SHIMADZU

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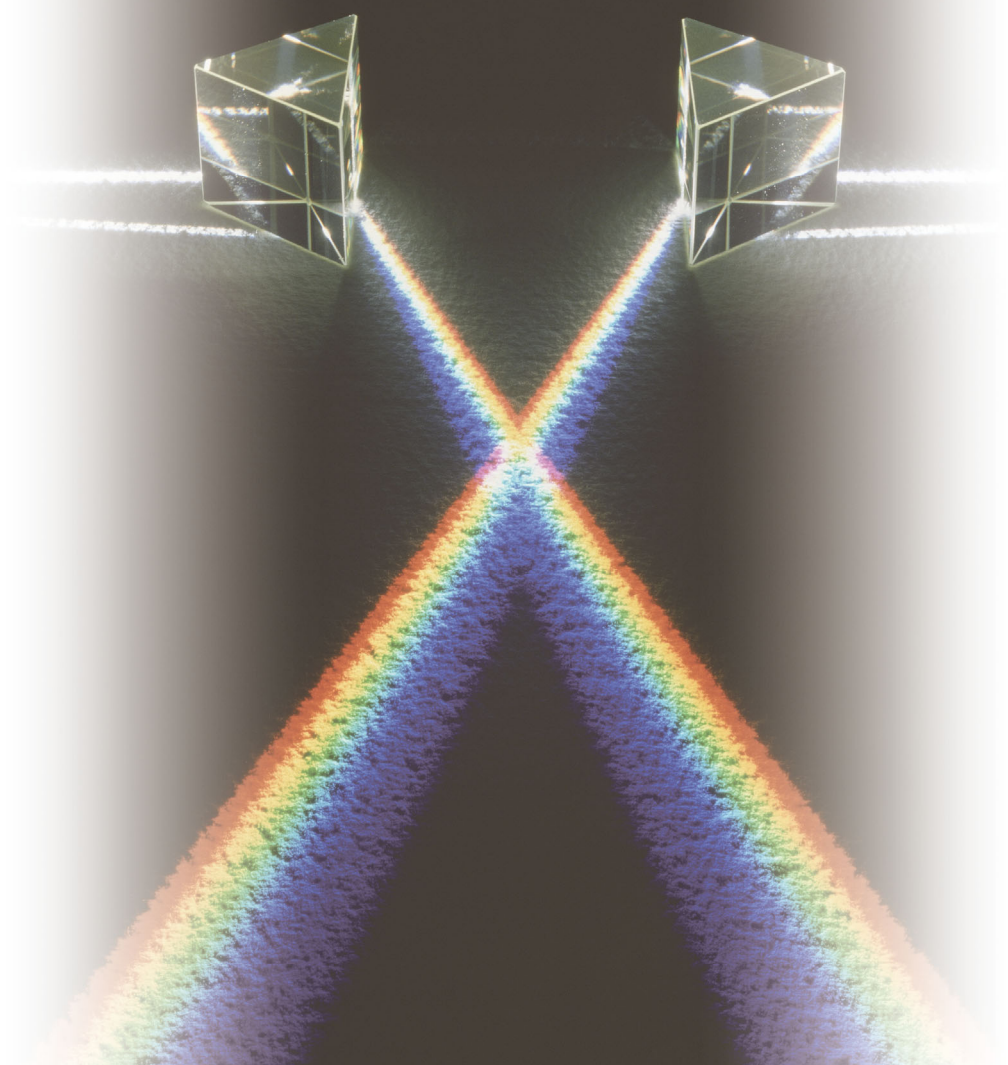
UV Talk Letter

Vol.8



UV Talk Letter

Instrument Validation and Inspection Methods

The theme of this UV TALK LETTER is the instrument validation of UV-VIS spectrophotometers. Instrument validation is essential for determining the condition of your instrument. Here, we will give details of what validation is and how to do it.

1. What is Validation?

At sites where products are developed and manufactured, a variety of equipment is used to create products through a complex series of work processes. To state that such products meet the expected quality standards, it is important to verify that the plant, equipment, and operational procedures are free of problems.

Verification procedures must be established to ensure that the details of the verification are consistent, regardless of who performs it. The series of processes from performing the verification according to a defined procedure to documenting the verification results is generally known as "validation."

