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UV
Talk Letter

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The Structure of a Spectrophotometer

1.The Measurement Principle Used by a Spectrophotometer

The basic measurement principle used by a spectrophotometer is relatively simple and easy to understand. I will explain the principle as it applies to solid samples and solution samples separately.

(1) Solid Samples

As shown in Fig. 1, first the intensity of the measurement light beam, I_0, is measured without the sample set. Then the sample is set in the path of the measurement light beam, and the intensity of the light beam after it passes through the sample, It, is measured.

$$T = \frac{I_t}{I_0} \qquad (1)$$

The product of the transmittance, T, and 100 is the percent transmittance (%T).

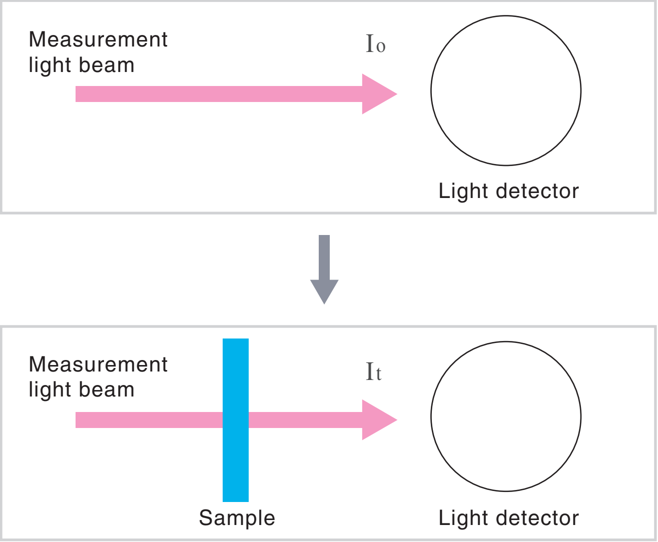

Fig.1 Measurement Principle for Solid Samples

(2) Solution Samples

As shown in Fig. 2, a cell containing solvent is set in the path of the measurement light beam, and the intensity of the light beam after it passes through the cell, I_0, is measured. Next, a cell containing a solution produced by dissolving the sample in the solvent is set in the path of the measurement light beam, and the intensity of the light beam after it passes through the cell, It, is measured. The transmittance, T, is given by equation (1), but with solution samples, it is more common to use the absorbance, Abs, which is given by equation (2).

$$Abs = Log_{10}\frac{1}{T} \qquad (2)$$

Equation (3), which expresses the relationship between the absorbance, Abs, and the sample concentration, C, is called the "Lambert-Beer law". There is a proportional relationship between the absorbance and concentration, and this forms the basis of quantitative analysis.

$$Abs = \varepsilon CL \qquad (3)$$

Here, ε is the sample's absorption coefficient and L is the cell's optical path length. The measurement method shown in Fig. 2 eliminates the influence of reflection from the cell surface and absorption by the solvent, and ensures that only the absorption due to the sample is measured.

Monochromatic light is usually used for the measurement light beam shown in Fig. 1 and Fig. 2. Monochromatic light is light that consists of a single wavelength. To be precise, it has a spectral bandwidth (slit width). For example, monochromatic light with a wavelength of 500 nm and a spectral bandwidth of 2 nm is light that covers a wavelength interval (full width at half maximum) spanning 499 and 501 nm.

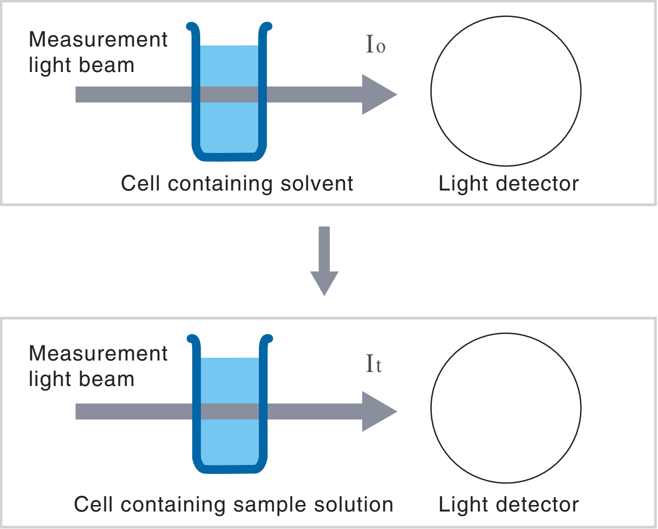

Fig.2 Measurement Principle for Solution Samples

2.The Configuration of a Spectrophotometer

You will find from the above explanation that the indispensable elements of a spectrophotometer consist, as shown in Fig. 3, of a light source, a spectrometer, a sample compartment, and a detector. Although I said in the previous section that the sample is exposed to monochromatic light, there are instruments in which white light is passed through the sample before being passed into the spectrometer. This method is employed in high-speed photometry instruments that use array detectors.

