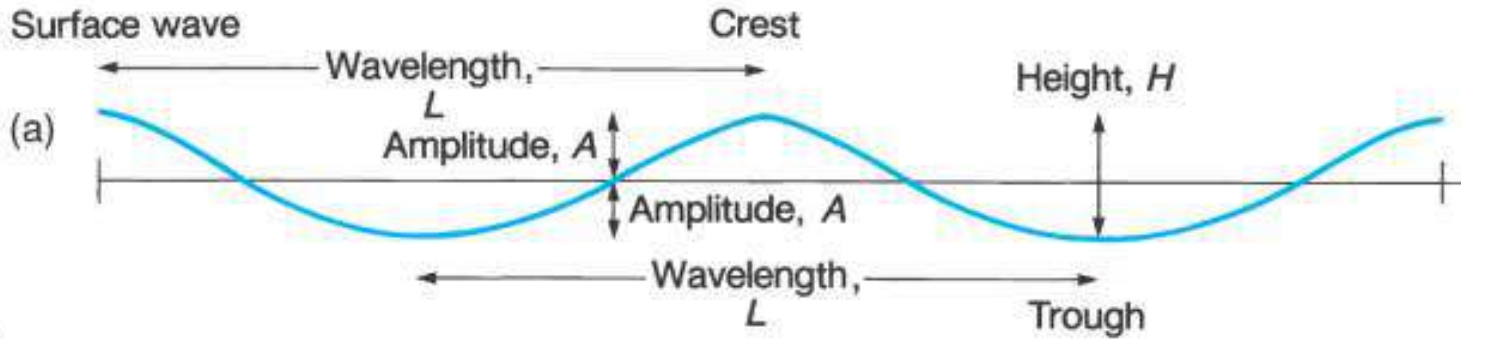
Introduction to Spectrophotometry

Spectroscopy

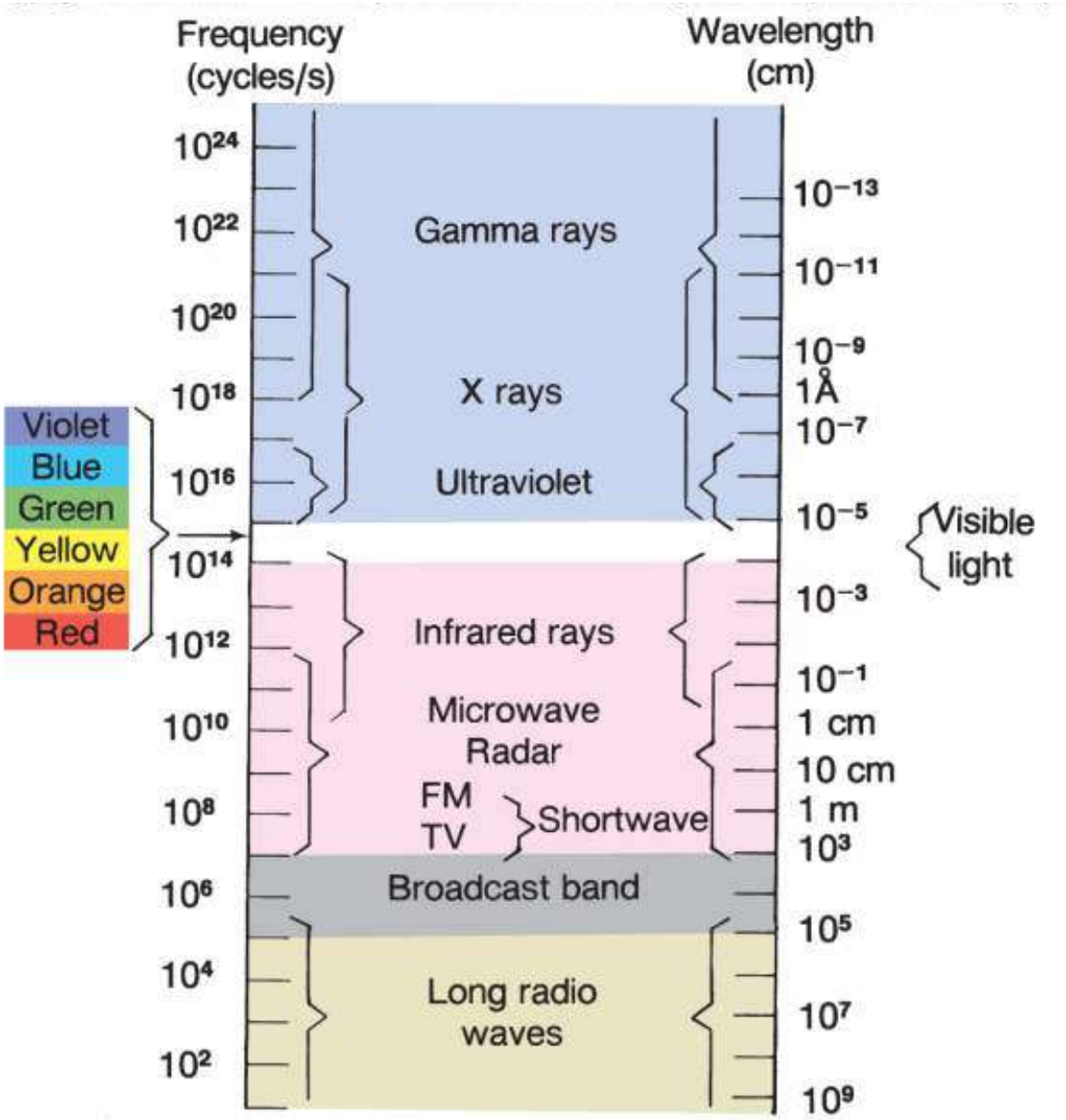
- Is the study of the interaction of light & matter
- Spectrophotometer – instrument that uses electromagnetic radiation from UV, visible or IR to analyze the absorption or transmission of a sample
- We will use visible in our lab