Validation can be performed on a diverse range of objects, from tangible items such as plant and equipment to intangible work procedures and processes. This UV TALK LETTER describes details about the instrument validation of UV-VIS spectrophotometers.

2. Instrument Validation of a UV-VIS Spectrophotometer

A spectrophotometer shines light at various wavelengths onto the sample and investigates the degree of absorption, reflection, and transmission of the light to perform qualitative or quantitative analysis of the sample. So, what sort of performance does a spectrophotometer offer?

JIS K0115 "General rules for molecular absorptiometric analysis" prescribes the performance items that should be displayed by the instrument, as shown in Table 1.

It can be seen from Table 1 that the generic term "performance" encompasses various conditions depending on the point of focus. Instrument validation of a spectrophotometer involves selecting the items required to manage and determine the status of the instrument from among these performance items, and verifying them.

Table 1 Performance Items Listed in JIS K0115

Performance Items	
Wavelength accuracy	Stray light
Wavelength repeatability	Baseline stability
Photometric accuracy	Baseline flatness
Photometric repeatability	Noise level
Resolution	

3. Performing Instrument Validation

The actual philosophy and method of performing instrument validation are described below using several performance items as examples.

Wavelength Accuracy

The emission lines of a deuterium or low-pressure mercury lamp or the absorption peaks of an optical filter for wavelength calibration are generally used to verify the wavelength accuracy. Fig. 1 shows the energy spectrum of a deuterium lamp. A deuterium lamp is known to exhibit sharp energy peaks (emission lines) at 656.1 nm and 486.0 nm wavelengths. Consequently, the instrument's wavelength accuracy can be verified by measuring the energy spectrum of a deuterium lamp, investigating the wavelength of the peak near 656.1 nm, and then comparing its wavelength value to 656.1 nm.

For example, if the detected peak wavelength is 656.2 nm, the error from the true 656.1 nm value is 0.1 nm, and this becomes the wavelength accuracy of the instrument.

How many multiple emission lines (or absorption peaks) should be used to confirm the wavelength accuracy and what error is permitted depends on the level of instrument performance required for the development and manufacture of the product. In cases where a sample spectrum is measured and the peaks must be specified within an error of 1 nm, a wavelength accuracy of 0.1 nm is probably adequate.

Stray Light

Stray light is light outside the specified wavelength that shines onto the sample. For example, when measuring the absorbance using light at 220 nm, accurate measurements are not possible if a lot of light at wavelengths other than 220 nm hit the sample.
Let us consider the case of 0.01 % stray light outside the specified wavelength. Due to the effects of the stray light, a sample with 1 % transmittance (2 Abs) appears to have a transmittance of 1.01 % (1.9957 Abs). Alternatively, a sample with 0.01 % transmittance (4 Abs) appears to have a transmittance of 0.02 % (3.6990 Abs). That is, errors of 0.0043 Abs and 0.3010 Abs, respectively, occur. This case reveals that the effects of stray light increase as the sample absorbance increases.
The presence of stray light causes distortion in the calibration curve. Consequently, an instrument with low stray light is required for the quantitation of high-concentration unknown samples (with high absorbance) using a calibration curve created with standard samples.

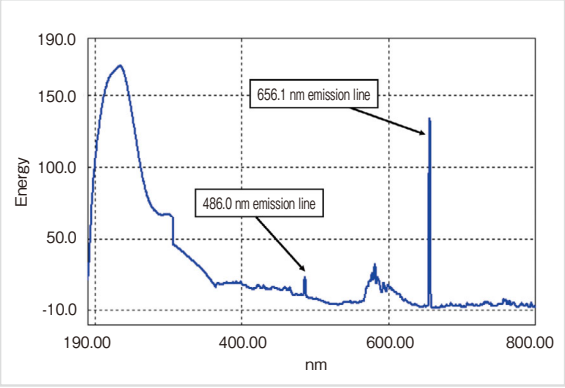


Fig. 1 Emission Lines of a Deuterium Lamp

An aqueous solution that is known to not transmit light at a specific wavelength, such as sodium iodide (NaI), is used to evaluate the amount of stray light. For example, an aqueous solution of NaI does not transmit light at 220 nm. Initially, the transmittance is measured with a shutter block that completely shuts out all light mounted in the sample compartment (transmittance X). Next, the transmittance is measured with the aqueous solution of NaI in the sample compartment (transmittance Y). The instrument's stray light is defined as (Y – X). This value is used to evaluate the level of stray light. Fig. 2 shows a schematic diagram of this situation.
The aim of instrument validation is to confirm that the instrument offers adequate performance for the inspection and manufacture of products. When performing an actual validation, it is important to select the appropriate inspection items and to set the evaluation criteria based on a sound understanding of the required instrument performance.

