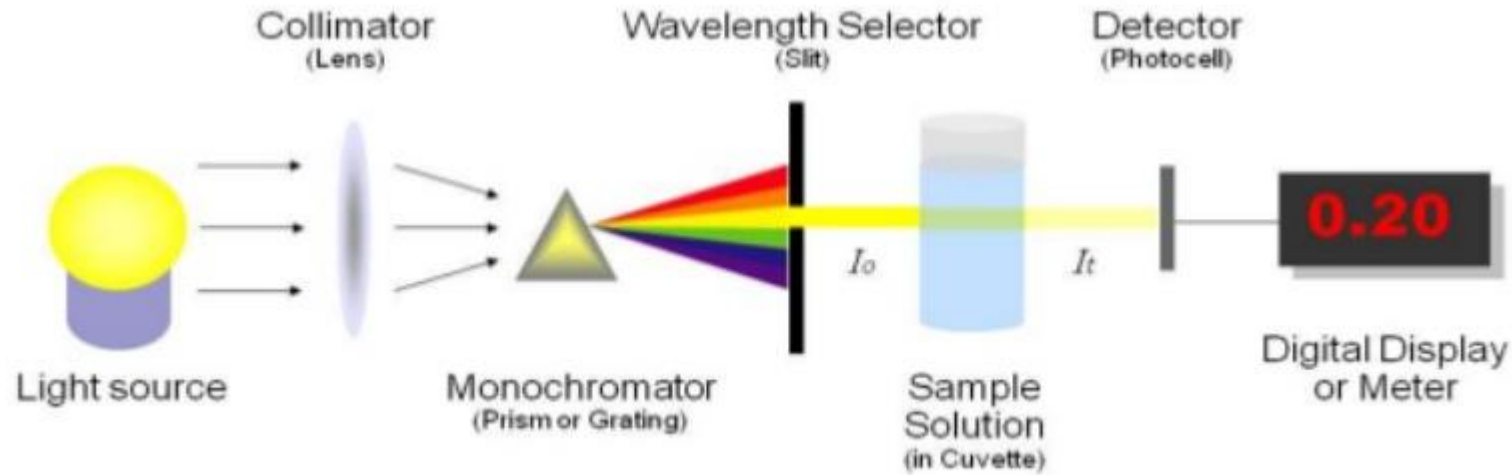


Spectrophotometry

Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution.

Principle



When a light passes through a solution, a certain fraction is absorbed. This fraction is detected, measured and used to relate the light absorbed or transmitted to concentration of substance. The light intensity is measured as a function of wavelength.

The intensity of light is symbolized as I_0 measure the number of photons per second. When the light is passed through the blank solution, it does not absorb light and is symbolized as (I) . Other important factors are Absorbance (A) and Transmittance (T).

$$T = I/I_0$$

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

Here, we need to measure the intensity of light that passes a blank solution, and later measures the intensity of light passing a sample.

Calculate the transmittance and the absorbance. A number of [protons](#) transmit and absorb totally depended on the length of the cuvette and the concentration of the sample.

The transmittance and absorption relation is:

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

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

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

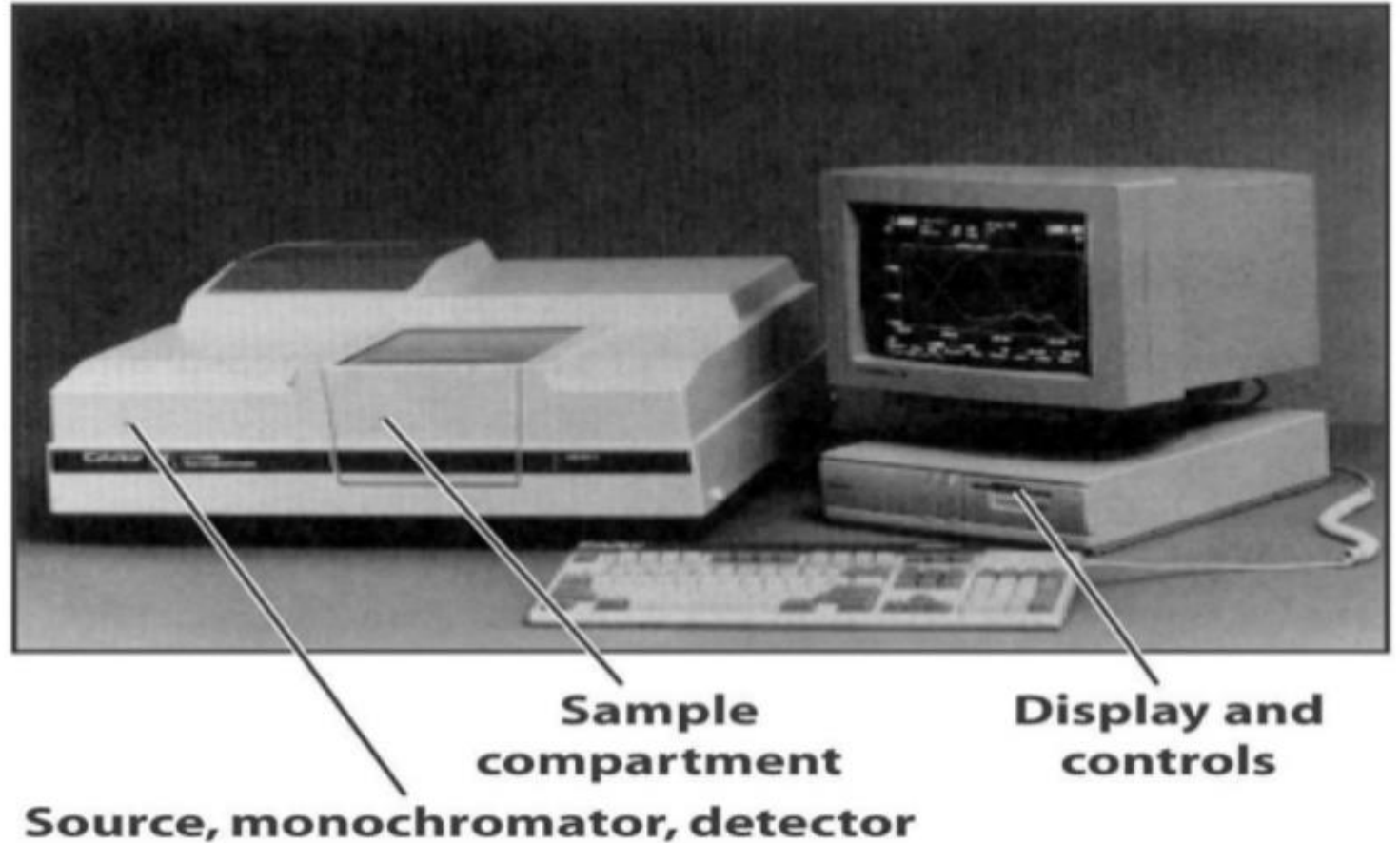
Here,

I_t = Light intensity after
passing via cuvette

I_0 = Light intensity before
passing via cuvette

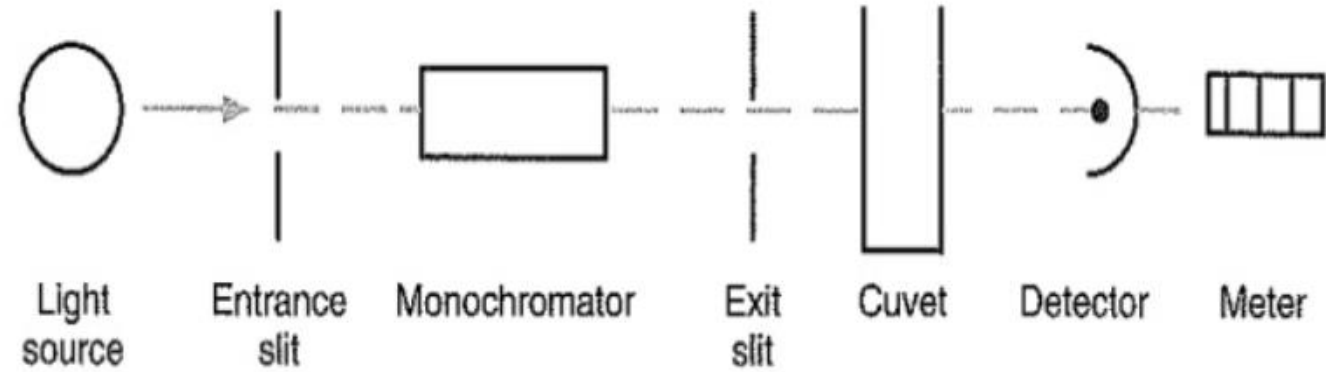
Instrumentation

- A light source
- A means to isolate a light of desired wavelength
- Fiber optics
- Cuvets
- A photodetector

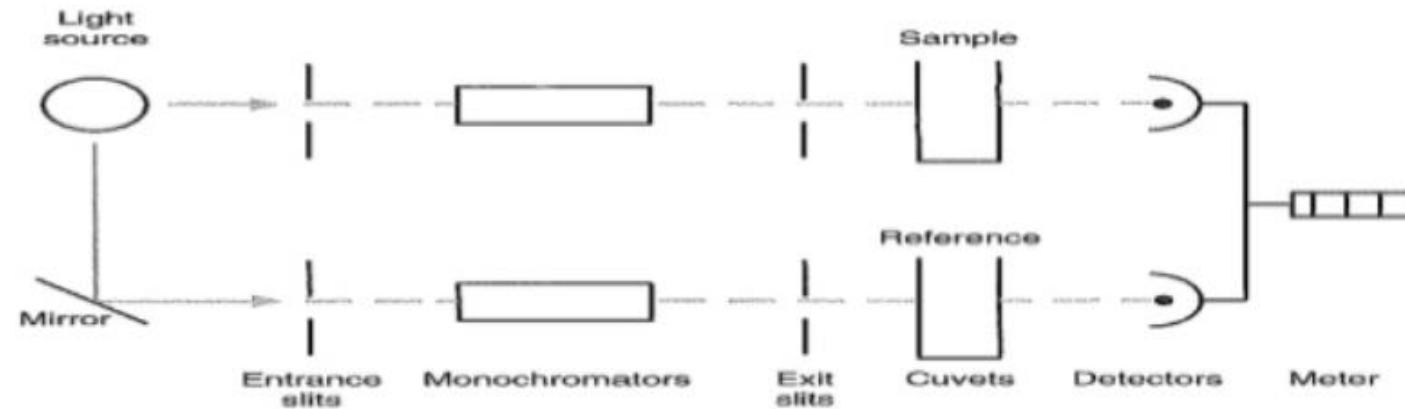


Types of spectrophotometer

The Single Beam Spectrophotometer:



Double-beam-in-space Spectrophotometer:



Double-beam-in-time Spectrophotometer:

