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UV Talk Letter

Diffuse Reflectance Measurement of Powder Samples and Kubelka-Munk Transformation

The diffuse reflection method is used to measure powder samples with a UV-VIS spectrophotometer. It involves using an integrating sphere to collect and measure the diffuse reflected light. In diffuse reflectance measurements, diffuse reflectance is proportional to concentration and the absorption coefficient when converted by Kubelka-Munk transformation, in the same way that transmittance is proportional to concentration and the absorption coefficient when converted to absorbance. Therefore, the following explanation of the Kubelka-Munk transformation compares how the transformation differs from the transmittance-to-absorbance conversion.

1. Transmittance Measurement and the Lambert-Beer Law

First, transmittance measurements are explained in relation to the Lambert-Beer Law (indicated in Japanese Industrial Standards). Fig. 1 shows a diagram of measuring the transmittance of a liquid sample. Actually, some surface reflection from the cell and absorption by the solvent are involved, but these factors can be compensated for using baseline correction or the auto-zero function. Therefore, they have been omitted in this figure.

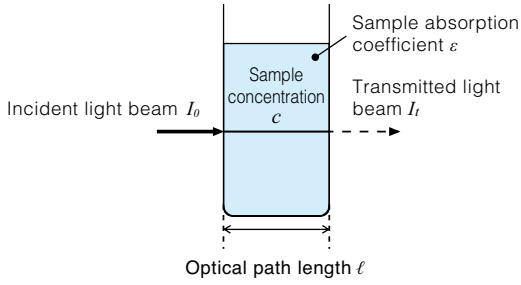

Fig. 1 Diagram of Monochromatic Light Transmitted Through a Sample Solution

When monochromatic light passes through a sample, the ratio between the intensity of the incident light beam (I_0) and the intensity of the light beam transmitted through the sample (I_t) is referred to as the transmittance (t). The percent transmittance (%T) indicates transmittance as a percentage. This relationship is shown in Equations 1.1.

Given a sample absorption coefficient ε, sample concentration c, and length (optical path length) ℓ, I_0 and I_t are related by Equation 1.2. The absorbance (A) value indicates the level of light absorption. It is expressed by Equation 1.3, which represents the negative common logarithm of transmittance.

By substituting Equation 1.2 into Equation 1.3, Equation 1.4 shows that absorbance is the multiplicative product of the absorption coefficient (ε), concentration (c), and optical path length (ℓ). This relationship is referred to as the Lambert-Beer Law. Though this example is for measuring transmittance through a solution, absorbance is similarly proportional to the absorption coefficient (ε), concentration (c), and optical path length (ℓ) when measuring the transmittance through solids as well.

$$\text{Transmittance (t)} = \frac{I_t}{I_0} \qquad \cdots\cdots \text{Eq. (1.1)}$$

$$\text{Percent transmittance (\%T)} = \frac{I_t}{I_0} \times 100$$

$$I_t = I_0 \times 10^{-\varepsilon cl} \qquad \cdots\cdots \text{Eq. (1.2)}$$

$$\text{Absorbance (A)} = -\log t = -\log \frac{I_t}{I_0} = \log \frac{I_0}{I_t} \qquad \cdots \text{Eq. (1.3)}$$

$$\text{Absorbance (A)} = \log \frac{I_0}{I_0 \times 10^{-\varepsilon cl}} = \log \frac{1}{10^{-\varepsilon cl}} = \log 10^{\varepsilon cl} = \varepsilon cl \qquad \cdots\cdots \text{Eq. (1.4)}$$

2. Measuring Diffuse Reflectance of Powder Samples

In contrast, the optical path is different when measuring the diffuse reflectance of powder samples. A diagram of light shining on a powder sample is shown in Fig. 2. Two types of light are reflected from powder samples, specular reflected light that is reflected from the sample surface and diffuse reflected light that is emitted out of the surface after penetration and repeated refraction, transmission, and scattering inside the sample.

