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Characteristics of Single and Double-Beam UV-VIS Spectrophotometry

Two types of UV-VIS spectrophotometers are available, single-beam and double-beam systems. In single-beam systems, only one beam of light passes through the sample compartment, whereas two beams pass through the compartment on double-beam systems. Why do two different types of systems exist in the world of spectrophotometers? The following explains the reasons for and describes the characteristics of each.

1. Single-Beam and Double-Beam Methods

In single-beam systems, monochromatic light from a monochromator (only a sample beam) enters the sample compartment and hits the detector directly. In a double-beam system, however, the monochromatic light from the monochromator is split into a sample beam (S) and reference beam (R) before entering the sample compartment and hitting the detector. [1] Each of these methods is illustrated in Fig. 1.

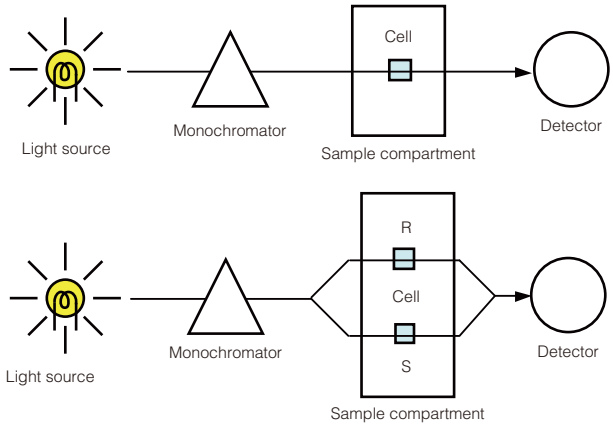

Fig. 1 Single-Beam (upper) and Double-Beam (lower) Methods

The single-beam configuration has a simpler design because it does not need a mechanism for splitting the beam into sample and reference beams. Therefore, single-beam designs tend to be used in lower priced systems.

2. Differences in How Each Type Processes Photometric Values

Both types measure samples after performing blank correction. The following compares some of the differences, such as differences in how measurement data is handled internally.

① **Single-Beam Spectrophotometers**

Single-beam spectrophotometers perform blank correction and sample measurements using only the sample beam. First the instrument records the intensity of the sample beam during blank correction (S_{Blank}). Then, during sample measurement, the instrument records the intensity of the sample beam (S_{Meas}). Measurement results are then calculated using S_{Blank} and S_{Meas}. For example, transmittance (%T) is calculated using the following formula.

$$\%T = \frac{S_{Meas}}{S_{Blank}} \times 100 \quad \cdots(\mathrm{A})$$

However, if the intensity of the light source varies between the blank correction and sample measurement, it can appear as variations in S_{Blank} and S_{Meas} values. As a specific example, consider the case of measuring a sample with 40 % transmittance, given the light source intensity during sample measurement is 90 % of the level during blank correction. In this instance, the intensity during sample measurement (S_{Meas}) is 0.9 × S_{Blank} before it enters the sample. The light transmitted through the sample is 0.4 × S_{Meas}, which results in transmittance (%T) measurements of 36 % instead of 40 %, based on equation (A) below.

$$\begin{aligned}\%T &= \frac{0.4 \times S_{Meas}}{S_{Blank}} \times 100 \\ &= \frac{0.4 \times (0.9 \times S_{Blank})}{S_{Blank}} \times 100 \\ &= 36\end{aligned}$$

In other words, if the light source intensity varies between the time the blank correction and sample measurement are performed, inaccurate data may be acquired.

Thus, in single-beam systems, fluctuations in the light source intensity can have a major effect on measurement results. Therefore, single-beam systems require waiting until the system stabilizes before starting measurements. As a general rule, they require waiting about one to two hours after switching the power ON.

② **Double-Beam Spectrophotometers**

Double-beam spectrophotometers perform blank correction and measure samples using both sample and reference beams. During blank correction, the instrument records the S_{Blank}/R_{Blank} ratio, based on the sample beam intensity (S_{Blank}) and the reference beam intensity (R_{Blank}). Next, during sample measurement, the instrument records the S_{Meas}/R_{Meas} ratio, based on the sample beam intensity (S_{Meas}) and the reference beam intensity (R_{Meas}).

Measurement results are then calculated using S_{Blank}/R_{Blank} and S_{Meas}/R_{Meas}.

