Basics of UV-VIS spectrophotometer

Hitachi High-Technologies Corporations

Ultraviolet and Visible Spectrophotometer

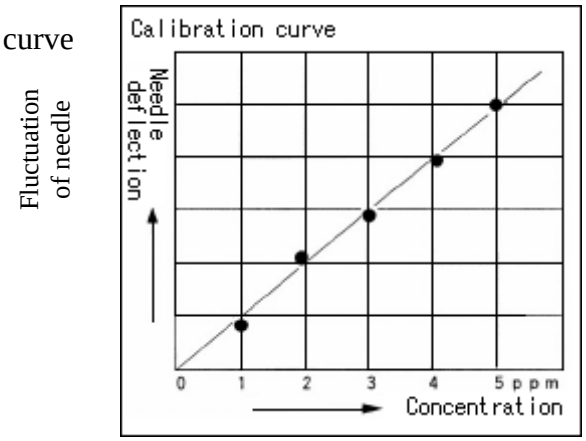
1. What can be done with an ultraviolet-visible spectrophotometer?

- **The concentration of a solution can be determined (what and how much is contained in the solution?).**

  An ultraviolet-visible spectrophotometer is also used for "quantitative analysis," which is its most popular application.

  This is a method to determine the concentration of a sample of unknown concentration in comparison with a solution of known concentration.

Calibration curve

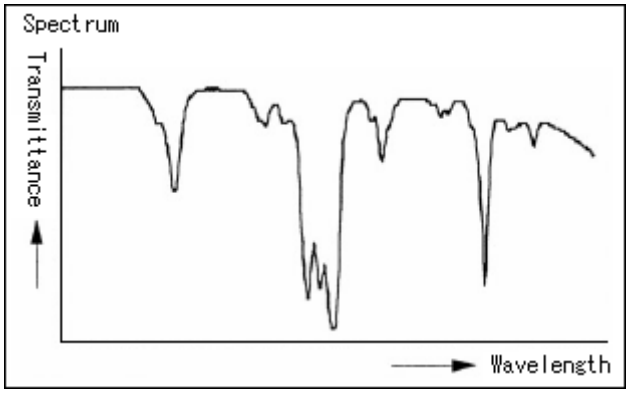


Fluctuation of needle

Concentration

- **The properties of a substance can be determined.**

  - For example, regarding sunglasses, UV-protective cosmetics and clothes that we use in mountain areas and by the sea in summer, and in ski areas in winter, UV protection becomes quite obvious by measuring "transmittance" to determine the actual level protection.
  - Each substance has it intrinsic "spectrum." It is also possible to compare the spectrum of an unknown substance with that of a familiar substance (for identification).



● **The molecular structure can be determined.**

A substance is made of molecules. Do you know a molecule is a collection of atoms?

This molecule has its characteristic spectrum (position, intensity, etc.).

This is in practical use in universities, research institutes of companies, and so forth, in spite of some difficulties.

2. Colorimetric analysis

Colorimetric analysis is also known as "absorptiometry," which refers to a method for observing a color and analyzing the substance.

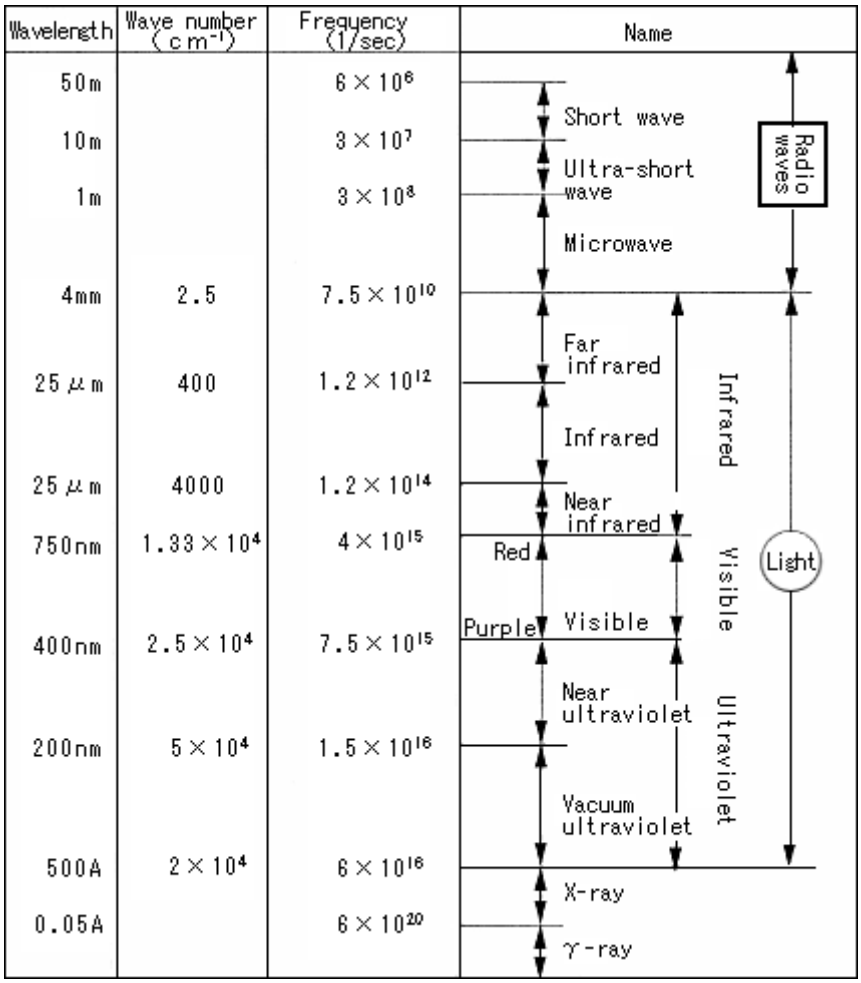
First, think about what is "color" and what is "light" that shows the color.

2-1. Light and color

2-1-1. Relationship between light, radio waves, and x-rays (what is light?)

