Spectrophotometry

Spectrophotometry is a method to measure how much a chemical substance absorbs light (at one or more wavelengths) by measuring the intensity of light as the beam of light passes through a sample solution. This depends on the concentration of that chemical substance. The basic principle is that **each compound absorbs light over a certain range of wavelength.**

Spectrophotometer is an instrument that measures the intensity of light after it passes through a sample solution.

Figure 1. Spectrophotometer device

Depending on the range of wave length of the light source, spectrophotometers can be classified into two different types (see Fig. 2):

- **UV range** spectrophotometer: Uses light over the ultraviolet range, and wave length ranges between 185 - 400 nm.
- **Visible range** spectrophotometer**:** Uses a tungsten light range and wave length ranges between 400 - 700 nm.

Note: Notation of wavelength is Lambda (λ)

Nanometer: It is the unit of measuring wavelength in nano-meter (nm = $1*10^{-9}$ m).

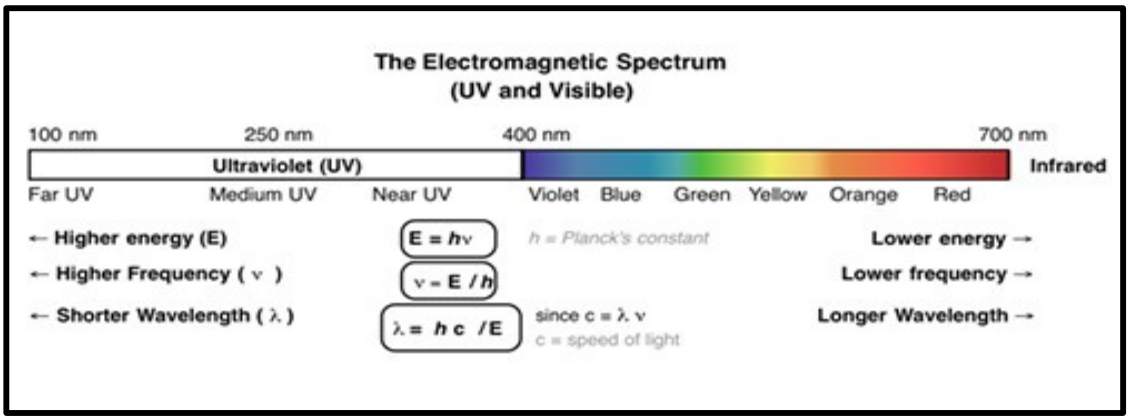

Figure 2. Range of wave lengths of spectrophotometers

The need for a spectrometer is to produce various wavelengths since the absorbance depends on the compatibility of components with different wavelengths. For example, the highest absorption of p-nitrophenol (acid form) occurs at approximately 320 nm, while p-nitrophenolate (basic form) takes place at 400 nm. See Fig.3.

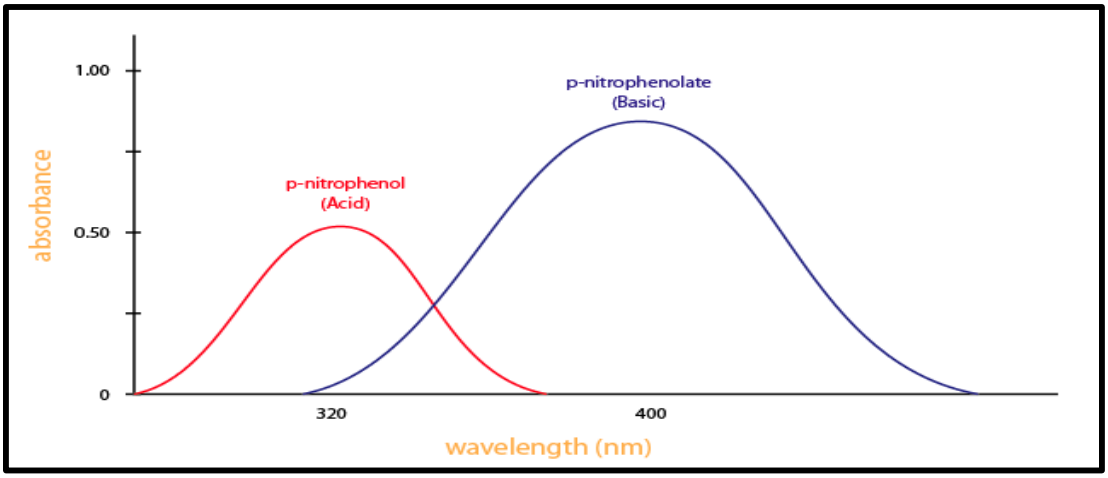

Figure 3. Peak absorbances of p-Nitrophenol (acid form) and p-Nitrophenolate (basic form)

Principles of Spectrophotometry

Spectrophotometry depends on Beer-Lambert Law

Beer-Lambert Law (also known as Beer's Law) states that there is a linear relationship between the absorbance and the concentration of the substance in the sample solution.

The equation below represents the origin of absorbance:

$$A = a\ b\ c$$

where

- ***A*** **:**indicates the measure of absorbance (without unit).
- ***a*** **:**is the molar extinction coefficient or molar absorptivity (or absorption coefficient).
- ***b*** :is the path length
- ***c*** :represents the concentration.