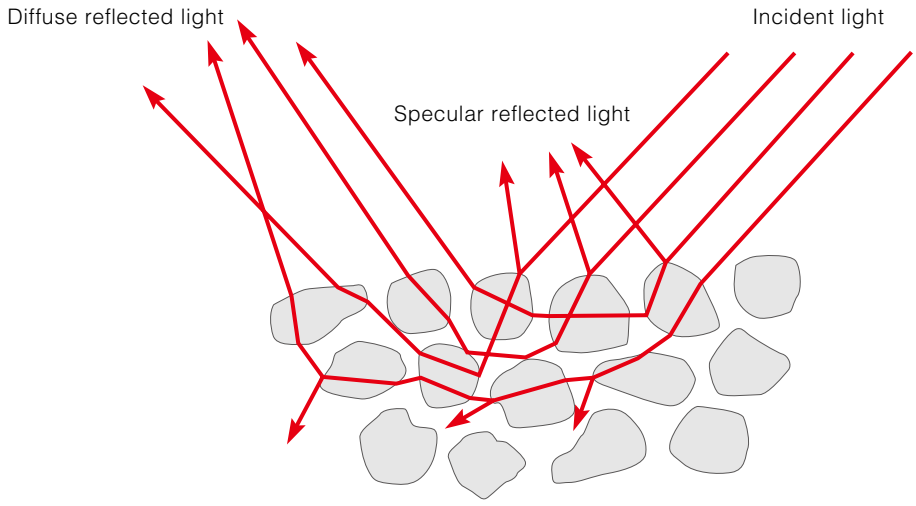

Fig. 2 Monochromatic Light Reflected from a Powder Sample (Diagram)

Powder sample measurements detect reflected light that is a mixture of specular and diffuse reflected light. However, if the particle size in the sample is sufficiently small, the effect of specular reflected light is not large. Because diffuse reflected light repeatedly passes through the sample before exiting the surface, it results in a similar absorption spectrum as transmittance measurements. However, the direction and distance the light travels is different, so the Lambert-Beer Law used for transmittance measurements cannot be used. The absorption of diffuse reflected light was analyzed by Kubelka and Munk[1] based on a model of exposing a layer of light-scattering and absorbing particles packed to a given thickness to incident light from a specific direction. For more information about that analysis, refer to the source indicated in the References section. The resulting relationship between diffuse reflectance and the absorption coefficient in this analysis is expressed by Equation 2.1.

$$f(R_\infty) = \frac{(1-R_\infty)^2}{2R_\infty} = \frac{K}{S} \qquad \cdots\cdots \text{Eq. (2.1)}$$

$f(R_\infty)$ is referred to as the Kubelka-Munk function, where R_∞ is the absolute reflectance of the sample, K is the absorption coefficient, and S is the scattering coefficient. The scattering coefficient varies depending on the size and density of the particles.
For actual measurements, the absolute reflectance is difficult to determine. Therefore, barium sulfate or another substance with an absorption coefficient K that is close to zero in the measurement range (i.e. with an R_∞ value close to 1) is used as a standard powder and then the relative reflectance described by Equation 2.2 is measured.

$$r_\infty = \frac{R_\infty\,(\text{Sample})}{R_\infty\,(\text{Standard powder})} \qquad \cdots\cdots \text{Eq. (2.2)}$$

Based on the relative reflectance (r_∞), the value is determined in Equation 2.3 below.

$$f(r_\infty) = \frac{(1-r_\infty)^2}{2r_\infty} = \frac{K}{S} \qquad \cdots\cdots \text{Eq. (2.3)}$$

The validity of this equation was confirmed experimentally by Kortum[2]. A comparison of the transmittance spectrum and diffuse reflectance spectrum for the didymium glass filter shows that, expect on the short wavelength end that is more easily affected by scattering, spectra are a close match.
Though this involves the scattering coefficient, which is not included in transmittance measurements, using the Kubelka-Munk transformation to convert diffuse reflectance values allows obtaining values that are proportional to the absorption coefficient and concentration.
Typically, an integrating sphere attachment is used to measure the relative diffuse reflectance (r_∞) values of a powder sample required for the Kubelka-Munk transformation. Fig. 3 shows an example of an integrating sphere attachment.

