrometer
ISN-723 60 mm dia. Integrating sphere (UV/VIS/NIR)
Standard white plate for integrating sphere

**Measurement/Analysis programs**
VWST-774 Solar/visible light measurement program
VWCD-790 Color diagnosis program

**Sample**
Two aluminum plates were painted by both general water paint and solar reflective material paint in two different colors such as Gray and Red and dried completely for 7 days.

**Figure 1** Standard solar intensity

**Figure 2** Conduction of solar thermal energy

**Figure 3** Integrating sphere unit

**Figure 4** Sample surface (Left : Gray / Right : Red)
1. Spectral measurement result
Diffuse reflectance of samples was measured using [Spectral measurement] program under measurement condition shown in Table 1. By comparing the results shown in Figure 5, it is known that the solar reflective material paint has remarkably higher reflectance in NIR region than general water paint material.

![Figure 5](image)

**Figure 5** Diffuse reflectance spectra of solar reflective paint material and general water paint

2. Color Analysis result
By using the spectra obtained, the color analysis of both different paint materials was implemented using Color Analysis program and the results are shown in Figure 6, indicating the very similar color positions for both Gray and Red on chromaticity diagram. Even colors of two kinds of paint material are quite similar, the characteristics are completely different. Spectral measurement in UV/Vis/NIR region is a very useful method to obtain various important information to investigate such differences.

![Figure 6](image)

**Figure 6** Chromaticity diagram

3. Calculation result of reflectance of solar light
Reflectance of solar light in three different wavelength ranges was calculated using Solar/visible light measurement program based on JIS K5602. It is clearly seen that solar reflective paint material has higher reflectance in all wavelength region and in NIR region.

![Figure 7](image)

**Figure 7** Result of reflectance of solar light

<table>
<thead>
<tr>
<th>Table 1 - Measurement condition</th>
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<tbody>
<tr>
<td><strong>Measurement range</strong></td>
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<td><strong>Data interval</strong></td>
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<tr>
<td><strong>UV/Vis bandwidth</strong></td>
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<td><strong>NIR bandwidth</strong></td>
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<table>
<thead>
<tr>
<th></th>
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<tr>
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**Table 1**
Introduction
UPF (UltraViolet Protection Factor) is used to indicate the UV shielding performance of sun protection for fabric products. The ‘UPF value’ represents the ratio of time for sunburn by UV with and without the protection of the fabric material or product. For example, in the case of skin irradiated by ultraviolet light in 10 minutes with a UPF 50 cloth, it takes 500 min (50 (UPF) x 10 min) to obtain the same amount of sunburn to the skin without using the cloth product. The test method of for UPF calculations when using a UV-Vis spectrophotometer is defined in AS/NZS 4399:1996, BS EN 13758-1:2002, the AATCC Test Method 183:2010, and GBT18830:2009.

In this application data, the evaluation of the UPF, UPF rating, UVA transmittance, and UVB transmittance of sun protection fiber products defined in AS/NZS 4399:1996 by using the UPF calculation system of a UV/Vis spectrophotometer is explained. Also, the fluorescence properties are explained by using a spectrofluorometer because fabric products sometimes emit fluorescence by UV light irradiation.

Calculation Method - UPF
UPF is calculated by equation (1).

\[
UPF = \frac{\sum_{\lambda=200}^{400} E(\lambda) \cdot S(\lambda)}{\sum_{\lambda=200}^{400} E(\lambda) \cdot S(\lambda) \cdot T(\lambda)} \times 100
\]  

(1)

E(\lambda): CIE reference erythema dose spectrum  
S(\lambda): radiation intensity distribution of sunlight  
T(\lambda): diffuse reflectance spectra (%T)

UPF rating
To calculate the UPF rating measure the transmittance spectrum at more than four different points for the same sample and round down the value, calculated by equation (2), by 5.

\[
UPF_{ave} = \frac{U_{PF1} + U_{PF2} + \ldots + U_{PFN}}{N}
\]

\[
E = \frac{t_{ka}}{\sqrt{N}} \times SD
\]

\[
SD = \sqrt{\frac{\sum_{i=1}^{N} (U_{PFi} - U_{PFave})^2}{N-1}}
\]

If the UPF rating is smaller than the minimum of each UPF, the value which is calculated by equation (3) are rounded down by 5.

\[
UPF_{rating} = \text{lowest UPF}
\]  

(3)

If UPF rating is more than 50, UPF rating is defined as 50+

UVA transmittance, UVB transmittance
UVA transmittance is calculated by equation (4) by using the average of the transmittance in the range from 315 nm to 410 nm.  
UVB transmittance is calculated by equation (5) by using the average of the transmittance in the range from 290 nm to 315 nm.

\[
U_{PF} = \sum_{\lambda=315}^{410} T(\lambda) \times 100
\]

(4)

\[
U_{PF} = \sum_{\lambda=290}^{315} T(\lambda) \times 100
\]

(5)
Evaluation of sun protection fabrics by using a UPF evaluation system

Sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>T shirt (black)</td>
<td>polyester 100%</td>
</tr>
<tr>
<td>Sports shirt (black)</td>
<td>polyester 100%</td>
</tr>
<tr>
<td>Arm cover (black)</td>
<td>rayon 55% polyester 45%</td>
</tr>
</tbody>
</table>

Measurement

3D fluorescence measurement

3D fluorescence measurements of the samples were conducted using a spectrofluorometer to identify the fluorescence property using excitation wavelengths in the range from 290 nm to 400 nm.

Transmittance spectra measurement

1. Baseline measurements were performed using a Spectralon reference tile.
2. Transmittance spectra measurements were performed at 4 different points in the same sample.

* If fluorescence was observed in the 3D fluorescence measurement, a Fluorescence Cut Filter Block and a Fluorescence Cut Filter (U-330) are required to measure samples which emit fluorescence in the wavelength range from 450 to 650 nm.

Measurement parameters

Spectrofluorometer
- Excitation bandwidth: 5 nm
- Emission bandwidth: 5 nm
- Scan speed: 5000 nm/min
- Response: 10 msec
- Data interval: 0.5 nm
- Spectra correction: ON

Spectrophotometer
- UV/Vis bandwidth: 5.0 nm
- Scan speed: 100 nm/min
- Response: 0.96 sec
- Data interval: 1 nm

Results of 3D fluorescence measurements

Figure 1 illustrates the 3D fluorescence measurement of the T shirt. No fluorescence was observed for the excitation wavelengths from 290 to 400 nm. No fluorescence was observed for the sports shirt and arm cover samples.