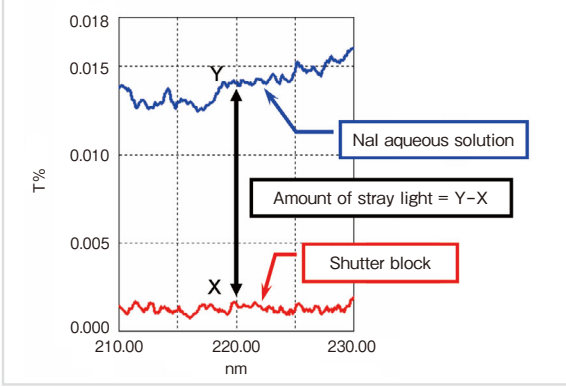


Fig. 2 Stray Light Measurements Using NaI Aqueous Solution

4. Validation Is Diagnosis of the State of the Instrument

A spectrophotometer is made up of many components, some of which are consumables that deteriorate according to the time and frequency of operation. Instrument validation is also useful for determining the state of the constituent components of the instrument.
Let's consider the noise level as an example. The noise level is one indicator of the condition of the light source (lamp) in the spectrophotometer. The noise level is defined as the maximum deviation (maximum distance between peak and trough) of the absorbance measured over one minute at a specific wavelength near 0 Abs. Fig.3 shows the graph of a measured noise level.
As the relative noise increases when the emitted light intensity of the lamp drops off over time, the noise level becomes higher. A higher noise level means that the data reproducibility is lower. This has a negative effect when accurate photometric values are required.
When it is necessary to detect extremely small absorption peaks, it may not be possible to detect the peaks correctly if the noise level is so high that they become buried in the noise.
The optical system of a spectrophotometer contains various mirrors for focusing and forming a spectrum. The surfaces of these mirrors can deteriorate over time. It is also possible for dust and dirt from the atmosphere to adhere to the mirror surfaces due to the environment where the instrument is installed. For example, the deterioration of a mirror that forms the spectrum can be one cause of the increase in stray light described above.
Therefore, instrument validation can provide valuable information to diagnose the condition of an instrument. It is recommended to perform periodic instrument validation to continuously understand and manage the state of the instrument. It is also important to validate an instrument after consumables are replaced or the instrument is moved to a different place.

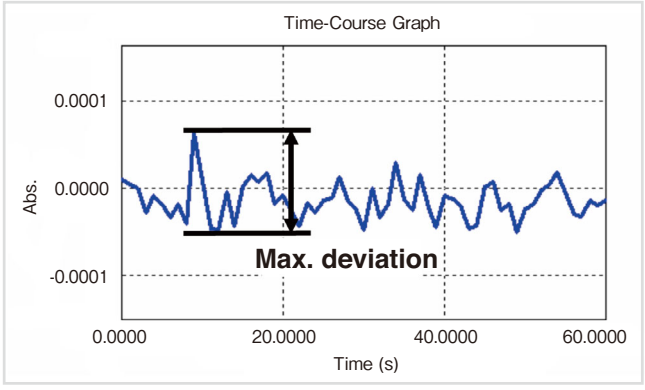


Fig. 3 Noise Level Measurement Results

5. Automated Instrument Validation Using Software

A diverse range of performance items must be checked during instrument validation. Doing so manually takes a lot of time. It is easy for errors to occur during such a complex inspection procedure.

A program designed to automate the measurements and calculations necessary for validation operations can significantly reduce the effort needed for instrument management.

Shimadzu supplies dedicated UV Performance Validation Software for the instrument validation of UV-VIS spectrophotometers. Fig. 4 shows a screen shot from the UV Performance Validation Software.

This software simplifies setup of the inspection items, inspection conditions, and the evaluation criteria and automates the process through measurement, calculation, and evaluation.