Device Contents

It consists of:

1. **Light source**: It is usually a tungsten lamp, where the light emits in the visible range only.
2. **Monochromator**: It is a coloured glass filter that absorbs most of the light and allows only light of the complementary colour with a sufficiently narrow wavelength. It should be noted that the selection of filters depends on the colour of the solution under test.
3. **Wavelength selector**.
4. **Cuvette for sample solution**: These are glass tubes of usually 1-cm diameter and uniform thickness in which absorbance is measured.
5. **Photoelectric detector**: Either a photocell or a phototube may be used to convert the transmitted light into electrical energy.
6. **Digital Display unit**.

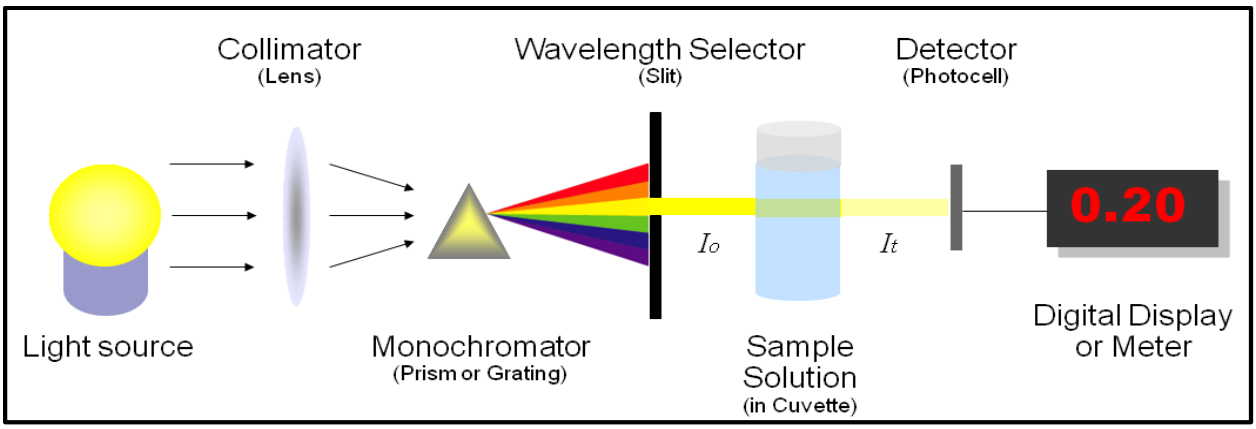

Figure 4. The components of the spectrophotometer

Table1. Complementary colours of the filters used in a spectrophotometer

Colour of solution	Colour of filter	Wavelength range (nm)
Bluish green	Red	650–700
Green blue	Orange	600–650
Blue	Yellow	575–600
Violet	Yellow green	555–575
Purple	Green	505–555
Red	Blue green	495–505
Orange	Green blue	475–495
Yellow	Blue	430–475
Yellowish blue	Violet	350–430

The intensity of incident light is symbolized as (I_0) which is the original light or "total light". When the light passes through a blank solution, it is only minimally absorbed and is symbolized as transmitted light (I_t). There are important values which are Transmittance **(T)** Absorbance **(A).**

$$\mathbf{T = \frac{I_t}{I_0}}$$

$$\mathbf{A = -\log_{10} T}$$

$\mathbf{I_t}$ = the intensity of light after passing through the cuvette.
$\mathbf{I_0}$= the intensity of light before passing through the cuvette.

In spectrophotometry, we need to measure the intensity of light that crossed a blank solution, and then measure the intensity of light that crossed or passed through a sample.

Calculation of Transmittance and Absorbance

The number of photons transferred or absorbed totally is dependent on the concentration of the sample and the length of the cuvette .

The transmittance and absorption relation is:

Absorbance (A) = −log (T)

–log (T)= - log (l_t/l_0)

The absorbance of an unknown sample can be calculated using the formula given below.

$$C\ (Test) = \frac{\text{A (Test)} - \text{A (Blank)}}{\text{A (Standard)} - \text{A (Blank)}} \ * \ C\ (Standard)$$

Or by symbols: $C\ (T) = \frac{\text{A (T)} - \text{A(B)}}{\text{A (S)} - \text{A(B)}} \ * \ C(ST)$

where :

C (T) or C (Test) = the concentration of the sample
C (ST) or C (Standard) = the concentration of the standard
A (T) or A (Test) = absorbance of the test
A (ST) or A (standard) = absorbance of the standard
A (B) or A (Blank) = absorbance of the blank

In an experiment, three different solutions are prepared in three tubes and are referred to as ‘Blank’, ‘Test’, and ‘Standard’ and marked by the first capital letter of each word as B, T, and S, respectively.

Sample (test): is made from the biological fluid being analyzed and subjected to all steps of the analysis to determine the concentration of an analytic in it.

Standard solution (s) (ST): are defined as the solutions that contain a known accurate amount (s) (i.e. concentration) of a substance or element. These solutions are used to compare a known concentration of a substance with the unknown concentration of the same substance in the sample.

Blank solution (B): Is a solution that contains all of the reagents needed in analysis of a substance except the substance under test or the standard.

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