Results of transmittance measurements

Figure 2 shows the transmittance spectrum of each sample.
Evaluation of sun protection fabrics by using a UPF evaluation system

Figure 3 illustrates the UPF measurement software display and Table 1 provides the analysis results for the samples. As shown in Figure 3, the [UPF measurement] program can objectively compare the performance of the ultraviolet shielding as a result of the numerical calculation of the UV shielding performance of fabric products. Moreover, the program corresponds to various standards including BS EN 13758-1:2002, AATCC Test Method 183:2010, and GBT18830:2009.

![Figure 3 UPF Measurement Program](image)

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.</th>
<th>UPF</th>
<th>UPF rating</th>
<th>UVA Transmittance (%)</th>
<th>UVB Transmittance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T shirt</td>
<td>1</td>
<td>30.0</td>
<td>25</td>
<td>4.4</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>30.0</td>
<td></td>
<td>4.4</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>30.0</td>
<td></td>
<td>4.3</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>30.0</td>
<td></td>
<td>4.4</td>
<td>3.1</td>
</tr>
<tr>
<td>Sports shirt</td>
<td>1</td>
<td>114.3</td>
<td>50+</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>114.2</td>
<td></td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>114.1</td>
<td></td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>114.4</td>
<td></td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Arm cover</td>
<td>1</td>
<td>68.2</td>
<td>50+</td>
<td>2.1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>68.0</td>
<td></td>
<td>2.1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>68.1</td>
<td></td>
<td>2.1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>68.0</td>
<td></td>
<td>2.1</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Table 1 Analysis result based on AS/NZS 4399:1996
Evaluation of the privacy film using an automated absolute reflectance measurement accessory

Introduction
A privacy film which is used for smartphone displays has a characteristic structure in which clear layers and light shielding layers are interlaminated. This structure prevents a smartphone display from bystanders ‘peeking’ at the screen while the viewing angle depends on the height and pitch of the light shielding layers in the louver layers. To evaluate the viewing angle or the transmittance of the louver layer, an absolute reflectance measurement accessory is an effective tool. The accessory can be used to set samples at a specified angle by rotating the sample to the source incidence and/or detector angles. In this application, the angle dependence of the transmittance spectra of the privacy film for a smartphone is explored.

Sample
Privacy film for smartphones (Figure 2)
Specification: View angle 65° Anti-glare processing

Measurement condition
- Position: transmittance, asynchronous
- Detection angle: 0°
- Incidence angle: −60° to 60°
- Measurement interval: 2°
- Measurement mode: %T
- Wavelength range: 380 to 780 nm
- Bandwidth: 5 nm
- Scan speed: 400 nm/min
- Response: 0.96 sec

Result
Figure 5 illustrates the interval data in which spectra were acquired every 2° from -60° to 60°. Figure 6 outlines the transmittance spectra at 0°, 10°, 20°, 30°, 40°, 50°, and 60°. Figure 7 provides the angle scanning transmittance data from −60° to 60° at 550 nm. Figures 5 and 6 indicate that the film absorbs blue light at less than 400 nm and keeps the transmissivity constant at more than 400 nm, which means that the film displays the light to eyes without a large color change. As illustrated in Figure 7, the transmittance is approximately 5% near the nominal view angle of ±32.5°. This characteristic is quite suitable for user privacy. As indicated in this report, the absolute reflectance measurement system is best suited to evaluate the incident angle dependence properties of various transmittance spectra.
Thickness Analysis of natural oxide film on microscopic Si pattern

Introduction
The MSV-5000 series microscopic spectrophotometer is for transmission/reflection measurement in a wide wavelength range from ultraviolet to near-infrared. It allows the measurement of the area of as small as 10 µm diameter and the built-in high-resolution camera enables to observe the samples precisely to determine the area to be measured. This instrument is most suitable to measure the minute samples or samples having microstructure. This time, the sample on which Si patterns of 35 µm widths are lined up on Ti substrate with 14 um intervals was measured as a microstructure sample. Actually, the thickness of SiO2 formed upon Si was analyzed from the obtained reflectance spectrum, because Si is easily oxidized in the air to form thin oxide film of SiO2.

Measurement System
MSV-5200 Microscopic spectrophotometer VWML-791 [Multi-Layer Analysis] program

Sample: Si and Si oxide film on the Ti substrate

Measurement condition
UV/Vis spectral bandwidth: 5.0 nm
NIR spectral bandwidth: 20.0 nm
Scan speed: 100 nm/min
Response: Slow
Data interval: 0.5 nm
Cassegrain objective mirror: 16x
Incidence angle: 23º
IN aperture: 10 umφ
OUT aperture: 10 umφ

Measurement
1. Baseline: Al vapor-deposited mirror as a reference is used for baseline measurement.
2. Measurement area: The sample is observed by the high-resolution camera to determine the measurement area (Figure 1). The red spot in Figure 1 shows the size and position of detected light.
3. Sample measurement: The reflectance spectrum is measured.
4. Transforming into absolute reflectance: The absolute reflectance spectrum of the sample is calculated by multiplying the obtained relative reflectance by the absolute reflectance of Al vapor-deposited mirror.

Analysis
Reflectance(R) is expressed by the equation of refractive index of the film (n_i), extinction coefficient (k_i), the angle of incidence (θ_i), wavelength (λ) and the film thickness (d_i). This time, optical constants of Si and SiO2 are used from the literature value. and then the film thickness of SiO2 is estimated by using [Multi-Layer Analysis] program by fitting the calculated reflectance spectrum to the measured one to make the thickness value reasonable.

Measurement Results
Measured absolute reflectance spectrum is shown in Figure 2. MSV-5000 series adopts the confocal optical system, which enables the measurement eliminating the influence of back side reflection. In the range over 1100 nm where the light passes through Si, the spectrum would not be influenced by the back side reflection.

