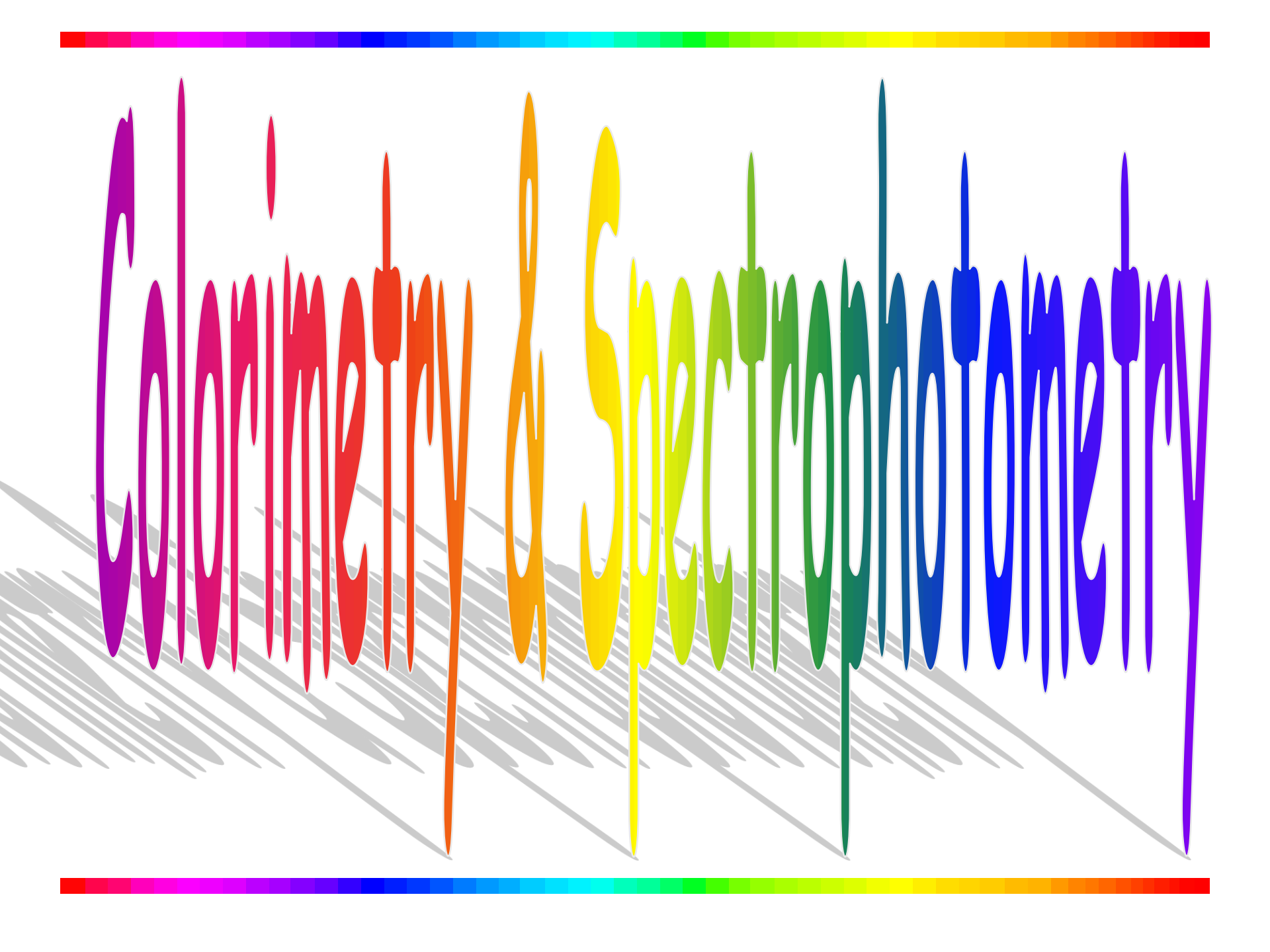
Colorimetry and Spectrophotometry

Presented by

Ku. Kavita G. More

B.Sc.III /Sem-V (Chemistry)


