Spectrophotometry

Spectrophotometry is a method used to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength. This measurement can also be used to measure the amount of a known chemical substance. Spectrophotometry is one of the most useful methods of quantitative analysis in various fields such as chemistry, physics, biochemistry, material and chemical engineering and clinical applications. In biochemistry, for example, it is used to determine enzyme-catalyzed reactions. In clinical applications, it is used to examine blood or tissues for clinical diagnosis. There are also several variations of the spectrophotometry such as atomic absorption spectrophotometry and atomic emission spectrophotometry.

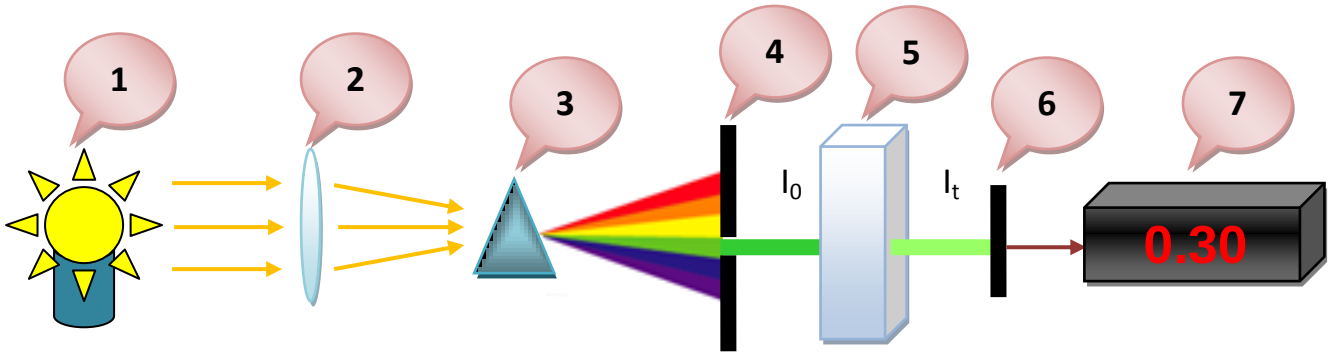
A spectrophotometer is an instrument that measures the intensity of light absorbed after it passes through sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can also be determined by measuring the intensity of light detected. Depending on the range of wavelength of light source, it can be classified into two different types:

- UV-visible spectrophotometer: uses light over the ultraviolet range (185 - 400 nm) and visible range (400 - 700 nm) of electromagnetic radiation spectrum.
- IR spectrophotometer: uses light over the infrared range (700 - 15000 nm) of electromagnetic radiation spectrum.

In visible spectrophotometry, the absorption or the transmission of a certain substance can be determined by the observed color. For instance, a solution sample that absorbs light over all visible ranges (i.e., transmits none of visible wavelengths) appears black in theory. On the other hand, if all visible wavelengths are transmitted (i.e., absorbs nothing), the solution sample appears white. If a solution sample

absorbs red light (~700 nm), it appears green because green is the complementary color of red. Visible spectrophotometers, in practice, use a prism to narrow down a certain range of wavelength (to filter out other wavelengths) so that the particular beam of light is passed through a solution sample.

Spectrophotometer consists of a light source, a collimator, a monochromator, a wavelength selector, a cuvette for sample solution, a photoelectric detector, and a digital display or a meter.



1: Light source.

2: Collimator (lens).

3: Monochromator (prism).

4: Wavelength selector (slit).

5: sample container (cuvette).

6: Detector.

7: Meter (digital or scale display).

I_0: incident light and I_t: transmitted light.

The amount of photons that goes through the cuvette and into the detector is dependent on the length of the cuvette and the concentration of the sample. Once you know the intensity of light after it passes through the cuvette, you can relate it to transmittance (T).

Transmittance is the fraction of light that passes through the sample. This can be calculated using the equation:

$$\text{Transmittance (T)} = I_t / I_0$$

Where I_t is the light intensity after the beam of light passes through the cuvette and I_o is the light intensity before the beam of light passes through the cuvette. Transmittance is related to absorption by the expression:

$$\text{Absorbance (A)} = -\log (T) = -\log (I_t / I_0)$$

With the amount of absorbance known from the above equation, you can determine the unknown concentration of the sample by using Beer-Lambert Law which states that there is a linear relationship between the absorbance and the concentration of a sample. Beer's Law is written as:

$$A = \epsilon l c$$

- A is the measure of absorbance (no units).
- ϵ is the absorption coefficient.
- l is the path length.
- c is the concentration.

The absorption coefficient is given as a constant and varies for each molecule. Since absorbance does not carry any units, the units for ϵ must cancel out the units of length and concentration. As a result, ϵ has the units: $L \cdot mol^{-1} \cdot cm^{-1}$. The path length is measured in centimeters. Because a standard spectrometer uses a cuvette that is 1 cm in width, l is always assumed to equal 1 cm. Since absorption, ϵ, and path length are known, we can calculate the concentration of the sample.