Lab (9):
Measurement of colors Spectrophotometry

Analytical biochemistry lab

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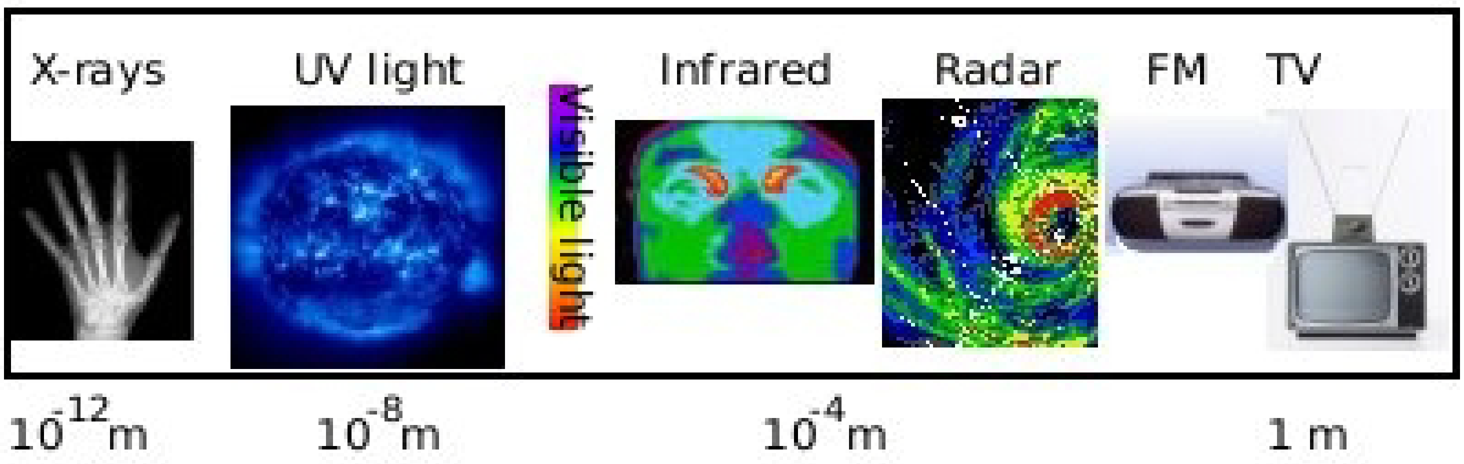
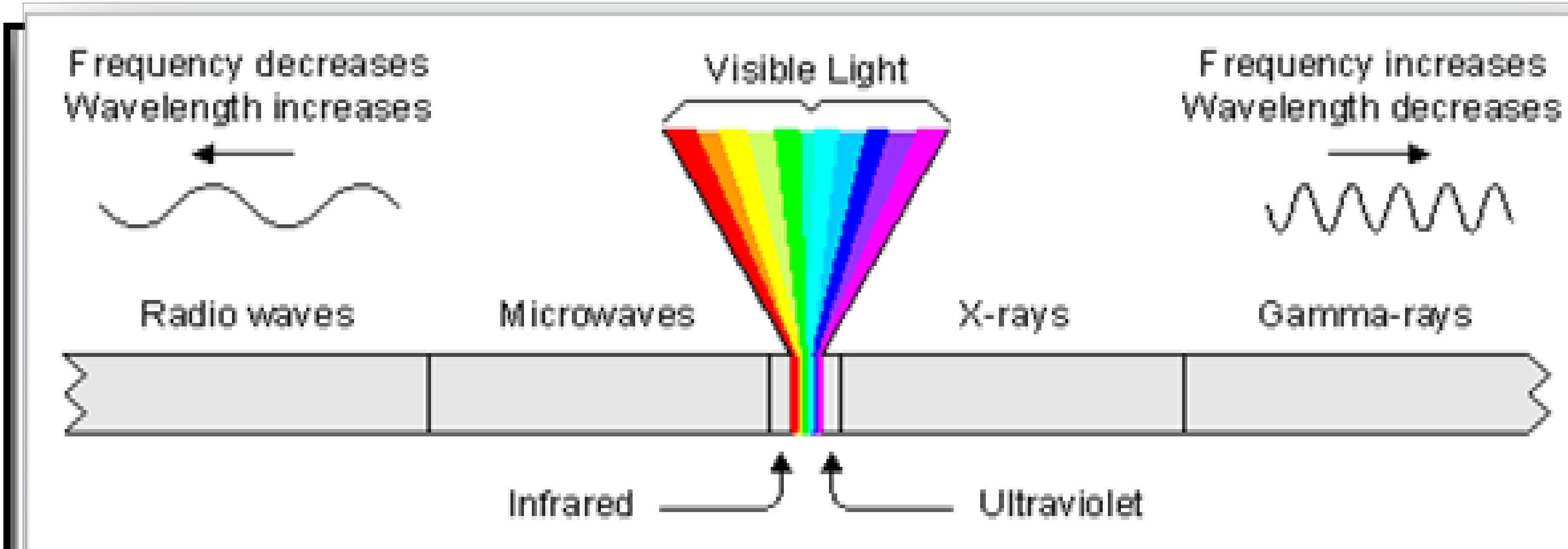
Electromagnetic spectrum (EMS)

- **electromagnetic spectrum (EMS):** is the full range of wavelengths of light

- EMS includes: very low frequency radio-wave, microwaves, infrared, visible and UV light to x-rays and gamma rays.