It offers a variety of functions to support instrument validation work, including management of inspection tools such as optical calibration filters, and printout of validation results reports.

Fig. 5 shows an example of the instrument validation workflow using the software.

It can be seen that the software permits accurate and efficient instrument validation.

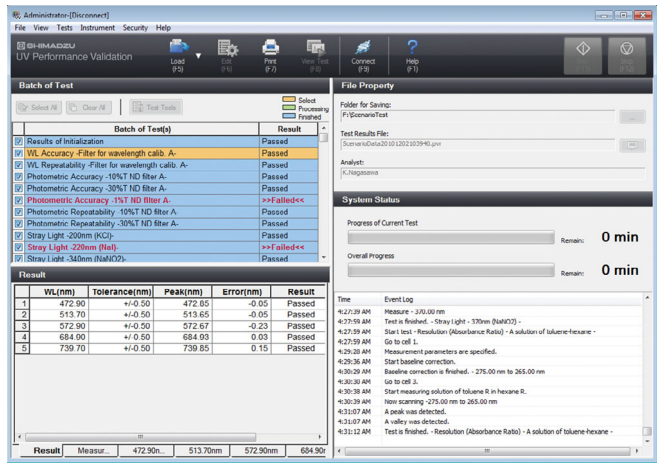


Fig. 4 UV Performance Validation Software Main Window

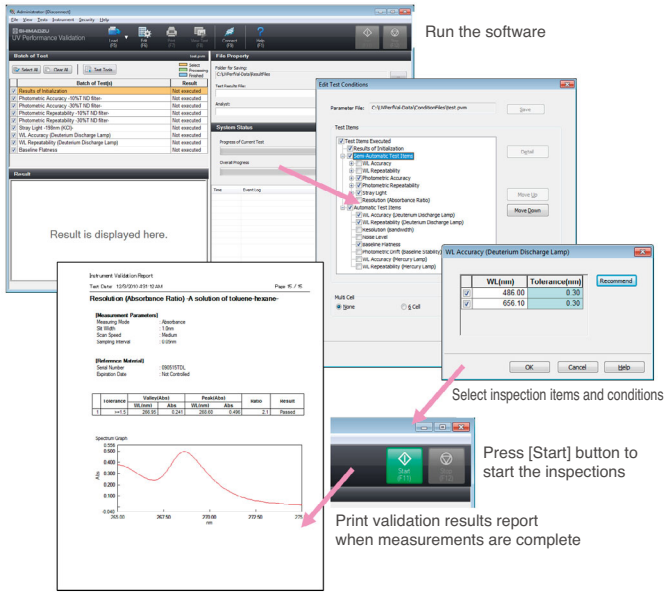


Fig. 5 Instrument Validation Workflow Using Software

6. Conclusions

This UV TALK LETTER introduced the following points:

- Instrument validation is important to determine the status of instruments used for the development and manufacture of products.
- To perform an actual instrument evaluation, select the required performance items and set the appropriate evaluation criteria for the inspection.
- The effective application of a validation program permits accurate and efficient instrument validation.

This UV TALK LETTER described instrument validation. We hope it gives you an understanding of how to manage and monitor the condition of your instrument.

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Introduction of Sippers That Are Effective for Multisample Continuous Measurements

A sipper is an accessory that can be used to measure solutions using a UV-VIS spectrophotometer. The sipper is an extremely convenient accessory that directly supplies the solution from a test tube or beaker into the sample compartment. A variety of sippers, available with or without thermostatic functions and with different types of flow cells, which are chosen according to the standard required sample volume, is available. The features of each sipper and the precautions when using them are escribed below.

1. Sipper Types and Selection

Fig. 1 shows the measurement of a sample solution introduced by a sipper from a test tube. When the lever is pressed on the sipper accessory, the sample is drawn in and measurements start automatically. Various sippers are available.

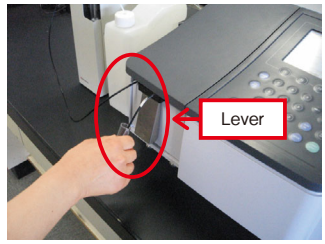

Fig. 1 Measurement Using a Sipper

Sippers can be broadly divided into two types, depending on the method of drawing in the sample solution. Sipper 160 models use a peristaltic pump, while Syringe Sippers use a syringe pump. The models are further classified according to the absence or presence of thermostatic functions and the type of flow cell. Table 1 shows a list of sipper models. The standard required sample volume is the volume of sample needed to perform measurements that are unaffected by the previously measured sample.