In the next sections, I will give an explanation of each element.

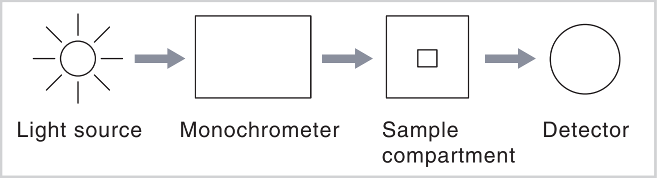

Fig.3 Configuration of Spectrophotometer

3.Light Source

The desirable properties of a light source are as follows:

a) Brightness across a wide wavelength range
b) Stability over time
c) A long service life
d) Low cost

Although there are no light sources that have all of these properties, the most commonly used light sources at the moment are the halogen lamps used for the visible and near-infrared regions and the deuterium lamps used for the ultraviolet region. Apart from these, xenon flash lamps are sometimes used.

(1) Halogen Lamp

The principle for light emission is the same as that for a standard incandescent bulb. Electric current is supplied to a filament, the filament becomes hot, and light is emitted. The bulb in a halogen lamp is filled with inert gas and a small amount of a halogen. While the tungsten used as the filament evaporates due to the high temperature, the halide causes the tungsten to return to the filament. This helps create a bright light source with a long service life. The emission intensity distribution of a halogen lamp can be approximated using Planck's law of radiation. Fig. 4 shows the emission intensity distribution for a temperature of 3,000 K. A halogen lamp offers superior temporal stability, a service life of approx. 2,000 hours, and a relatively low cost. It has relatively high levels of each of the properties a) to d) mentioned above.

(2) Deuterium Lamp

A deuterium lamp is a discharge light source in which the bulb is filled with deuterium (D_2) at a pressure of several hundred pascals. Fig. 5 shows the emission intensity distribution for a deuterium lamp. Although 400 nm is, in general, an approximate usage limit at the long wavelength end, because the degree of attenuation at this end is quite low, light of wavelengths greater than 400 nm is used. In the region beyond 400 nm, there are also large numbers of bright line spectra. Among these, the bright line spectra at 486.0 nm and 656.1 nm are particularly intense, and can be used for the wavelength calibration of spectrophotometers. The usage limit at the short wavelength end is determined by the transmittance of the window material. In Fig. 5, the graphs for cases where synthetic silica and UV glass are used for the window material are given as examples.

4.Monochrometer

Spectroscopy is the technique of splitting light that consists of various wavelengths into components that correspond to those wavelengths. The element that splits this light is called a dispersive element. Prisms and diffraction gratings are typical dispersive elements. Prisms used to be commonly used as the dispersive elements in spectrometers, but recently, diffraction gratings have become the most commonly used type of dispersive element. The diffraction gratings used in spectrophotometers have from several hundred to approximately 2,000 parallel grooves per millimeter cut into them at equal intervals. An example of a cross-section is shown in Fig. 6. If this diffraction grating is exposed to white light, because of interference, the white light is dispersed in a direction perpendicular to the grooves, and light components of specific wavelengths are reflected only in specific directions. This is illustrated in Fig. 7. λ_1 to λ_3 represent wavelengths. The wavelengths change continuously and so if a diffraction grating is exposed to white light, it appears iridescent. The way that the clear side of a CD appears to glitter with iridescence when it is exposed to light is based on the same mechanism as the spectroscopy performed with a diffraction grating.

A monochrometer consists of an entrance slit, an exit slit, and a diffraction grating, as well as the mirrors and other parts that come with them. Although various types of monochrometers, which vary according to the arrangement of the elements, have been devised, Fig. 8 shows an example of the simplest monochrometer configuration, which uses a concave diffraction grating. Light of varying wavelengths is projected from the output slit by rotating the concave diffraction grating.