Colorimetry & Spectrophotometry

Useful Terminology

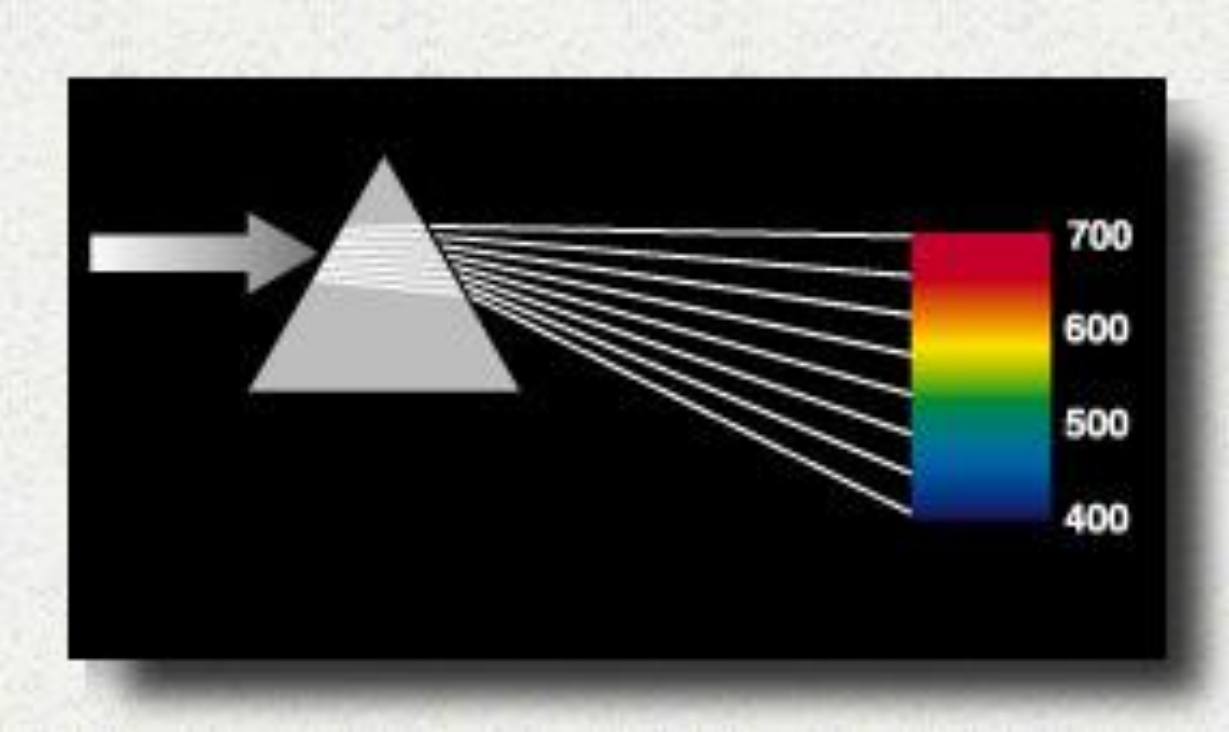
- **Colorimetry** is the use of the human eye to determine the concentration of colored species.

- **Spectrophotometry** is the use of instruments to make the same measurements. It extends the range of possible measurements beyond those that can be determined by the eye alone.