Table 1 List of Sipper Models

	Model name	Cell type/ thermostatic function	Standard required sample volume
Peristaltic pump types	Sipper Unit 160L	Standard	2.0 mL
	Sipper Unit 160T	Triple-pass type	1.5 mL
	Sipper Unit 160C	Constant-temperature type	2.5 mL
	Sipper Unit 160U	Supermicro type	0.5 mL
Syringe pump types (Flow cell sold separately)	Syringe Sipper N	Normal-temperature type	0.9/ 1.0/ 5.0 mL (Depending on flow cell)
	Syringe Sipper CN	Constant-temperature water circulator type	

Fig. 2 shows the appearance of Sipper Unit 160. The Sipper Unit 160 is an extremely compact unit that can be mounted inside the sample compartment. Four models are available, varying according to the flow cell used. Fig. 3 shows the shapes of the Sipper Unit 160 flow cells. The L-Type is the standard flow cell. The T-Type is a triple-pass flow cell. These flow cells have an approximately straight construction that allows a smooth sample flow during intake and discharge. The C-Type is a constant-temperature flow cell that permits measurements at constant temperature. The U-Type is the supermicro flow cell, which can measure smaller sample volumes than other flow cells.

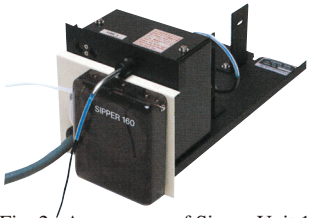

Fig. 2 Appearance of Sipper Unit 160

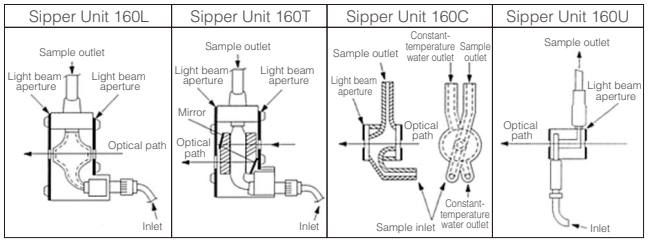

Fig. 3 Shapes of Sipper Unit 160 Flow Cells

Fig. 4 shows the appearance of a Syringe Sipper installed on a UV-1800 instrument. As a Syringe Sipper uses a syringe to draw in the sample, it achieves extremely high suction volume repeatability (repeat precision: ±0.03 mL). When choosing a Syringe Sipper, first select the N-Type (normal-temperature type) or the CN-Type (constant-temperature water circulator type). Next, select the standard required sample volume of the flow cell. Normally, a sample volume larger than the standard required sample volume is introduced for measurements. One of the features of Syringe Sipper flow cells is that they can be easily replaced. This simplifies maintenance. Table 2 shows the flow cells recommended for Syringe Sippers.

Fig. 4 Appearance of Syringe Sipper

Table 2 Flow Cells Recommended for Syringe Sipper

Shape	Optical path length	Aperture dimensions	Standard required sample volume
Square flow cell (supermicro)	10mm	2 mm dia.	0.9 mL
Square flow cell (micro)	10mm	3 mm dia.	1.0 mL
Square flow cell (semimicro)	10mm	H11 × W3.5 mm	5.0 mL

2. Chemical Resistance

The chemical resistance differs according to the type of sipper. As the Sipper Unit 160 uses a PVC tube in the peristaltic pump, the standard configuration cannot handle strong acids, strong alkalis, or some organic solvents. Therefore, the All-fluoropolymer Solenoid Valve and SWA-2 Sample Waste Unit are available as options. These options allow the analysis of diverse sample types. In contrast, the liquid-contact parts in the Syringe Sipper are made of PTFE, glass, and quartz. These offer superb chemical resistance that permits the measurement of most samples.