5.Sample Compartment

Fig. 9 shows an example of a standard sample compartment. You can see that two light beams (indicated by red arrows in Fig. 9) pass through the compartment, and that this is therefore the sample compartment of a "double-beam spectrophotometer". The monochromatic light that leaves the spectrometer is split into two beams before it enters the sample compartment. A spectrophotometer in which only one beam passes through the sample compartment is called a "single-beam spectrophotometer". An explanation of the difference between single-beam and double-beam spectrophotometers is given in the Q&A of previous issue of UV Talk Letter. Refer to this explanation if necessary.

In a standard configuration, the sample compartment contains cell holders that, as shown in Fig. 9, hold square cells with optical path lengths of 10 mm. The various accessories are attached by replacing these cell holder units or by replacing the entire sample compartment. Among spectrophotometers of medium or higher grade that use photomultipliers, which will be described later, as detectors, there are models for which large sample compartments are made available in order to allow the analysis of large samples or the attachment of large accessories.

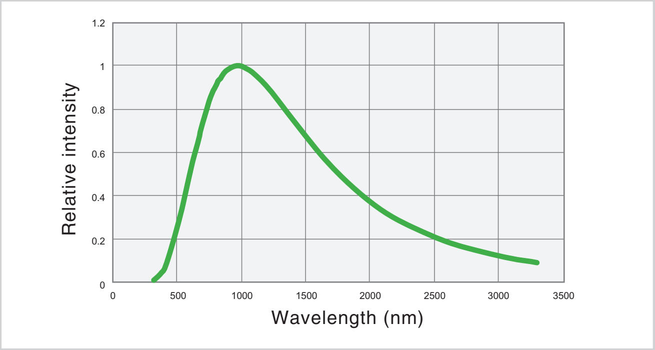

Fig.4 Emission Intensity Distribution of Halogen Lamp (3,000K)

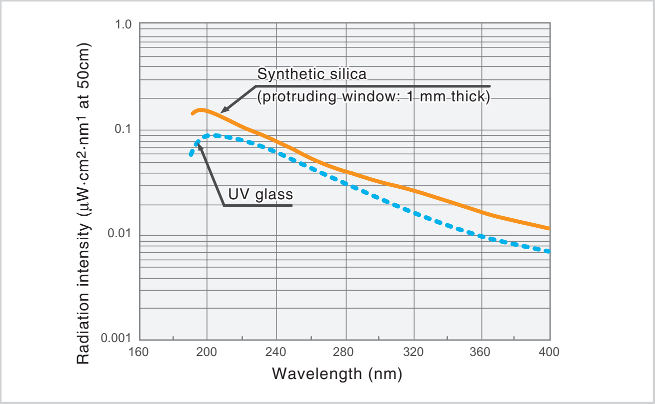

Fig.5 Emission Intensity Distribution of Deuterium Lamp[1)]