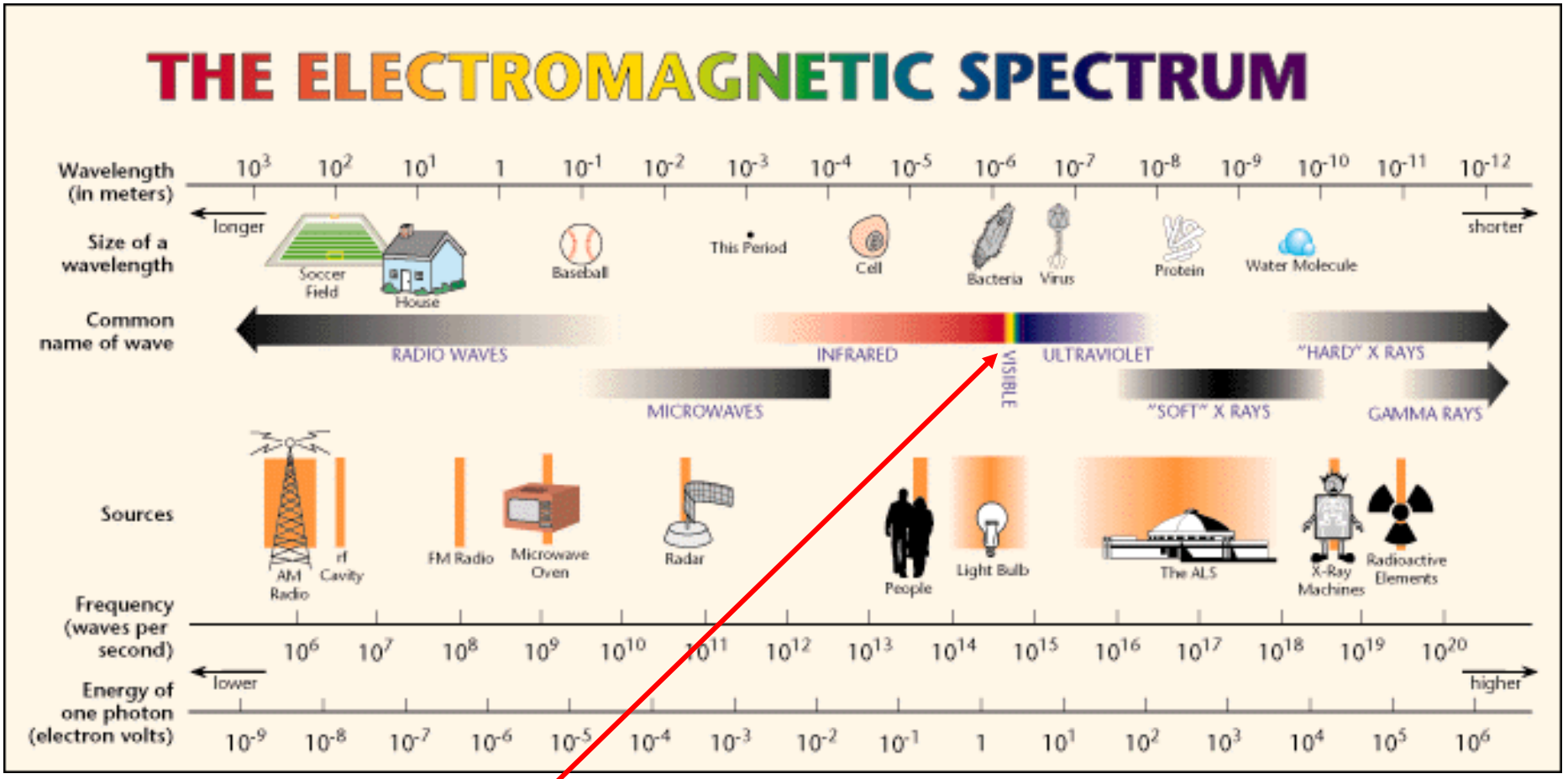
Note: This experiment will demonstrate **both techniques** on the **same set of dyes**.

Colorimetry

- **Visual Observations** – Because colorimetry is based on inspection of materials with the human eye, it is necessary to review aspects of visible light.
- **Visible light** is the narrow range of electromagnetic waves with the wavelength of **400-700 nm**.



ROY G BIV = the mnemonic used to remember the colors of the visible spectrum.



Visible light is only a very small portion of the electromagnetic spectrum.

Note: Frequency (υ) and Energy (E) are directly proportional whereas Frequency (υ) and Wavelength (λ) are inversely proportional.