3. Measuring Bubble-Prone Samples

Bubbles may form when viscous samples are measured using a sipper. It is important to avoid bubble formation in the flow cell as the bubbles can affect the measured values. If bubbles do form, first reduce the suction speed in the measurement method. In addition, setting the discharge volume to the minimum value (0 mL) or extending the stabilization time can improve the situation. If the sample is highly viscous, the effects of the previously measured sample are greater if the standard required sample volume is used. It may be beneficial to increase the suction volume.

4. Automated Continuous Measurements

The sipper can be combined with an ASC-5 Auto Sample Changer to handle the automatic measurement of multiple samples. It permits the automated continuous measurement of up to 100 samples. Fig. 5 shows the appearance of the ASC-5 Auto Sample Changer. The ASC-5 comes with a test-tube rack and test tubes as standard. However, it can also handle non-standard sample containers, such as beakers. The installation footprint does not exceed 200 mm (D) × 200 mm (W) × 150 mm (H).

Fig. 5 Appearance of ASC-5 Auto Sample Changer

5. Carryover and Standard Required Sample Volume

When continuously performing a series of measurements, a portion of the previous sample may remain in the flow cell and affect the measurement of the next sample. This is called "carryover."
The suction volume that achieves carryover not exceeding 1.0 % is set as the standard required sample volume of the sipper. In practice, the required sample volume differs according to the length of the intake tube leading up to the flow cell. Therefore, when an ASC-5 Auto Sample Changer is used, the suction volume must be larger than the standard required sample volume.
We followed the method described in the instruction manual to test the carryover of a UV-1800 with Syringe Sipper N and a square flow cell (micro) with 1.0 mL standard required sample volume. Table 3 shows a list of the sipper setting conditions. The sample was an aqueous solution of potassium dichromate prepared to approximately 0.5 Abs. The absorbance at 350 nm was measured three times each for water (blank) and the sample. The sequence of measurements was: (1) Water 1, (2) Water 2, (3) Water 3, (4) Sample 1, (5) Sample 2, and (6) Sample 3.

The carryover is defined as the difference between the absorbance of (4) Sample 1 (immediately after switching from water to the sample) and the absorbance of (6) Sample 3, divided by the absorbance measured for (6) Sample 3. If no carryover occurs, the absorbance of (4) Sample 1 and (6) Sample 3 should both have the same value, within the range of measurement error. However, if (4) Sample 1 is affected by (3) Water 3 that immediately precedes it, the absorbance measured for (4) Sample 1 will be less than the absorbance measured for (6) Sample 3.
The measured results are shown in Fig. 6. It was confirmed that almost no carryover occurred.
Next, baseline correction was performed using water (blank), and the absorption spectra of (a) Sample and (b) Water were measured in sequence. The measured results are shown in Fig. 7. The spectrum for (b) Water exhibits 0.001 Abs absorption at 350 nm, which is virtually unobservable. We confirmed that accurate measurements are possible using the sipper set conditions shown in Table 3.

Sample Table - (Active)

	Sample ID	Type	Ex	WL350.0	Comments
1	Water1	Unknown		0.001	
2	Water2	Unknown		-0.000	
3	Water3	Unknown		-0.000	
4	Sample1	Unknown		0.503	
5	Sample2	Unknown		0.502	
6	Sample3	Unknown		0.503	
7					

Fig. 6 Measurement Results at 350 nm

Table 3 Syringe Sipper Set Conditions

Set Item	Setting
Suction speed	0.6
Suction volume (mL/s)	1.0
Discharge volume (mL)	1.0
Stabilization time (s)	2
No. of rinses	0

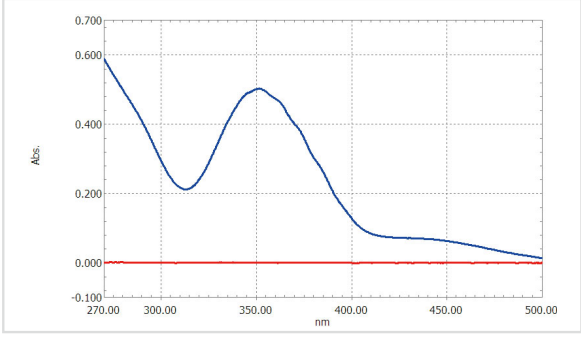

Fig. 7 Measured Spectra (— : (a) Sample — :(b) Water)

6. Maintenance

When operation is complete, immediately pump water or detergent to thoroughly rinse out the sipper. If detergent is used, subsequently rinse it out with water. Sample remaining in the flow cell can result in contamination. Bubbles occur more readily in a dirty flow cell.
For the Sipper Unit 160, periodic replacement of the peristaltic pump tube is required in addition to rinsing the flow cell. Refer to the instruction manual for details about the replacement procedure.
For a Syringe Sipper, it is adequate to thoroughly rinse the flow cell and other liquid-contact parts.