Analysis Results
The result of fitting the reflectance spectra using [Multi-Layer Analysis] program is shown in Figure 3. The error between measured spectrum and calculated one was within 2% (Figure 3) and the film thickness of SiO2 was calculated to be 7.6 nm.
Luminous Color Measurement by using UV/Vis Spectrophotometer

Lately, products such as LEDs, Organic EL displays, and plasma display panels (PDP) that applied luminous phenomenon are in widespread use and the developments in this field are rapidly proceeding. Starting with the blue LED developed in 1993, engineers finally succeeded in developing the white LED that is now the mainstream illuminant of the mobile phone backlight, flashlight, and so on. In display field, next-generation display with luminous body, such as the Organic EL displays having superior performances on luminous efficacy, flat-screen, and power consumption and the PDP for large-sized flat TV screen are actively developing.

For these developments, numeric evaluations of colors and color rendering of the luminous body itself and of the manufactured display are required. Here, the methods of evaluating luminous colors and color rendering by JASCO spectrophotometer are introduced for the cutting edge technologies of LED, Organic EL, and PDP. Moreover, the method of evaluating colors of the liquid crystal display (LCD) by the same system is also introduced.

System Configuration for the Measurement/Analysis

- V-650/660/670 UV/Vis/NIR spectrophotometer
- Model ELM-742 External Source Fiber Optic Interface
- Calibrated lamp unit
- VWLU-788 Luminous Color Measurement/Analysis Program
Main Functions of the [Luminous Color Measurement/Analysis]

- Color Calculation Functions
  - Tristimulus Values X, Y, Z JIS Z 8701-1999
  - Chromaticity coordinate (x, y), (u, v), (u’, v’) JIS Z 8701-1999
  - Dominant Wavelength λd (Complementary Wavelength λc), Excitation purity pe JIS Z 8701-1999
  - Correlated Color Temperature Tcp and Deviation duv JIS Z 8725-1999
  - Color Rendering Index Ra, R1 to R15 JIS Z 8726-1990
  - Classification of the Fluorescent lamps JIS Z 9112-1990, 2004
- Pass/Fail Criteria Function

Measuring Method

1. Correction Coefficient Spectrum Measurement
Spectrophotometer has different grating efficiency and detector sensitivity according to the wavelength, so a gained spectrum reflects the instrument characteristics. To remove the instrument characteristics, measure the standard light source having a known emitting pattern in order to obtain the instrument characteristics (Correction Coefficient Spectrum). Here, the emitting pattern of the standard light source is called as the “Standard light source data”, and a spectrum of the standard light source measured by the spectrophotometer is a “Standard light source spectrum”. “Correction Coefficient Spectrum” can be calculated by dividing “standard light source raw spectrum” by “Standard light source data” (Figure 3).

2. Sample Measurement
Measure sample after obtaining the Correction Coefficient Spectrum. Here, a spectrum measured by spectrophotometer is called as a “Raw spectrum of sample”, and the “Spectrum Correction” means removing the instrument characteristics from the spectrum. The “Spectrum Correction” is executed by dividing the “Raw spectrum of sample” by the “Correction Coefficient Spectrum”.

![Figure 3 Calculation of the Correction Coefficient Spectrum](image)

![Figure 4 Methods of the Spectrum Correction](image)
Example 1 - Color Rendering and Correlated Color Temperature of Illuminations

White LED, fluorescent lamp, and sunlight were measured to calculate the Color Rendering Index and the Correlated Color Temperature. The white LEDs show relatively favorable Color Rendering Index (Ra) with high Correlated Color Temperature (Tcp). The color of the white LEDs differs according to their types, some of them show deeper blue color. Sunlight shows almost Ra 100 and the fluorescent lamp shows smaller Ra.

Example 2 - Color Calculation of Liquid Crystal Display

The screens of a liquid crystal display were measured when it shows white, red, blue, green, yellow, light blue, and deep red. The spectra were calculated by the color calculation program.

Table 1 Correlated Color Temperature and Color Rendering for each illumination

<table>
<thead>
<tr>
<th>No.</th>
<th>Illumination (Spectrum Color)</th>
<th>Tcp [K]</th>
<th>duv</th>
<th>λd [nm]</th>
<th>Ps (%)</th>
<th>Reference illuminants</th>
<th>Ra</th>
<th>Classification of FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>White LED1 (Blue)</td>
<td>9055</td>
<td>-0.013</td>
<td>469.37</td>
<td>19.28</td>
<td>D9055</td>
<td>86</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>White LED2 (Light Blue)</td>
<td>8563</td>
<td>0.003</td>
<td>482.54</td>
<td>17.62</td>
<td>D8563</td>
<td>80</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>White LED3 (Yellow Green)</td>
<td>19359</td>
<td>-0.014</td>
<td>470.37</td>
<td>31.23</td>
<td>D19358</td>
<td>80</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>Fluorescent lamp (Green)</td>
<td>4632</td>
<td>0.005</td>
<td>572.78</td>
<td>19.05</td>
<td>P4632</td>
<td>82</td>
<td>N</td>
</tr>
<tr>
<td>5</td>
<td>Sunlight (Red)</td>
<td>5158</td>
<td>0.003</td>
<td>566.74</td>
<td>9.1</td>
<td>D5158</td>
<td>98</td>
<td>N</td>
</tr>
</tbody>
</table>

Figure 5
 Emitting spectra of the illuminations (above)
data plotted on the chromaticity diagram (below)

Figure 6
 Spectra for each screen and (above)
data plotted on the chromaticity diagram (below)
The XYZ values were converted into RGB values, and compared the values with that of display settings. Although the values G and B showed relatively higher number than set value, we consider the results match reasonably.

The conversion of the XYZ values into RGB values is executed according to the method of Colors & Dyeing Club in Nagoya/Osaka. (http://www005.upp.so-net.ne.jp/fumoto/index.htm).