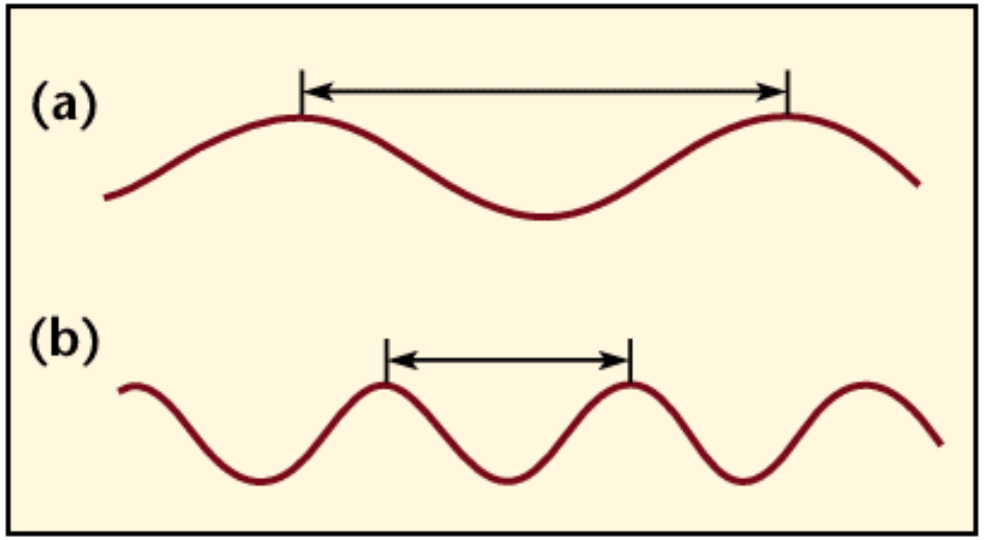
Electromagnetic Spectrum

Type of Radiation	Frequency Range (Hz)	Wavelength Range	Type of Transition
gamma-rays	10^{20}-10^{24}	<1 pm	nuclear
X-rays	10^{17}-10^{20}	1 nm-1 pm	inner electron
ultraviolet	10^{15}-10^{17}	400 nm-1 nm	outer electron
visible	**4-7.5x10^{14}**	**750 nm-400 nm**	**outer electron**
near-infrared	1x10^{14}-4x10^{14}	2.5 μm-750 nm	outer electron molecular vibrations
infrared	10^{13}-10^{14}	25 μm-2.5 μm	molecular vibrations
microwaves	3x10^{11}-10^{13}	1 mm-25 μm	molecular rotations, electron spin flips*
radio waves	<3x10^{11}	>1 mm	nuclear spin flips*

Electromagnetic radiation is characterized by its wavelength, λ, Frequency, ν and energy, E:

$$E = h\nu = hc / \lambda \qquad c = \nu \lambda$$

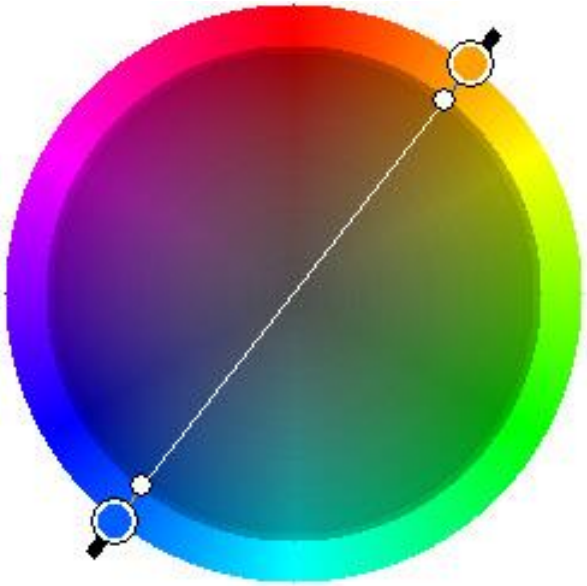
Where h = Planck's constant & c = speed of light in a vacuum.



(a) longer wavelength, lower energy;
(b) shorter wavelength, higher energy.

Color Wheel

(ROYGBIV)



Complementary colors lie across the diameter on the color wheel and combine to form **"white light"**, so the color of a compound seen by the eye is the complement of the color of light absorbed by a colored compound; thus it **completes** the color.

Observed Color of Compound	Color of Light Absorbed	Approximate Wavelength of Light Absorbed
Green	Red	**700 nm**
Blue-green	Orange-red	**600 nm**
Violet	Yellow	**550 nm**
Red-violet	Yellow-green	**530 nm**
Red	Blue-green	**500 nm**
Orange	Blue	**450 nm**
Yellow	Violet	**400 nm**

Visual Colorimetry

Intensity: For light shining through a colored solution,the observed intensity of the color is found to be **dependent on both the thickness of the absorbing layer (pathlength) and the concentration of the colored species.**

←Side view

←Top view
(a.k.a. Bird's eye view)

For One Color: A series of solutions of a single color demonstrates the **effect of either concentration or pathlength**, depending on how it is viewed.

Visual Colorimetry

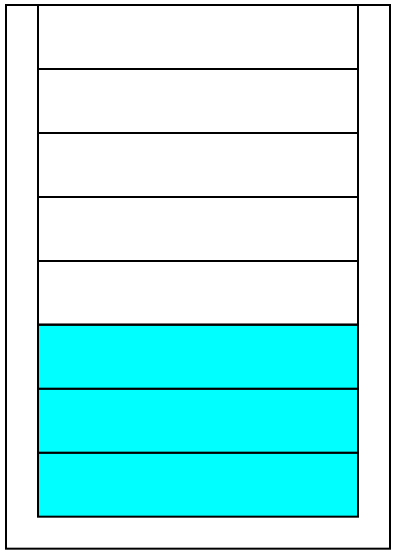
←**Ratio used**

←**Purple produced**

For more than one color: the ratio of an unknown mixture can also be determined by matching the shade of the color to those produced from known ratios.

In this example, the ratio of a mixture of **red** and **blue** can be determined visibly by comparing the mixture to **purples** produced from known ratios of red and blue.

Dilution Factor (constant pathlength)



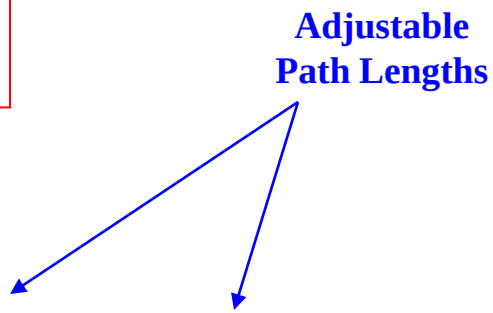
3 drops of dye std
+ 5 drops water
8 drops total volume

$C_{(diluted)}$ = (Vol Dye / Total Vol) x $C_{(std)}$

= (3 drops / 8 drops) x $C_{(std)}$

Intensity: When the product of the concentration and the pathlength of any two solutions of a colored compound are the same, the same intensity or darkness of color is observed.

Duboscq visual colorimeter



Spectrophotometry

- **Spectrophotometer** - an instrument that measures the amount of light absorbed, or the intensity of color at a given wavelength.

- The **intensity of color** can be given a numerical value by comparing the amount of light prior to passing it through the sample and after passing through the sample.

- These **quantitative measurements** of light absorbed are the **Transmittance** and the **Absorbance**.

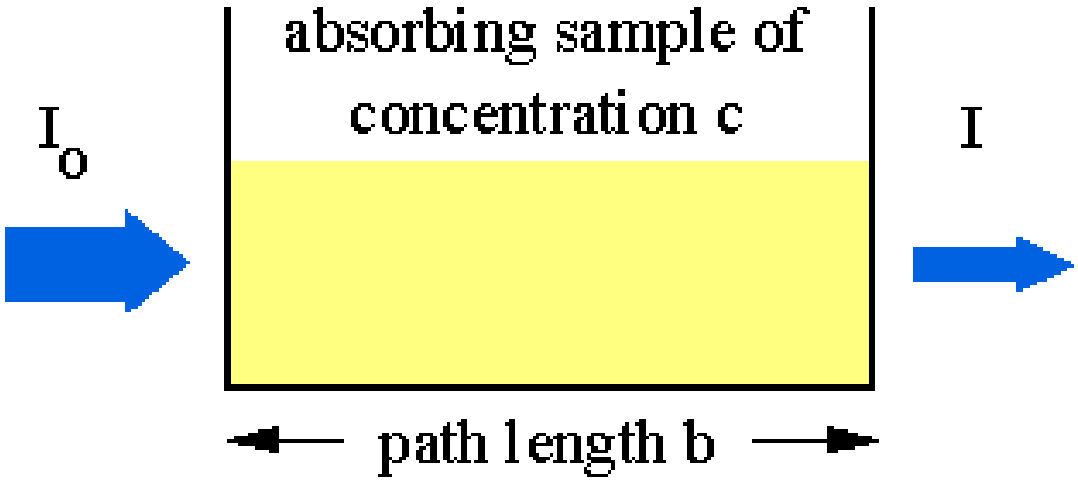
Transmittance is given by the equation:

$$T = I/I_o$$

where I is the intensity of the light after it has gone through the sample & I_o is the initial light intensity.