The visible portion of the electromagnetic spectrum

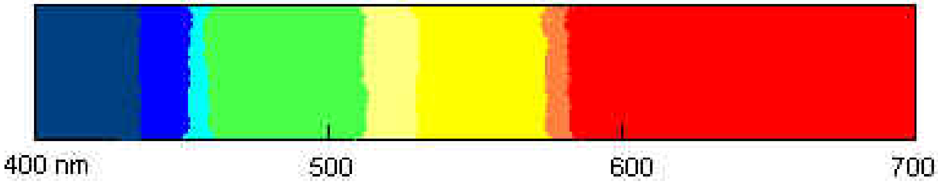
Light

- Is type of electromagnetic radiation consists of different WL.
- is decomposed by **prism** produces **color spectrum**.

Wavelength

- Is the length of light wave which determines its color.
- Commonly designated by Greek letter **Lambda (ʎ).**
- WL measured by (unit of measurement): angstroms, nanometers (nm), or microns.

colour region	wavelength (nm)
violet	380 - 435
blue	435 - 500
cyan	500 - 520
green	520 - 565
yellow	565 - 590
orange	590 - 625
red	625 - 740



Color:

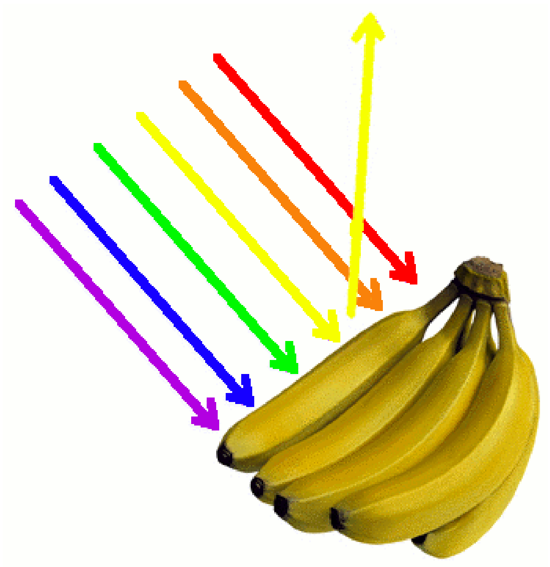
Why colored solution appears colored?

- molecules in solution absorb light of certain wave length (color).
- color (WL) of the residual light (not absorbed), which escapes (reflected or transmitted= not absorbed) enters our eyes, **determine** the color of the object.

So, what color we see depends on WL of light our eyes absorb.

Example:

yellow banana does not have color in itself.
It appears yellow because it absorb other colors except yellow
(reflected yellow is absorbed by our eyes)



- **Black:** absorbs all colors

- **White:** reflects all colors

- **Colorless:** absorb UV (400 nm) and IR (800 nm)
  outside visible region

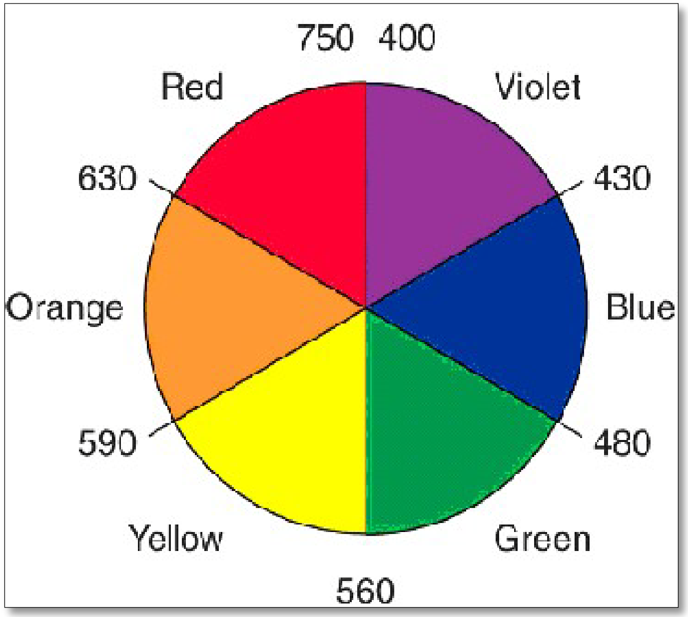
depends on structure of substance

- We see the **complementary** color of the absorbed color.