**Table 2** Color Calculation Result for each color
(Two-degree standard observer is applied to the calculation)

<table>
<thead>
<tr>
<th>No.</th>
<th>Color</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>x</th>
<th>y</th>
<th>Tcp (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>White</td>
<td>18.95</td>
<td>20.56</td>
<td>22.01</td>
<td>0.308</td>
<td>0.3342</td>
<td>6714</td>
</tr>
<tr>
<td>2</td>
<td>Red</td>
<td>8.66</td>
<td>4.51</td>
<td>0.19</td>
<td>0.648</td>
<td>0.3376</td>
<td>------</td>
</tr>
<tr>
<td>3</td>
<td>Blue</td>
<td>3.56</td>
<td>1.95</td>
<td>19.37</td>
<td>0.1431</td>
<td>0.0784</td>
<td>------</td>
</tr>
<tr>
<td>4</td>
<td>Green</td>
<td>1.37</td>
<td>2.9</td>
<td>0.67</td>
<td>0.277</td>
<td>0.5868</td>
<td>------</td>
</tr>
<tr>
<td>5</td>
<td>Yellow</td>
<td>15.3</td>
<td>18.54</td>
<td>2.82</td>
<td>0.4173</td>
<td>0.5057</td>
<td>------</td>
</tr>
<tr>
<td>6</td>
<td>Light Blue</td>
<td>10.2</td>
<td>15.96</td>
<td>22.06</td>
<td>0.2115</td>
<td>0.331</td>
<td>------</td>
</tr>
<tr>
<td>7</td>
<td>Deep Red</td>
<td>2.74</td>
<td>1.4</td>
<td>5.1</td>
<td>0.2963</td>
<td>0.1518</td>
<td>------</td>
</tr>
</tbody>
</table>

**Table 3** RGB values comparison between display settings and converted XYZ values

<table>
<thead>
<tr>
<th>Color</th>
<th>Display' RGB settings</th>
<th>RGB converted from XYZ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>G</td>
</tr>
<tr>
<td>White</td>
<td>255</td>
<td>255</td>
</tr>
<tr>
<td>Red</td>
<td>255</td>
<td>0</td>
</tr>
<tr>
<td>Blue</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Green</td>
<td>0</td>
<td>128</td>
</tr>
<tr>
<td>Yellow</td>
<td>255</td>
<td>255</td>
</tr>
<tr>
<td>Light Blue</td>
<td>0</td>
<td>255</td>
</tr>
<tr>
<td>Deep Red</td>
<td>128</td>
<td>0</td>
</tr>
</tbody>
</table>
Introduction
The bandgap energy for semiconductor materials can be determined from the transmittance or reflectance spectrum using a JASCO spectrophotometer.

Diffuse transmittance spectra for rutile and anatase titanium dioxide powder were measured. Since for titanium dioxide, absorption occurs due to forbidden direct transitions, plotting $h\nu$ against $(h\nu\alpha)^{2/3}$ allows the bandgap energy to be determined.

Although, in principle, the bandgap should be calculated using the absorption coefficient, when only bandgap absorption occurs, the bandgap energy can be determined directly from the transmittance or reflectance spectrum. If the sample is a thin film and an interference pattern is present in the transmittance or reflectance spectrum, use an absolute reflectance measurement unit to measure the spectrum. The interference effect can be eliminated by calculating $100-\%T-\%R$.

Apparatus
- V-770 - UV/Vis/NIR Spectrophotometer
- ISN-923 - Integrating sphere unit
- PSH-002 – Powder Cell
- VWBG-773 - Band Gap Calculation program
Reflection Object Color Measurements using Color Diagnosis System

Introduction
The color diagnosis system for the V-700 series can evaluate the reflection or transmission object color determined based on the CIE (Commission Internationale de l’Éclairage) standard. Reflectance spectra for colored plastic reflective plates were measured with or without the specular component using an integrating sphere unit. If the spectrum includes the specular component, it is found that the lightness is exaggerated and the chroma is diminished. Accordingly, to evaluate the color of a mirror or a mirror-like sample surface, it is necessary to remove the specular component during measurement.

L*a*b* chromaticity diagram

<table>
<thead>
<tr>
<th>Standard</th>
<th>Standard Name</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>JIS Z 8722:2009</td>
<td>Methods of colour measurement -- Reflecting and transmitting objects.</td>
<td>Reflection object color: the φ60-mm or φ150-mm integrating sphere unit corresponds to geometric condition d.</td>
</tr>
<tr>
<td>CIE No.15:2004</td>
<td>COLORIMETRY, THIRD EDITION</td>
<td>Transmission object color: the standard cell holder or film holder corresponds to geometric condition e. The φ60-mm or φ150-mm integrating sphere unit corresponds to geometric condition f.</td>
</tr>
</tbody>
</table>

Light Source  | Standard                                    |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>JIS Z 8701: 1982</td>
</tr>
<tr>
<td>F1, F3, F4, F5, F6, F8, F9, F10</td>
<td>CIE 15: 2004</td>
</tr>
<tr>
<td>F2, F7, F11, F12</td>
<td>CIE 15: 2004</td>
</tr>
</tbody>
</table>

Colored plastic reflective plates

Reflectance spectrum for blue reflective plate
# Reflection Object Color Measurements using Color Diagnosis System

## Color System

<table>
<thead>
<tr>
<th>Color System</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Munsell</td>
<td>JIS Z 8721: 1993</td>
</tr>
</tbody>
</table>

## Color-matching Function


## Color Difference

<table>
<thead>
<tr>
<th>Color Difference</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta E_{ab} ) ( \Delta L^* ), ( \Delta a^* ), ( \Delta b^* )</td>
<td>JIS Z 8730: 2009, CIE 15: 2004, ISO 7724/3: 1984, ASTM D2244: 2011</td>
</tr>
<tr>
<td>( \Delta E_{ab} ) ( \Delta L^* ), ( \Delta C_{ab}^* ), ( \Delta H_{ab}^* )</td>
<td>JIS Z 8730: 2009, CIE 15: 2004, ISO 7724/3: 1984, ASTM D2244: 2011</td>
</tr>
<tr>
<td>( \Delta E_{00} ) ( \Delta L' ), ( \Delta C' ), ( \Delta H' )</td>
<td>JIS Z 8730: 2009, CIE 15: 2004 / ASTM D2244: 2011</td>
</tr>
<tr>
<td>( \Delta E_{94} ) ( \Delta L^* ), ( \Delta C_{ab}^* ), ( \Delta H_{ab}^* )</td>
<td>JIS Z 8730: 2009, ISO 105-J03: 2009, ASTM D2244: 2011</td>
</tr>
<tr>
<td>( \Delta E_{CMC(l:C)} ) ( \Delta L^* ), ( \Delta C_{ab}^* ), ( \Delta H_{ab}^* )</td>
<td>JIS Z 8730: 2009, ISO 105-J03: 2009, ASTM D2244: 2011</td>
</tr>
<tr>
<td>( \Delta E_{uv} ) ( \Delta L^* ), ( \Delta u^* ), ( \Delta v^* ), ( \Delta C_{uv}^* ), ( \Delta H_{uv}^* )</td>
<td>JIS Z 8730: 2009, CIE 15: 2004 / ASTM D2244: 2011</td>
</tr>
</tbody>
</table>