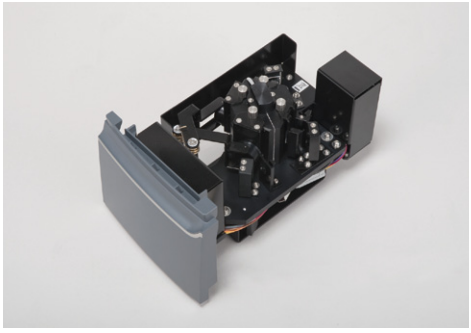

Fig. 3 Integrating Sphere Attachment (ISR-2600Plus)

Diffuse reflectance is measured by placing the measurement sample next to the reflectance measurement window on the side of the integrating sphere. If the sample can be compressed, it is compressed in a sample cell. If not, it is placed in a powder sample holder. If necessary, adjust the concentration. Fig. 4 shows the measurement procedure.

First, use the barium sulfate standard white plate to correct the baseline.
Then swap the standard white plate with the measurement sample and measure the sample. This procedure results in obtaining relative diffuse reflectance (r_∞) values.

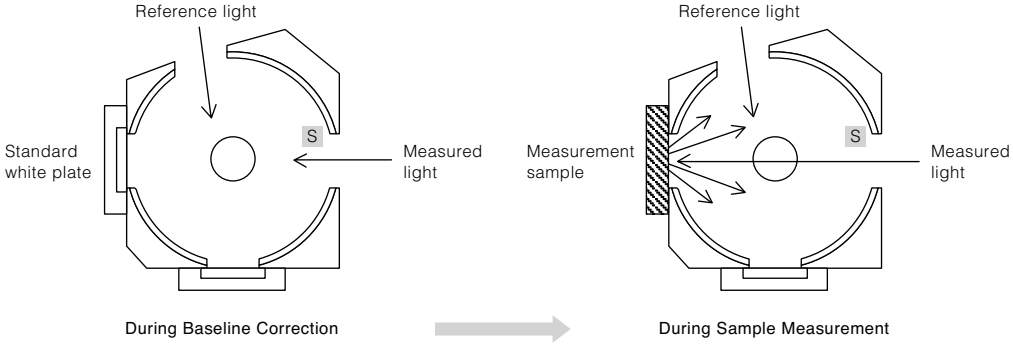

Fig. 4 Measuring Diffuse Reflectance

3. Comparison of Transmittance and Diffuse Reflectance Measurements

An integrating sphere was used to actually measure the diffuse reflectance spectrum of caffeine powder. For comparison purposes, the transmittance spectrum of an aqueous caffeine solution was also measured. Fig. 5 shows the diffuse reflectance spectrum obtained by Kubelka-Munk transformation and the transmittance spectrum converted from absorbance. To facilitate comparison, the spectra were normalized to a maximum peak value of 1. These results show that the peak positions approximately match. Though there are some minor differences between the spectra, the differences are probably due either to differences in the excitation state as a consequence of using an aqueous solution for the transmittance measurement sample or due to scattering effects in the short-wavelength UV region. As this shows, the Kubelka-Munk transformation can be used to obtain absorption spectra in the same manner as absorbance.

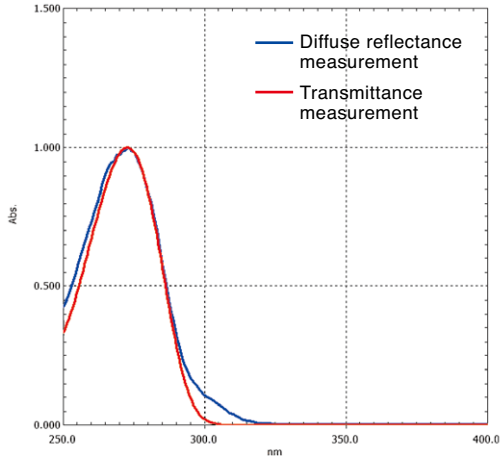

Fig. 5 Comparison of Diffuse Reflectance Spectrum (by Kubelka-Munk Transformation) and Transmittance Spectrum (by Absorbance)

4. Differences Between Transmittance and Diffuse Reflectance Measurements

Transmittance measurements indicate absorbance values that are proportional to sample concentration, based on the Lambert-Beer Law. In contrast, diffuse reflectance measurements allow obtaining values that are proportional to sample concentration, based on the Kubelka-Munk transformation. However, because light paths, scattering effects, and other factors are different between transmittance measurements and diffuse reflectance measurements, the relationship between absorbance and concentration is not the same.
Fig. 6 shows the relationship between the amount of light absorbed and the concentration for transmittance measurements and diffuse reflectance measurements.