For example, transmittance (%T) is calculated using the following formula.

$$\%T = \frac{\dfrac{S_{Meas}}{R_{Meas}}}{\dfrac{S_{Blank}}{R_{Blank}}} \times 100 \quad \cdots(\mathrm{B})$$

Unlike single-beam systems, double-beam systems determine measurement values using the ratio of sample beam intensity to reference beam intensity during both sample measument blank correction. Consider the same example described above, where a sample with 40 % transmittance is measured with the light source intensity during sample measurement at 90 % of the level during blank correction. In this instance, the sample and reference beam intensities during sample measurement are S_{Meas} = 0.9 × S_{Blank} and R_{Meas} = 0.9 × R_{Blank}, respectively, before they enter the sample. Since the light transmitted through the sample is 0.4 × S_{Meas}, measured transmittance (%T) is described by equation (B) below.

$$\begin{aligned}\%T &= \frac{\dfrac{0.4 \times S_{Meas}}{R_{Meas}}}{\dfrac{S_{Blank}}{R_{Blank}}} \times 100 \\ &= \frac{\dfrac{0.4 \times (0.9 \times S_{Blank})}{0.9 \times R_{Blank}}}{\dfrac{S_{Blank}}{R_{Blank}}} \times 100 \\ &= \frac{0.4 \times \dfrac{S_{Blank}}{R_{Blank}}}{\dfrac{S_{Blank}}{R_{Blank}}} \times 100 \\ &= 40\end{aligned}$$

As shown, fluctuations in the light intensity did not affect measurement results. Essentially, using the ratio of sample and reference beam intensities in double-beam systems cancels out any fluctuations in the intensity of the light source and reduces the potential of such fluctuations affecting measurement values. To summarize, data is not affected by light source fluctuations because the reference beam continuously compensates for any fluctuations in the intensity of the light source in real time. [2] In other words, **double-beam spectrophotometers measure samples assuming the intensity of the light source is always constant.** This also means double-beam systems offer superior time stability.

As an example of the difference in time stability between the configurations, the fluctuations in measurement values over time (drift) were compared using a single-beam UVmini-1240 system and a double-beam UV-1800 system. Fig. 2 shows the results from placing the UVmini-1240 and UV-1800 in the same room and using each to obtain time-course measurements for one hour at 5-second intervals.
The double-beam UV-1800 system had less time variability than the single-beam UV-1240 system.
This means the double-beam system provides more stable measurement values than the single-beam system. The single-beam system requires waiting until the light source and detector stabilize, performing frequent blank corrections, etc to minimize such variability.

A summary of single-beam and double-beam characteristics is shown in Table 1.

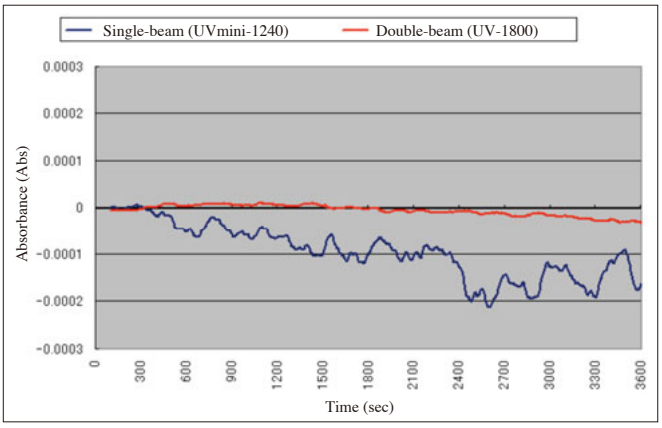

Fig. 2 Comparison of Fluctuations in Measurement Values

Table 1 Comparison of Single-Beam and Double-Beam Characteristics

	Single-Beam	Double-Beam
Price	Low	High
Construction	Simple	Complicated
Measurement	Poor time stability	Good time stability

3. Measurement Procedure Using Single- and Double-Beam Systems

The following describes the measurement procedures involved in using single-beam and double-beam systems. In this case, they were both used to measure a liquid sample.

① Measuring a Solution Using a Single-Beam Spectrophotometer

1: Place the solvent in the cell and place the cell in the cell holder.
2: Perform blank correction. [3)]
3: Remove the cell and dispose of the solvent. Then add the measurement solution and place the cell in the cell holder. (Rinse the cell with the sample solution two times, then use the third solution for measurement.)
4: Measure the solution.

② Measuring a Solution Using a Double-Beam Spectrophotometer

1. Place the solvent in two cells and place the cells in the sample beam and reference beam cell holders.
2. Perform blank correction. [3)]
3. Remove the cell from the sample beam holder and dispose of the solvent. Then add the measurement solution and place the cell in the sample beam cell holder. Leave the reference side the same as in step 1. (Rinse the cell with the sample solution two times, then use the third solution for measurement.)
4. Measure the solution.

As described above, measuring samples in a double-beam system involves placing solvent in the reference beam side as well. **Note that the solvent is placed in the reference beam side to balance the light intensity with the sample beam side, not to serve as a blank (reference).** [4)]

4. Summary

This article discussed the characteristics of single-beam and double-beam spectrophotometers. Overall, a double-beam spectrophotometer allows more stable measurements. Though there are also differences in measurement procedures, the author hopes the procedures indicated in this article will provide a useful reference for such differences.
Note, however, that to ensure more precise measurements with both single-beam and double-beam systems, it is recommended that samples are measured in a location with minimal variations in the surrounding environment, such as room temperature, and that measurements be performed at least an hour after switching the spectrophotometer power ON. (For more information on the recommended time to wait after switching the power ON, refer to the spectrophotometer's instruction manual.)