## Calculation Items

<table>
<thead>
<tr>
<th>Calculation Items</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant wavelength: ( \lambda_0 ), excitation purity: ( p_0 )</td>
<td>JIS Z 8701: 1999, CIE 15: 2004</td>
</tr>
<tr>
<td>Lightness index: ( L ), chromaticity coordinates: ( a ), ( b )</td>
<td>JIS Z 8730: 2002, ASTM D2244: 2011</td>
</tr>
<tr>
<td>Hue: ( H ), lightness: ( V ), chroma: ( C )</td>
<td>JIS Z 8721: 1993</td>
</tr>
</tbody>
</table>
Introduction
The Gardner color measurement system can evaluate the Gardner color as a measure of the degree of coloration of samples such as boiled oil, varnish, petroleum, liquids used in the production of chemicals, and substances that become molten when heated.

This system is compliant with ISO 4630-2:2004 Clear liquids - Estimation of colour by the Gardner colour scale - Part 2: Spectrophotometric method.

The Gardner scale is defined based on the chromaticity of glass or liquid standards numbered from 1 for the lightest (light yellow) to 18 for the darkest (brownish red). By identifying the standard solution that is closest in color to the sample solution, the standard color number for the sample can be evaluated. In this program, the chromaticity coordinates x and y, and the tristimulus value Y for the standard samples can be registered by entering them directly or by calculating them from the spectrum for the standard sample. The sample spectrum is then measured and its Gardner color is calculated either from the color difference between the sample and the standard, or by comparing their x and y values.
Introduction
The Hazen color measurement system can evaluate the Hazen color of samples such as drying oil, varnish, petroleum, liquids used in the production of chemicals, and substances that become molten when heated.

1. The color difference between standard and sample solutions
2. A calibration curve for the relation between the yellowness index YI and Hazen units for standard solutions
3. A calibration curve for the relation between the chromaticity coordinates b* and Hazen units
4. A calibration curve for the relation between the absorbance at a specified wavelength and Hazen units for standard solutions

The Hazen scale is defined based on the chromaticity of numbered standard solutions with different concentrations and yellow hues. By identifying the standard solution that is closest in color to the sample solution, the standard color number for the sample can be evaluated. In the [Color Evaluation–Hazen Color] program, any of the following methods can be used to determine the color in Hazen units.

This system is compliant with ISO 6271-2:2004 Clear liquids – Estimation of colour by the platinum-cobalt scale–Part 2: spectrophotometric method. In this program, the visual test defined in JIS K 0071-1:1998, ISO 6271-1:2004 or ASTM D 1209-05 is applied to the UV/Vis spectrophotometer.
Introduction
This system measures the transmittance for each pixel in RGB color filters used for flat panel displays, and analyzes the color. The transmittance spectrum for the three colors in the filter was measured, and the calculation results were plotted as a chromaticity diagram using the [Color Diagnosis Analysis] program.

<table>
<thead>
<tr>
<th>Model No.</th>
<th>Product name</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>MSV-5200-16</td>
<td>UV/Vis/NIR Micro-spectrophotometer</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Wavelength range: 200 to 2700 nm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Supplied with 16x Cassegrain objective, converging mirrors, and manual stage.</td>
</tr>
<tr>
<td>Optional Program</td>
<td>MCAN-581</td>
<td>[Color Diagnosis Analysis] program</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Dedicated analysis program for Spectra Manager Ver.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Data in multiple file formats (*.jwa, *.jwb, *.jwd) can be analyzed.</td>
</tr>
<tr>
<td>Optional Accessories</td>
<td>MAXY-501-F</td>
<td>Automatic XYZ stage</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Optionally installed in factory Movable distance*: X: 76 mm Y: mm, 52 mm, Z: 25 mm. * Varies depending on the magnification of the objective or convergence mirror, or the type of sample.</td>
</tr>
</tbody>
</table>

Description
Available models are the MSV-5100 (200 to 900 nm) and MSV-5300 (200 to 1600 nm). Using the automatic XYZ stage, mapping, line and multipoint measurements can be performed, in addition to automatic focusing and multiple-image acquisition. The joystick is an optional extra.
Estimation of refractive index of monocrystalline sapphire by polarization measurement using MSV-5000 series

**Introduction**

The MSV-5000 series microscopic spectrophotometer is for transmission and reflection measurements of a sample as small as 10 µmφ in a wide wavelength range from ultraviolet to near-infrared. The MSV-5000 has a built-in the Glan-Taylor polarizer as standard and can obtain optical constant such as refractive index (n) and extinction coefficient (k) by measuring reflectance spectrum of small monocrystalline having birefringence. This time, the monocrystalline sapphire (measurement area size: 50 um in diameter), which has two types of crystal axis (c-axis or a-axis) and whose refractive index is already known, was measured and the dispersion of the refractive index was calculated.

**Measurement system**

MSV-5200 UV/Vis/NIR Microscopic spectrophotometer

VWML-791 Multi-layer analysis program

**Sample**

Monocrystalline sapphire

**Measurement**

1. Baseline: Two baselines of Al vapor deposited mirror were measured with polarizer angle at 0 and 90 degrees as a reference.

2. Determination of crystalline axis: Under the condition of the polarizer angle at 0 degree and wavelength at 550 nm, the sample was rotated to find the angle of sample where the sample showed maximum reflectance. Then c-axis was determined by this angle and as orthogonal of c-axis, a-axis was determined as.

3. Sample Measurement: After determination of c-axis, the reflection spectra were measured at polarizer angle at 0 and at 90 degree respectively.

4. Conversion into absolute reflectance: The absolute reflectance spectrum of the sample was calculated by multiplying the obtained relative reflectance by the absolute reflectance spectrum of Al vapor deposited mirror.

**Analysis**

Two kinds of calculation methods were used for obtaining the refractive index and the results were compared.