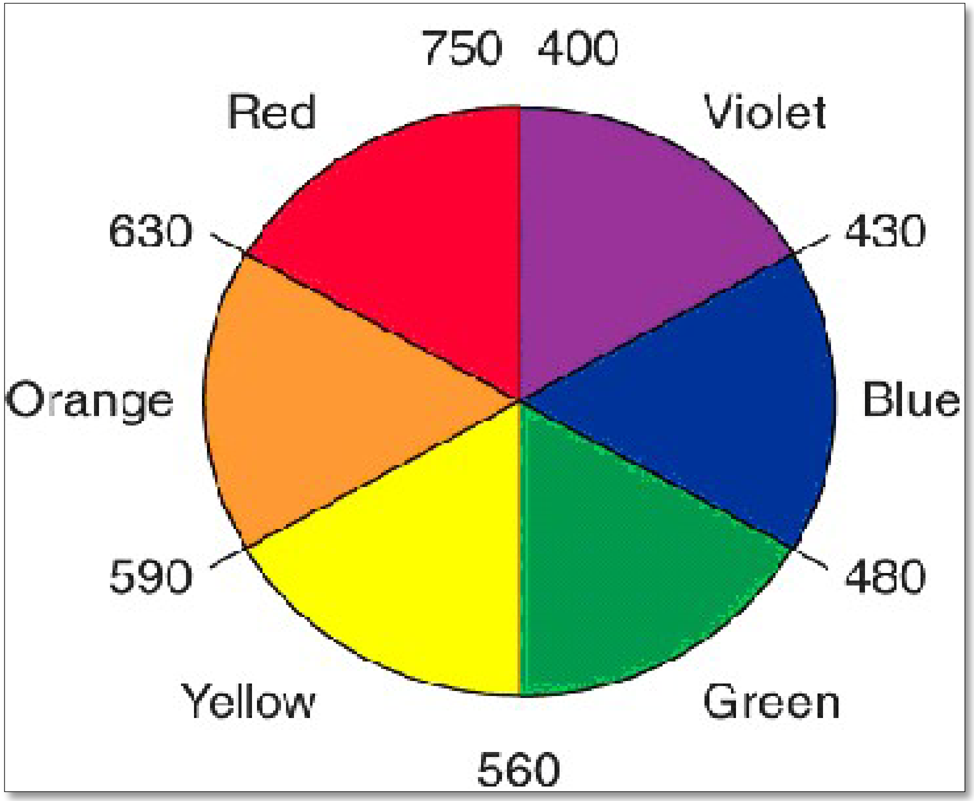
  (complementary color = color that is reflected completely)

- Each color found in visible light spectrum has its own **wavelength.**

- Primary colors: red, yellow, and blue, they from other colors, every other color can be made fro these primary.

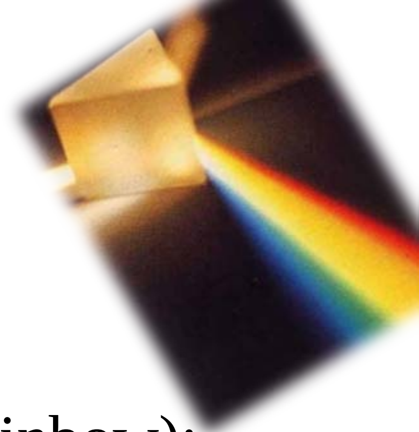


If a solution sample absorbs red light (700nm), it appears green because green is the complementary color of red.



Visible light

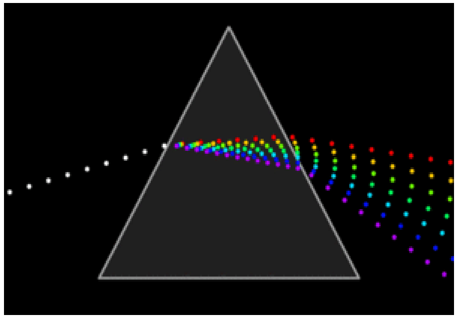
- Visible light is a very small part of the EMS that human eye can only see (400 to 700 nm).



- It is made of seven wavelength groups (colors of rainbow):

Starts from violet **ends** with red:

Red, Orange, Yellow, Green, Blue, Indigo and Violet.



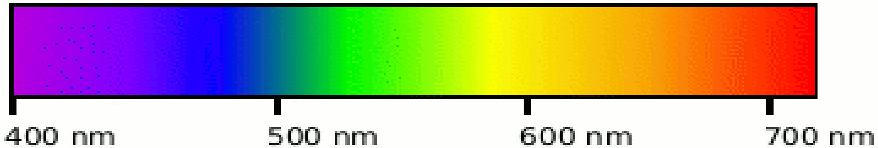
✓reddish color: is the longest WL

✓greenish color: is the mid-size WL

✓violet color: is the shortest WL

- above red WL is called infra-red (long)
- below violet WL is called ultraviolet (short)

Human eyes un able see them. Animals can.



Infrared IR :

- long WL (700 nm) after red in the visible spectrum.

- Infrared IR can be felt, but can't be seen by naked eye.

- Everything emits infrared light. Because of this, **movement** can be **detected** in the dark with infrared detectors.

Ultraviolet:

- short WL of light (below 400 nm) beyond violet in visible spectrum.
- It is often given off by sun (source: sunlight).
- reflected by ozone layer, but some do pass to our atmosphere.
- It damage unprotected skin and known to cause skin cancer.

Colorimetry and Spectrophotometry

- science that measure colors in numbers.
- Measure amount of light that sample absorb using different instruments.