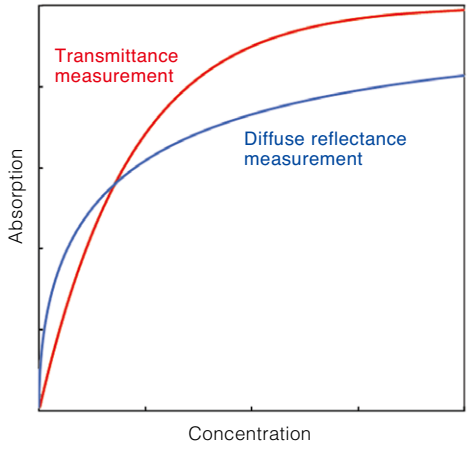

Fig. 6 Relationship Between Light Absorption and Sample Concentration

As shown in Fig. 6, if the concentration is low, diffuse reflectance measurements result in higher absorption values than transmittance measurements. This means that small absorption peaks will appear bigger in diffuse reflectance measurements than in transmittance measurements. Though both indicate the amount of light absorption inside the sample, transmittance and diffuse reflectance measurements have differences, as indicated.
In some cases, solutions can result in spectra that do not represent the powder status, due to the samples interacting with solvents or other factors. For example, in the case of phenolphthalein solution, absorption peaks can vary depending on the pH level, which can result in significantly different measurement results than when measuring a powder sample.

5. Summary

Using the Kubelka-Munk transformation for diffuse reflectance measurements allows values that are proportional to concentration and the absorption coefficient to be obtained. This can be utilized for various applications such as for the quantitation of samples that cannot be prepared as a solution. However, using the Kubelka-Munk transformation involves an additional scattering coefficient, which can cause resulting values to vary depending on the particle size and density. Therefore, to ensure accurate quantitative values, the scattering coefficient must be constant. Therefore, these factors should be taken into consideration when using the method.

References

1) P. Kubelka, F. Munk, *Z. Tech. Phys.*, 12, 593 (1931)
2) G. Kortüm, W. Braun, G. Herzog, *Angew. Chem., Int. Ed. Engl.*, 2, 333 (1963)

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Measuring Food Color

A variety of coloring is used for foods. As an important element for judging the external appearance of foods, color can even make foods more or less appetizing. Though color has sensory aspects, a spectrophotometer can be used to express it numerically. The following describes measuring the color of various sugars. It also shares expertise on measuring powder samples and explains how to determine the color value of such samples.

1. Measuring Sugar

Six sugar samples were prepared and labeled A to F as indicated in Table 1. Fig. 1 shows a photograph of the measured sugars. The photograph shows that all the sugars except sugar A have a yellow-based hue. Fig. 2 shows the powder sample holder used for measurements. The sample cell with a window is shown on the left side of the photograph. The holder used to hold the cell with a window against the integrating sphere attachment ("integrating sphere" below) is shown on the right. Fig. 3 shows a photograph of the cell with a window packed with sample B. Attempting to pack a powder such as sugar that does not clump easily into a regular integrating sphere sample cell could result in the sample spilling out, which can be prevented by using a cell fitted with a window.

Table 1 Six Kinds of Sugar Measured

Sample	Name of Sugar
A	Superfine sugar
B	Sugar
C	Sugar
D	Light brown sugar
E	Dark brown sugar
F	Dark brown sugar

Fig. 1 Sugar Samples (A to F)

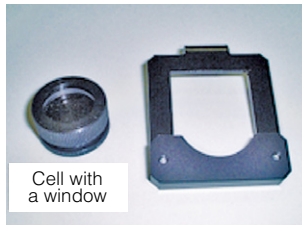

Fig. 2 Powder Sample Holder

Fig. 3 Cell with a Window Packed with Sample B

2. Measurement Results

Samples were mounted to the integrating sphere as shown in Fig. 4 and then the reflectance of all light in the visible range from 380 nm to 780 nm was measured. Then each sample was repacked in the cell and re-measured twice. A cell with a window was packed with barium sulfate powder as a reference for reflectance and used for baseline correction.
Fig. 5 shows measurement results and Table 2 shows the measurement conditions. Sample A showed almost no change in reflectance within the measurement range, which indicates it has an achromatic color.
Samples other than A showed relatively low reflectance in the blue region below 500 nm and higher reflectance in green and red regions above 500 nm, as indicated by its yellowish color, which is a mixture of green and red.