"What is light?" - Can you give a prompt answer to this question? "Light" exists all around us.
In fact, light is a kind of electromagnetic wave, comparable with the radio waves used for radio and television broadcasting or the X-rays used for radiography.

★ Light is a kind of electromagnetic wave.



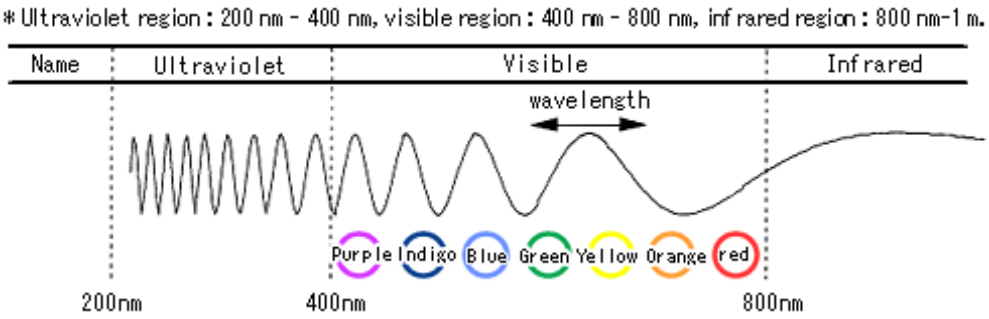
2-1-2. Sun and laser beams (white light and monochromatic light).

In the electromagnetic spectrum shown in the table in the previous page, light with a wavelength of 200-400 nm is called ultraviolet (UV) light, that with 400-800 nm as visible (VIS) light, and that with 800 nm - approximately 1 mm as infrared (IR) light.

Among these, only visible light can be seen as "color" to our eyes as its name suggests. "Wavelength" determines colors, like red and blue.

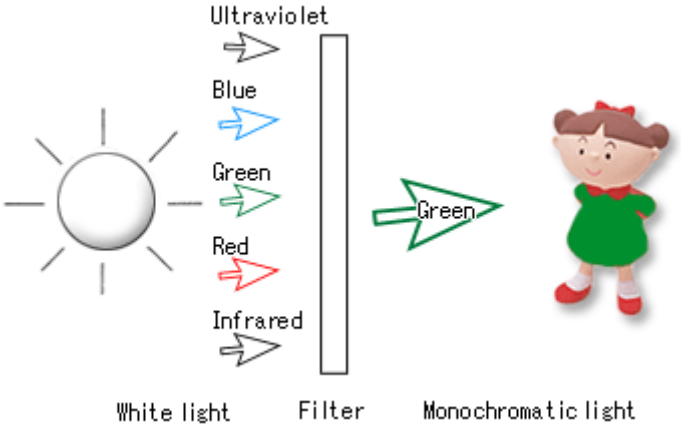
This is why the colors of a rainbow always line up in the same way.

* Ultraviolet range: 200 nm - 400 nm, Visible range: 400 nm - 800 nm, Infrared range: 800 nm - 1 mm



A light containing all the wavelengths, including the ultraviolet, visible light, and infrared lights, is called "white light," which includes sunray, incandescent lamp, and so forth. A single individual light, like red and blue, is called "monochromatic light," which includes light (color) split using a device like a "filter" or "prism," so that the white light, "sunlight," becomes a rainbow of seven colors, laser beam that outputs only one wavelength (called "single wavelength"), and so forth.

✱ White light: A light containing all wavelengths, Monochromatic light: A light containing a single wavelength.



2-1-3. Why is an apple red? (What is color?)

"Why does an apple fall from a tree?" Newton thought.

Here, think about "why does an apple look red?"

Does an apple look red in the pitch darkness of a room at night? The answer is NO. But, an apple looks red when a light is on, or in the brightness of daytime. It can be said that light (white light) is necessary for us to see "color."

An apple looks neither blue nor yellow. Why does it look red?

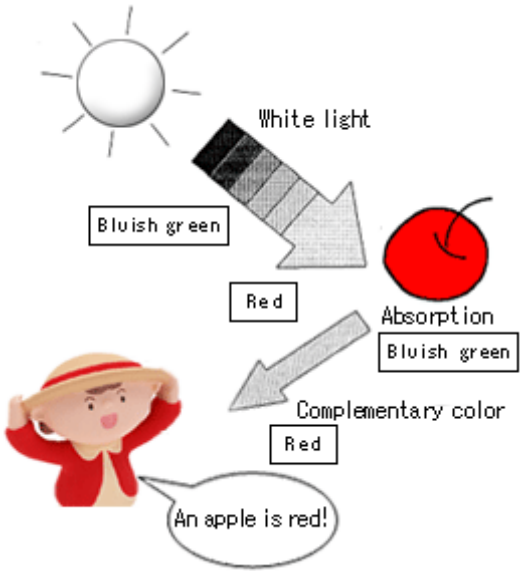
We have our own likes and dislikes of color, and so do substances such as those contained in apples, cars, and clothes.

When light (white light) containing various colors is irradiated onto a substance, the substance absorbs only the favorite colors among them (this phenomenon is called "absorption"), and the color that the substance dislikes is visible to our eyes as the color of that substance. (This is called "complementary color".)

Specifically, an apple likes a color called blue green and dislikes red; when white light is irradiated, the apple absorbs bluish green, and its complementary color, red, can be seen.
Besides, keep in mind that all the things we call "color" are related to "wavelength."

★ A substance absorbs the light of a specific wavelength, and thus the visible color is its complementary color.

Wavelength (nm)	Color	Complementary color
-400	Ultraviolet	
400-435	Purple	Yellow green
435-480	Blue	Yellow
480-490	Patina	Orange
490-500	Blue green	Red
500-560	Green	Red purple
560-580	Yellow green	Purple
580-595	Yellow	Blue
595-610	Orange	Patina
610-750	Red	Blue green
750-	Infrared	



2-1-4. Invisible light (ultraviolet and infrared lights)

Did you understand what visible light is like, from the above explanations? Those who could not understand, need to review the lesson.

