

Spectrophotometer

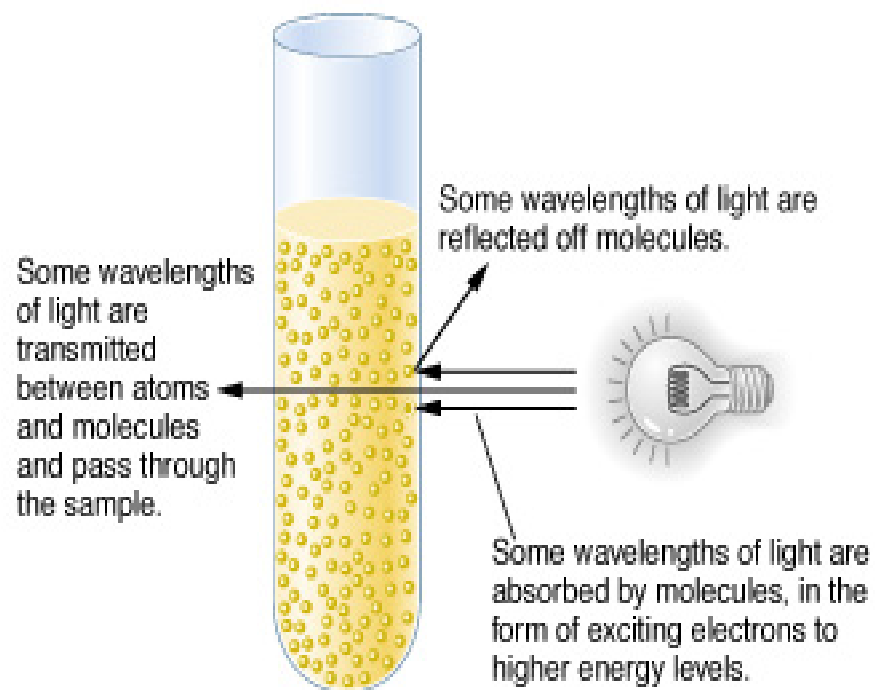
- Use to quantify the amount of molecules in a liquid sample (solution).
- Measure the amount of light that can pass through a solution

Process

- Spectrophotometer shines a light on the sample & molecules in the sample interact with the light.

