

Application News

No. A574

Spectrophotometric Analysis

Micro-Volume Measurement of Food Dye and Amino Acid Compound – Application of TrayCell and NanoStick –

A UV-Visible spectrophotometer is used for the quantitative and the qualitative analysis of a substance in various fields, in which food and environmental fields need a micro-volume measurement to reduce the amount of waste solution after the measurement. Pharmaceutical, chemical and life science fields also require it because a sufficient amount of a sample used for a measurement can not be collected in some cases. In the case of a cell with a conventional quadrangular shape, its width or height is decreased to reduce the volume keeping the optical path of 10 mm and such a cell allows sample quantities of a few tens of microliters to be measured.

In this report, the micro-volume measurements of food dyes and amino acid compounds using the TrayCell™ (Hellma Analytics) and the Nano Stick (SINCO Inc.) which are micro-volume cells and need sample quantities of only a few micro liters for a measurement and the UV-1900 UV-VIS spectrophotometer are demonstrated.

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■ TrayCell and Nano Stick

The external appearance of the UV-1900 is shown in Fig. 1. The UV-1900 is a small-footprint UV-Visible spectrophotometer (450(W) × 501(D) × 244(H) mm) and its hardware design is done based on ergonomics. The UV-1900 uses a color touch panel as a control panel and features the easy-to-navigate user interface which can grasp the current status and operating procedures at a glance. Fig. 2 shows the external appearance of TrayCell and Nano Stick. The left side in Fig. 3 shows the sample dropping to the Traycell. The Traycell comprises a cap and a cell in which the fiber optical system is included. A small drop of sample is pipetted onto the window in the cell. After putting the cap on it, the Traycell is set in the cell holder in a UV-visible spectrophotometer to measure the sample. After the measurement, the sample is only wiped to measure the next sample. The right side in Fig. 3 shows the optical path of the Traycell. The incident light from a light source is reflected by a mirror located at the bottom of the cell and guided to the window. The light transmits the sample and is reflected by a mirror located on the back of the cap. It transmits the sample again and is reflected by another mirror located at the bottom and guided to a detector in the UV-visible spectrophotometer. The optical path is twice the length between the window and the mirror inside the cap. Two kinds of caps are available to select the optical path.

Fig. 4 shows the procedures to measure a sample with the Nano Stick. (1) A small amount of a sample (minimum: 2 μ L) is dropped on the window in the separated Nano Stick. (2) Another part of the Nano Stick is put on it (optical path: 0.5 mm). (3) The combined Nano Stick is set in the cell holder to measure the sample. (4) After the measurement, the sample is only wiped to measure the next sample. The specifications of TrayCell and Nano Stick are shown in Table 1.

Table 1 Specifications of TrayCell and Nano Stick

Usable Wavelength Range	: 190 to 1100 nm
Optical Path	: 0.2 mm, 1.0 mm (TrayCell) 0.5 mm (Nano Stick)
Minimum Sample Volume	: 0.7 to 4 μ L (TrayCell, Optical path: 0.2 mm) 3 to 5 μ L (TrayCell, Optical path: 1.0 mm) 2 μ L (Nano Stick)



Fig. 1 External Appearance of UV-1900



Fig. 2 Left: TrayCell Right: Nano Stick

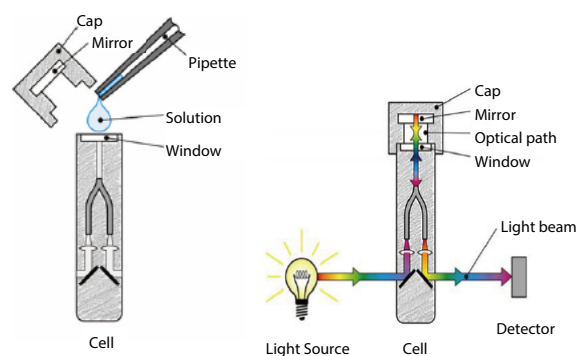


Fig. 3 Left: Dropping of Solution to TrayCell Right: Optical Path

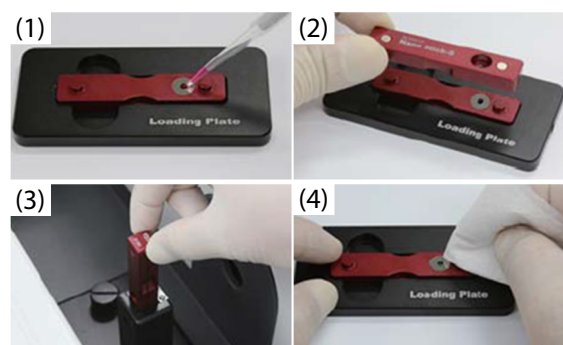


Fig. 4 Procedures to Measure Sample with Nano Stick

■ Micro-Volume Measurement of Food Dyes

Food dyes are often added to foods to show them deliciously. Commercial food dyes were measured with the TrayCell whose cap had 1.0 mm of optical path. Fig. 5 shows UV-Visible spectra of their aqueous solutions. Each food dye has the absorptions at wavelengths based on the color. Five green food dye aqueous solutions with concentration of 25, 50, 100, 250 and 500 mg/L were prepared to obtain the calibration curve. The calibration curve obtained with their absorbance values at 629 nm is shown in Fig. 6. It is expressed by $Abs = 0.1304 \times 10^{-2} Conc.$ and the square of the correlation coefficient stood at 0.9999. The results demonstrated that the high accuracy measurement could be performed for a small amount of solution using the TrayCell.

Table 2 Measurement Conditions

Wavelength Range	: 350 to 900 nm
Scan Speed	: Medium
Sampling Pitch	: 1.0 nm

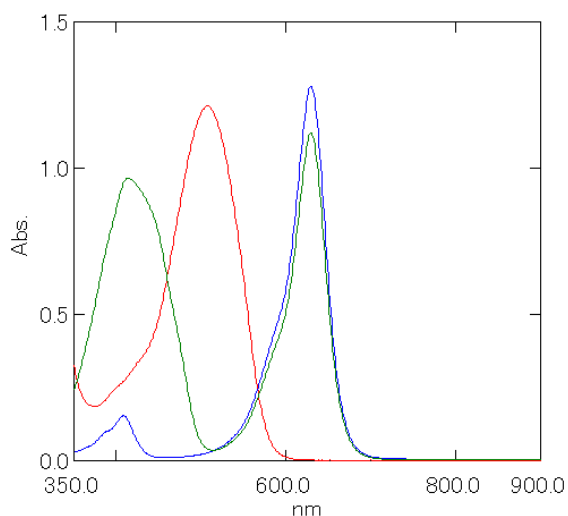


Fig. 5 Absorption Spectra Blue: Blue Dye (1000 mg/L), Green: Green Dye (2000 mg/L), Red: Red Dye (2000 mg/L)

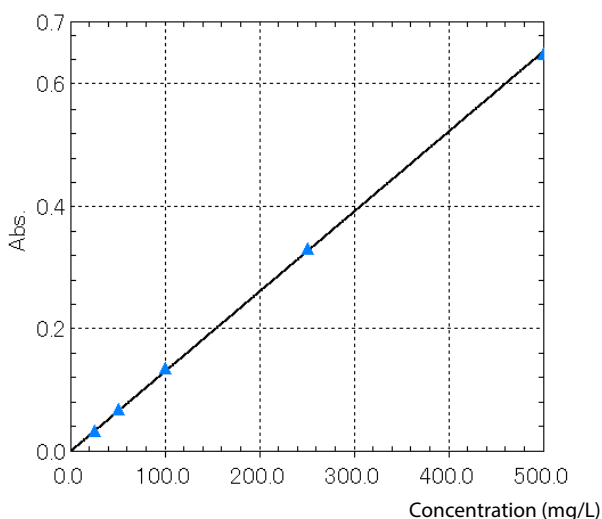


Fig. 6 Calibration Curve of Green Dye

■ Micro-Volume Measurement of Amino Acid Compounds

All of tyrosine, tryptophan and phenylalanine which are amino acid compounds constituting proteins have the absorption near 280 nm, which is used for the quantitation of proteins. Fig. 7, which is drawn with a screen shot function of the UV-1900, shows UV-visible spectra of them dissolved in sodium hydroxide solution. They were measured with the Nano Stick. The measurement conditions are shown in Table 3. While they have the absorption peaks at their specific wavelengths, all of them show the absorption near 280 nm. Six tryptophan solutions with concentration of 0.125, 0.25, 0.5, 1.25, 2.5, 5 Mmol/L were prepared to obtain the calibration curve. The calibration curve obtained with their absorbance values at 280 nm is shown in Fig. 8. It is expressed by $Abs = 0.2597 Conc.$ and the square of the correlation coefficient stood at 0.9996. The results demonstrated that the high accuracy measurement could be performed for a small amount of solution using the Nano Stick.

Table 3 Measurement Conditions

Wavelength Range	: 235 to 350 nm
Scan Speed	: Medium
Sampling Pitch	: 1.0 nm



Fig. 7 Absorption Spectra Blue: Tyrosine (0.01 mol/L), Yellow: Tryptophan (0.005 mol/L), Red: phenylalanine (0.1 mol/L)

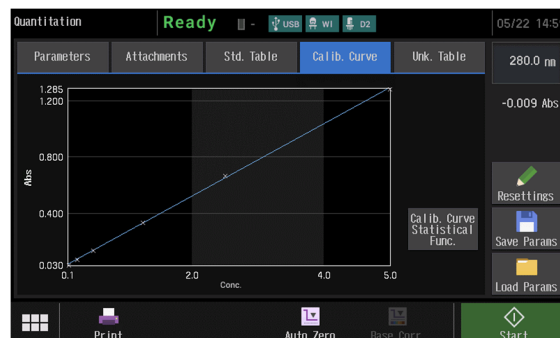


Fig. 8 Calibration Curve of Tryptophan

■ Conclusion

This report demonstrates that the use of the UV-1900 and a micro-volume cell such as the TrayCell and the Nano Stick allows the high accuracy measurement to be performed even for a few microliters of sample which is extremely low volume.

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