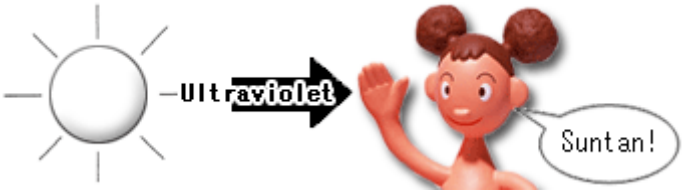
What is ultraviolet and infrared light, lights invisible to our eyes?

- **Ultraviolet light**

Ultraviolet light causes skin cancer and suntan. Ultraviolet light is electromagnetic waves with a maximum wavelength of approximately 400 nm and a minimum wavelength of roughly 100 nm (at a wavelength of several tens of nm or less, we find soft X-rays), abbreviated as UV.

In the field of spectrometry, an ultraviolet range of 200 nm or less is expressed as far ultraviolet, and that of 300 nm or more as near ultraviolet.

Generally, an ultraviolet visible light spectrophotometer allows measurement at a minimum of 200 nm.



- **Infrared light**

We often hear the word, "infrared," in our everyday life, e.g., far-infrared grill, far-infrared kotatsu, etc.

An infrared light consists of electromagnetic waves with a maximum wavelength of approximately 1 mm (partly overlapping with the submillimeter waves of microwave radiation) and a minimum wavelength of approximately 800 nm, abbreviated as IR. An infrared light is also called a heat ray since it has a thermal action. Now, this explains why infrared light is used for a grill or a kotatsu.

There is no single pattern for distinguishing infrared light; however, an infrared light of less than 2.5 μm is usually called near-infrared light, that of 2.5-25 μm infrared light, and that of more than 25 μm far-infrared light.

Although an infrared spectrophotometer is used for the measurement of infrared light, some ultraviolet-visible spectrophotometers allow measurement in the near-infrared range.

2-2. Neglecting cleaning increases absorbance (Lambert-Beer's law).

Are you a tropical fish-lover? Caring for an aquarium is somewhat troublesome, although the fish are lovely.

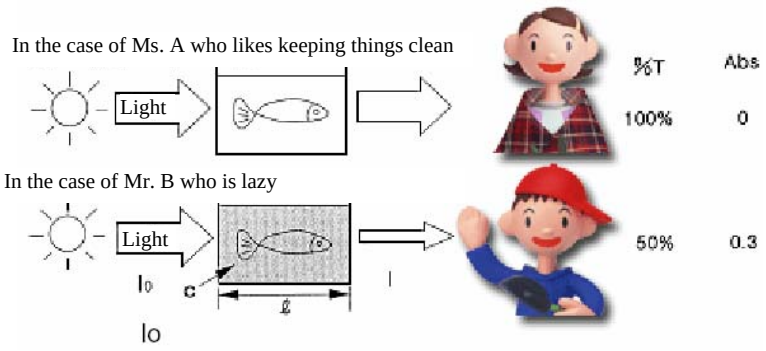
Here are Ms. A and Mr. B who keep tropical fish. Ms. A likes keeping things clean, and the aquarium in which the tropical fish are kept is always clear and beautiful. On the other hand, Mr. B is lazy, and the aquarium tends to become muddy.

The aquariums of these two persons are placed by the window. Since the water of Ms. A's aquarium is clear, the light that comes in from a window can mostly be seen even through the aquarium. However, since the water of Mr. B's aquarium becomes muddy, light hardly comes in through the aquarium.

Of the light that comes in from the window, the percent of light that comes in through the aquarium is called "transmittance (T)."

Generally, transmittance is more commonly expressed as a percentage "transmittance (%T)". The part of the light that comes in from the window and is absorbed into the water of the aquarium is calls "absorbance (Abs)."

The water of the aquarium that is not cleaned becomes muddy and absorbs light preventing its transmittance, resulting in an increase in absorbance and a decrease in transmittance. In contrast, since the water of the aquarium that is always clean clear lets the light pass through well, the transmittance is close to 100%, and the absorbance is close to zero.



Let's make this theory into a mathematical formula. When the light that comes in from the window is defined Io, and the light that comes out from the aquarium is defined as I, the transmittance is I/Io. Furthermore, when the width of the aquarium is defined as ℓ (optical path length), the degree of muddiness of the water (concentration) as c, and a constant (called "molar absorption coefficient") unique to the substance (in this case, the substance that causes muddiness) as ε, the transmittance will become $10^{-\varepsilon c \ell}$ (Formula 1). Absorbance can be obtained with the formula 3, since it is a logarithm of the inverse of the transmittance, suggesting that the absorbance is proportional to the molar absorption coefficient, concentration, and optical path length. This formula 3 is called Lambert-Beer's law.

Lambert-Beer's law

The absorbance (Abs) of a solute (coloring substance) is proportional to the concentration of a solution (C) and the thickness of a solution layer (ℓ).

$$T = \frac{I}{I_0} = 10^{-\varepsilon c \ell} \quad \cdots\cdots \text{Formula (1)}$$

$$\%T = T \times 100 \quad \cdots\cdots \text{Formula (2)}$$

$$Abs = \log\frac{1}{T} = \log\frac{I_0}{I} = \varepsilon c \ell \quad \cdots\cdots \text{Formula (3)}$$

Notes 1) Lambert-Beer's law is called "Bouger-Beer's law" in JIS.

Notes 2) Molar absorption coefficient is a constant unique to a substance (solute), and is needed to quantify each substance by spectrometry.

2-3. Measurement by color strength (quantitative analysis)

A quantitative analysis refers to an assay to measure the content of a certain substance (in a solution state).

For example, the water that we drink every day contains metals, small quantities of agricultural chemicals, microorganisms, and so forth.

However, the amounts contained cannot be determined visually.