1) The light is split using devices such as half and chopper mirrors.
2) In addition to light source fluctuations, the double-beam configuration offers similar benefits with respect to other fluctuations over time, such as fluctuations in detector sensitivity.
3) For spectral measurements, this is sometimes expressed as baseline correction.
4) If measured without placing solvent on the reference side, the balance between reference and sample beam intensities cannot be kept, causing measurement data to fluctuate more easily. In general, nothing needs to be placed on the reference side if measuring a solid sample, such as measuring film using a film holder.

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Analyzing Mineral Water Using Multivariate Analysis

In recent years, multivariate analysis has been commonly used in chemical measurement fields for quantitative analysis of mixtures or for classifying multiple samples. Using multivariate analysis enables more easily quantifying the content of target components in mixture samples and classifying a larger number of samples. This article describes utilizing mineral water's characteristic absorption spectrum in the UV region to perform various kinds of analyses using multivariate analysis.

1. Overview of Multivariate Analysis

Multivariate analysis is a technique of statistically analyzing multiple sets of analytical data to provide information not available using previous data analysis methods. It is sometimes called chemometrics when used in chemical measurement fields.

Multivariate analysis comprises a wide variety of techniques, but these can be categorized as either quantitative methods or classification methods.

Quantitative methods include multiple regression (multiple linear regression (MLR) or inverse least squares (ILS)), principal component regression (PCR), and partial least squares (PLS). Using such techniques allows determining the quantity of each component (or target components) in a sample containing a mixture of components. Multiple regression requires that the analyst select specific data (descriptive variables), but that is not required using PCR and PLS techniques, which allow using all data. However, PCR and PLS are more susceptible to the effects of noise (data variability) than multiple regression.

Classification methods include principal component analysis (PCA), cluster analysis, discriminant analysis, and factor analysis. Using such techniques allows classifying multiple samples based on similarities to understand their characteristics or help identify where they were produced. Fig. 1 shows an overview of multivariate analysis.

In this article, multiple regression was selected as the quantitative method and principal component analysis (PCA) and cluster analysis were selected as the classification methods to illustrate multivariate analysis.

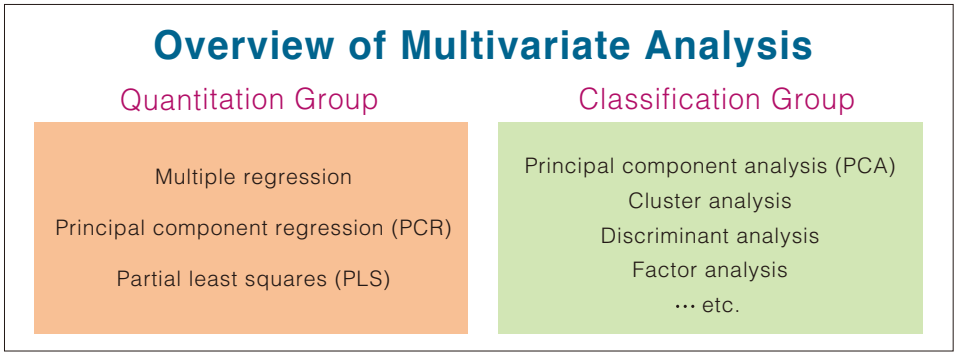

Fig. 1 Overview of Multivariate Analysis

2. Simultaneous Quantitation of Mineral Water Mixture Samples Using Multiple Regression

Multiple regression allows determining the quantity of each component (or target components) in a sample containing a mixture of components. In this example, three commercial brands of bottled mineral water – A, B, and C, were mixed in various proportions, then multiple regression was used to determine the mixture ratio of each sample.

First a calibration model was created using standard samples prepared by varying the mixture ratio. Then samples with known mixture ratios were prepared for verification of the calibration model. The UV absorption spectra of nine standard samples and three verification samples were measured using a UV-3600 UV-VIS-NIR spectrophotometer. The absorption spectra for each brand of mineral water, measured using a quartz cell with a 10 mm optical path length and ion-exchange water as a reference, are shown in Fig. 2. Mixture ratios of the standard and verification samples are indicated in Tables 1 and 2. Results from measuring these mixtures are shown in Fig. 3 (standard samples ① to ⑤), Fig. 4 (standard samples ⑥ to ⑨), and Fig. 5 (verification samples ① to ③). Calibration models (multiple regression) were created for each mineral water using the absorption at four wavelengths – 200 nm, 205 nm, 210 nm, and 215 nm. [1] The multiple regression equation for each model is shown in Table 3. The multiple correlation coefficients for the calibration models for mineral water A, B, and C are 0.999921, 0.999986, and 0.999973, respectively. The multiple correlation coefficients indicate the correlation between the actual and predicted measurement values, where predicted values are calculated using the multiple regression equations.