1. Method using [UVVIS K-K Conversion] Program: The refractive index (n) is expressed by specular reflectance spectrum (R) and phase change (φ) (Equation 1). Since the Kramers-Kronig(K-K) equation can be applied to specular reflectance spectrum (R) and phase change (φ) (Equation 2), phase change (φ) was calculated by K-K conversion of specular reflectance spectrum (R) and then, the refractive index (n) was calculated.

![Equation 1](image1)

![Equation 2](image2)

2. Method using [Multi-layer Analysis] Program (Application of Fresnel equation): Reflectance spectrum is expressed by the refractive index of the air and the sample (n1, n2), the incident angle (θ1) and the reflection angle (θ2) (Equation 3). By applying this equation, the wavelength dispersion of the refractive index was calculated using [Multi-layer Analysis] Program by fitting the calculated reflectance spectrum using Equation 3 to the measured spectrum.

![Equation 3](image3)
Estimation of refractive index of monocrystalline sapphire
by polarization measurement using MSV-5000 series

Parameters
Spectral bandwidth: 5.0 nm
Response: Slow
Accumulation: 3 times
Cassegrain objective: 16 times
Angle of polarizer: 0, 90 degree
Scan speed: 200 nm/min
Data interval: 0.1 nm
Incident angle: 23 degree
IN aperture: 50 umφ
OUT aperture: 50 umφ

Measurement Results
Absolute reflectance spectrum of monocrystalline sapphire is shown in Figure 1. Reflectance of ordinary light (c-axis) is approximately 0.15% higher than that of extraordinary light (a-axis).

Analysis Results
By using [UVVis K-K Conversion] and [Multi-layer Analysis] Program, the wavelength dispersion of the refractive index was obtained (Figure 2). Table 1 shows the result compared with the literature value of the reflective index of ordinary light and extraordinary light. The refractive index was determined with precision of two decimal places in a small area of several tens of microns, by either calculating method.

Table 1 Comparison with literature value of the refractive index of monocrystalline sapphire

above: by using [UVVis K-K Conversion] Program
below: by using [Multi-layer Analysis] Program
Introduction
Small type lens is widely used in various products as smart phone, tablet, PC etc. This note show the transmittance and reflectance measurement for 1-mm-diameter lenses used in mobile-phone cameras.

For samples that refract light such as lenses, the transmittance can be measured using an integrating sphere. The reflectance can also be measured if the aperture size is sufficiently reduced so that the area being analyzed can be considered to be flat.

<table>
<thead>
<tr>
<th>Model No.</th>
<th>Product name</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Instrument</strong></td>
<td>MSV-5200-16</td>
<td>UV/Vis/NIR Micro-spectrophotometer</td>
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<tr>
<td></td>
<td></td>
<td>• Wavelength range: 200 to 2700 nm</td>
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<tr>
<td></td>
<td></td>
<td>• Supplied with 16x Cassegrain objective, converging mirrors, and manual stage.</td>
</tr>
<tr>
<td><strong>Optional Accessories</strong></td>
<td>MISP-552</td>
<td>Integrating sphere for MSV-5200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Wavelength range: 250 to 2000 nm</td>
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<tr>
<td></td>
<td></td>
<td>• Only for the dedicated manual stage for the integrating sphere. Not applicable for diffuse transmittance or reflectance measurements.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Normal (non-diffuse) reflectance measurements can be performed using the reflectance optical path.</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td></td>
<td>Available models are the MSV-5100 (200 to 900 nm) and MSV-5300 (200 to 1600 nm). The type of integrating sphere varies depending on the instrument model, and determines the wavelength range that can be used.</td>
</tr>
</tbody>
</table>
Evaluation of antireflection film
by using absolute reflectance measurement accessory

Introduction
Antireflection film is widely used in many different products of various fields. For example, it is used for window and lens in visible region and also for near IR region laser diode in optical communication purpose and optical materials. The performance of antireflection film is getting higher recently, which is less than 0.1% reflectance level. And it is the one of reason for above expanded markets and fields. This application note proved that JASCO Automated absolute reflectance measurement accessory can be applied to the reflectance measurement with less than 0.1%. For this proof statement, several measurement data are shown in below about film sample which has been designed less than 0.1% reflectance both in visible and near infrared regions.

Sample
1) Measurement for linearity
   • Visible region: KMnO4 solution (66.7, 133.3, 200.0, 266.7 mg/L)
   • Infrared region: ND filter A, ND filter B
2) Reflectance measurement of antireflection film*1
   • AR coat VIS (430 - 600 nm)
   • AR coat NIR-1 (980 - 1140 nm)
   • AR coat NIR-2 (1320 - 1600 nm)
*1 The base of antireflection film is quartz.

Measurement
Measurement for linearity
1.1 Visible region
   (1) Dark measurement is carried out with setting the masking shield to the cell holder of Automated absolute reflectance measurement accessory.
   (2) Baseline measurement is carried out with setting water set into the rectangular cell holder.
   (3) Sample measurement is carried out with setting sample into the rectangular cell holder.

1.2 Near infrared region
   (1) Dark measurement is carried out with setting the masking shield to the sample holder of Automated absolute reflectance measurement accessory.
   (2) Baseline measurement is carried out with air.
   (3) Sample measurement is carried out with ND filter A set into the sample holder.
   (4) Sample measurement is carried out with ND filter B, not parallel to ND filter A.
   (5) Sample measurement is carried out with setting off ND filter A.

2.0 Reflectance measurement of antireflection film
   (1) Dark measurement is carried out with setting the masking shield to the sample holder of Automated absolute reflectance measurement accessory.
   (2) Baseline measurement is carried out with air.
   (3) Sample measurement is carried out with setting sample to sample holder.

Result
1) Measurement for linearity
1.1 Visible region
Figure 1 shows the absorption spectra of KMnO4 solution, and Figure 2 shows the calibration curve at 526 nm where is peak wavelength. As shown in Figure 2, in absorbance range from 1 to 4, R2 shows the high correlation (more than 0.999). This indicates that Automated absolute reflectance measurement accessory can provide absorbance up to 4, which means that transmittance and reflectance are obtained up to 0.01%.

Figure 1 Absorbance spectrum KMnO4 solution
Evaluation of antireflection film
by using absolute reflectance measurement accessory

1.2 Near infrared region
Figure 3 shows the absorption spectrum of ND filter. The spectrum of ND filter A and B agrees in that of sum of filters. This indicates that Automated absolute reflectance measurement accessory can provide absorbance up to 4, which means that transmittance and reflectance are obtained up to 0.01 %.