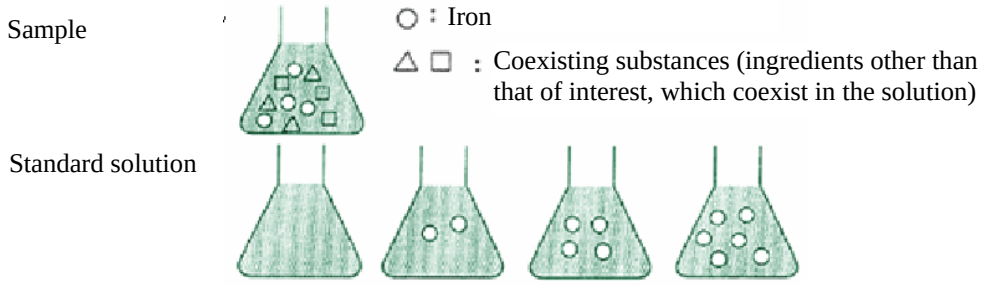
Generally, in absorptiometry where a coloring reagent is added to a sample to see the degree of coloring in the reaction with the substance of interest, we use a method consisting of preparing a standard solution and a sample, adding the reagent for coloring the substance of interest, and comparing the absorbance between the standard solution and the sample using a calibration curve to determine the sample concentration.

Let's think about the procedures for measuring iron in tap water as an example.

2-3-1. Sample and standard solution

To measure the concentration of a substance, a sample of unknown concentration to be measured ("sample") is compared with a sample of known concentration ("standard solution") to determine the concentration. To analyze iron in tap water, tap water is used as a sample, and iron of known concentration as a standard solution. Standard solutions of different iron concentrations are prepared as standard solutions.

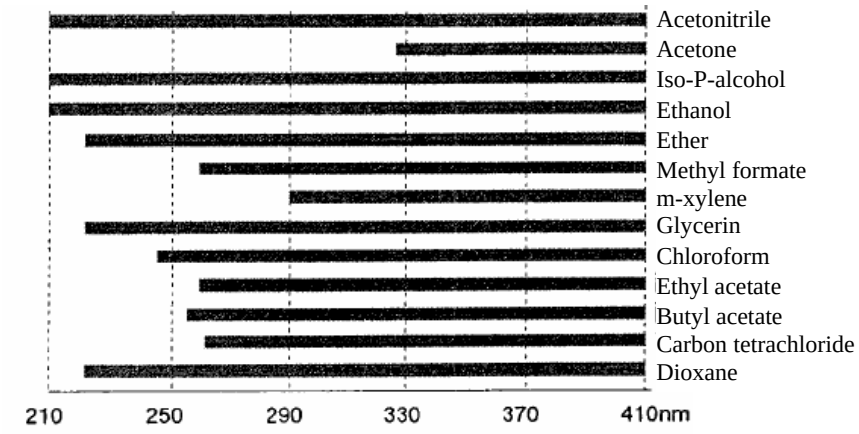
Accurate results cannot be obtained unless the sample and the standard solution are prepared and measured under the same conditions. For example, when the sample is tap water, an aqueous solution is used as a standard solution instead of an alcoholic solution; when an acid is added to the sample, the acid should be added also to the standard solution.



A liquid that dissolve a substance, such as water and alcohol, is called "solvent," and a substance that is dissolved into a liquid, such as iron, is called "solute." Accurate results cannot be obtained when absorption occurs at the same wavelength for the solute and the solvent.

Generally, water is often used since there is no absorption in the visible-ultraviolet range. An organic solvent is also used commonly; however, take care about its use due to absorption in the invisible ultraviolet range.

Use of an organic solvent in the ultraviolet range
Water is used as a control, with the absorbance of 1 or less.

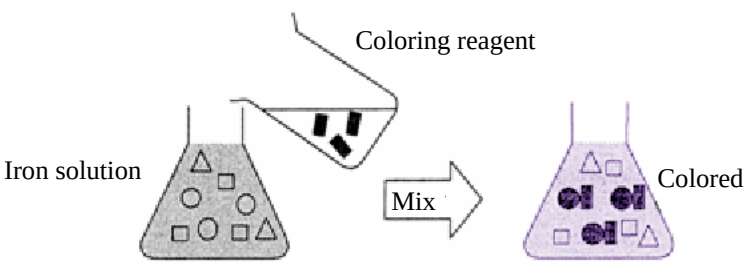


2-3-2. Magic drug (coloring reagent)

Can you see the color of iron in tap water? Colorimetric analysis is a method to analyze a color. The assay is impossible if a color cannot be seen (in the case of visible ranges). Then, what should be done.....? Color iron with a magic drug.

The magic drug that colors the substance of interest in a solution is called "coloring reagent" or "color reagent."

The higher the iron content in tap water, the darker becomes the color (high absorbance). The lower the iron content in tap water, the lighter becomes the color (low absorbance). Since various color reagents are available for each component, they can be used according to the analytical components.



2-3-3. Calibration curve

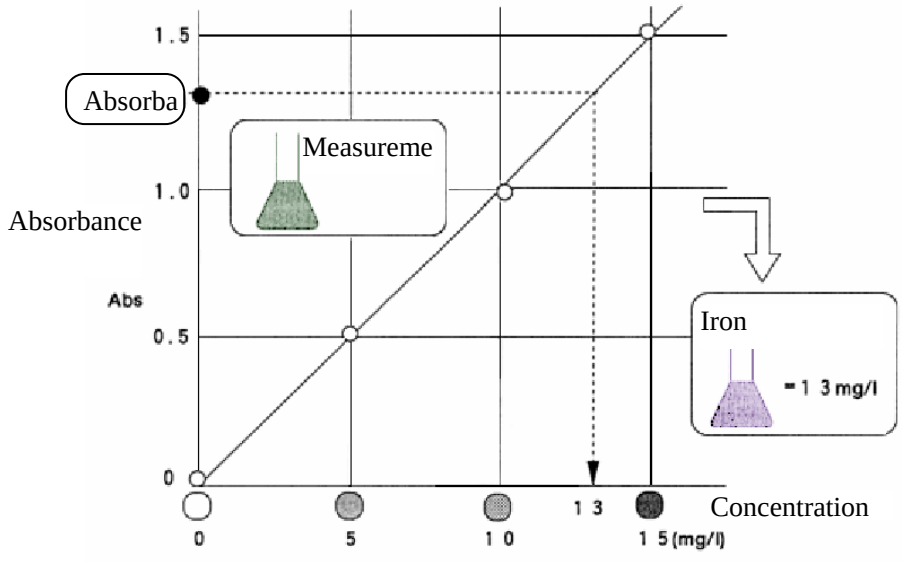
This is the end of the preparation of solutions. Finally, start measurement. First, determine the wavelength for measurement.

This was explained in the chapter on the spectrum. Let's get ahead. Next, measure the absorbance of a standard solution to prepare a calibration curve. The horizontal axis indicates concentration, and the vertical axis absorbance. By measuring standard solutions in the order of iron concentrations (from a low concentration), and plotting the concentrations and absorbance, we obtain the graph shown in the figure below.

This is the "calibration curve." The calibration curve is usually a straight line, but may become a quadratic curve that is slightly curved up or down if the concentration is too low or too high.

When the standard solution is measured and the calibration curve is prepared, measure a sample. Measurement provides absorbance, although the concentration is unknown. See the calibration curve. The absorbance of the vertical axis provides the concentration.

In a quantitative analysis, the concentration of a sample is determined in this manner.



2-4. See a color (spectrum)

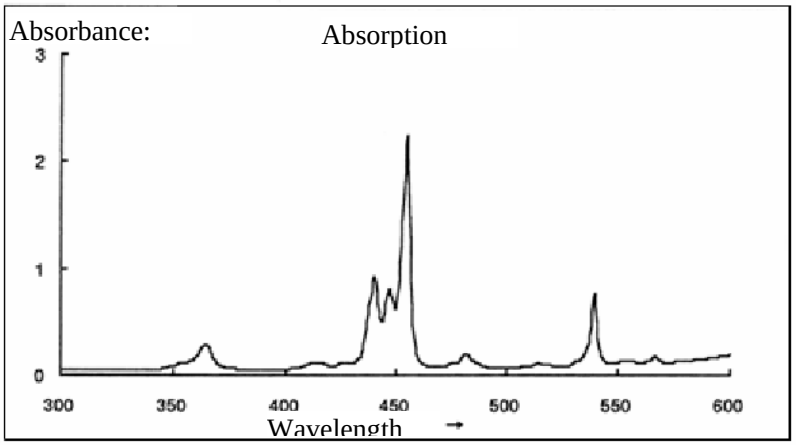
2-4-1. What's a spectrum?

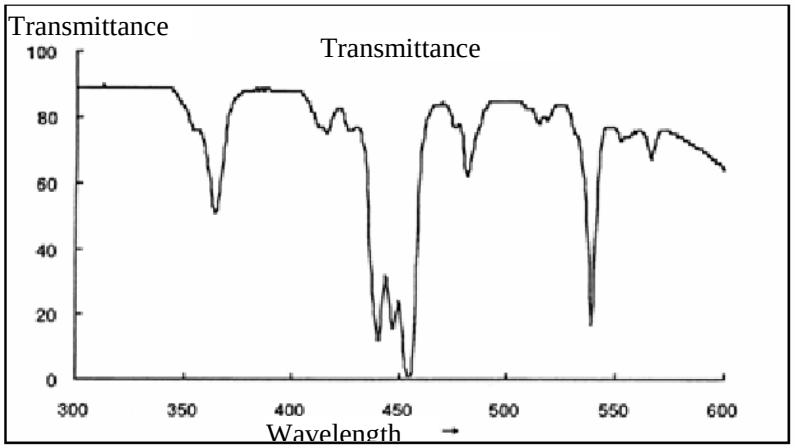
In a quantitative analysis, the amounts of light absorption of a substance were compared at a certain wavelength to determine concentration. Then, how much is the absorption at other wavelengths? "Spectrum" is a graph of the absorption of the substance at each wavelength.

Transmittance and absorption spectra can be measured with an ultraviolet-visible spectrophotometer.

In the spectrum, the horizontal axis indicates wavelength, and the vertical axis indicates transmittance and absorbance.

Look at the transmittance spectrum and the absorption spectrum indicated below to recall the relationship between transmittance and absorbance.





2-4-2. What can be determined from a spectrum?

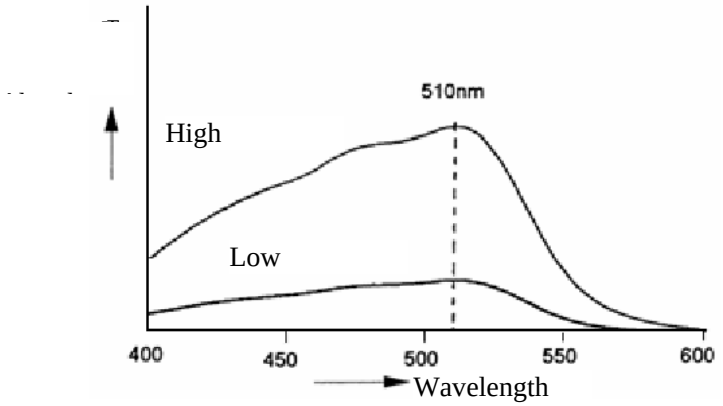
Quantitative analysis of iron was performed in the preceding chapter. At which wavelength (nm) should measurement be conducted?

Higher absorption of the wavelength provides higher sensitivity; thus, it is desirable to select a wavelength with maximum absorption...

Here, the absorption spectrum comes in. See the spectrum below. This is an iron solution to which a coloring reagent was added. The absorption is highest at around 510 nm (the wavelength at which absorption reaches its peak is called absorption maximum wavelength). This tells us that the iron solution should be measured at around 510 nm.