The mixture ratio of each sample was calculated by replacing the absorbance in the multiple regression equations with absorbance values obtained from verification samples at four wavelengths. Quantitative analysis results are indicated in Table 4. A comparison with Table 2 shows that good results were obtained.

Multiple regression methods, which are able to utilize absorbance from multiple wavelengths, are especially useful for simultaneous quantitative analysis of multiple components, as shown in this example.

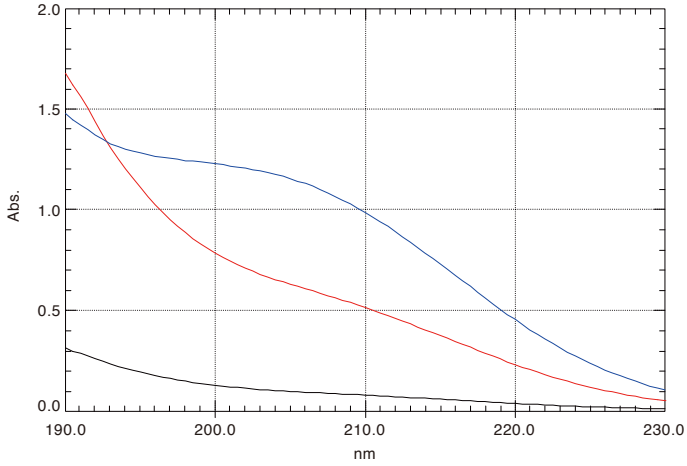

Fig. 2 Absorption Spectra of Mineral Waters A, B, and C
(Red: A, Blue: B, and Black: C)

Table 1 Standard Sample Mixture Ratios

	A (%)	B (%)	C (%)
Standard Sample ①	20	30	50
Standard Sample ②	50	20	30
Standard Sample ③	30	50	20
Standard Sample ④	0	50	50
Standard Sample ⑤	50	0	50
Standard Sample ⑥	50	50	0
Standard Sample ⑦	100	0	0
Standard Sample ⑧	0	100	0
Standard Sample ⑨	0	0	100

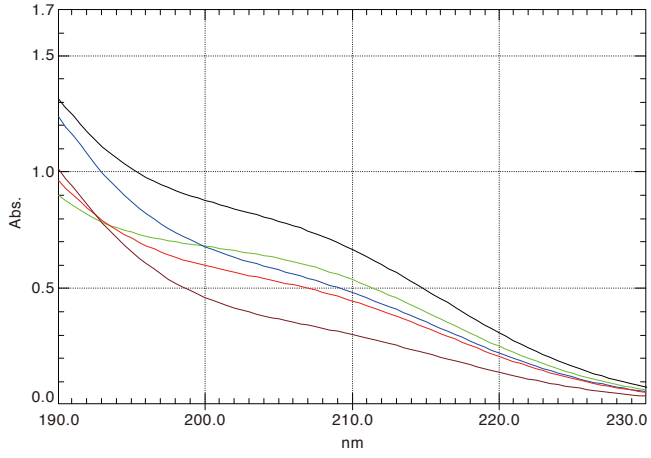

Fig. 3 Absorption Spectra of Standard Samples ① to ⑤
(Red: ①, Blue: ②, Black: ③, Green: ④, and Brown: ⑤)

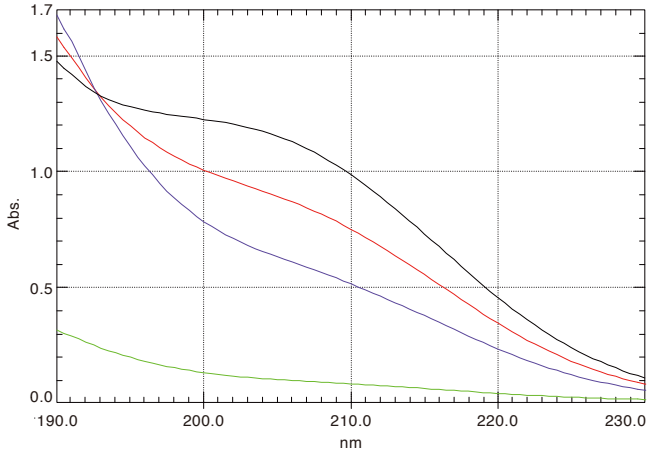

Fig. 4 Absorption Spectra of Standard Samples ⑥ to ⑨
(Red: ⑥, Blue: ⑦, Black: ⑧, and Green: ⑨)

