

# Experiment (1)

## SPECTROPHOTOMETER

### ➤ The Objective:

The spectrophotometer is a device designed to measure the amount of light absorbed by a solution at various wave lengths and determine the transmitted light.

### ➤ Theory:

A spectrophotometer is an electromechanical device that can optically determines the absorbance or transmission of characteristic wavelengths of radiant energy (light) by a chemical species in solution. According to the Beer-Lambert law, the amount of light absorbed at these wavelengths is directly proportional to the concentration of the chemical species. As light passes through a compound in solution the intensity is reduced. The longer the path length the more light is absorbed. Also, the higher the concentration of compound in solution the more light is absorbed. Absorbance is proportional to path length and the concentration. When light is absorbed by a sample, the radiant power or intensity of the light beam decreases.

### ➤ The Configuration and Parts of the System:

All spectrophotometers have the following fundamental parts:

- A light source.
- The dispersion devices (a prism or grating )
- Focusing devices (lenses, slits, mirrors)
- A device for holding the sample (cuvettes).
- A photoelectric cell for measuring light intensity (detectors).
- Display devices. See Fig.(1)a,b

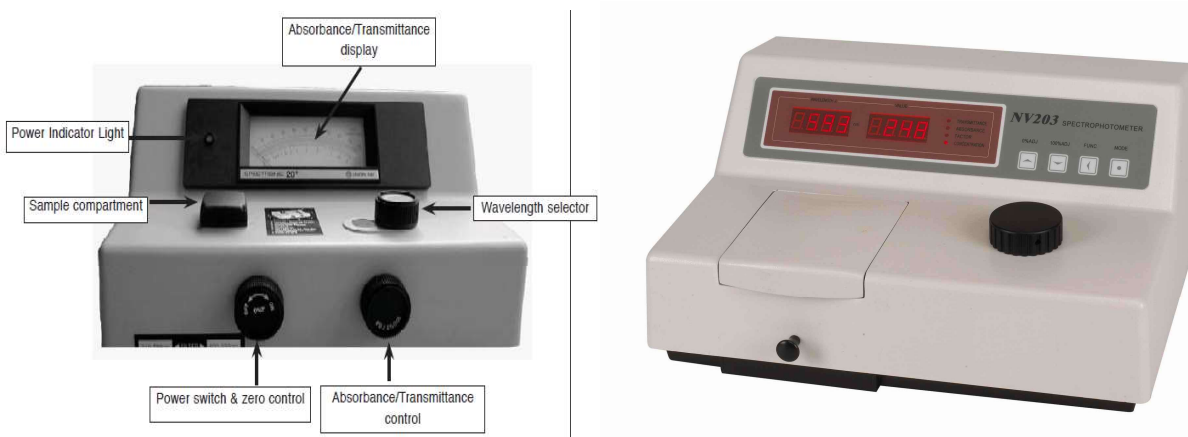


Fig.(1) spectrophotometer device

## ➤ System Block Diagram

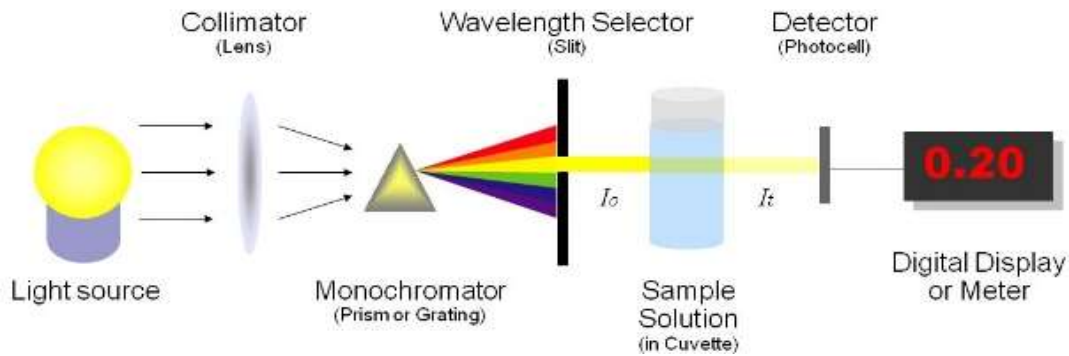


Fig.(2) Spectrometer Block Diagram

## ➤ The Principle Work of the System

First, a light source generates light at a specific wavelength or wavelengths, the light passes through a dispersion device that separates the light into its component wavelengths. Slits then isolate a bandpass (the segment of the spectrum isolated by the monochromator) of the wavelength needed for measurement, improving its purity. Next, the light passes through the sample, and a portion of the radiant energy is absorbed; the amount depends on the nature and concentration of the sample. The portion of the bandpass that is not absorbed is transmitted to a photodetector, which converts light energy to electrical energy that can be registered on a meter or digital readout.

## ➤ The Procedure of Operation



Fig. (3) Spectrophotometer Laboratory System

**A.** Turn on the spectrophotometer (left-hand knob on the front of the instrument (**A**)) and allow it to warm up for at least 15 min.

**B.** Adjust the wavelength to the appropriate value. The knob on the right top (**C**) of the instrument controls the wavelength, which is indicated at the left of the digital display.

**C.** With the sample holder empty and the lid closed, adjust the Zero Adjust Knob (**A**) until the instrument reads 0% on the transmittance scale. Be sure that the display function is set to transmittance, if not push the "Mode" button until the display is set to transmittance.

**D.** Carefully insert the appropriate blank tube (cuvette) into the sample holder (**E**) and close the cover. Be sure you are using a cuvette with white markings. The cuvette's outside surface must be dry and clean, including free of fingerprints!! Use a Kim wipe to clean the cuvette before inserting. The white markings should line up with the notch on the sample holder. It is important to line up the markings. The cuvettes will be scratched otherwise.

**E.** Adjust the 100% Adjust Knob (**B**), on the right front of the instrument, until the display reads 100% on the transmittance scale.

**F.** Remove the blank cuvette and immediately insert the sample cuvette as described in step (**D**) above. Do not change any instrument setting! Switch the display to read absorbance by pushing the "Mode" (**D**) button.

**G.** Record the value indicated on the absorbance scale.

**H.** Repeat this procedure for additional cuvettes or wavelengths as required. Always adjust the blank transmittance to 100% before inserting and reading a new set of cuvettes.

## ➤ **Discussion**

- 1- Give the relationship between absorbance and transmittance.
- 2- What is the specification of light in the system?
- 3- What is the purpose of detector?
- 4- Why the system uses monochromator?