The shape of this spectrum is unique to the iron solution to which a coloring reagent was added (the whole absorption intensity changes with concentration); thus, an unknown solution can be demonstrated to be an iron solution to which a coloring reagent was added if its spectrum measured corresponds to that of the following figure.

Thus, the properties of a substance can be investigated by measuring the spectrum.



In addition, the chemical structure of a substance can also be known from absorption maximum wavelength, molar absorption coefficient, etc. of the ultraviolet-visible spectrum. (Qualitative analysis)

Absorption of main compounds

Compounds	Examples of compounds	Absorption maximum wavelength	Maximum molar absorption coefficient	Solvent
Alkene (R-CH=CH-R)	Ethylene	165	15000	Gas
		193	10000	Gas
Alkyne (R-C≡C-R)	2-octine	195	21000	Heptane
		223	160	Heptane
Ketones (R-CO-R)	Acetone	189	900	Heptane
		279	15	Heptane
Aldehyde (R-CHO)	Acetaldehyde	180	10000	Gas

3. Structure of a spectrophotometer

3-1. Differences between a man and a machine (rough structure)

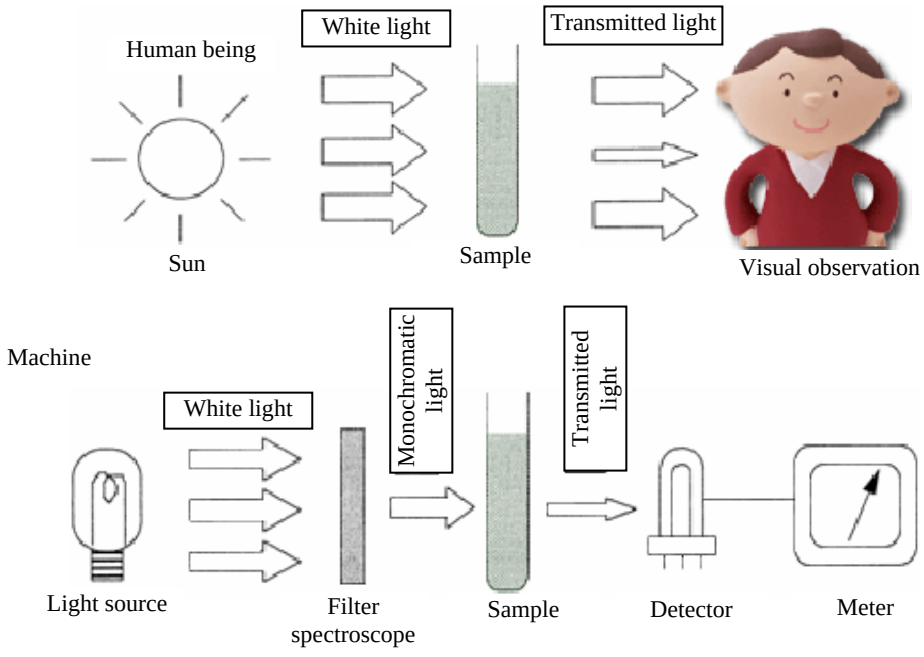
How can we know whether a color is deep or light, which color (wavelength) this substance absorbs in the wavelength range (200-800 nm) of the ultraviolet-visible range that we have studied until now?

Even human beings can roughly estimate the absorption wavelength from the color of a substance, or recognize color density, etc., however, we cannot know the exact wavelength, and also we have individual differences. Besides, the ultraviolet range is not visible to our eyes.

Here, an "ultraviolet-visible spectrophotometer," a device that can measure even in the ultraviolet range without individual differences, comes in.

With the spectrophotometer, we measure a color (wavelength) using an artificial source instead of the sun, and a detector and a meter instead of our eyes. The biggest difference between the spectrophotometer and macroscopic observation is that the spectrophotometer measures the degree of absorption of each color, using prism or diffraction grating (not irradiating white light on a substance directly) to split the light into each color and irradiate each of the colors (monochromatic light) on the substance in order (called "scanning").

★ **The spectrophotometer splits white light into monochromatic lights to measure absorption.**



3-2. Optical system

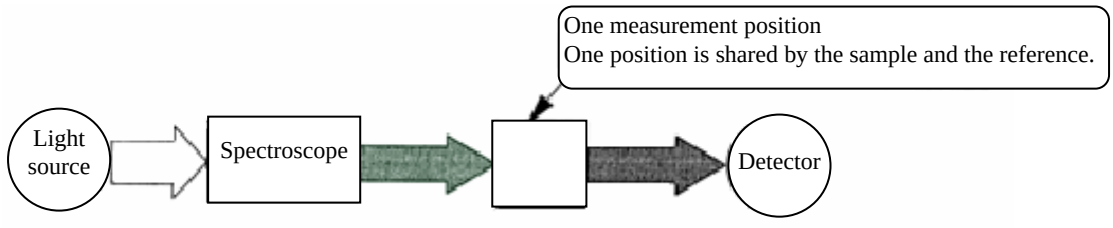
3-2-1. Is there a dedicated position for a sample? (Single beam and double beam)

- **Single beam system**

  In the measurement of absorption, a sample and a reference are measured each time to compare these measurement results.

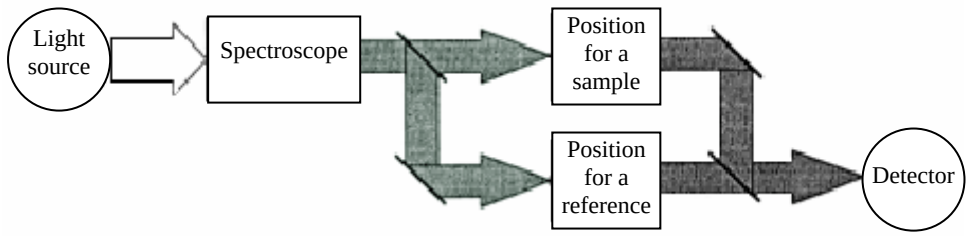
  Since a single beam system has only one position for measurement, the sample and the reference should be replaced, and 100% adjustment (or 0 Abs adjustment) should be conducted whenever measurement wavelength is changed.