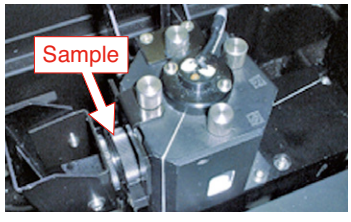

Fig. 4 Sample Mounted to the Integrating Sphere

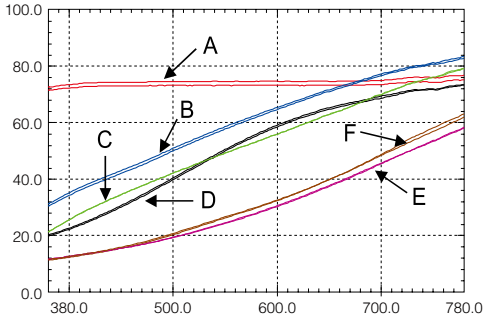

Fig. 5 Total Reflectance Spectrum of Sample (Red: A; Blue: B; Green: C; Black: D; Violet: E; and Brown: F)

Table 2 Measurement Conditions

Instruments Used	UV-3600 UV-VIS-NIR spectrophotometer and MPC-3100 large sample compartment (built into the integrating sphere)
Measurement Wavelength Range	380 to 780 nm
Scan Speed	Medium
Sampling Interval	2.0 nm
Photometric Parameter	Reflectance
Slit Width	8 nm

3. Calculating Color

Based on the measurement results in Fig. 5, L*a*b* color values were calculated using the optional color measurement software. Table 3 shows these results. The colors were calculated using D65 lighting and a 10 degree field of view. Fig. 6 shows the Table 3 values expressed in the L*a*b* chromaticity diagram. Fig. 7 is an enlargement of the area near the points plotted on the a*b* graph (with a* values plotted on the horizontal axis and b* values on the vertical axis) on the right side of Fig. 6.
In the L*a*b* chromaticity diagram, the L* values are plotted as vertical bars on the left side and a* and b* values are plotted on the graph on the right side. The L* graph indicates the sample lightness, where the lighter the color the higher the brightness, and the darker the color the lower the brightness. The higher a sample is plotted on the L* graph, the brighter its color. Based on Fig. 6 and Table 3, Sample A is relatively bright and Samples E and F relatively dark. In the a*b* color chart on the right, hue varies in the angular direction and chroma saturation varies with the distance from the center. The hue indicates the type of color and chroma the intensity of the color. In the angular direction, when facing the chart, hues are more red toward the right side of the chart, more yellow toward the top, green toward the left, and blue toward the bottom. Based on Figs. 6 and 7, the samples other than A have a reddish yellow hue. In terms of chroma, colors closer to the center are duller and colors farther from the center are more vivid.
Representing colors on a two-dimensional chromaticity diagram in this way allows the relative differences in color between samples to be understood.

Table 3 L*a*b* Values (D65 lighting and 10 degree field of view)

Data Name	L*	a*	b*
A–1	89.15	–0.06	0.22
A–2	88.48	–0.04	0.14
B–1	80.93	3.62	15.72
B–2	80.46	3.76	16.07
C–1	75.55	4.06	17.23
C–2	75.56	4.09	17.20
D–1	75.75	5.01	23.28
D–2	76.12	4.93	23.11
E–1	57.12	7.59	18.03
E–2	56.92	7.58	17.67
F–1	58.84	6.86	19.85
F–2	58.54	7.16	20.17

