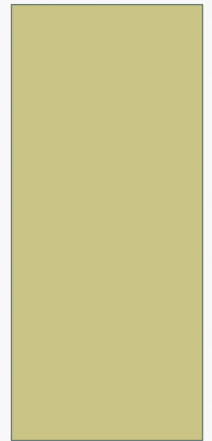


SPECTROPHOTOMETER I

LAB 3

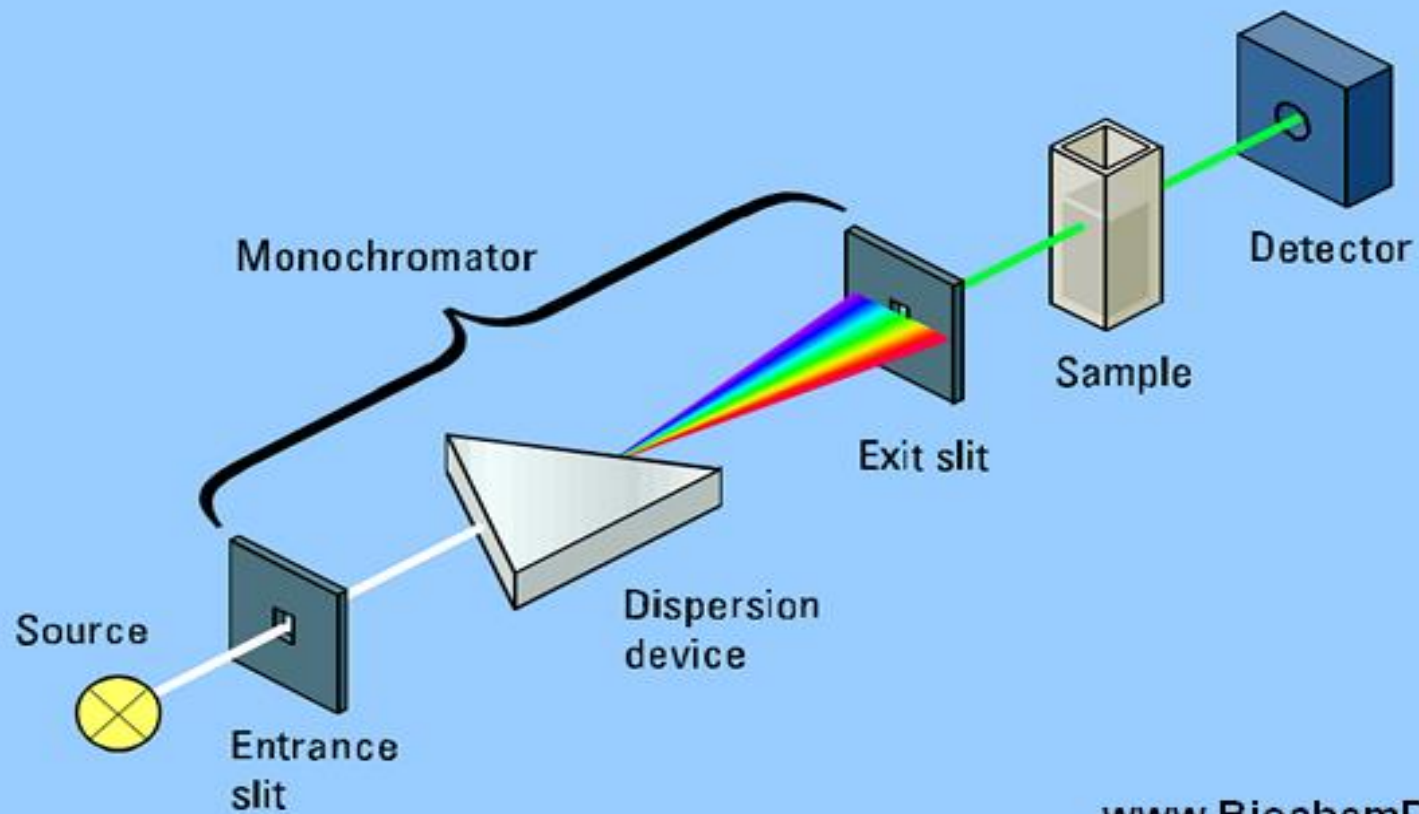


DEFINITION

- A spectrophotometer is an instrument that measures the amount of light absorbed by a sample.
- Spectrophotometer techniques are used to measure the concentration of solutes in solution by measuring the amount of the light that is absorbed by the solution in a cuvette placed in the spectrophotometer