2) Reflectance measurement of antireflection film
Figure 4 shows the reflectance spectrum of antireflection film in visible region and Figure 5, 6 show in near infrared region. Table 1 shows the reflectance of the bottom peak. As shown in Figure 4, 5, and 6, V-770/780 allow measurement with less than 0.1 % reflectance in the wavelength range from visible to near infrared. V-770 and V-780 provides almost similar spectrum in terms of S/N ratio in the wavelength range from 980 nm to 1140 nm. But, in wavelength range from 1320 nm to 1600 nm, V-780 provides the more high-quality spectrum with high S/N ratio due to the difference of installed detector. V-770 equips the PbS detector which has high sensitivity in the wide wavelength range. On the other hand V-780 equips the InGaAs detector which has more high sensitivity. Thus, this means V-770 is suitable for measurement with wide wavelength range and V-780 is suitable for high sensitivity measurement.
Evaluation of antireflection film by using absolute reflectance measurement accessory

Measurement parameters

1 Measurement for linearity

1.1 Visible
- UV/Vis bandwidth L5.0 nm
- UV/Vis response 3.84 sec
- Scan speed 100 nm/min
- Data interval 1 nm

1.2 Near-infrared
- NIR bandwidth 40.0 nm
- NIR response 3.84 sec
- Scan speed 100 nm/min
- Data interval 1 nm

2 Reflectance measurement of antireflection film

AR coat VIS
- UV/Vis bandwidth L5.0 nm
- UV/Vis response 0.96 sec
- Scan speed 200 nm/min
- Data interval 0.5 nm
- Incident angle 5°
- Polarization N-polarized light

AR coat NIR-1
- UV/Vis bandwidth L5.0 nm
- UV/Vis response 3.84 sec
- Scan speed 100 nm/min
- Data interval 1 nm
- Incident angle 5°
- Polarization N-polarized light

AR coat NIR-2
- UV/Vis bandwidth L5.0 nm
- UV/Vis response 3.84 sec
- Scan speed 100 nm/min
- Data interval 1 nm
- Incident angle 5°
- Polarization N-polarized light

Table 1
Reflectance of bottom peak in reflectance spectrum

<table>
<thead>
<tr>
<th>Wavelength [nm]</th>
<th>Reflectance [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>V-770 450.5</td>
<td>0.03681</td>
</tr>
<tr>
<td>V-770 558.0</td>
<td>0.03144</td>
</tr>
<tr>
<td>V-780 1049</td>
<td>0.03655</td>
</tr>
<tr>
<td>V-780 1406</td>
<td>0.03703</td>
</tr>
<tr>
<td>V-770 1406</td>
<td>0.04235</td>
</tr>
</tbody>
</table>
Introduction of Haze value measurement system for UV/Vis spectrophotometer

**Introduction**

Haze value is an indicator to show fogged degree of samples and recently is often used to evaluate the transparence and diffuseness of touch panel and solar cell materials.

In this application note, the measurement method of Haze value and total light transmittance, and measurement of Haze value in diffuser panels are reported. Measurement methods on JIS, ISO, and ASTM applied to the UV-visible spectrometer in this application.

**Measurement Method**

With using integrating sphere, total transmittance (Tt), sample diffusion rate (T4) and scattering rate (T3) which is required to calibrate the diffusion by instruments are measured in the wavelength range from 380 to 780 nm (figure 1). Using equation (4) provides the diffuse transmittance (Td) of sample, and Haze value can be calculated by the ratio between Tt and Td (equation (5)).

![Image of Haze value measurement](image)

**Sample**

Six quartz diffuser panels.

**Measurement Results**

The spectrum of total light transmittance (Tt) and of sample diffusion rate (T4) are shown in figure 3. The calculated Haze value is shown in table 1. As shown in the figure and the table, the difference of fog degree between 3 and 4, 5 and 6 are distinguish clearly. As result, in this system, the fogged degree of samples which is difficult to be detected by visual observation can be evaluated by numerical value. So, this system can be effective to comparison of products or control of quality.
Introduction of Haze value measurement system for UV/Vis spectrophotometer

Figure 3a
Transmittance spectrum of six plate samples - Sample 1

Figure 3b - Sample 2

Figure 3c - Sample 3

Figure 3d - Sample 4

Figure 3e - Sample 5

Figure 3f - Sample 6

<table>
<thead>
<tr>
<th>No.</th>
<th>$T_3$ [%]</th>
<th>$T_4$ [%]</th>
<th>$T_4$ [%]</th>
<th>$T_4$ [%]</th>
<th>Haze [%]</th>
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<tbody>
<tr>
<td>1</td>
<td>0.08</td>
<td>93.06</td>
<td>0.71</td>
<td>0.63</td>
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<td>2</td>
<td>0.08</td>
<td>92.13</td>
<td>4.94</td>
<td>4.86</td>
<td>5.3</td>
</tr>
<tr>
<td>3</td>
<td>0.08</td>
<td>90.88</td>
<td>18.7</td>
<td>18.62</td>
<td>20.5</td>
</tr>
<tr>
<td>4</td>
<td>0.08</td>
<td>90.7</td>
<td>23.12</td>
<td>23.04</td>
<td>25.4</td>
</tr>
<tr>
<td>5</td>
<td>0.08</td>
<td>87.75</td>
<td>69.34</td>
<td>69.27</td>
<td>78.9</td>
</tr>
<tr>
<td>6</td>
<td>0.08</td>
<td>85.46</td>
<td>70.56</td>
<td>70.49</td>
<td>82.5</td>
</tr>
</tbody>
</table>

Table 1 Calculation results of Haze value

Measurement Condition
Band width 5.0 nm
Scan speed 400 nm/min
Response 0.24 sec
Data interval 1 nm

Calculation Condition
Light source D65
Color-matching function JIS Z 8781-1
View angle 2°
Introduction
Dielectric multi-layer mirrors are laminated optics comprising of a combination of high refractive index and low refractive index materials. Using the interference effect, this mirror can provide extremely high reflectance (near 100%) in a specific wavelength range. (Figure 1)

![Diagram of the dielectric multi-layer mirror](image)

*Figure 1 Diagram of the dielectric multi-layer mirror*

\[ n_1: \text{high refractive index} \]

\[ n_2: \text{low refractive index} \]

This type of mirror is widely used in cameras, telescopes and optics for optical communication to reduce loss in light intensity. Therefore, in the quality control and R&D of dielectric multi-layer mirrors, it is very important to evaluate the reflectance with very high accuracy. JASCO provides a high performance absolute reflectance measurement system using the V-700 series UV/Vis spectrophotometer with double-beam optical system, which has high photometric stability.