Absorbance is related to the %T:

$$A = -\log T = -\log(I/I_o)$$



Absorbance

Beer-Lambert Law (a.k.a. Beer's law) - the linear relationship between absorbance and concentration of an absorbing species.

$$A = abc$$

A is the absorbance

"a" is molar absorptivity in L/[(mole)(cm)]

"b" is the path length in cm

"c" is the concentration of the analyte (sample) in mol/L

"a" or **molar absorptivity** (1/M.cm)

It is sometimes called "**extinction coefficient**"

A wavelength dependent constant for the species being analyzed

"**ε**" is also used in some texts for "**a**".

"b" or **path length** (cm)

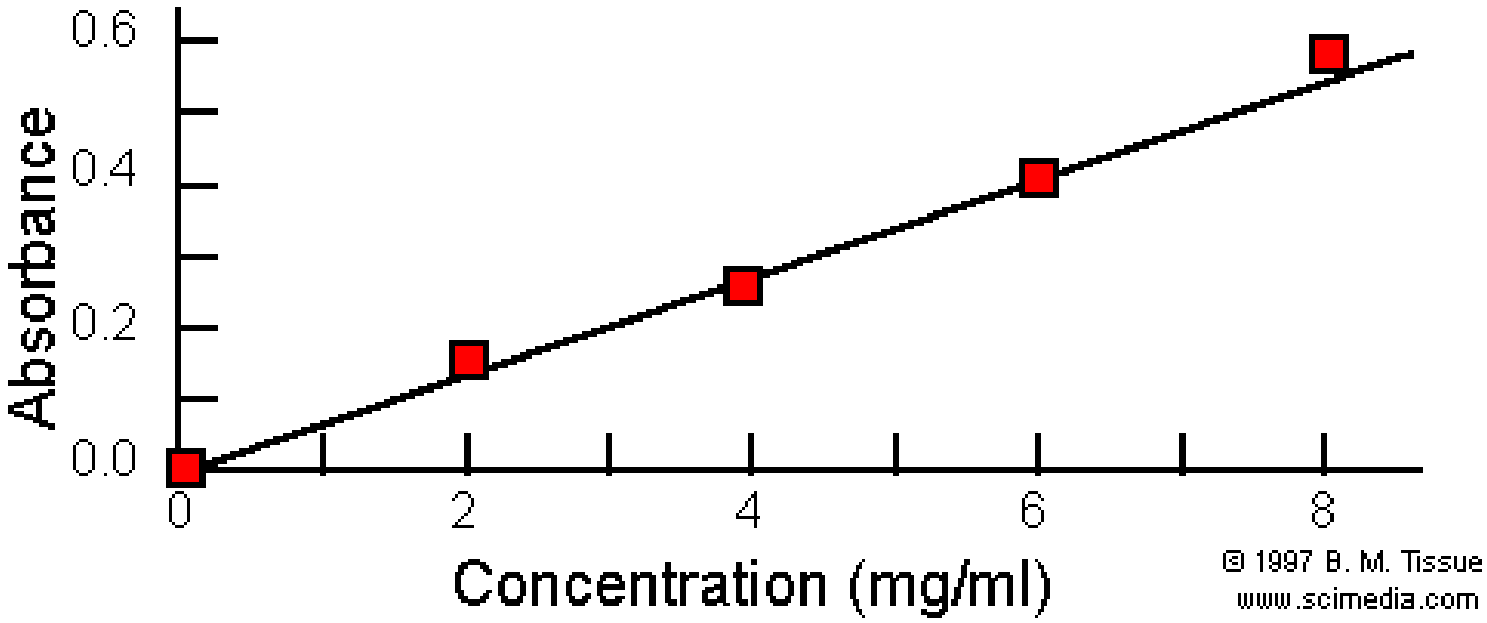
The diameter of the cuvette or sample holder which is the distance the light travels through the absorbing sample.

Becomes a constant when the same cuvette is used for all samples

"c" or **concentration** (M or mol/L)

Generally the main use of Beer's Law is to determine the concentration of various solutions

A Working Curve is produced by plotting the Absorbance vs. the Concentration.