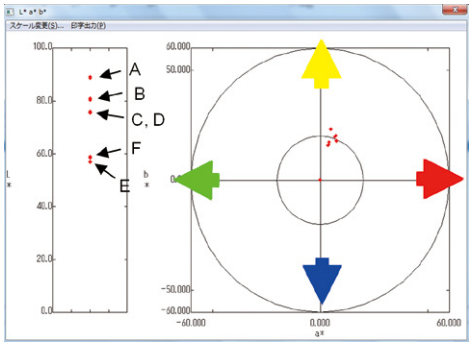

Fig. 6 L*a*b* Chromaticity Diagram

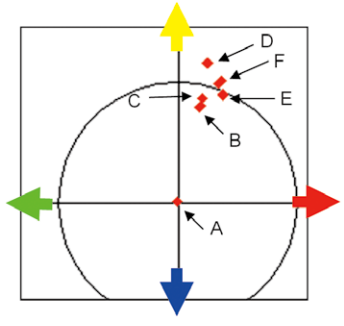

Fig. 7 Enlarged View of a*b* Chart in Fig. 6

4. Summary

In this example, color measurement software was used with an integrating sphere and powder sample holder to measure the color of sugar. Integrating sphere attachments are particularly helpful for measuring samples with especially rough surfaces, such as powders. For more detailed information about integrating spheres, refer to *UV Talk Letter* Vol. 5.
Color measurements are used not only in the food industry, but also in a wide variety of other fields, such as chemicals and agriculture. Hopefully, this article will serve as a useful reference for measuring the reflectance or color of solid samples.

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Q

What effect does the scan speed setting in analytical conditions have on data when performing analysis using a UV-VIS spectrophotometer?

A

Scan speed is one of the settings specified in analytical conditions for UV-VIS spectrophotometers.
The scan speed setting choices include fast, medium, slow, and very slow speeds, which determine the number of times measurement data are acquired (number of scans) at each measurement wavelength.
Fig. 1 shows a baseline obtained using each scan speed setting using a Shimadzu UV-2600 UV-VIS spectrophotometer. As evident from Fig. 1, the slower the scan speed, the more scans are performed at each measurement wavelength, which provides spectra with lower noise.
The medium speed setting is recommended for typical analysis. However, to obtain spectra with lower noise levels, select slow or very slow scan speed settings, in exchange for longer measurement times.

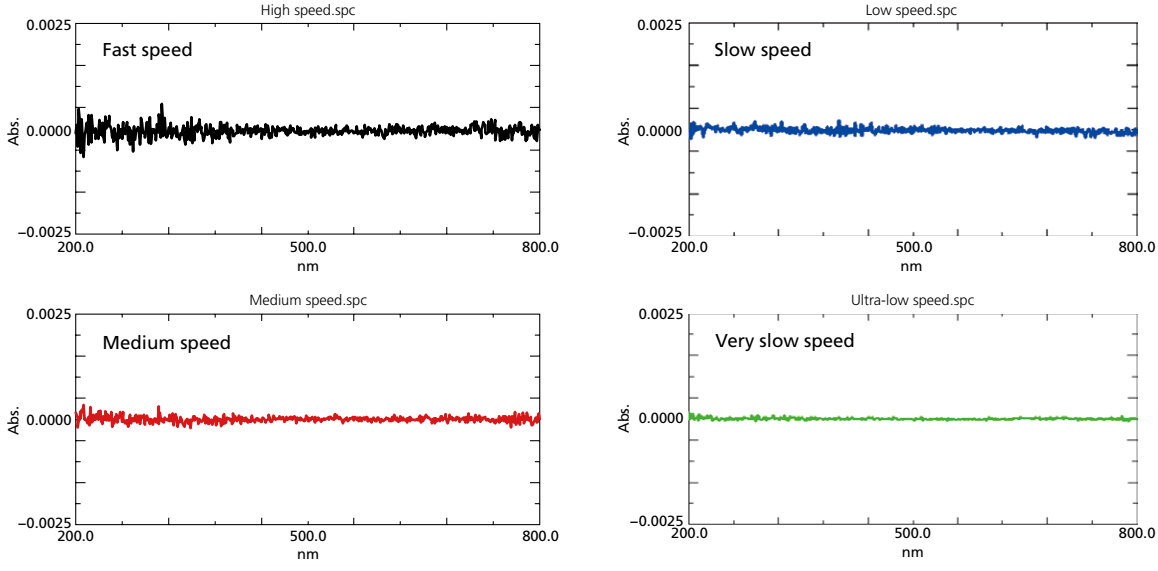

Fig. 1 Baselines Obtained at Each Scan Speed Setting Using the UV-2600

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