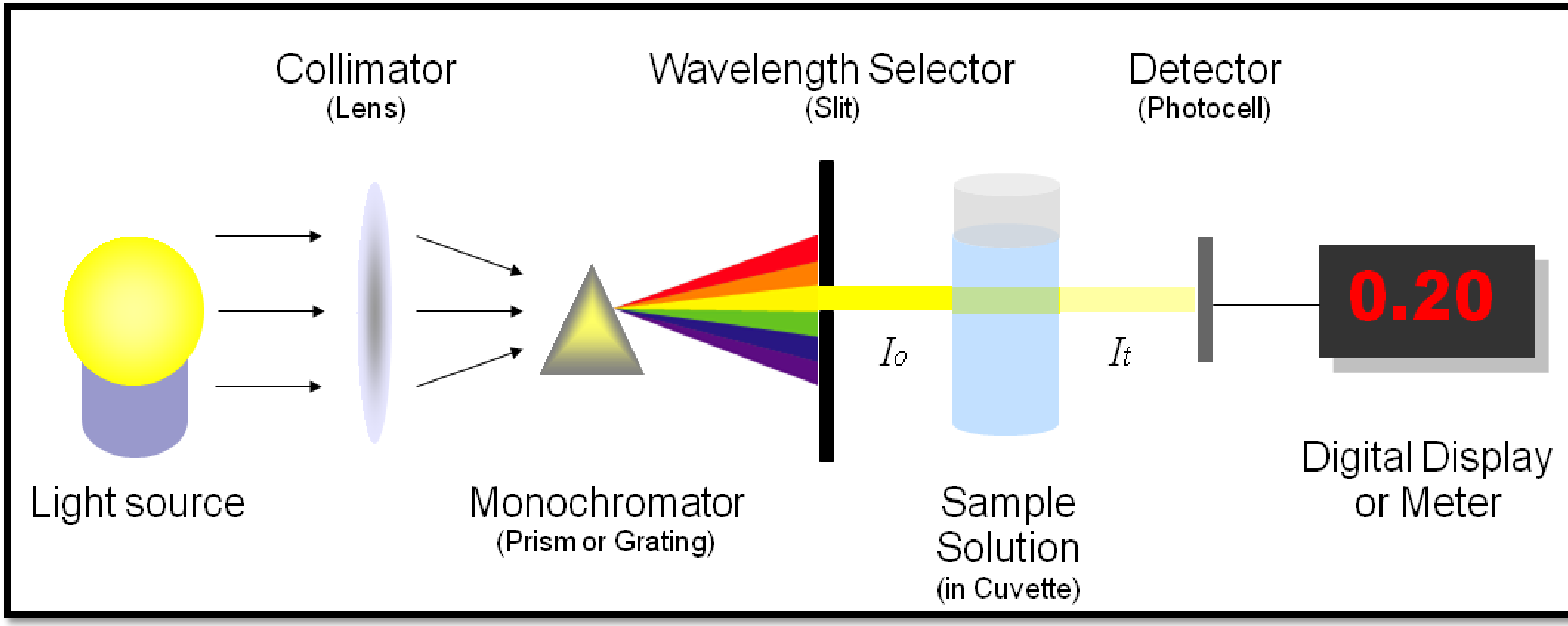
Most important idea in measurement of color is:

1- color intensity is directly proportional to the concentration.
2- concentration is proportional to the absorbance (Beer’s law).

- Most widely used method for determining the concentration of biochemical compounds are colorimetry and spectrophotometry.

Components of colorimetric instrument:

- Sources of light: (UV and visible)
- Collimator: Condenser lens (collect light into one direction)
- Monochromator: analyze the spectrum
- Wavelength selector
- Sample containers: (cuvettes)
- Photoelectric detector (convert light to electrical signals)
- Digital display or readout device that displays the signal from the detector.



Collimator
(Lens)
Wavelength Selector
(Slit)
Detector
(Photocell)
0.20
Io
It
Light source
Monochromator
(Prism or Grating)
Sample
Solution
(in Cuvette)
Digital Display
or Meter

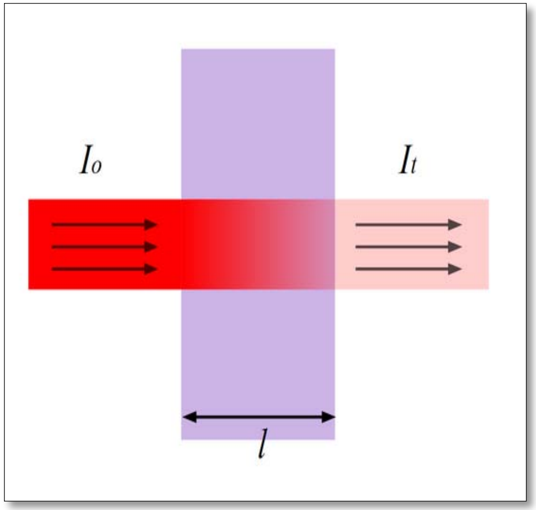
The sequence of events in a spectrophotometer

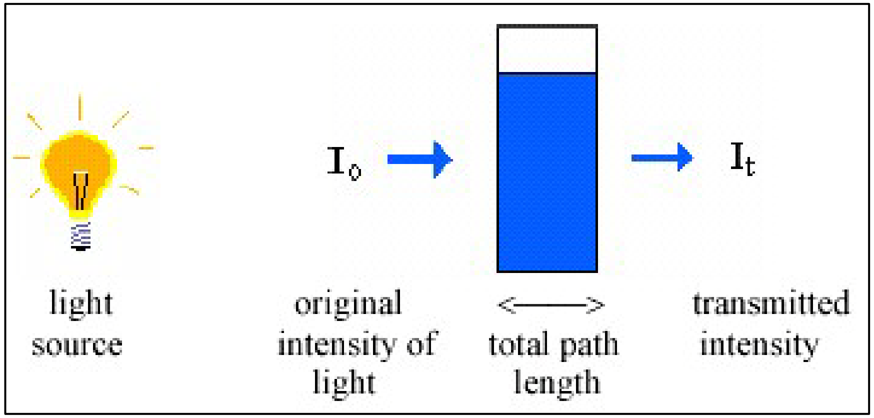
- light source enters the sample.
- sample absorbs light.
- Light detector detects the intensity of light received and convert it into an electrical signal.
- then sends a signal to a galvanometer or a digital display

So, basically all spectrophotometer reads **transmittance** not absorbance, then it converts it to absorbance if you choose abs. mode.

$$T = I_o / I_i \times 100$$

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





Cuvettes:

- containers of sample and reference solution
- must be transparent (pass not absorb) to the radiation which will pass through them.

Three kinds of cuvettes:

1- **Quartz** or fused silica: used in UV-VIS region (200 nm to 800 nm) because of its high grade of transparency.

2- **Silicate glasses:** used for WL > 350 nm.
Plastic and glass absorb UV, so they can only be used for visible light spectroscopy.

3- **Disposable** cuvettes

Difference between colorimeter and spectrophotometer

<table>
<tr><th>Colorimeter</th><th>Spectrophotometer</th></tr>
<tr><td>Colorimeter is the general type</td><td>Spectrophotometer is the <u>specific</u> type.</td></tr>
<tr><td colspan="2">Both of them measure color and intensity of color through light.</td></tr>
<tr><td colspan="2">Basic method of operation is similar for all instruments.</td></tr>
<tr><td>colorimeter utilizes a three color source (Red, green, and blue) generated by either a color wheel with colored filters or, sets of specially designed LEDs.</td><td>Spectrophotometer utilizes either a diffraction grating or prism in the sensor</td></tr>
<tr><td>Colorimeter is <u>limited</u> to the visible light only with WL 400-700 nm</td><td>spectrophotometer can be extended to x-ray, UV light, infrared and radiofrequencies</td></tr>
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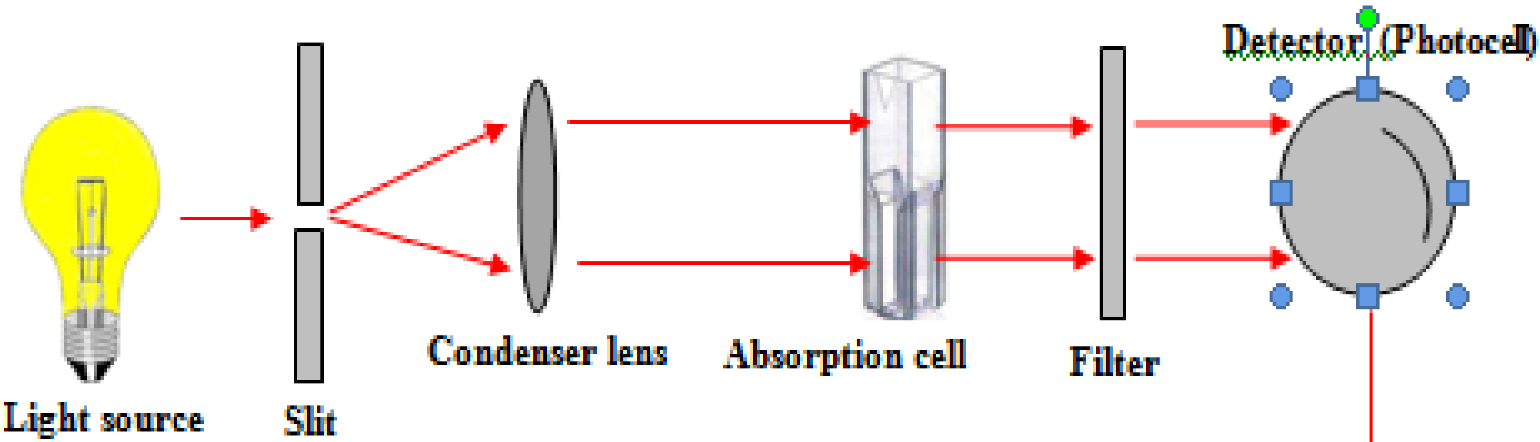
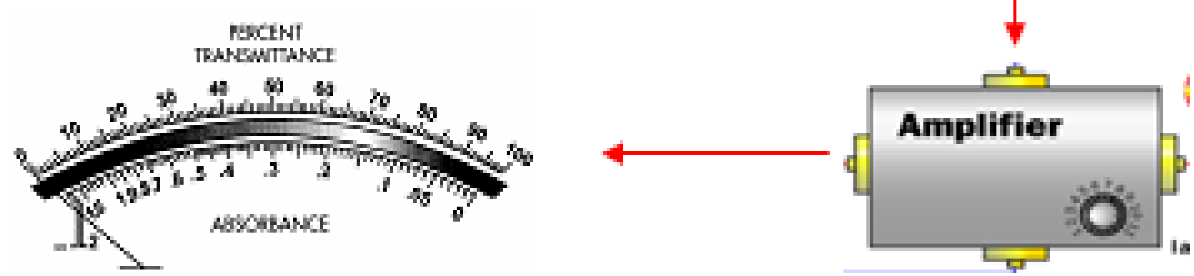
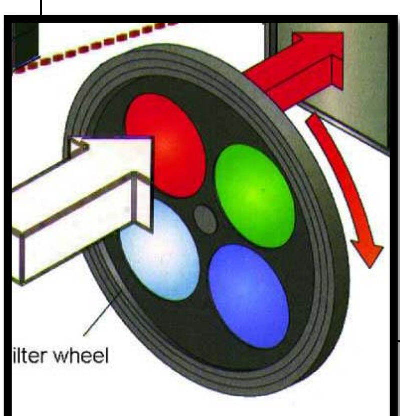
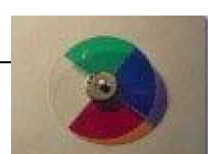
A) Schematic diagram of the components of colorimeter

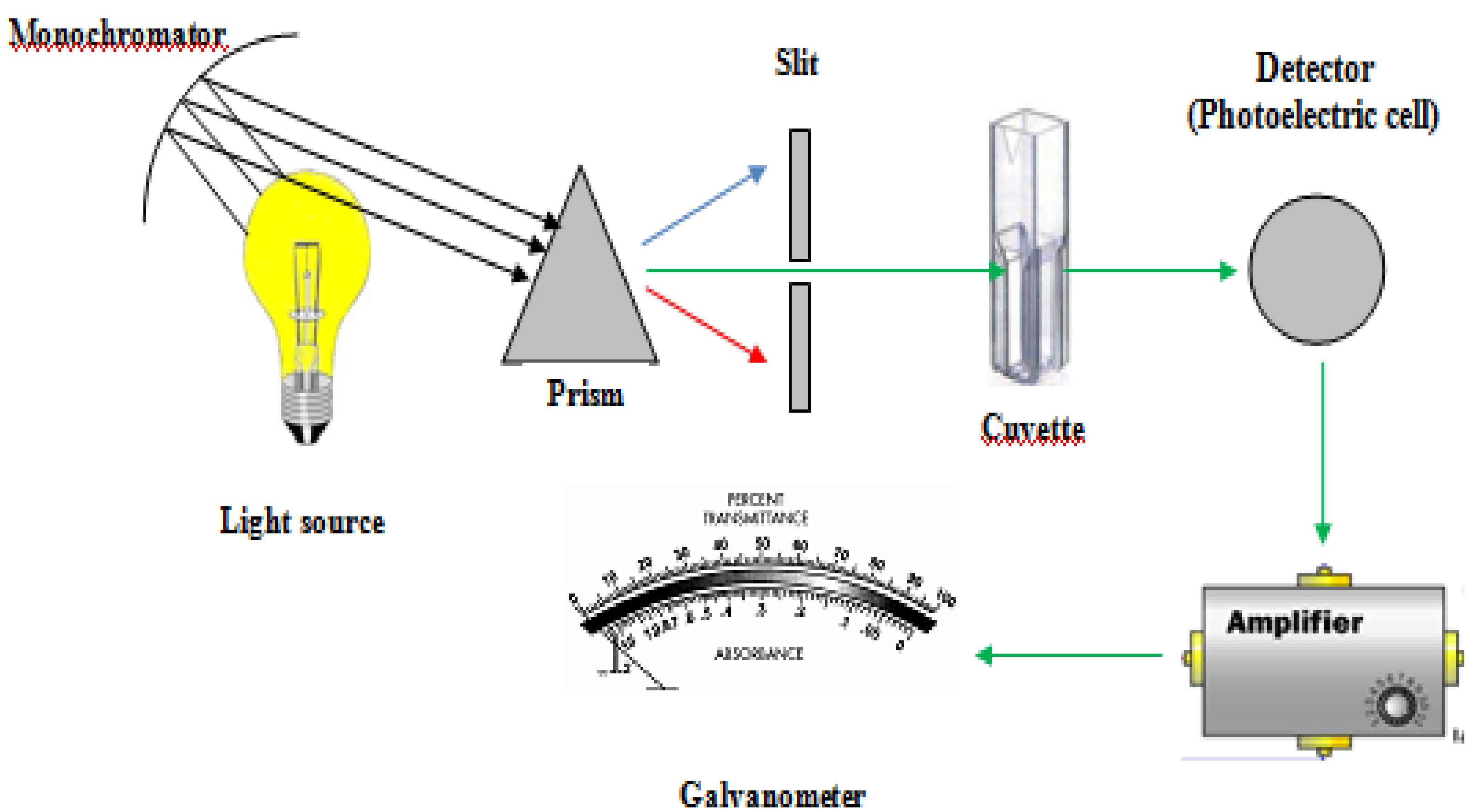


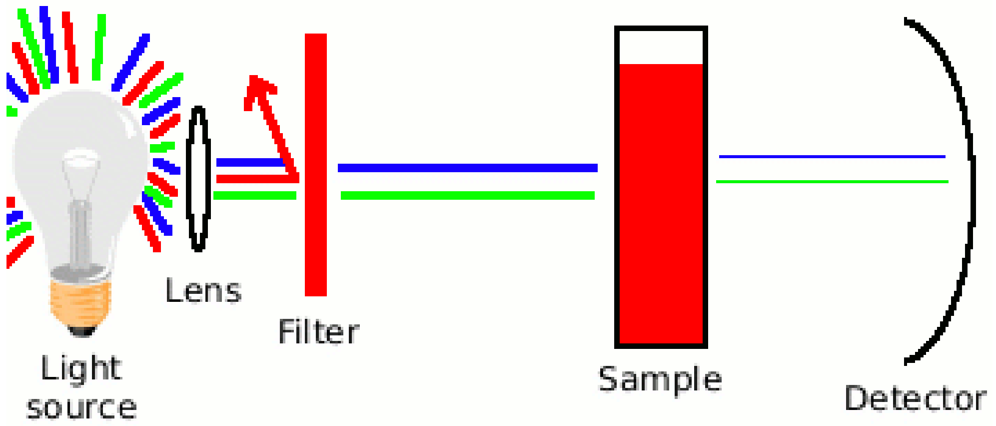




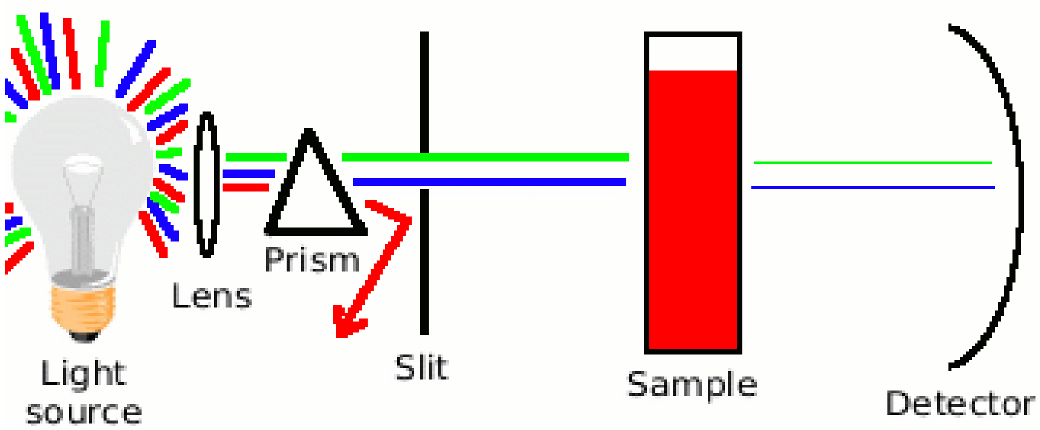


B) Schematic diagram of the components of spectrophotometer





Light path in a colorimeter



Light path in a spectrophotometer

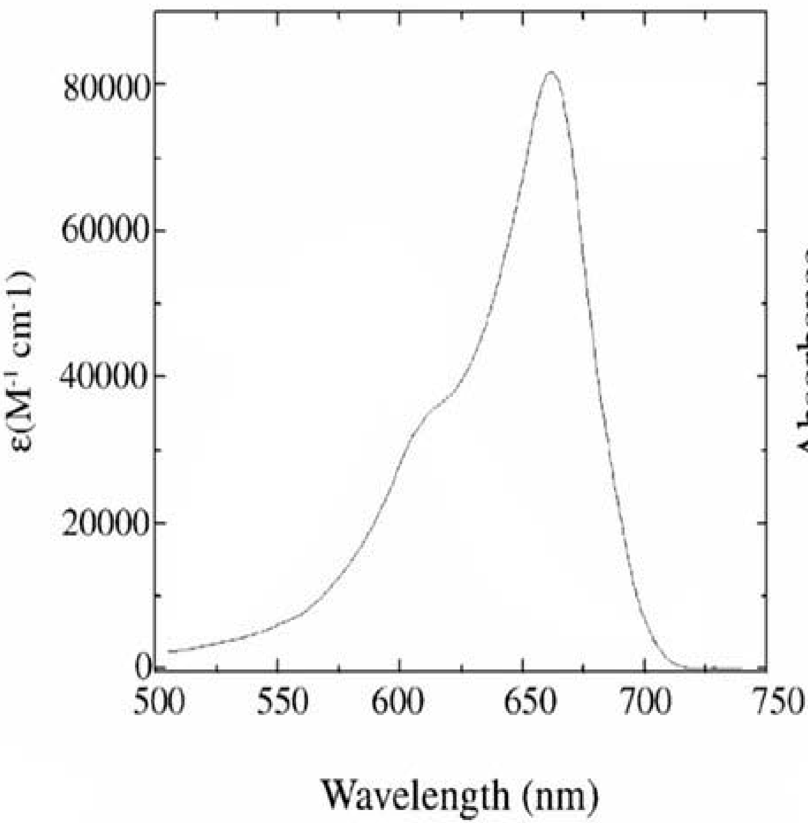
Lab practice:

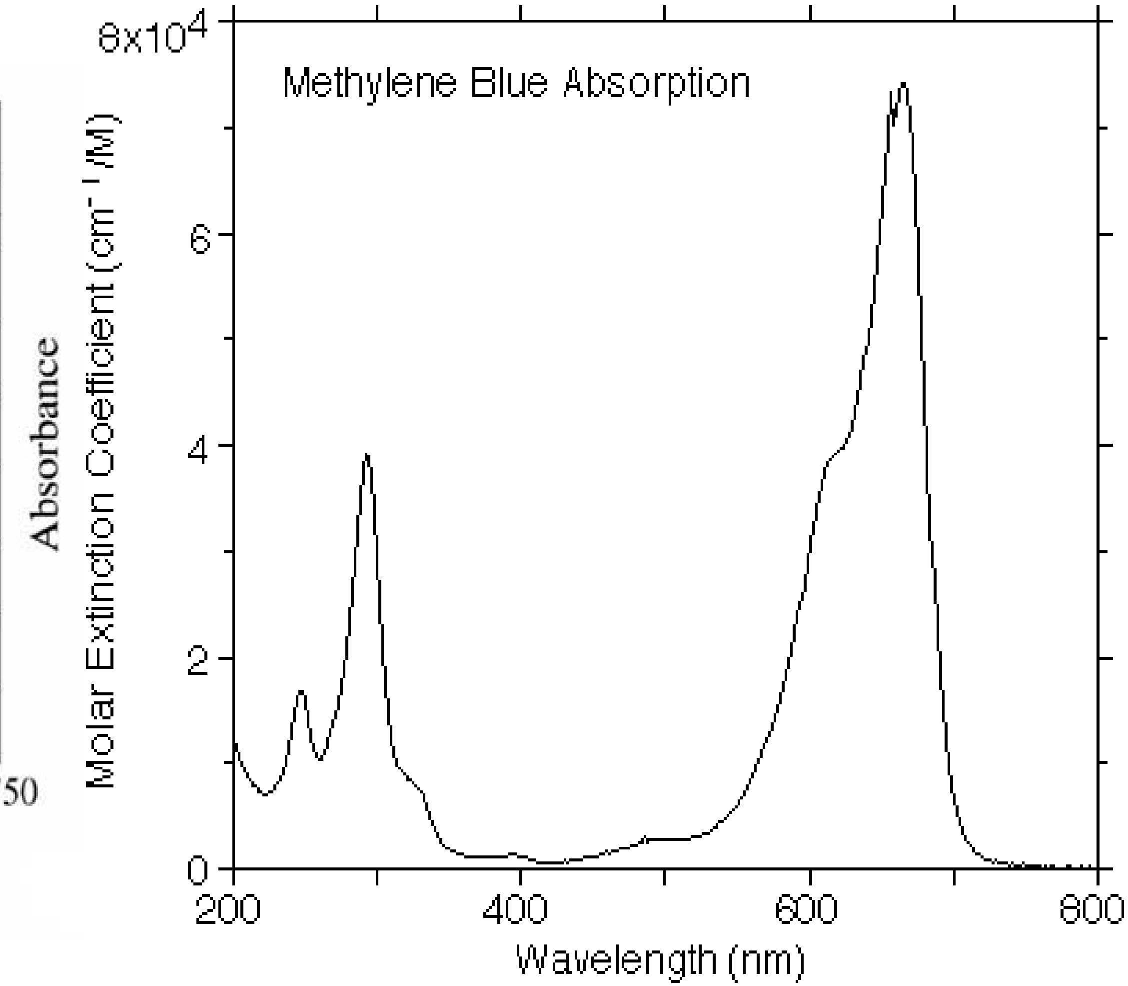
Determination of λ_{max} or (Absorption spectrum) of certain dyes

Aim:

- Construct absorption spectrum of two dyes: Methylene orange and methylene blue.
- To find wavelength of maximum absorbance of

Wavelength (nm)	Absorbance		Wavelength (nm)	Absorbance	
	1	2		1	2
400 nm			560 nm		
410 nm			570 nm		
420 nm			580 nm		
430 nm			590 nm		
440 nm			600 nm		
450 nm			610 nm		
460 nm			620 nm		
470 nm			630 nm		
480 nm			640 nm		
490 nm			650 nm		
500 nm			660 nm		
510 nm			670 nm		
520 nm			680 nm		
530 nm			690 nm		
540 nm			700 nm		
550 nm					


ε(M-1 cm-1)
80000
60000
40000
20000
0
500
550
600
650
700
750
Wavelength (nm)


Absorbance
Methylene Blue Absorption
Molar Extinction Coefficient (cm-1/M)
8x10^4
6
4
2
0
200
400
600
800
Wavelength (nm)