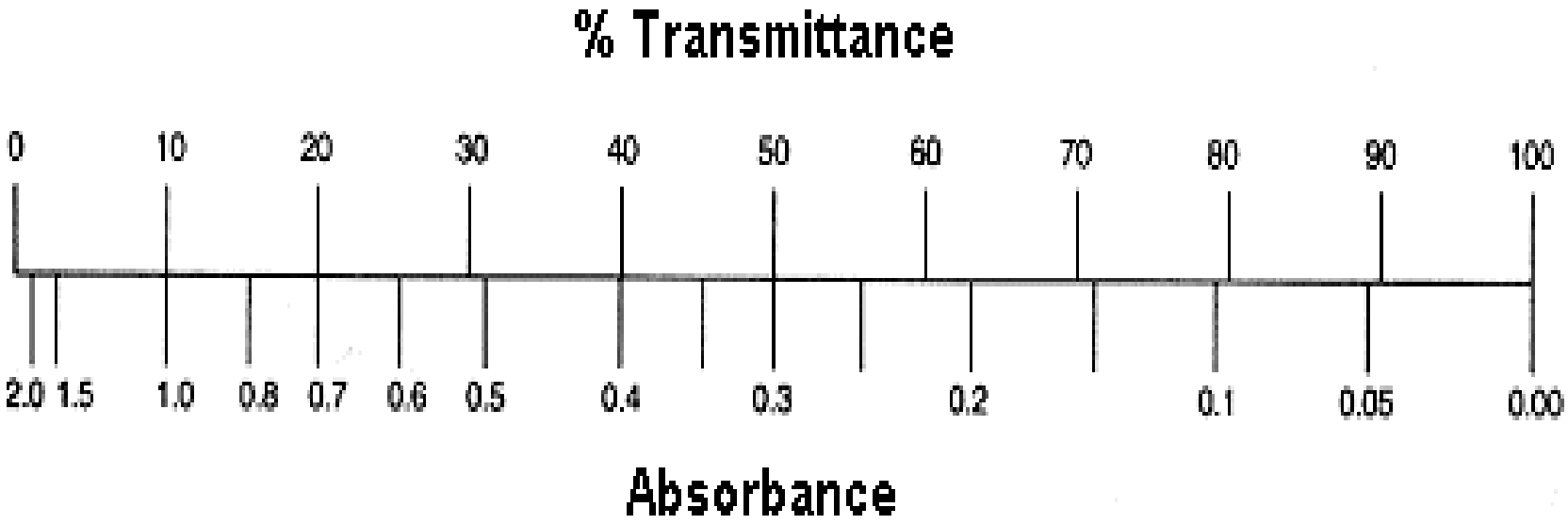
From **A Working Curve** – one can determine the concentration of an unknown sample by knowing the absorption.

Equation Summary:

$$T = (I/I_o) = 10^{-A}$$

$$\%T = (I/I_o) \times 100$$

$$A = -\log T = \log(1/T)$$

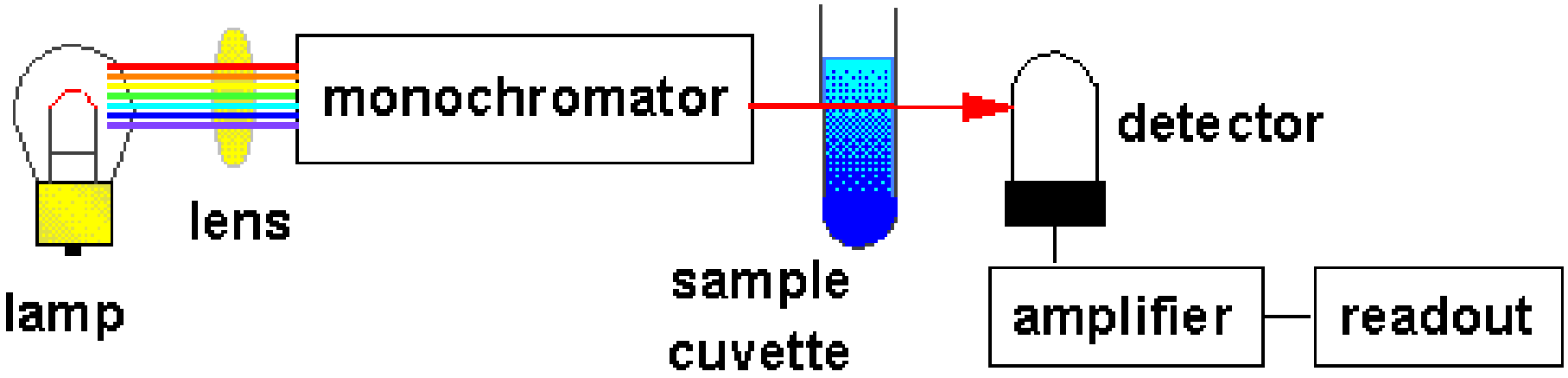


Note the scale for Absorbance: 9/10th of the scale is from 0-1 and 1/10th is from 1-2. For this reason, the spectrometers have been calibrated in % Transmittance and all readings will be taken in %Transmittance.