Properties of Light

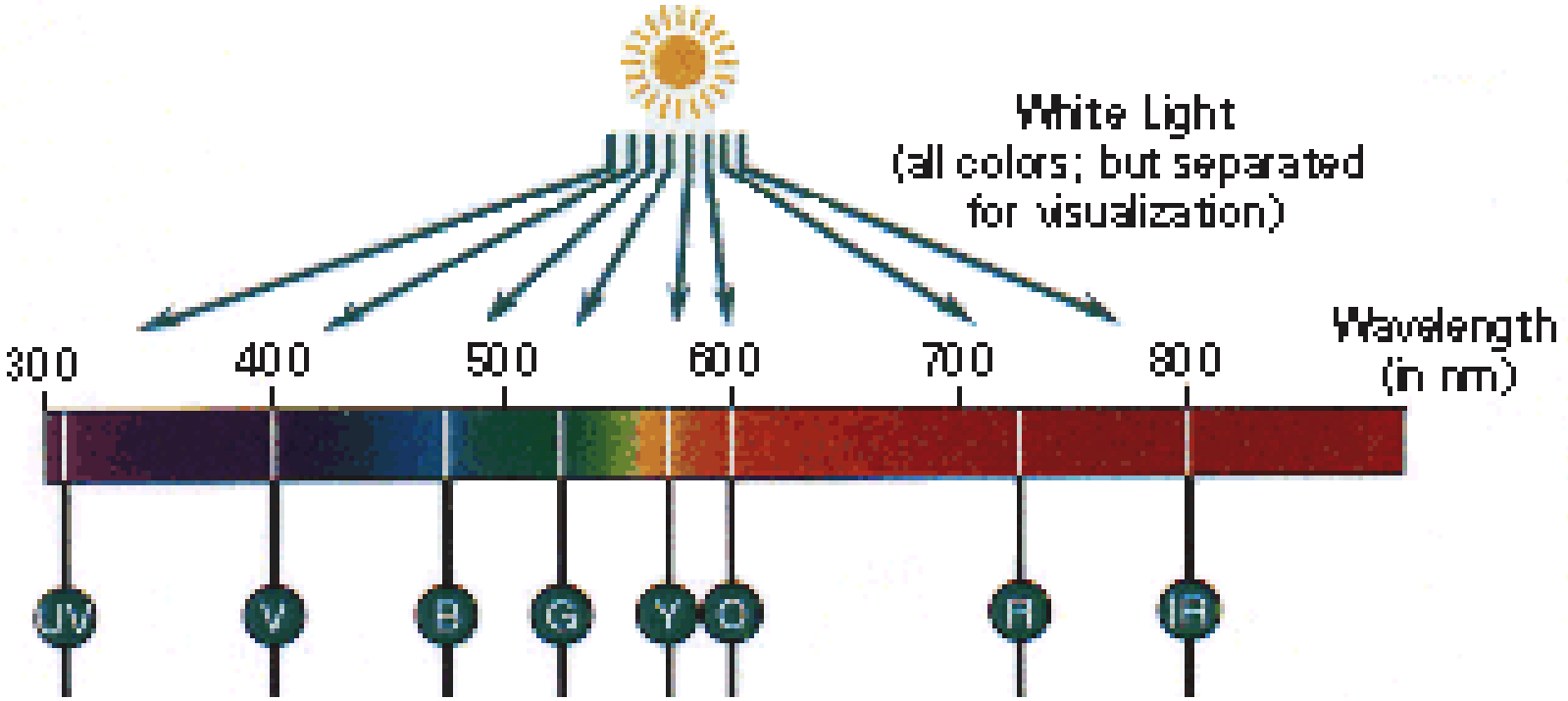
- *Electromagnetic radiation* moves in waves



Electromagnetic Spectrum

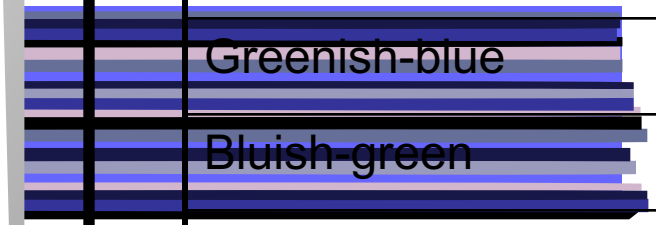


Electromagnetic Spectrum



Colors & Wavelengths

Visible Light



COLOR	WAVELENGTH (λ in nm)
Ultraviolet	< 380
Violet	380 – 435
Blue	436 – 480
Greenish-blue	481 – 490
Bluish-green	491 – 500
Green	501 – 560
Yellowish-green	561 – 580
Yellow	581 – 595
Orange	596 – 650
Red	651 – 780
Near Infrared	> 780

What is Colorimetry?

- The solutions of many compounds have characteristic colors.
- The intensity of such a color is proportional to the concentration of the compound.

What are Spectroscopy and Spectrophotometry??

- Light can either be ***transmitted*** or ***absorbed*** by dissolved substances
- Presence & concentration of dissolved substances is analyzed by passing light through the sample
- Spectroscopes measure electromagnetic ***emission***
- Spectrophotometers measure electromagnetic ***absorption***

Instruments of Measurement

- Two most common:

1. Visible Spectrophotometer
   - Spect 20, Spect 88
   - Uses Xe or W lamps as light sources
   - Glass cuvettes hold the sample