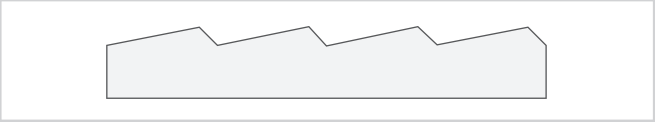

Fig.6 Cross Section of Diffraction Grating

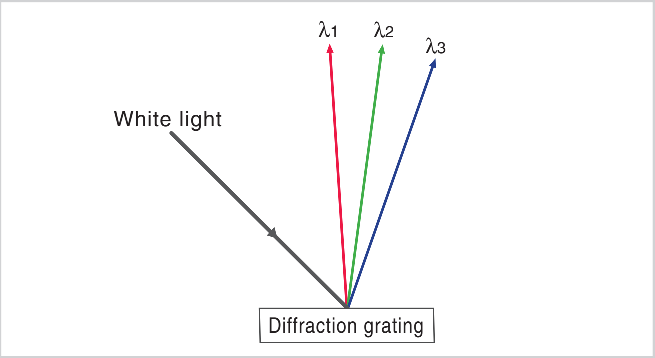

Fig.7 Dispersion of Light by Diffraction Grating

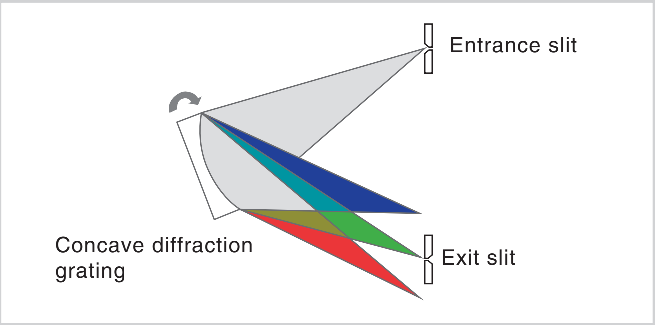

Fig.8 Graphic Illustration of a Concave-Grating Spectrometer

6.Detector

The light beams that pass through the sample compartment enter the detector, which is the last element in the spectrophotometer. Photomultipliers and silicon photodiodes are typical detectors used with spectrophotometers for the ultraviolet and visible regions. For the near-infrared region, PbS photoconductive elements have always been used in the past, but recently, instruments incorporating InGaAs photodiodes have been sold. Silicon photodiode array detectors are used, in combination with the back spectroscopy method, for high-speed photometry instruments. Photomultipliers and silicon photodiodes are described below.

(1) Photomultiplier

A photomultiplier is a detector that uses the fact that photoelectrons are discharged from a photoelectric surface when it is subjected to light (i.e., the external photoelectric effect). The photoelectrons emitted from the photoelectric surface repeatedly cause secondary electron emission in sequentially arranged dynodes, ultimately producing a large output for a relatively small light intensity. The most important feature of a photomultiplier is that it achieves a significantly high level of sensitivity that cannot be obtained with other optical sensors. If there is sufficient light intensity, this feature is not particularly relevant, but as the light intensity decreases, this feature becomes increasingly useful. For this reason, photomultipliers are used in high-grade instruments. The spectral sensitivity characteristics of a photomultiplier are mainly determined by the material of the photoelectric surface. Fig. 10 shows an example of the spectral sensitivity characteristics of a multi-alkali photoelectric surface, a type of surface that is often used in spectrophotometers.

(2) Silicon Photodiode

A silicon photodiode is a detector that uses the fact that the electrical properties of a detector change when it is exposed to light (i.e., the internal photoelectric effect). Solar cells, which have attracted much attention recently, use the same structure and principle as silicon photodiodes. In comparison with photomultipliers, silicon photodiodes offer advantages such as low cost, little locality of sensitivity in the light-receiving surface, and the fact that a special power supply is not required. Even regarding sensitivity, if the light intensity is relatively large, they can obtain photometric data that is no inferior to that obtained with photomultipliers. Fig. 11 shows an example of the spectral sensitivity characteristics of a silicon photodiode.

7.Summary

Here, I have given an overview of the structure of UV-VIS spectrophotometers. Due to space limitations, I have only described the basics. In the future, I plan to give more detailed explanations about specialized topics. I look forward to your continued interest.

References
1) Hamamatsu Photonics Deuterium Lamp Brochure
2) Hamamatsu Photonics Photomultiplier Brochure
3) Hamamatsu Photonics Photodiode Brochure