Table 2 Verification Sample Mixture Ratios

	A (%)	B (%)	C (%)
Verification Sample ①	10	80	10
Verification Sample ②	30	30	40
Verification Sample ③	20	60	20

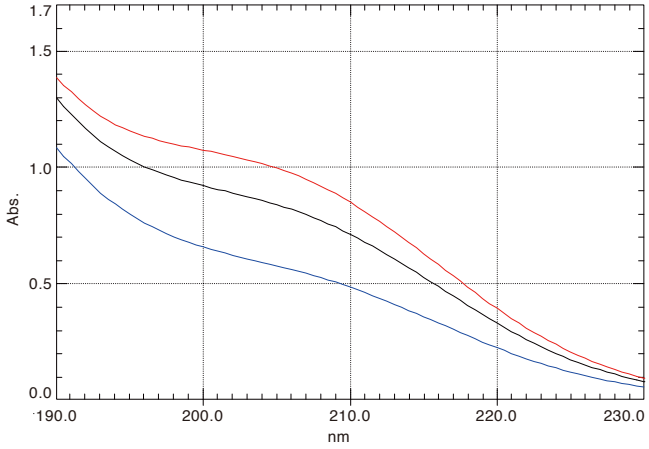

Fig. 5 Absorption Spectra of Verification Samples ① to ③ (Red: ①, Blue: ②, and Black: ③)

Table 3 Multiple Regression Equations for Each Calibration Model

Model A	$R_A = 1073.318 \cdot A_{200} - 1614.381 \cdot A_{205} + 1120.181 \cdot A_{210} - 731.675 \cdot A_{215} - 24.416$
Model B	$R_B = -541.210 \cdot A_{200} + 822.186 \cdot A_{205} - 91.882 \cdot A_{210} - 133.158 \cdot A_{215} + 3.725$
Model C	$R_C = -532.109 \cdot A_{200} + 792.195 \cdot A_{205} - 1028.299 \cdot A_{210} + 864.833 \cdot A_{215} + 120.690$

Note: A_{200}, A_{205}, A_{210}, and A_{215} indicate the absorbance at respective wavelengths and R_A, R_B, and R_C indicate the mixture ratios of mineral waters A, B, and C.

Table 4 Verification Sample Quantitative Analysis Results

	A (%)	B (%)	C (%)
Verification Sample ①	10.38	79.90	9.72
Verification Sample ②	30.12	29.96	39.92
Verification Sample ③	20.41	59.68	19.91

3. Classification of Mineral Waters Using Principal Component Analysis (PCA)

The following describes an example of using principal component analysis (PCA). A total of 20 absorption spectra (A to T) for 19 mineral water samples and one tap water sample is shown in Fig. 6 to Fig. 8.

Principal component analysis was used to analyze these 20 spectra. [(2)] All data obtained for the measurement range of 190 nm to 230 nm were used in the analysis. The score plot obtained is shown in Fig. 9. The score plot indicates the scores corresponding to principal component Groups 1 and 2, plotted on a coordinate plane, resulting in 20 points corresponding to measurement samples plotted on a graph. The horizontal axis corresponds to Group 1 principal components and the vertical axis corresponds to Group 2 principal components. In this case, "principal components" represent some sort of characteristic assigned in mathematical terms. It indicates that samples with principal component points plotted close to each other are similar. Fig. 10 shows spectra for samples in the circled showing how spectra within the same group are very similar.

Fig. 11 shows the spectra corresponding to plot points at select points along the horizontal axis (Group 1 principal components). Similarly, spectra for select plot points on the vertical axis (Group 2 principal components) are shown in Fig. 12. Presumably, the horizontal axis corresponds to total absorption (spectral area), whereas the vertical axis corresponds to spectral shape (based on whether or not there is a bulge near the center of the spectrum). (The validity of this presumption can be confirmed by viewing a graph called a loading plot, the explanation of which is omitted here.) Total absorption values probably correspond to the total ions and other items dissolved in the sample, whereas spectral shape probably corresponds to the type of ions and so on. By analyzing the principal components of multiple samples, it is possible to visually understand the similarities between samples.