  Although this system is used mainly for quantitative measurement, and offers a small and inexpensive device configuration, changes (drift) in the light source cannot be adjusted.



- **Double beam system**

  A double beam system has two dedicated positions for the sample and the reference, in contrast to the single beam system with only one measurement position. The double beam system does away with the troublesome exchange of sample and reference (100% adjustment or 0 Abs adjustment for each change of measurement wavelength) and is suitable for qualitative analysis, as well as quantitative analysis.

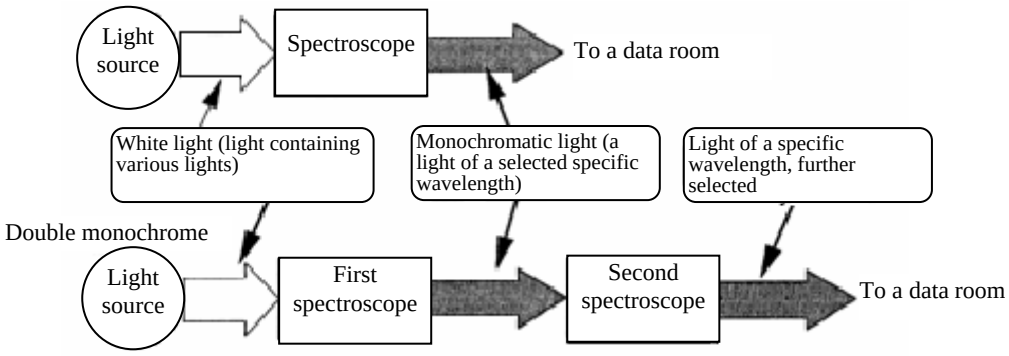


3-2-2. Careful selection of wavelength (single and double monochromes)

The hall staff is checking visitors at the entrance of an event hall with restricted admission (e.g., STUDENTS, NO ENTRANCE). Are there really no students in this hall? We often hear that there are students who slipped in unseen even if visitors were checked one by one carefully.

A monochrome (monochromator, spectroscope) plays the role of a sorter that picks up the light of the desired wavelength out of the light containing various wavelengths (i.e., the hall staff who conducts an entrance check). But, however carefully they are checked, wavelengths other than the wavelength of interest may slip in (this light that was lost or slipped in is called stray light). This stray light may affect the accuracy of the measurement. A double monochrome system conducts a second check on the light that passed the first check. The system that checks only once is called a single monochrome system.

Single monochrome

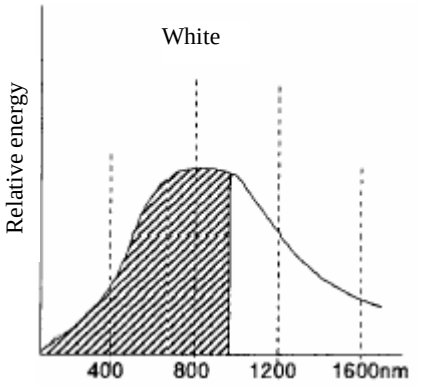
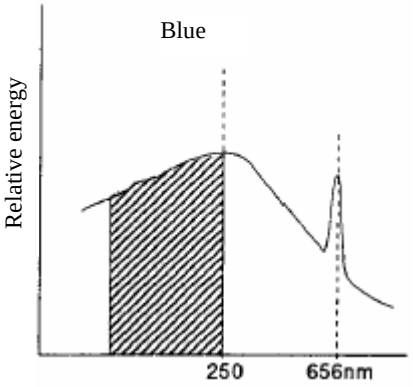


3-3. Components of spectrophotometer

3-3-1 Light source

Two kinds of lamps, a hydrogen discharge tube for measurement in the ultraviolet range and a tungsten lamp for measurement in the visible and near-infrared ranges, are used as the light sources of a spectrophotometer.

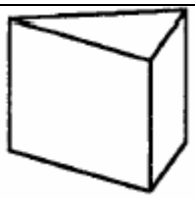
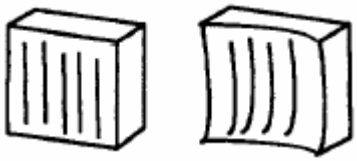
Types and properties of light source

Type	Tungsten	Hydrogen discharge tube
Sign	W WI	H2 D2
Property	A continuous spectrum of 300-3000 nm is emitted.	A continuous spectrum of 168-500 nm, with maximum energy at 250 nm, is emitted.
Wavelength range	340-1100 nm	185-360 nm
Spectrum energy		

3-3-2. Spectroscope

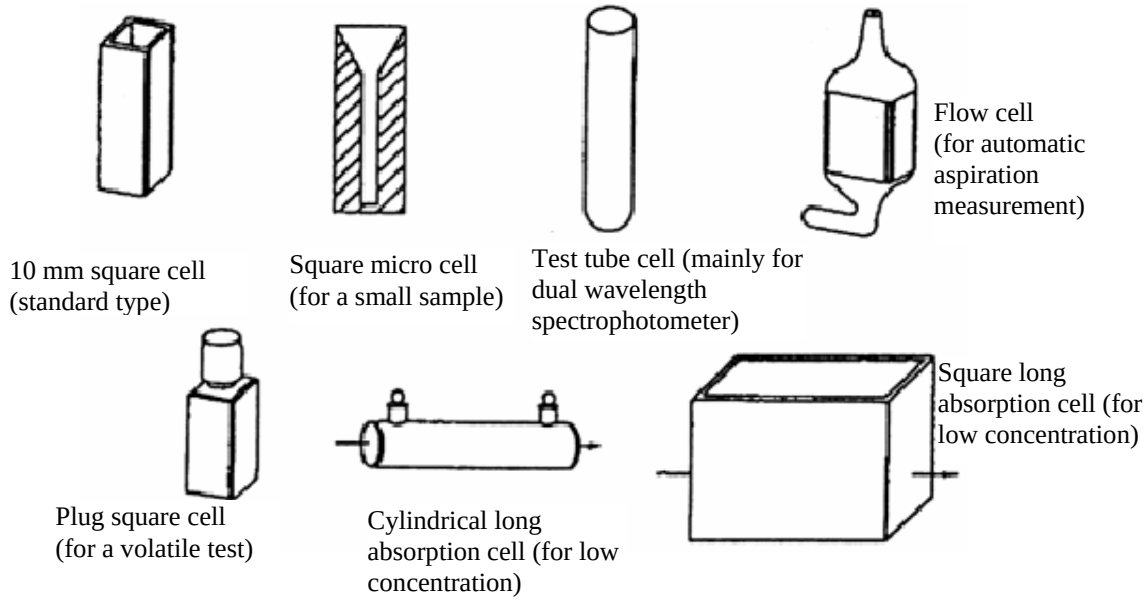
A spectroscope plays a role in selecting a monochromatic light from a light source (white light). Spectroscopes include a) filter type, b) prism type, and c) grating (diffraction grating) type.