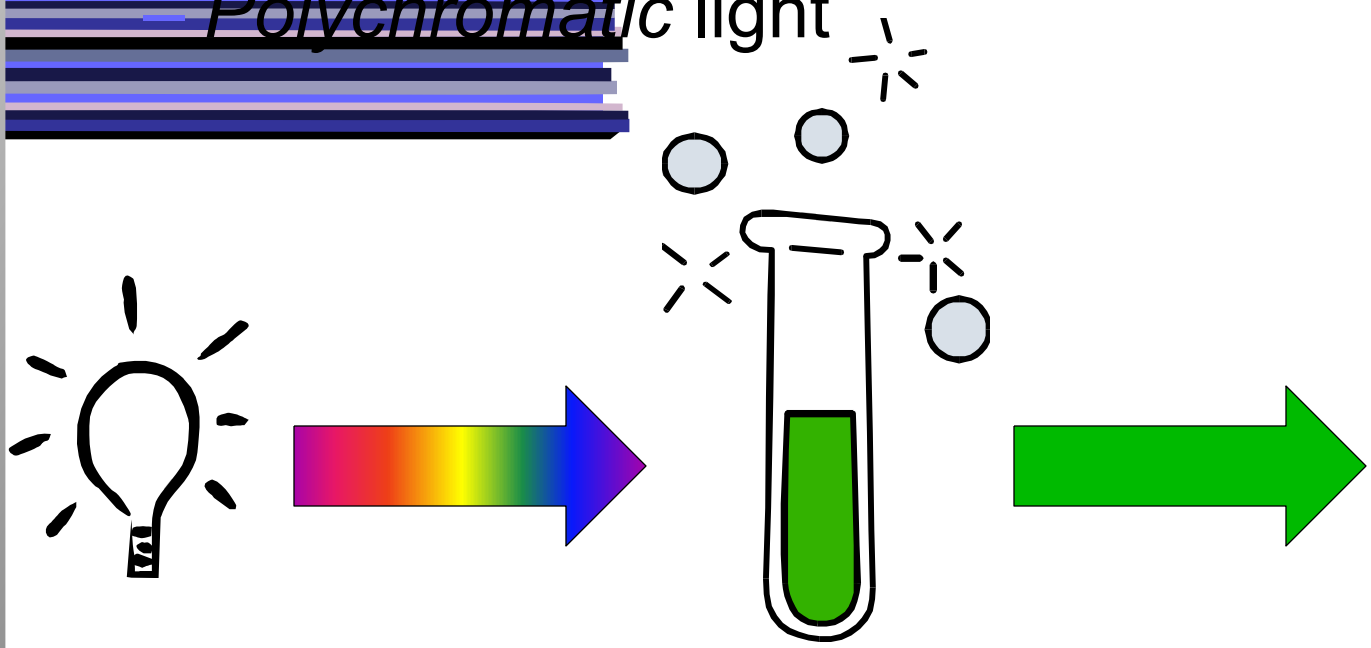
2. Atomic-Absorption Spectrophotometer

Instruments of Measurement

- What do visible spectrophotometers measure?
  - Amount of light absorbed by the dissolved substance
    - Qualitative – color gives info about the solution composition
    - Quantitative – provides numerical data for the concentration

Absorption of Light

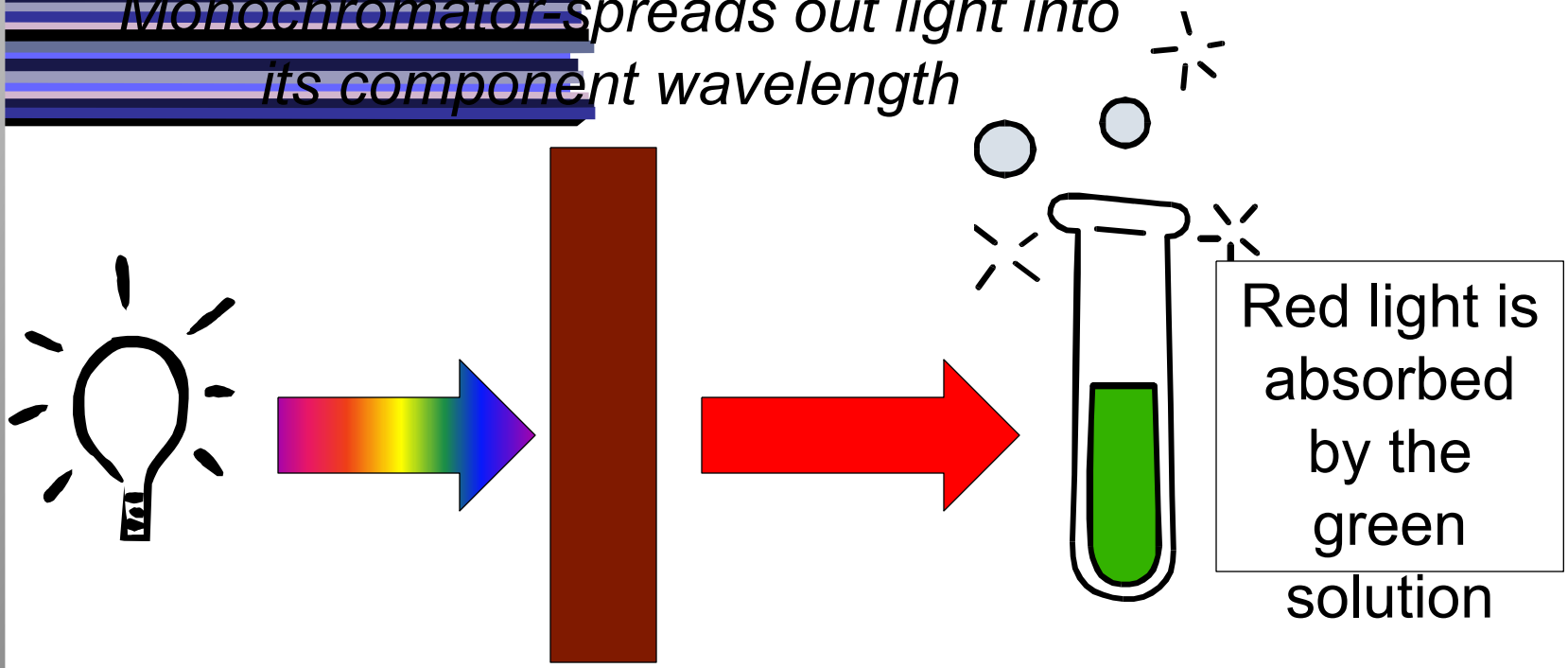
- White light
  - All colors
  - *Polychromatic* light



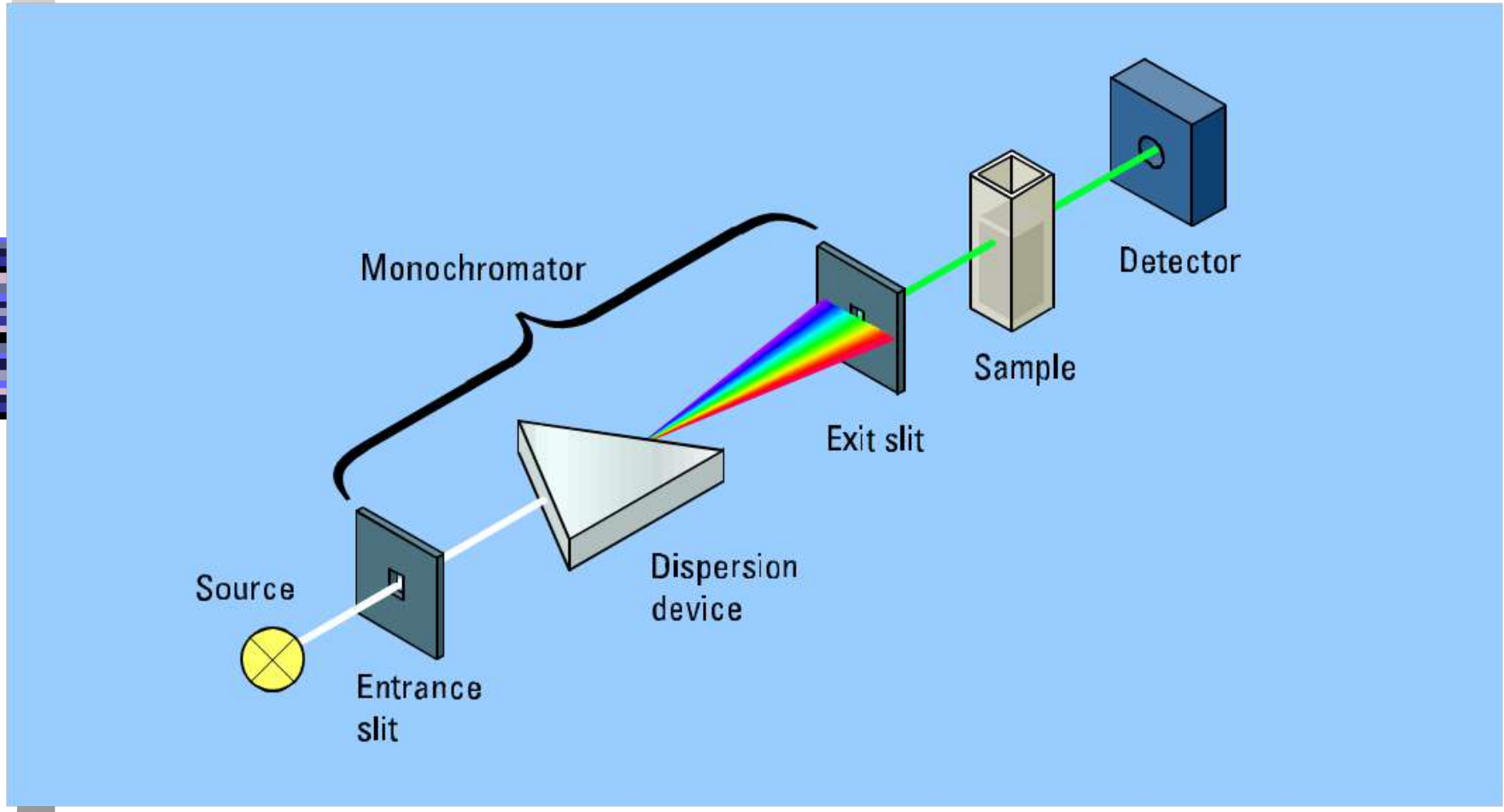
Absorption of Light

- Monochromatic light
  - Light of one color

Monochromator-spreads out light into its component wavelength



The Spectrophotometer

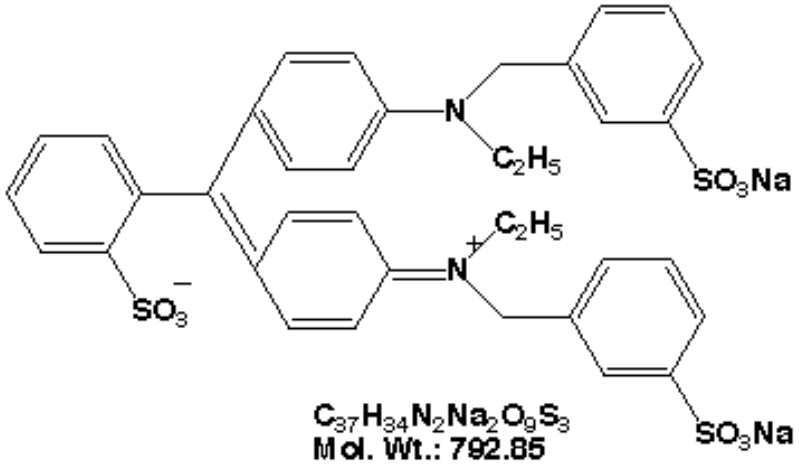


Success of spectrophotometry…

- Requires sample to absorb light differently to the other chemicals in the solution
- **How is the correct wavelength selected?**
  - The amount of light absorbed depends on the energy difference between 2 electron energy levels
  - Optimum wavelength for spectrophotometric analysis is selected by measuring the *visible spectrum* of the substance
  - This is done by plotting absorbance (A) versus wavelength (λ)

Food Dyes

- Only 7 dyes are approved by the FDA for use in foods, drugs & cosmetics
- All artificial food colors are mixtures of these 7 dyes
- We will be using FD&C Blue in this lab



FD&C Blue 1

- A solution containing this dye is blue in white light
- The colors absorbed by solution are complementary to the transmitted color
- Blue solution absorbs yellow, orange, & red light
- So expect dye solution to peak at 580 – 650 nm
- Optimum wavelength is determined from wavelength of max. absorption λ_{max} = 630 nm for Blue 1
- This is given for the blue solution but you will have to claculate this for the red

Wavelength of light absorbed:

- Is related to electronic structure of substance
- Intensity of light absorbed depends on the concentration of solution
  - More concentrated, the more intense color & the greater intensity of light absorbed
- When light is absorbed, the radiant power (P) of light beam decreases

Transmittance (T)

- This is the fraction of incident light (P/P_o) that passes through the sample

$$T = \frac{P}{P_o}$$

- Po = intensity of "blank"
- Blank – is solution identical to sample but without solute