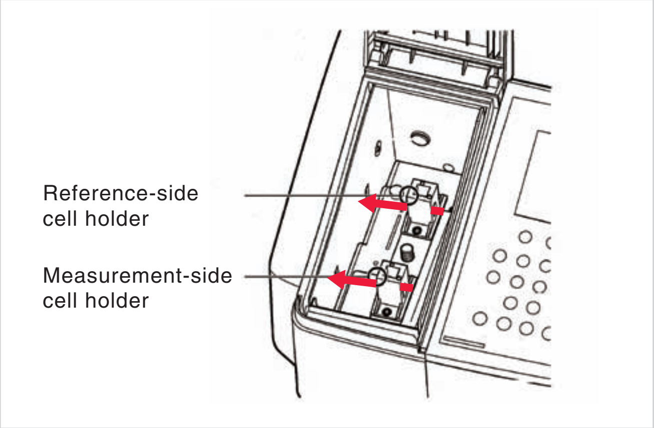

Fig.9 Sample Compartment

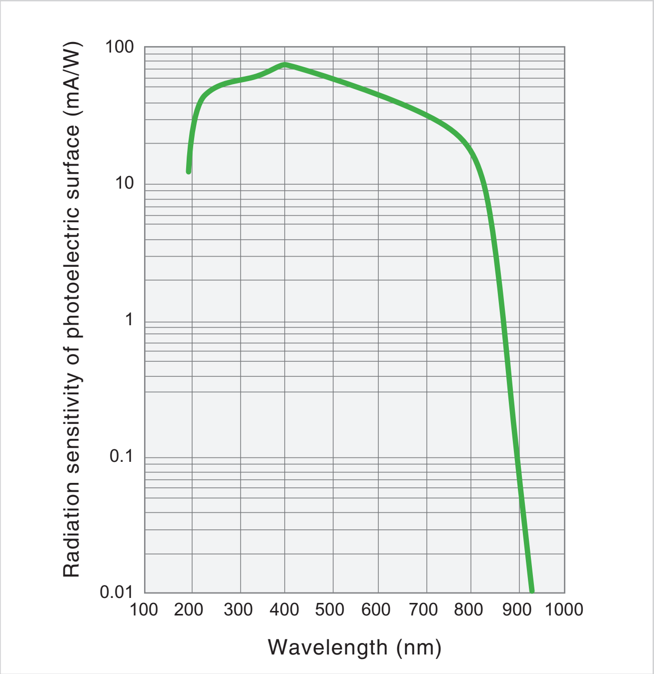

Fig.10 Spectral Sensitivity Characteristics of a Photomultiplier[2)]

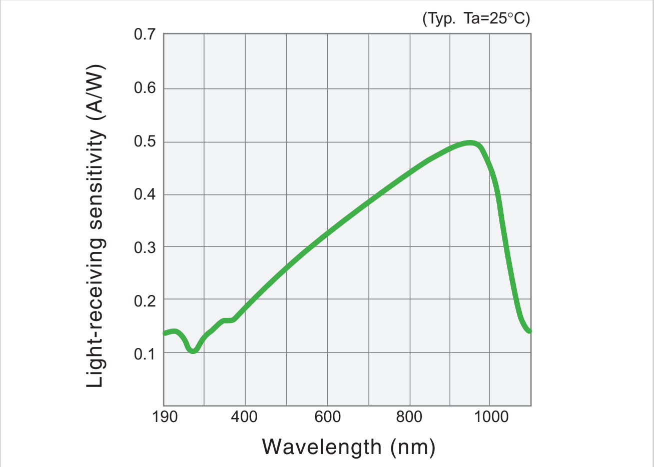

Fig.11 Spectral Sensitivity Characteristics of a Silicon Photodiode[3)]

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The Relationship Between UV-VIS Absorption and Structure of Organic Compounds

There are many colored organic compounds, such as dyes and pigments. How is it that these colors come about? There is a close relationship between the color of an organic compound and its structure. Here, I will explain this relationship using absorption spectra of organic compounds obtained with Shimadzu's UV-2550 UV-VIS spectrophotometer.

1.The Relationship Between Conjugated Double Bond Systems and Absorption Peaks

There are many organic compounds that have conjugated double bond systems (hereafter referred to as "conjugated systems"), in which every other bond is a double bond. These conjugated systems have a large influence on peak wavelengths and absorption intensities.

Fig. 1 shows the structures of benzene, naphthalene, and anthracene. Fig. 2 shows the absorption spectra obtained by dissolving these compounds in ethanol and analyzing the resulting solutions. The concentrations were adjusted so that the absorption intensities of the components were roughly the same. It can be seen in Fig. 2 that peak wavelengths tend to be shifted toward the long wavelength region as the conjugated system gets larger. Table 1[1)] gives the peak wavelengths and the molar absorption coefficients of various organic compounds. The molar absorption coefficient is a measurement of how strongly a substance absorbs light. The larger its value, the greater the absorption. With larger conjugated systems, the absorption peak wavelengths tend to be shifted toward the long wavelength region and the absorption peaks tend to be larger.

2.Absorption Spectra of Food Dyes with Large Conjugated Systems

Fig. 3 shows the structures of food dyes New Coccine (Red No. 102) and Brilliant Blue FCF (Blue No. 1) and Fig. 4 shows their absorption spectra. Food dyes tend to have large conjugated systems, like those shown in Fig. 3, and therefore their peak wavelengths tend to be shifted toward the long wavelength region, with peaks appearing in the visible region (400 to 700 nm). This is why they are recognized as colors. Incidentally, the color that we see is the color that is not absorbed by the substance (which is called the "complementary color"). As shown in Fig. 4, New Coccine absorbs blue and green light in the range 450 to 550 nm, and so the complementary color, red, is seen by the human eye. Brilliant Blue FCF absorbs yellow light in the range 560 to 650 nm and so blue is seen by the human eye.