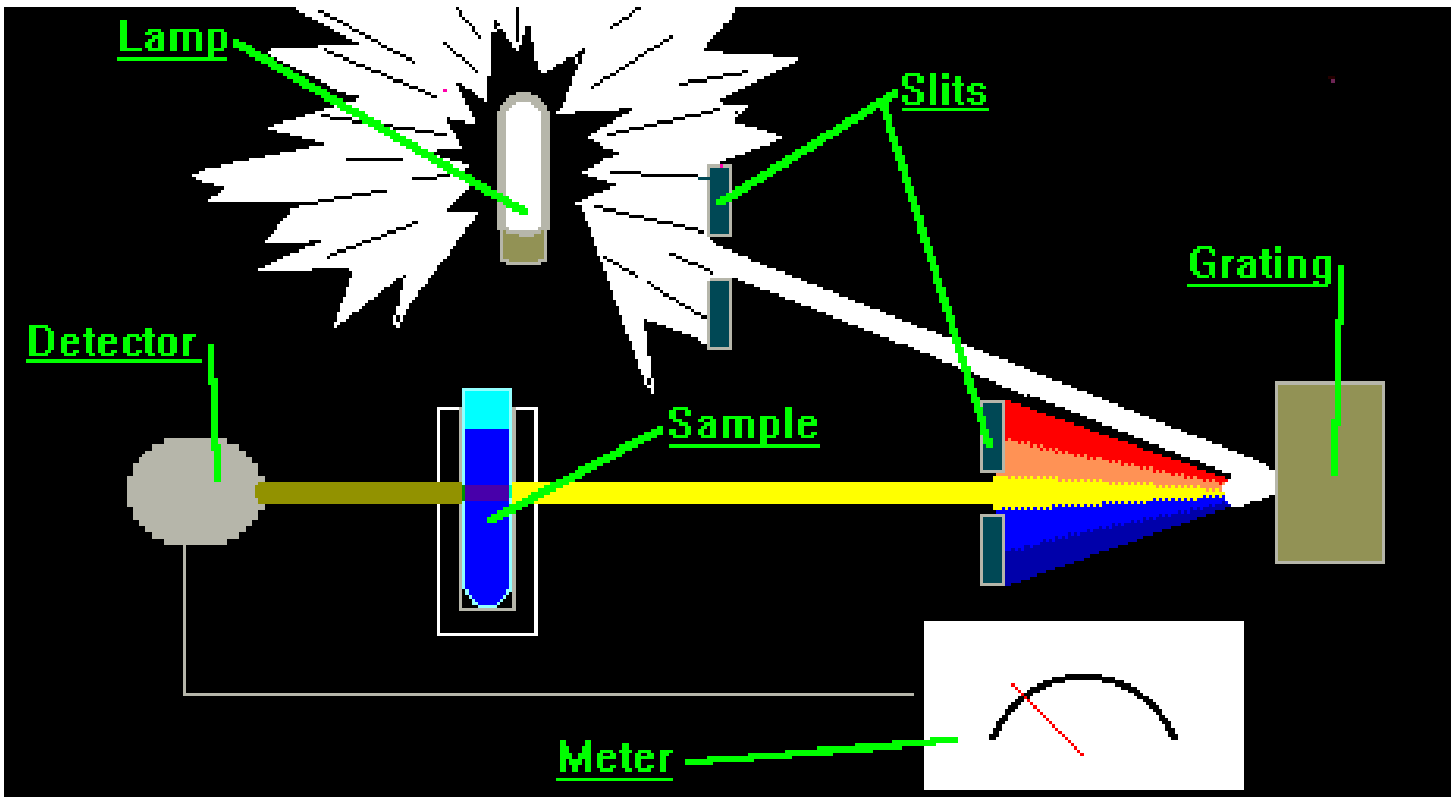
Measure

- Transmittance:
- Absorbance:
- Usually compared to a standard of known concentration



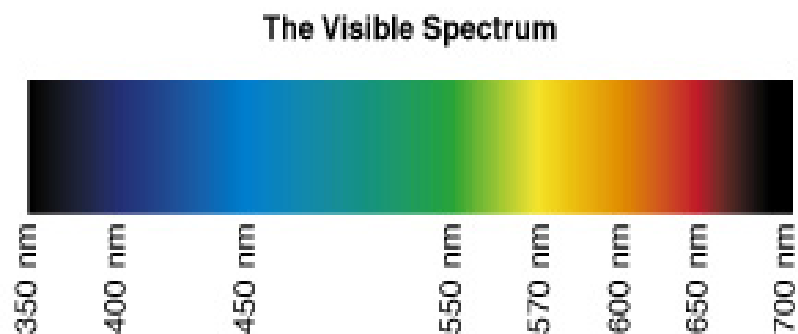
Visible Spectrum

- Use “White” light
- Prism adjusted to position a specific visible wavelength to interact with the sample
- Measures the amount of light being passed through the sample at that wavelength
 - Absorbance is based on a calculation



This diagram is a representation of the inside of a Spectronic 20™

Molecules are whatever color of light that they transmit and do not absorb.



The wavelength of different colors of light energy

Copper II Sulfate Pentahydrate

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$



Copper Sulfate

- Blue sample:
 - When white light shows on a blue molecule all wavelengths are absorbed except the blue wavelength
 - The blue wavelength passes through the sample & so it is detected
 - By our eyes or by the instrument
- Set measurement to 590 nm

Dilution Factor

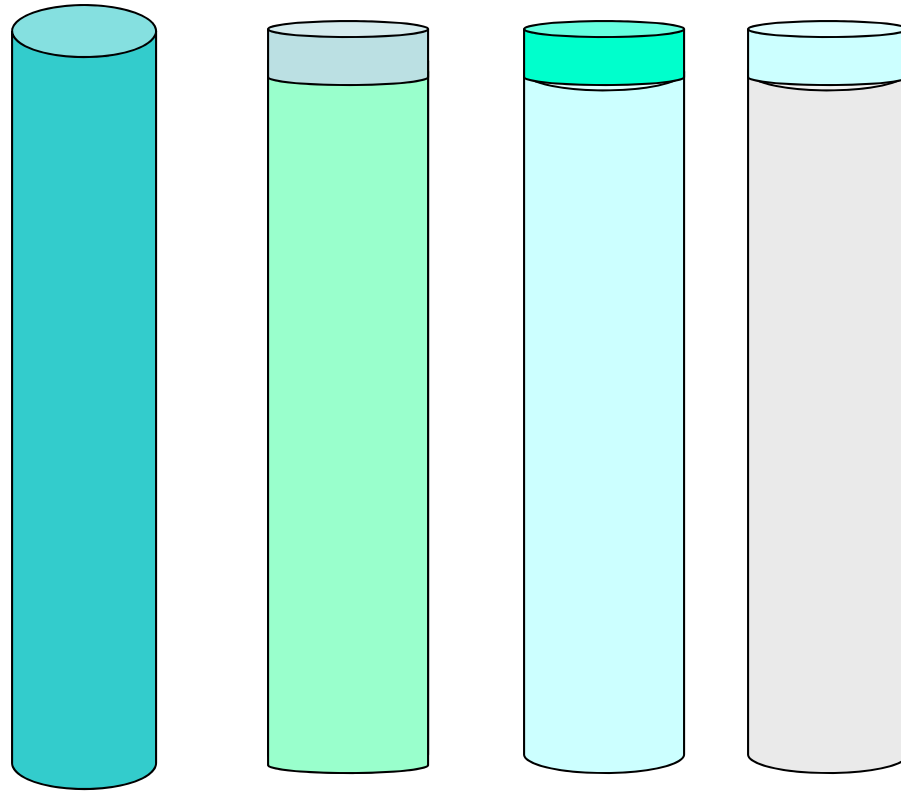
- Original Solution to Diluted Solution
 $1: 10 = 1 \text{ to } 10 \text{ dilution}$
- Combine 1 volume of original solution + 9 volumes of the solvent
- $1 + 9 = 10 \text{ dilution factor}$

Serial Dilution Example 2:

- 3 steps with each step at 1: 10 dilution

1 mL of the original solution

Add 9 mL of water to make 10 mL of the diluted solution



Final Dilution Factor = 10 x 10 x 10 = ?