Spectrophotometer

Spectrophotometer is an instrument that measures the amount of photons (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:

Types of Spectrophotometer: أنواع المطياف الضوئي

1- **UV (Ultraviolet) visible spectrophotometer:** مقياس المطياف الضوئي فوق البنفسجي المرئي

uses light over the ultraviolet range (180-400 nm) and visible range (400-750 nm) of electromagnetic radiation spectrum.

2- **IR (Infrared) spectrophotometer:**

uses light over the infrared range (750 - 15000 nm) of electromagnetic radiation spectrum.

- **Single beam spectrophotometer:**

It operates between 325-1000 nm wavelength using only a light beam. The light travels in a single direction, and the test solution and the blank solution are read in the same cuvette one after another.

- **Double beam spectrophotometer:**

It works between 185-1000 nm wavelength. The two photocells split the light from the monochromator into two beams. A beam is used for reference, and another is used for the reading samples.

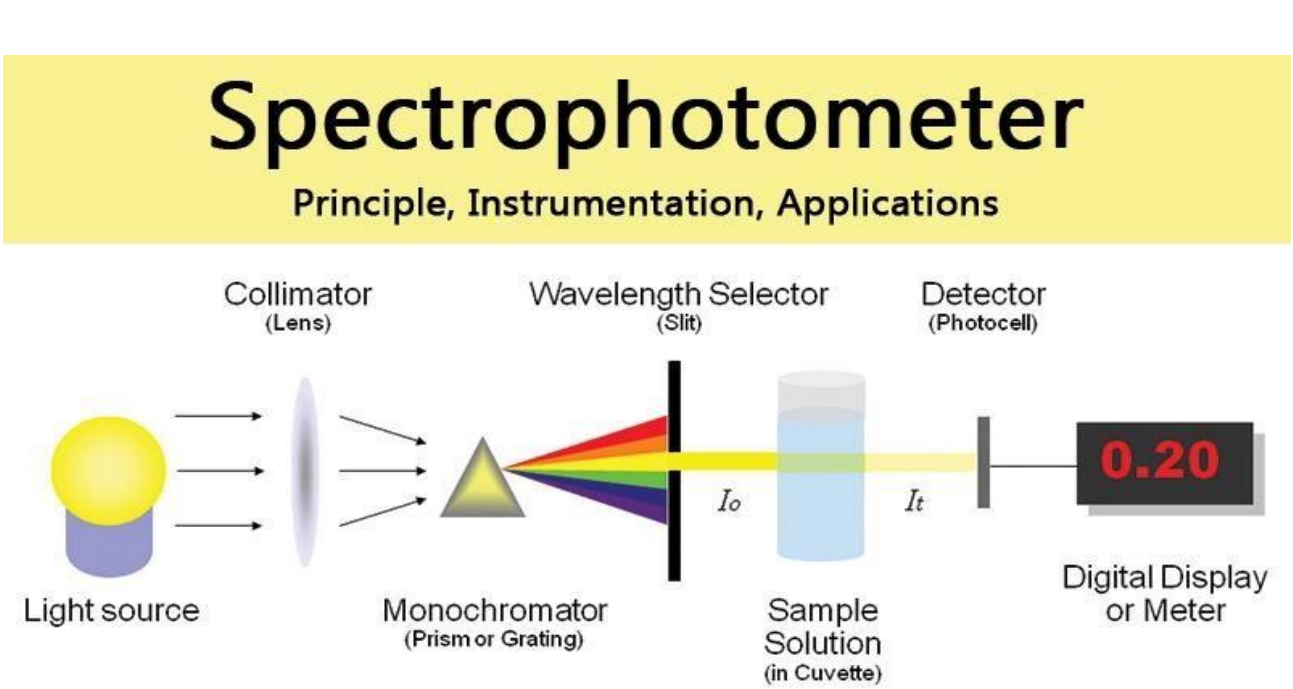


Figure (1): Component of Spectrophotometer

Parts of the spectrophotometer: there are six parts in the spectrophotometer

أجزاء جهاز المطياف الضوئي

1- light sources مصدر الضوء

2- condensing lens or collimator lens العدسة المكثفة

3- monochromator احادي لون

4- sample holders (Cuvettes) حامل العينة

5- sample detectors كاشف العينة

6- Recorder المسجل

Components of Spectrophotometer

1- **Light source: -** there are three different sources of light are commonly used to produce light of different wavelength. The most common source of light used for the visible spectrum is a **tungsten lamp.** For Ultraviolet radiation, commonly used sources of are the **hydrogen lamp** and the **deuterium lamp.** The deuterium lamb gives wider and more intense light in UV region than hydrogen lamp. Nernst filament or globar is the most sources of IR (Infrared) radiation.

2- **Collimator lens:** a collimator consists of a curved mirror or lens. This can be used to replicate a target focused to make collimated light or parallel rays.

3- **Monochromator:** to select the particular wavelength, prism or diffraction grating is used to split the light from the light source.