The absolute reflectance system can perform measurement at arbitrary and user-selectable incident angles; this allows the measurement of dielectric multi-layer mirrors at designated incident angles. As an example of high reflectance measurement using the JASCO absolute reflectance measurement system, this application note reports the results for a dielectric multi-layer mirror.

Sample
Dielectric multi-layer mirror

Measurement
1. Dark measurement is performed with a light shielding plate.
2. Baseline measurement is performed against air.
3. Sample measurement is performed.

The procedure detailed above was repeated three times.

Measurement Condition
- Bandwidth 5.0 nm
- Scanning speed 20 nm/min
- Response 3.84 sec
- Data interval 1 nm
- Incident angle 10 degree
- Polarization p-polarized light

![Absolute reflectance measurement accessory](image)

*Figure 2 Absolute reflectance measurement accessory*
Example of the measurement of a highly reflective material, a dielectric multi-layer mirror using absolute reflectance spectroscopy

Measurement result
Figure 3 shows an overlay of the absolute reflectance spectra of the dielectric multi-layer film mirror, and Figure 4 shows a zoomed view of the same spectra. As shown in the Figure 4, the measurement repeatability around 100%R is better than 0.1%R, which demonstrates that this system has very high measurement repeatability. Table 1 shows the comparison between the expected values and the measured values. The difference between the average of the measurement value and the expected value is better than 0.15%, which shows that this system has high accuracy and good correlation for reflectance measurement close to 100%R.

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
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<tr>
<td>680</td>
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<td>720</td>
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<td>99.9094</td>
<td>99.9316</td>
<td>99.8889</td>
<td>99.9100</td>
<td>0.021</td>
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</tbody>
</table>

Table 1 Comparison of theoretical and measured values
Measurement reproducibility of Color Diagnosis System

Introduction
Color is a visually perceived property that is derived from the reflected or transmitted spectrum of light interacting with the eye (Figure 1).

It is important to evaluate and obtain measurable factors describing color for color management during the industrial processes used in the manufacture of products.

JASCO has developed various applications that use the V-700 Series of UV/Vis spectrophotometers for color analysis using both reflectance and/or transmittance spectra of samples. The V-700 spectrophotometer Figure 1 Mechanism of color recognition by human eye uses a double-beam optical system, which offers high measurement accuracy and stability. Because of the high performance of the V-700 UV/Vis spectrophotometer color coordinates (results for color calculation) can be determined with high reproducibility.

This application note illustrates the reproducibility of color calculations made using JASCO’s proprietary color diagnosis system.

Sample
Color pellets (blue, red and yellow) (Figure 2)

Measurement
Measurement was performed using a V-750 UV-Vis spectrophotometer with ISV-922 integrating sphere and and VWCD-960 Color diagnosis program. Baseline measurement was performed using a standard white reference plate, then the sample was measured. Both reference and sample measurement was made with the specular reflection component included.

To confirm the measurement reproducibility 10 spectra were collected with the sample removed and replaced in between scans to assess the effects of changing the measurement position.

Measurement Condition
• Bandwidth 5.0 nm
• Scanning speed 400 nm/min
• Response 0.96 sec
• Data interval 1 nm

Measurement result
The reflectance spectra measured for each sample are shown in Figure 3. The right side of Figure 3 shows zoomed-in spectra in the wavelength range 740 - 780 nm.
As shown in each spectrum, the difference between maxima and minima of the photometric value is less than 0.15%R, this demonstrates that measurement system has high reproducibility.
Measurement reproducibility of Color Diagnosis System

Color pellet (Blue)

Color pellet (Red)

Color pellet (Yellow)

Figure 3 Reflectance spectra of color pellets
Analysis Results

The Color Diagnosis Program (Fig.4) is used to calculate color coordinates from measured spectra. Figure 5 shows the results plotted on XYZ, L*a*b*, Lab and L*u*v* chromaticity diagrams. The calculated values are shown in tables 1, 2 and 3. For the color coordinates that represent chroma and hue (xy, a*b*, ab, u*v*), the difference between maxima and the minima are; x and y, less than 0.0012, a* and b*, less than 0.31, a and b, less than 0.18, u* and v*, less than 0.50.

For the color coordinates that represent brightness (Y, L, L*), the difference between the maxima and minima is less than 0.1.

As shown in these results the JASCO Color Diagnosis system offers high reproducibility.
# Measurement reproducibility of Color Diagnosis System

## Table 1: Color calculation results of color pellet (blue)

<table>
<thead>
<tr>
<th>Number of times</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>x</th>
<th>y</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Lh</th>
<th>a</th>
<th>b</th>
<th>u*</th>
<th>v*</th>
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<tbody>
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<td>65.84</td>
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<td>0.2094</td>
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<td>56.03</td>
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<td>65.71</td>
<td>0.2095</td>
<td>0.2110</td>
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<td>0.2096</td>
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<td>56.06</td>
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Max-Min: 0.07 | Ave.: 23.78 | SD: 0.02289 | CV [%]: 0.10

## Table 2: Color calculation results of color pellet (red)

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<th>Z</th>
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Max-Min: 0.09 | Ave.: 27.74 | SD: 0.0270 | CV [%]: 0.10

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*Table 1* Color calculation results of color pellet (blue)

*Table 2* Color calculation results of color pellet (red)
Measurement reproducibility of Color Diagnosis System

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<th>Z</th>
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<th>y</th>
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SD 0.0218 0.0021 0.0023 0.00008 0.00006 0.00089 0.00118 0.00678 0.0111 0.0112 0.00241 0.00348 0.00457
CV [%] 0.03 0.02 0.16 0.02 0.01 0.01 -0.13 0.08 0.01 -0.12 0.05 0.15 0.05

Table 3 Color calculation results of color pellet (yellow)

Analysis Conditions
- Viewing angle 2º
- Data interval 5 nm