Spectronic 20 (a.k.a. Spec-20)

- **Spec-20** - A single-beam visible light spectrophotometer.
- Tungsten filament lamp emits visible wavelengths of light.
- Blank is inserted to adjust 100%Transmittance at each wavelength.

Simple Spectrophotometer Schematic

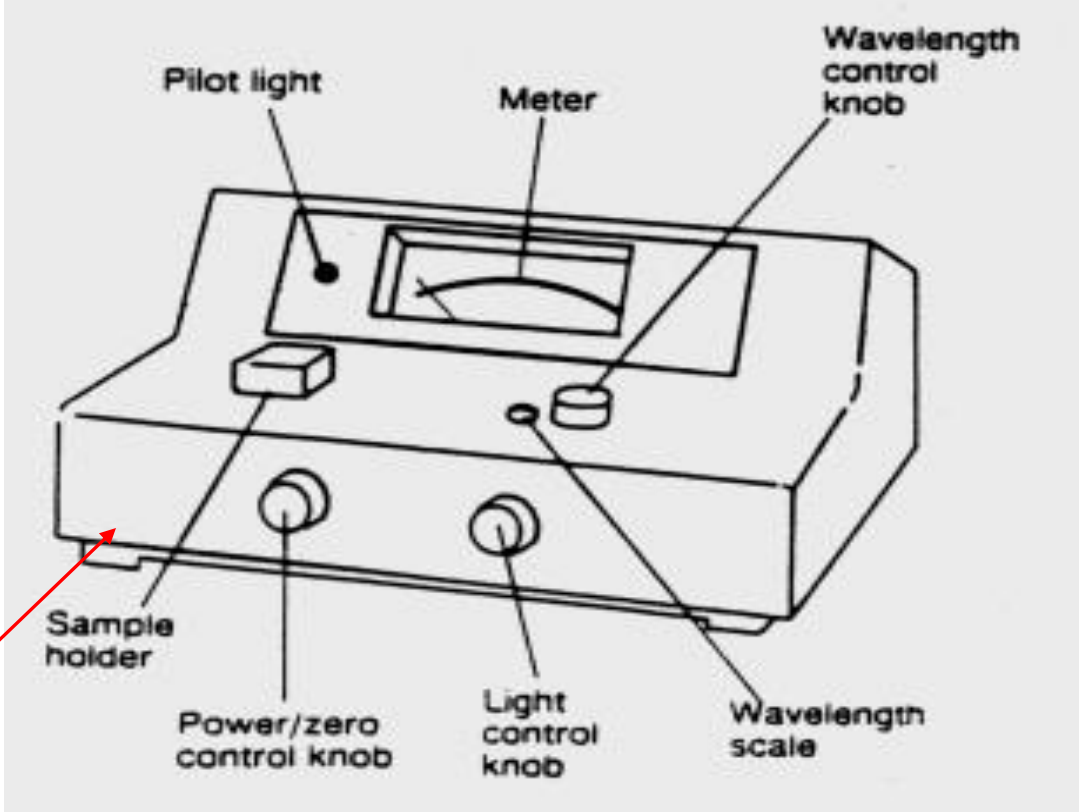


- **The lamp** emits all colors of light (i.e., white light).
- **The monochromator** selects one wavelength and that wavelength is sent through the sample.
- **The detector** detects the wavelength of light that has passed through the sample.
- **The amplifier** increases the signal so that it is easier to read against the background noise.

Spectronic 20 Instructions

(available next to each instrument)

1. With sample **chamber empty**, set desired wavelength then adjust to **0%T** with left knob on front panel.
2. **Insert blank solution**, close lid and adjust **100%T** with right knob on front panel.
3. **Insert dye solutions**, read and record **%T** values.
4. **Change wavelength**, **repeat steps 2-4.**



NOTE: Some digital instruments have a filter on the lower left of the front panel that must be changed midway through the wavelength range studied.

THANK YOU