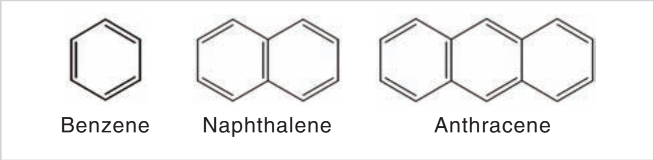

Fig.1 Structures of Benzene, Naphthalene, and Anthracene

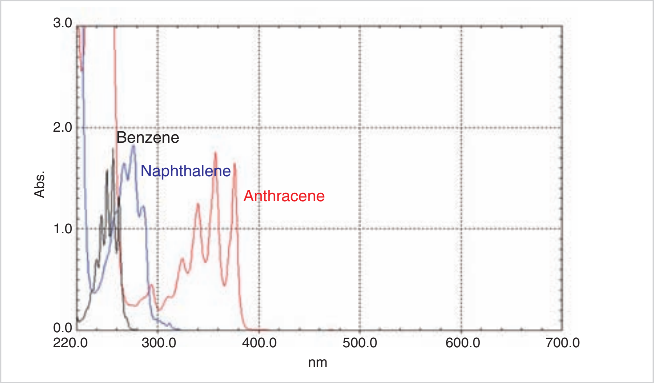

Fig.2 Absorption Spectra of Benzene, Naphthalene, and Anthracene

Substance	Absorption Peak	Molar Absorption Coefficient
Ethylene (CH_2=CH_2)	180nm	10000
1,3-butadiene	217nm	21000
Vitamin A	328nm	51000
β-carotene	450nm	140000
Benzene	255nm	180
Naphthalene	286nm	360
Anthracene	375nm	7100
Naphthacene	477nm	11000

Table 1 Absorption Peaks and Molar Absorption Coefficients of Various Organic Substances[1)]

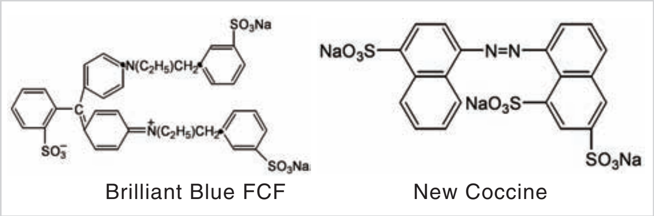

Fig.3 Structures of New Coccine and Brilliant Blue FCF

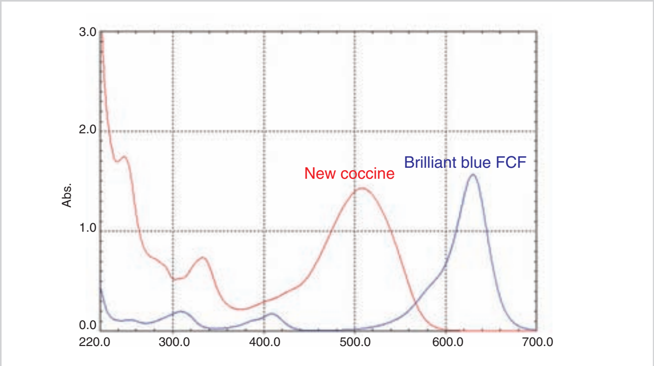

Fig.4 Absorption Spectra of Food Dyes New Coccine and Brilliant Blue FCF

3.The Influence of Functional Groups

Absorption peaks are also influenced by functional groups. Fig. 5 shows the absorption spectra of benzene, phenol, which consists of a hydroxyl group bonded to a benzene ring, and p-nitrophenol, which consists of a hydroxyl group and a nitro group bonded to a benzene ring. The functional groups influence the conjugated systems, causing the absorption peaks to appear at longer wavelengths than the peak wavelength of benzene, although they do not go beyond 400 nm and enter the visible region. The color of organic compounds, then, is influenced more strongly by the size of the conjugated system.