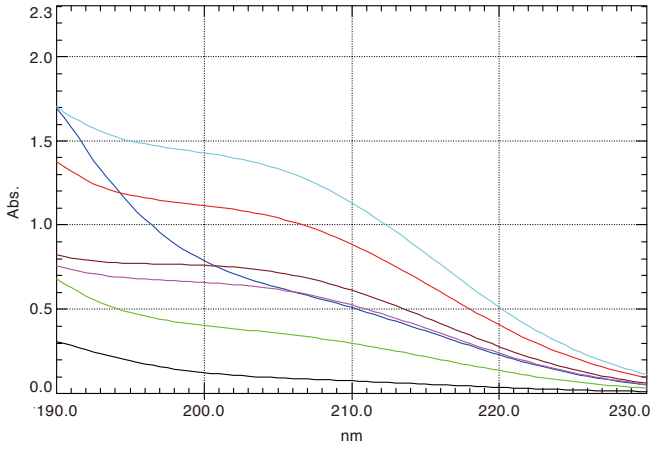

Fig. 6 Spectra of Mineral Waters A to G
(Blue: A, Red: B, Black: C, Green: D, Brown: E, Light Blue: F, and Violet: G)

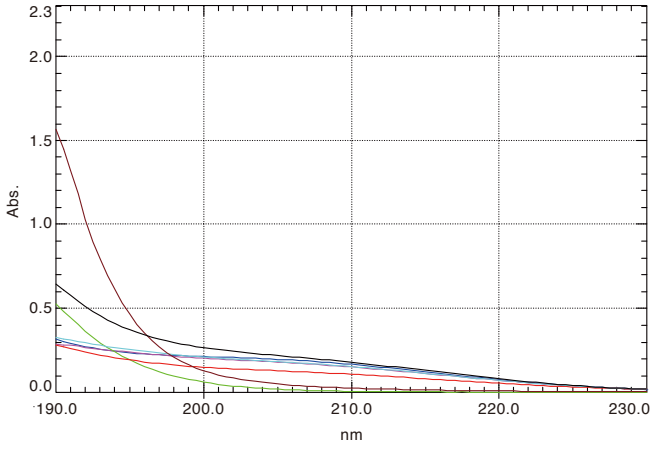

Fig. 7 Spectra of Mineral Waters H to N
(Blue: H, Red: I, Black: J, Green: K, Brown: L, Light Blue: M, and Violet: N)

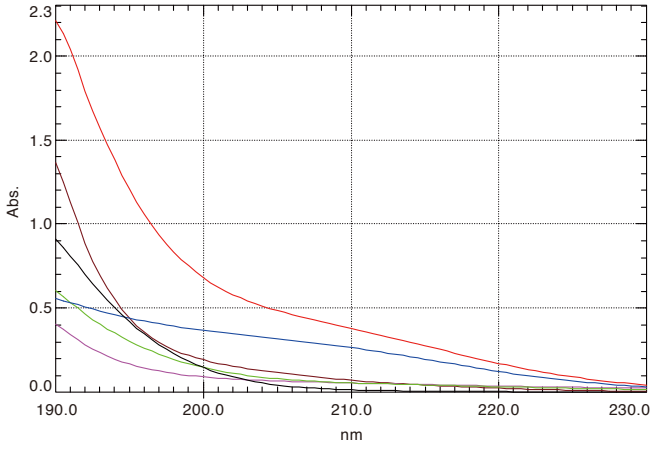

Fig. 8 Spectra of Mineral Waters O to S and Tap Water T
(Blue: O, Red: P, Black: Q, Green: R, Brown: S, and Violet: T [tap water])

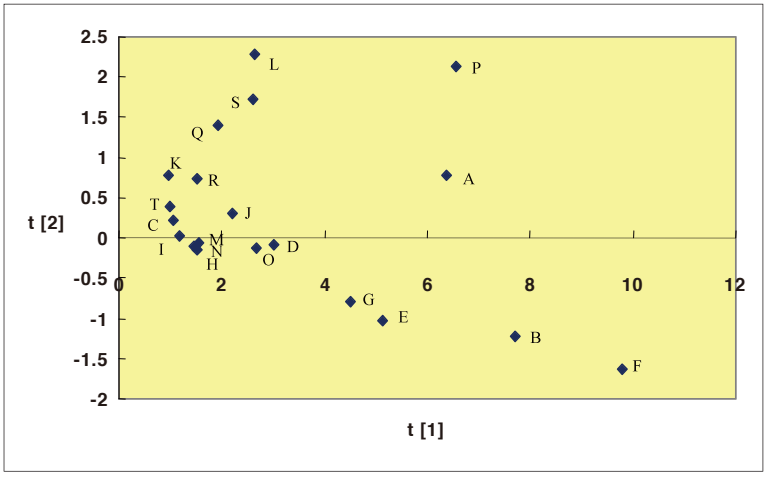

Fig. 9 Score Plot

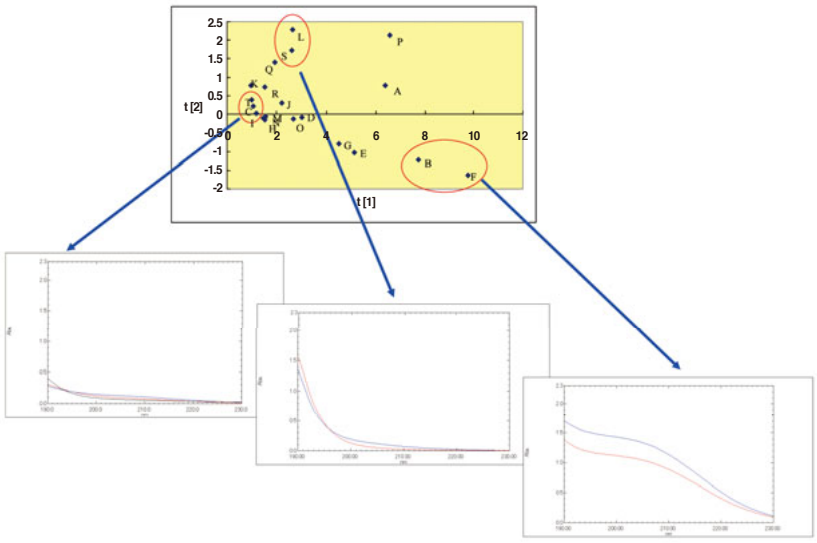

Fig. 10 Absorption Spectra of Different Groups in the Score Plot

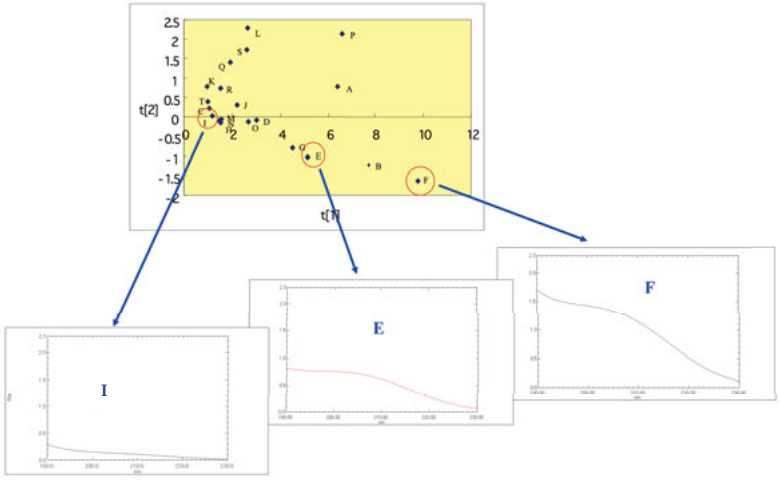

Fig. 11 Score Plot: Focus on Horizontal Axis (Group 1 Principal Components)

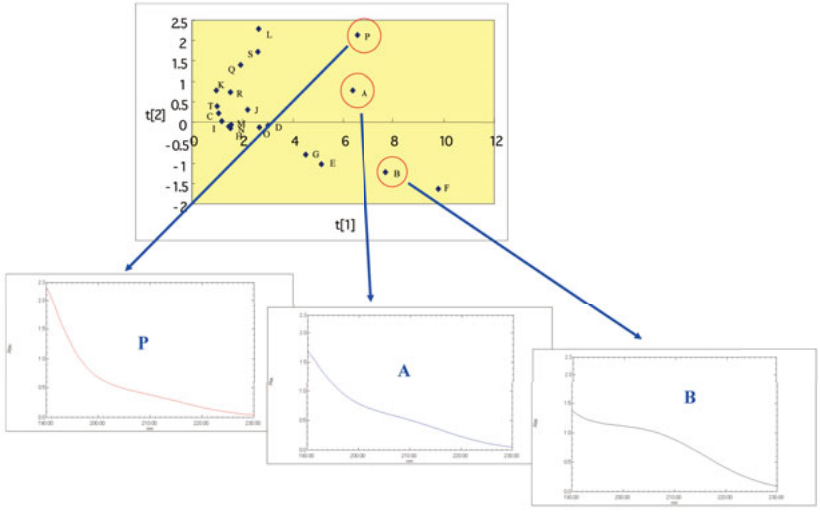

Fig. 12 Score Plot: Focus on Vertical Axis (Group 2 Principal Components)

4. Classification of Mineral Waters Using Cluster Analysis

Cluster analysis, which is a separate classification method than principal component analysis (PCA), allows displaying all samples by using a dendrogram to diagram the order of similar samples. Given the 20 kinds of mineral water described above, results from cluster analysis are shown in Fig. 13. (3)
In this example, the Ward's method was used for calculation in cluster analysis. Fig. 13 shows a cluster diagram along with the PCA. It shows how cluster analysis provided similar results to PCA. The dendrogram enables understanding the similarity between data and clusters.

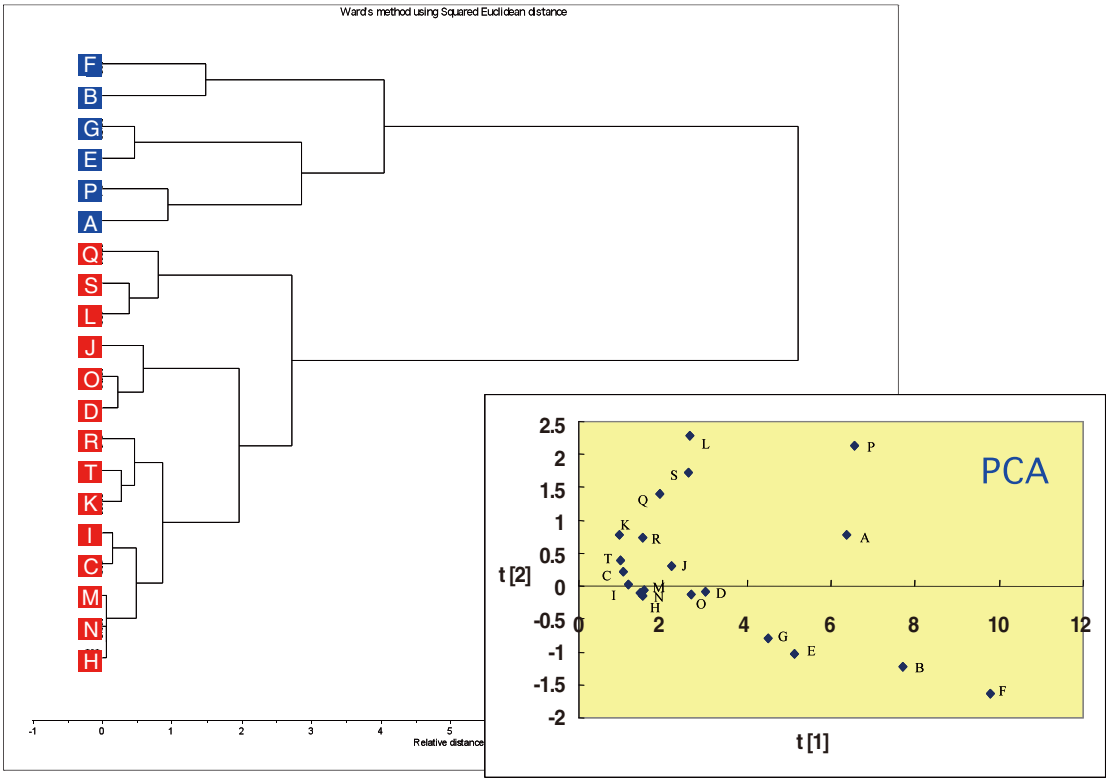

Fig. 13 Cluster Analysis Results and PCA Score Plot

5. Summary

This article described how using multiple regression enabled simultaneously quantitating mixtures of three kinds of mineral water. It also described using principal component analysis to express the characteristics of 20 kinds of mineral water in terms of a score plot and described using cluster analysis to further summarize characteristics in a dendrogram. These classification methods provided a visual understanding of the similarities between samples.
In addition to the analysis examples described above, multivariate analysis is also used for a wide variety of other situations. For example, multivariate analysis is used in simple spectrometers for scanning tangerines, apples, and other fruit, to measure their sugar and acid content. It is also used in many other applications, such as to identify the origin of vegetables, classify odors, evaluate the effectiveness of cosmetics, and measure the fat content of fish. Application fields are diverse as well, and include food products, chemicals, life sciences, pharmaceuticals, electrical, and semiconductors. With so many ways available to view analytical data, multivariate analysis will no doubt continue to be widely used in the future.

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Global Application Development Center, Analytical & Measuring Instruments Division
Mikio Sugioka

Q How should cells be cleaned?

A

This is a common question without a fixed answer. In fact, it is hard to find any specific published methods. Why are there no specified methods?

The reason is because of the diverse variety of samples that are analyzed. Due to the wide variety of analytical samples, a wide variety of corresponding cleaning methods are required. However, since describing all methods is not realistic, it does not help solve the problem. Therefore, a few of the common cleaning methods are described below.
These methods can be roughly categorized into two groups. One group uses water as the solvent and the other uses organic solvent.

【 Using Water as a Solvent 】
After cleaning with purified water, clean with ethanol and store dry. However, for more severe contamination, soak cells in a commercial cleaning solution made specifically for cleaning cells (for about 10 minutes at 30 to 50 °C). Then clean the cell with distilled water and soak them in a dilute solution of nitric acid and a small amount of hydrogen peroxide (for about 30 minutes). Finally, rinse the cell with distilled water and store cells dry.

【 Using Organic Solvents 】
After cleaning with the organic solvent being used, clean with ethanol or acetone and then clean using the same method as described for the aqueous solution above.

For stubborn contamination, the cell may be scrubbed lightly with a cotton swab. Avoid using alkaline cleaning solutions that can dissolve glass or ultrasonic cleaning devices that can damage the cell.
The above are only general recommendations. Use them as a reference for considering the best method to clean specific samples.

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