Types and properties of dispersion

	Filter	Prism	Grating (diffraction grating)
Properties	A single wavelength can be extracted with a filter. A filter is also used in combination with diffraction grating for filtering out stray light.	A spectrum of 175-2700 nm can be dispersed. The degree of dispersion varies with the wavelength; the dispersion worsens as the wavelength becomes longer.	Dispersion is homogeneous at the entire wavelength, and a wide-range wavelength can be obtained with a diffraction grating. In addition, a constant spectrum featuring a constant slit breadth can be obtained.
Types and materials			
	Colored glass filter Interference filter	Crystal or fused quartz	Plane diffraction grating Concave diffraction grating

3-3-3. Sample cell

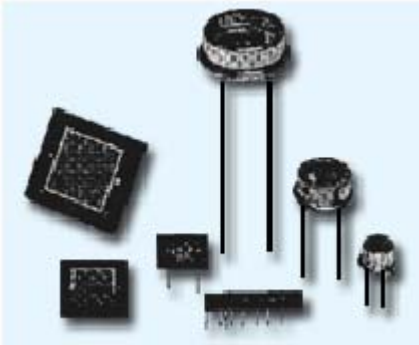
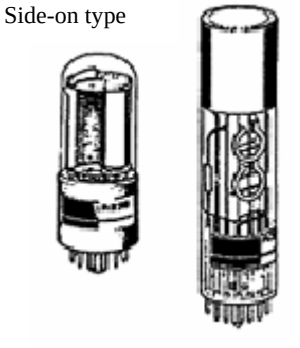
A container that contains a sample is usually called "cell"; two types are available, glass and quartz cells. Since light in the ultraviolet range with a wavelength of 340 nm or less hardly passes through a glass cell, it is used for measurement in the visible range of 340 nm or more. On the other hand, although a quartz cell allows passage of light in the entire wavelength in the ultraviolet and visible ranges, it is mainly used for the measurement in the ultraviolet range due to its high price.



3-3-4. Detector

A detector plays a role in changing the light transmitted from a sample into an electric signal.

a) Photo cell b) Types of photomultiplier, etc. are available.

Photo cell	Photomultiplier
A photo cell sensitive only in the visible range is used. A selenium (Se) photo cell is a representative one. It is small and also sensitive to light with a relatively short wavelength. 	This combines a photoelectric tube and an amplifier (approximately 10-fold amplification) sensitive in both the ultraviolet and visible regions, and the sensitivity can be changed sharply by increasing the applied voltage. The sensitivity decreases in the long wavelength range (900 nm or more). 

4. Keywords

Light:	A kind of electromagnetic wave. Light refers to the wavelength ranges of ultraviolet, visible, and infrared ranges.
Ultraviolet range:	Wavelength range of 200-400 nm.
Visible range:	Wavelength range of 400-800 nm. Visible range appears as a color to our eyes.
Infrared range:	Wavelength range of more than 800 nm.
White light:	Light that contains the light of visible to near-infrared ranges.
Monochromatic light:	Light of one wavelength.
Complementary colors:	Light paired with the color absorbed by a substance.
Transmittance rate (%T):	Passage of light through a substance expressed as percent. Transmittance rate, not expressed as percent, is called transmittance.
Absorbance (Abs):	Degree to which a substance absorbs light. Absorbance (Abs) is expressed as the common logarithms of the inverse of transmittance.
Lambert-Beer's law:	"The absorbance of a solution is proportional to the concentration of the solution and the thickness of the solution layer."
Quantitative analysis:	An assay to determine the concentration of a component of interest in a sample.
Coloring reagent:	A reagent that colors the component of interest in a sample.
Calibration curve:	A curve that indicates the relationship between the concentration of a measured component of known concentration and the measurements (transmittance, absorbance, etc.).
Spectrum:	A curve that indicates the relationship between each wavelength of the measured samples and measurements (transmittance, absorbance, etc.).
Single beam system:	A system that replaces a sample and a control solution into the same beam of light to measure each solution, and measures absorbance etc. from the ratio.
Double beam system:	A system that splits the light from a light source into two, places a sample into one sample beam, places a control solution into the control beam of light on the other side, measures each simultaneously, and measures absorbance etc. from the ratio of measurements.
Monochromator:	A device that disperses light and obtains monochromatic light. Monochromator is also called a spectroscope.
Single monochrome system:	A system that disperses white light with one spectroscope.
Double monochrome system:	A system that further disperses the light, dispersed with the first spectroscope, with the second spectroscope.
Filter:	An optical element that allows the passage of only a light of a specific wavelength from white light.
Diffraction grating:	One of the optical elements that disperse white light. Diffraction grating allows dispersion of an extensive wavelength range.
Sample cell:	A container to hold a sample for measurement. Quartz and glass cells are available.