4.Absorption Spectra of Compounds with a Large Molecular Framework and a Small Conjugated System

Fig. 7 shows the absorption spectra of prednisolone, which is used as a pharmaceutical, and benzene. Although prednisolone has a large molecular framework, its conjugated system is small and so its peak wavelengths are not shifted greatly toward the long wavelength region, and its peaks appear at roughly the same position as those of benzene.

5.The Reason for the Shift Toward the Long Wavelength Region

I have shown the relationship between molecular structure and absorption spectra. Why, then, does the peak wavelength tend to be shifted toward the long wavelength region as the size of the conjugated system increases? Let us consider the relationship between the energy of light and the movement of electrons.

Light exhibits properties of both waves and particles (photons). The energy of one photon is expressed as hc/λ, where h is Planck's constant, c is the speed of light, and λ is the wavelength.

Absorption in the ultraviolet and visible regions is related to the transition of electrons. "Transition" refers to the switching of an electron from one state of motion to another. The state of motion of the π electrons in the conjugated system changes more easily than that of the σ electrons that form the molecular frameworks. If a photon collides with a π electron, that π electron readily changes to a different state of motion. This is true even if the photon has only a small amount of energy. The π electrons in relatively large conjugated systems are more easily affected by low-energy photons. Transition expresses the way that the energy of photons is absorbed by electrons. If a photon has a relatively small amount of energy, the value of hc/λ for that photon is relatively small, and therefore the value of λ is relatively large. λ is observed as the absorption wavelength and so, if there is a conjugated system, peaks tend to appear in regions where λ is large, i.e., the long wavelength region.