COMPONENTS OF SPECTROPHOTOMETER



COMPONENTS OF SPECTROPHOTOMETER

- **Light Source** : to provide a sufficient of light which is suitable for marking a measurement.
- 1- Tungsten Lamp
- 2- Hydrogen Lamp
- 3- Xenon Lam
- Tungsten Lamp :It is the most common light source used in spectrophotometer wavelength , range of about 330 to 900 nm

DISPERSION DEVICES

Dispersion devices

*Monochromator Accepts polychromatic input light from a lamp and outputs monochromatic light.

- Dispersion devices causes a different wavelength of light to be dispersion at different angles.
- Types of Dispersion devices used are
 - 1- Prism
 - 2- Filters
- Prism is used to isolate different wavelength.
- Filters separate different parts of the electromagnetic spectrum by absorbing or reflecting certain wavelengths and transmitting other wavelengths.

CUVETTES

- Absorption cells(Cuvettes) A cuvette is a kind of cell (usually a small square tube) sealed at one end, made of Plastic, glass or optical grade quartz and designed to hold samples for spectroscopic experiments



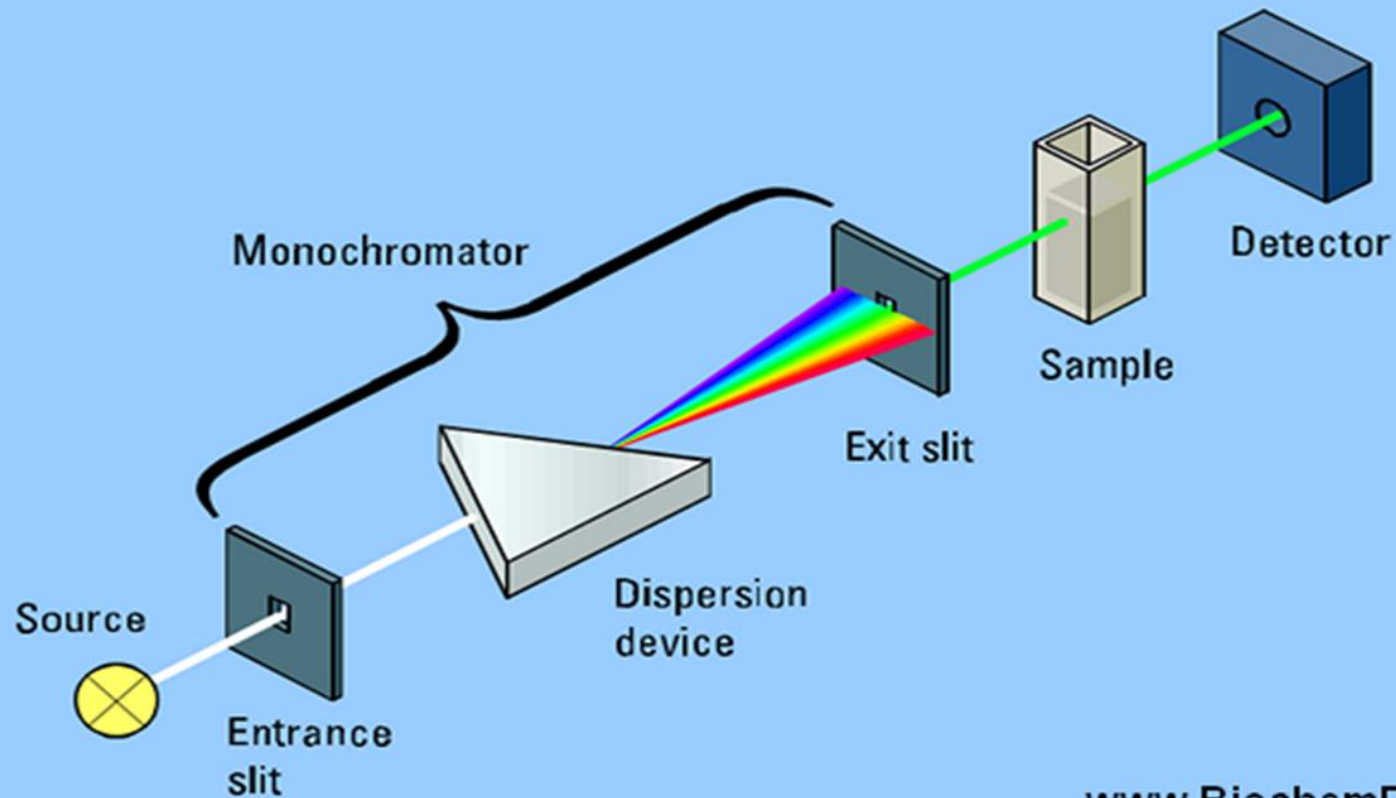
DETECTORS

- **Detectors** Any photosensitive device can be used as a detector of radiant energy .The photocell and phototube are the simplest photo detectors, producing current proportional to the intensity of the light striking them.

DISPLAY DEVICE

- **Display devices** The data from a detector are displayed by a readout device, such as an analog meter, a light beam reflected on a scale, or a digital display , Or liquid crystal display(LCD) .The output can also be transmitted to a computer.

COMPONENTS OF SPECTROPHOTOMETER



THE WORK OF SPECTROPHOTOMETER

- 1.A sample solution is placed inside the spectrophotometer.