Definitions & Symbols

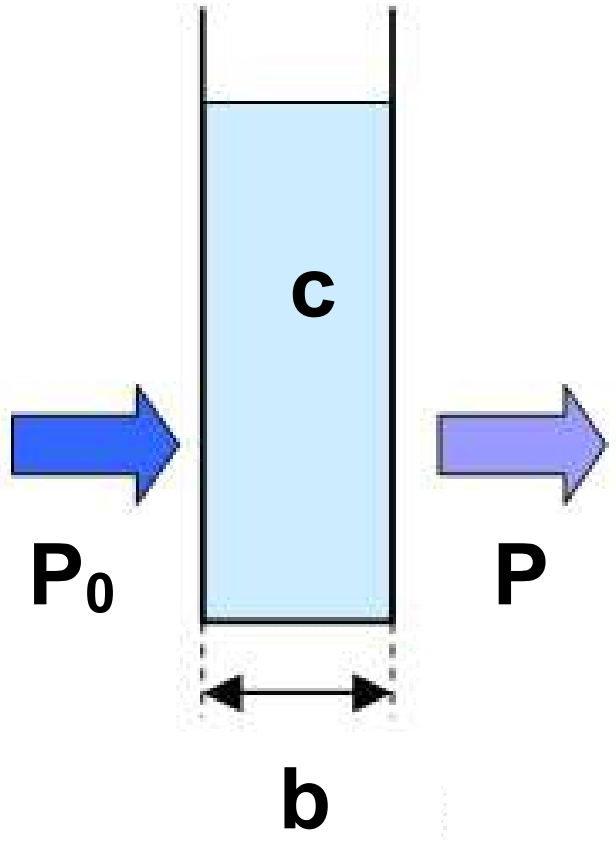
- Intensity (I)
- Transmittance (T)
  - It's also referred to as %T or T x 100
  - $T = P/P_o$
    - Where P_o is the intensity of the blank
    - Can also use I = Intensity instead of Power
    - $T = I / I_o$

Graphical Relationship

- % transmission and % absorption are not linearly related to concentration
- For a graph to be useful, a straight line is needed
- **ABSORBANCE = log(1/T) = -log(T)**

The amount of light absorbed depends upon:

- Concentration (c)
- Path length of sample cell (b) thru which light passes
- Defined by Beer's Law



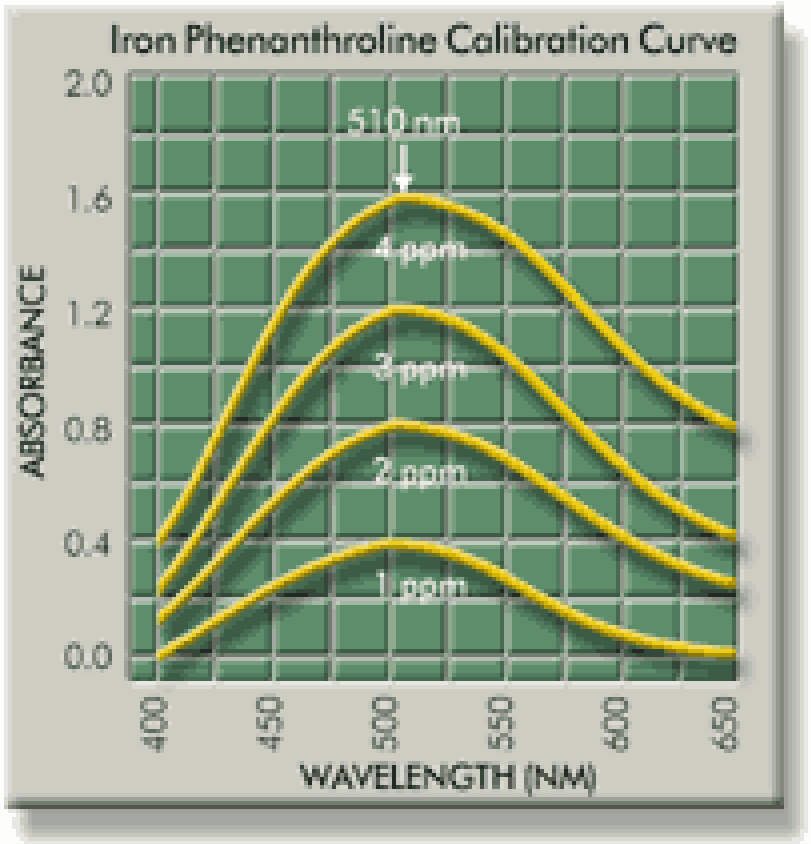
Beer's Law

- The intensity of a ray of monochromatic light decreases exponentially as the concentration of the absorbing medium increases
  - More dissolved substance = more absorption and less transmittance
- ε = molar absorptivity coefficient and is constant for a substance

- %T = Tx100 = P/P_0x100%
- A = - log T
- A = ε b c

Spectral Transmission Curve

- Optimum wavelength



Standardization Graph

- **Standards** (solutions of known concentration) of the compound of interest are made, treated, and their absorbances (ABS) and concentration values are used to create a **Standardization Graph**.

Standardization Graph