4. **Sample holder (cuvettes):** test tube or cuvettes are used to hold the colored solutions. They are made up of **plastic or glass or quartz** at a visible wavelength.

5. **Detector:** When light falls on the detector system, an electric current is generated that reflects the galvanometer reading.

6. **Recorder:** The current from the detector is fed to the measuring

device, the galvanometer. The meter reading is directly proportional to the intensity of light.

Uses of Spectrophotometer استخدامات الجهاز المطياف الضوئي

The uses of a spectrophotometer are as follows:

1- A spectrophotometer is used for the quantitation of nucleic acid (DNA or RNA). يستخدم جهاز المطياف لتقدير كمية الحمض النووي

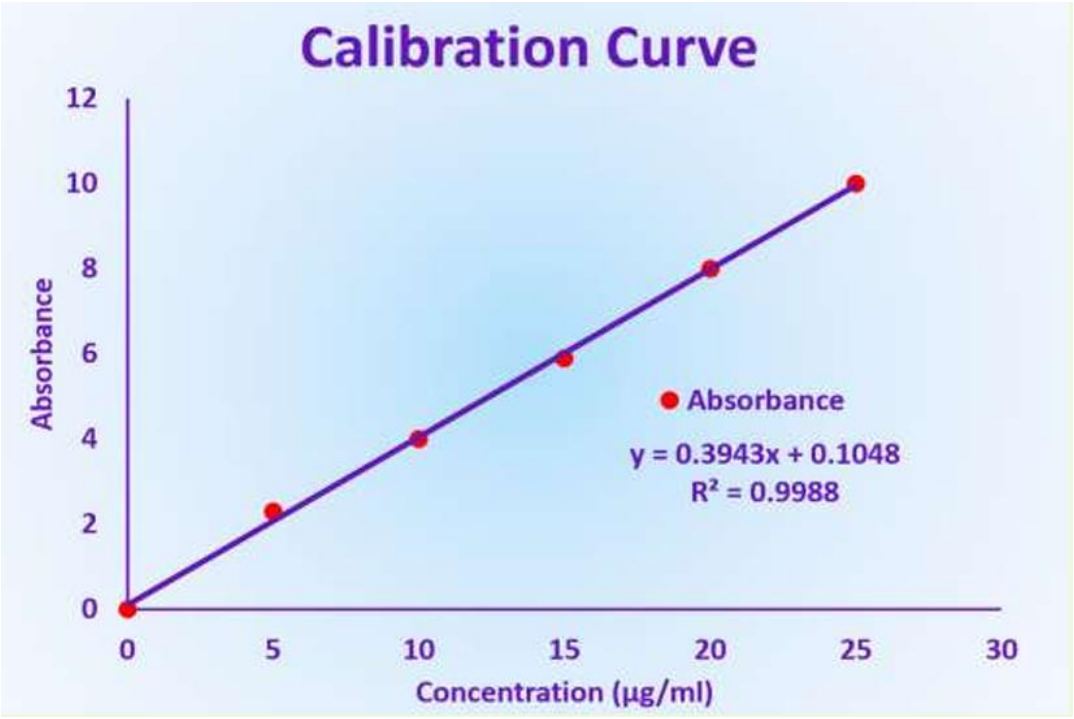
2- It determines the concentration of color and colorless compounds by measuring the absorbance of the solution. It can also determine the concentration of biological materials like nucleic acid and proteins.

3- Similarly, it determines the phase of reaction by measuring the formation and disappearance rate of the light-absorbing compounds in the range of the visible and UV region of the electromagnetic spectrum.

4- It also identifies compounds by determining the absorption spectrum in the visible region of the light and the UV region of the electromagnetic spectrum.

The calibration curve:

The calibration curve is a plot of instrumental signal vs. concentration. The plot of the standards should be linear, and can be fit with the equation y=mx+b, where m is the slope of the line and b is the intercept. x and y represent the distance of the line from the x-axis and y-axis, respectively. 4 The non-linear portions of the plot should be discarded, as these concentration ranges are out of the limit of linearity.



Blank solution and Standard solution

Blank solution: محلول الفارغ is a solution containing all the solution component except the one substance that interested, usually used to calibrate instruments such as a colorimeter .to use blank solution in spectrophotometer we have to adjust the meter needle of transmittance to (100 XT) and the absorption will be 0.

Standard solution:المحلول المعياري is a solution containing a known concentration of an element or a substance. the standard solution are used to determine the concentrations of other substances.

Preventive Measures: إجراءات وقائية

1. The spectrophotometer should be turned on 10 to 15 minutes before use.
2. The device should be calibrated each time.
3. The selected wavelength should be the maximum wavelength that the solution can absorb.
4. The sample used should not contain any substance which can dissociate, react or change during the measurement.
5. Since the absorbance depends on the concentration, sample preparation should be within the acceptable concentration range.

Figure (2): Spectrophotometer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