- 2.A light source shines light toward the sample.
- 3.A monochromator splits the light into each color, or rather, individual wavelengths. An adjustable slit allows only one specific wavelength of light through to the sample solution.
- 4.The wavelength of light hits the sample, which is held in a little container called a cuvette.
- 5.Whatever light passes through the sample is read and displayed on the output screen.

* استخلاص الكلوروفيل بطريقة الغمر في المذيب:-

- ١ - ضعي ٣ جرام ورق طازج في بيكر (سجلي الوزن بالضبط) .
 - ٢ - اضيفي اليها ٣٠ مل اسيتون ٨٠ % ثم اقلبي البيكر باحكام مستخدمه ورق الالمونيوم او البلاستيك حتى يمتع
فقد الاسيتون بالتطاير.
 - ٣ - اتركي التسيج متقوفا ومغمورا في الاسيتون لمدة ١٥ دقيقة مع الهز اليدوي البسيط مره كل ٥ دقائق.
 - ٤ - انقلي المستخلص الاسيتوني فقط الي انبوية اختيار جديد.
- ضعي المستخلص في الانابيب الخاصة لجهاز الطرد المركزي ويتم تسجيل قراءة الكثافة الضوئية التي رمزها O.D للمستخلص بعد وضعه في الخلايا الزجاجية الخاصة لجهاز السيكتروفوتوميتر ويتم استخدام مقاييس الطيف الضوئي على الموجات التالية :
- ← ٤٤٠ الكاروتين C
 - ← ٦٦٣ كلوروفيل A
 - ← ٦٤٥ كلوروفيل B

*** يمكن اتباع الخطوات السابقة باستخدام مذيب اخر وهو ثنائي ميثيل الفورماميد DMF

$$\text{Chlorophyll A} = 12.7 (\text{O.D (A)}) - 2.69 (\text{O.D (B)}) \frac{V}{w \times 1000} \text{ mg/g}$$

$$\text{Chlorophyll B} = 22.9 (\text{O.D (B)}) - 4.68 (\text{O.D (A)}) \frac{V}{w \times 1000} \text{ mg/g}$$

$$\text{Total} = 20.2 (\text{O.D (B)}) + 8.02 (\text{O.D (A)}) \frac{V}{w \times 1000} \text{ mg/g}$$

$$\text{Carotene} = 4.695 (\text{O.D (C)}) - 2.88 (\text{O.D (A)}) + (\text{O.D (B)}) \frac{V}{w \times 1000} \times \text{mg/g}$$

وزن التسيج القطن = W
حجم المستخلص النباتي = V