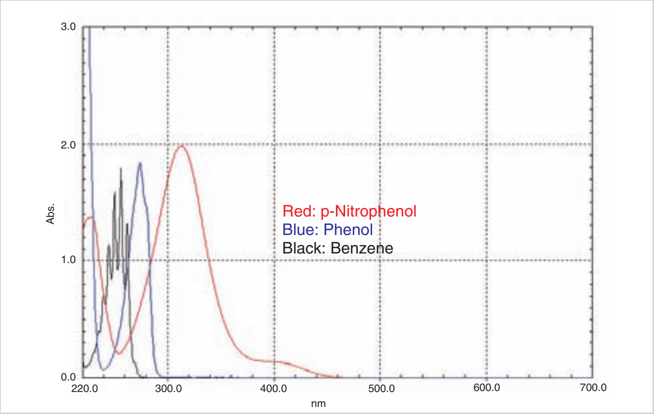

Fig.5 Absorption Spectra of Benzene, Phenol, and p-Nitrophenol

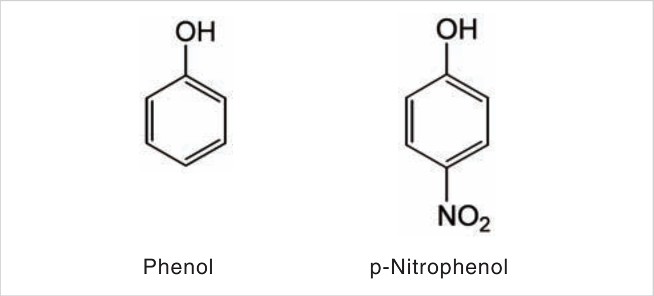

Fig.6 Structures of Phenol and p-Nitrophenol

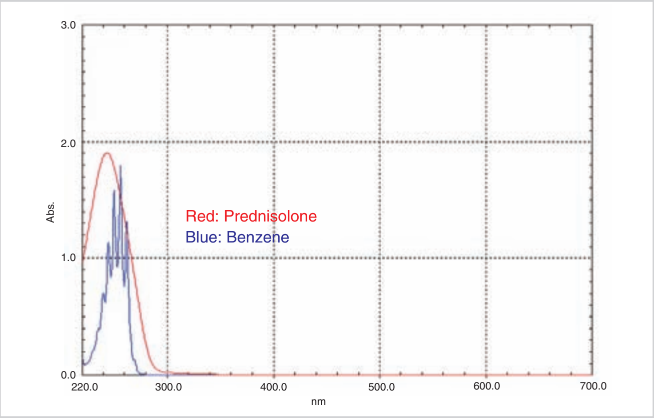

Fig.7 Absorption Spectra of Prednisolone and Benzene

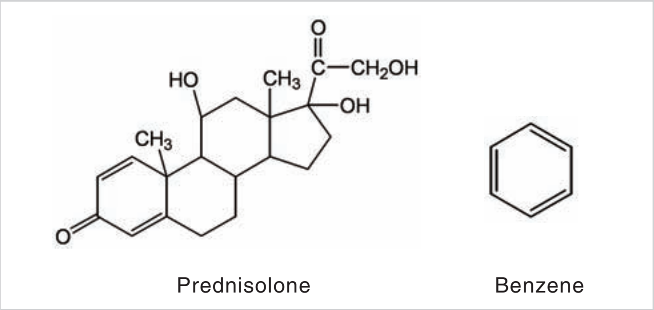

Fig.8 Structures of Prednisolone and Benzene

References
1) Masayoshi Nakahara:
"The Science of Color", Baifukan (2002), p. 108

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Q

The term “stray light” appears in product brochures. What is this exactly?

A

“Stray light” is light of any wavelength contained in the light used in a spectrophotometer that differs from the set target wavelength. It is expressed as the ratio (%) of the total amount of light of wavelengths other than the target wavelength to the amount of light of the target wavelength. Fig.1 shows the graphic illustration of stray light. In Fig. 1, the blue section corresponds to the target wavelength and the gray section corresponds to stray light. Checks for stray light are performed using filters (e.g., NaI solution filter and $NaNO_3$ solution filter) that do not allow light of specific wavelengths to pass through. The measurement wavelength is set to the wavelength that should be completely absorbed by the filter, the actual transmittance is measured, and from this the amount of stray light is calculated.

Stray light is a problem because it influences the linearity of the calibration curves used for quantitative analysis. This influence is hardly observed at all in low-absorbance regions, but if there is a lot of stray light in high-absorbance, high-concentration regions, the calibration curve is prone to bend. (See Fig. 2.) The wavelength of stray light differs from the target wavelength and so it is not absorbed by the sample. Light of wavelengths other than the target wavelength passes through the sample without being absorbed and so, in accordance with the Lambert-Beer law, the measured absorbance is less than the true value. Even if the calibration curve is bent, quantitative analysis is still possible using quadratic expressions. However, because the sizes of changes in absorbance with respect to the sizes of changes in sample concentration decrease, the quantitative error increases.

In general, the level of stray light is lower with double-monochromator instruments than it is with single-monochromator instruments.

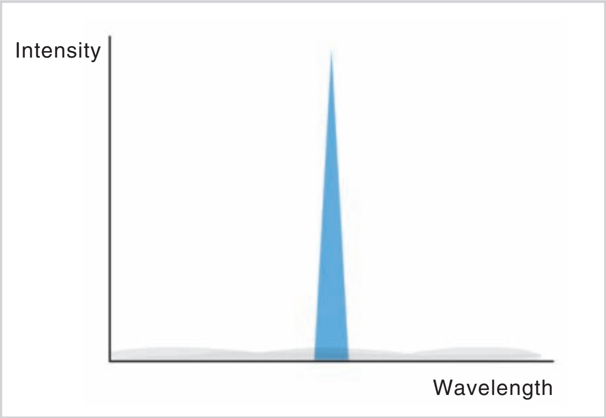

Fig.1 Graphic Illustration of Stray Light
■:Target wavelength ■:Stray light

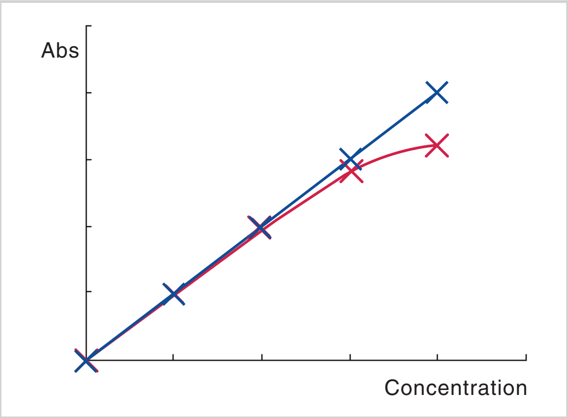

Fig.2 Change in Calibration Curve due to Stray Light
—:Low stray light instrument —:High stray light instrument

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*1 Survey by Shimadzu in November 2008.
*2 The optional auto atomizer changer is required.

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