7. Summary

A sipper can continuously supply sample to save the effort required to introduce the sample solution into the cell. Care must be taken about some important differences between cells. Particular care is required with bubble formation. Syringe Sippers offer excellent suction volume repeatability, chemical resistance, and ease of maintenance, making them extremely easy-to-use accessories.

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Q How can I determine the haze value of a resin sheet or film?

A

Measurements of the haze value of a resin sheet or film are very common. Haze is an indicator of the cloudiness of a sample. It is determined as the ratio of the diffuse transmittance to the total light transmittance.
Cloudier samples have a larger haze value. The Integrating Sphere attachment is used to determine the haze value.

The measurement procedure is shown below.

(1) Perform baseline correction with the integrating sphere inlet uncovered, as shown in Fig. 1.
(2) Place the sample over the integrating sphere measuring light inlet, as shown in Fig. 2, and measure the total light transmittance.
(3) Remove the standard white plate from the face opposite the sample*1), as shown in Fig. 3, and measure the diffuse transmittance*2).
(Removing the standard white plate from the face opposite the sample allows the linear transmitted light to exit the integrating sphere. Consequently, only the diffuse transmitted light captured in the integrating sphere is used to measure the diffuse transmittance.)
(4) Calculate the haze using the expression below.
Haze (%) = (diffuse transmittance / total light transmittance) × 100

Fig. 4 shows the results of total light transmittance and diffuse transmittance measurements on ethylene vinyl acetate copolymer (EVA) film. For example, the haze value at 550 nm from the expression above is 72.5 % (calculated using the total light transmittance and diffuse transmittance at 550 nm). The haze value is relatively large, as this is a cloudy sample.
Note that this value may differ from the dedicated haze meter reading, due to differences in the measuring light shining on the sample and the aperture ratio*3) of the integrating sphere.

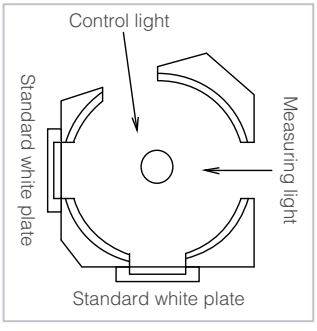

Fig. 1 Baseline Correction

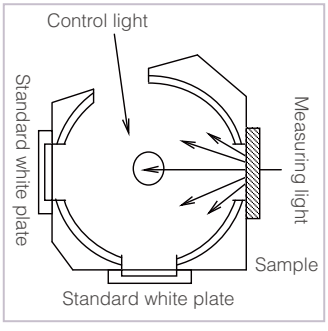

Fig. 2 Total Light Transmittance Measurement

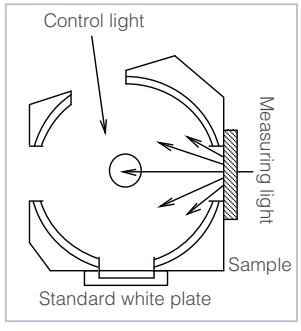

Fig. 3 Diffuse Transmittance Measurement

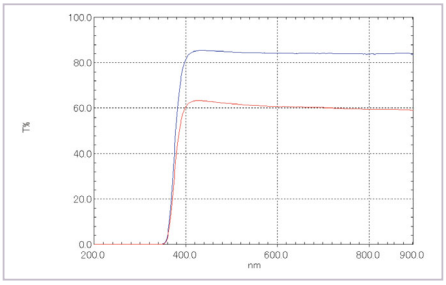

Fig. 4 Total Light Transmission Spectrum (Blue) and Diffuse Transmission Spectrum (Red) of EVA Film

*1: In some cases, an optical trap (tube that captures light) is used for this measurement.
*2: In some situations, 0 % correction is required.
*3: The aperture ratio is the total window area of the integrating sphere as a proportion of the sphere inner surface area.

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