Colorimetry

&

Spectrophotometry

Useful Terminology

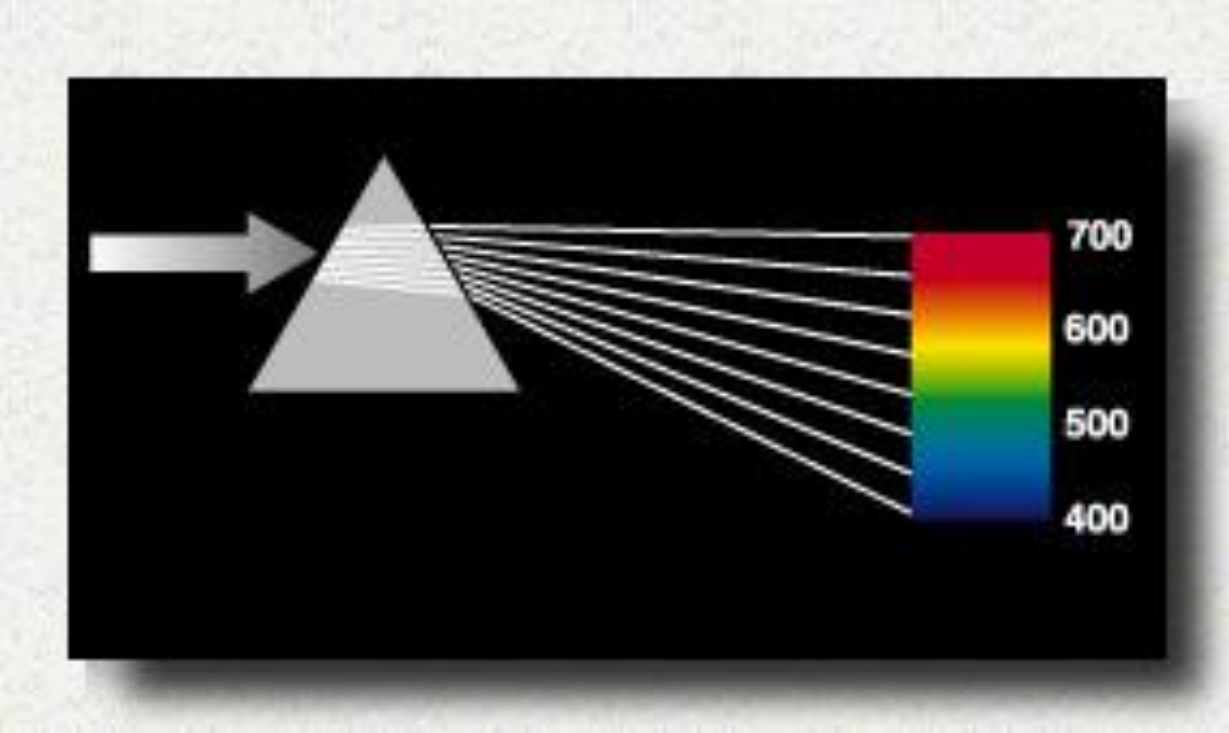
- **Colorimetry** is the use of the human eye to determine the concentration of colored species.

- **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.

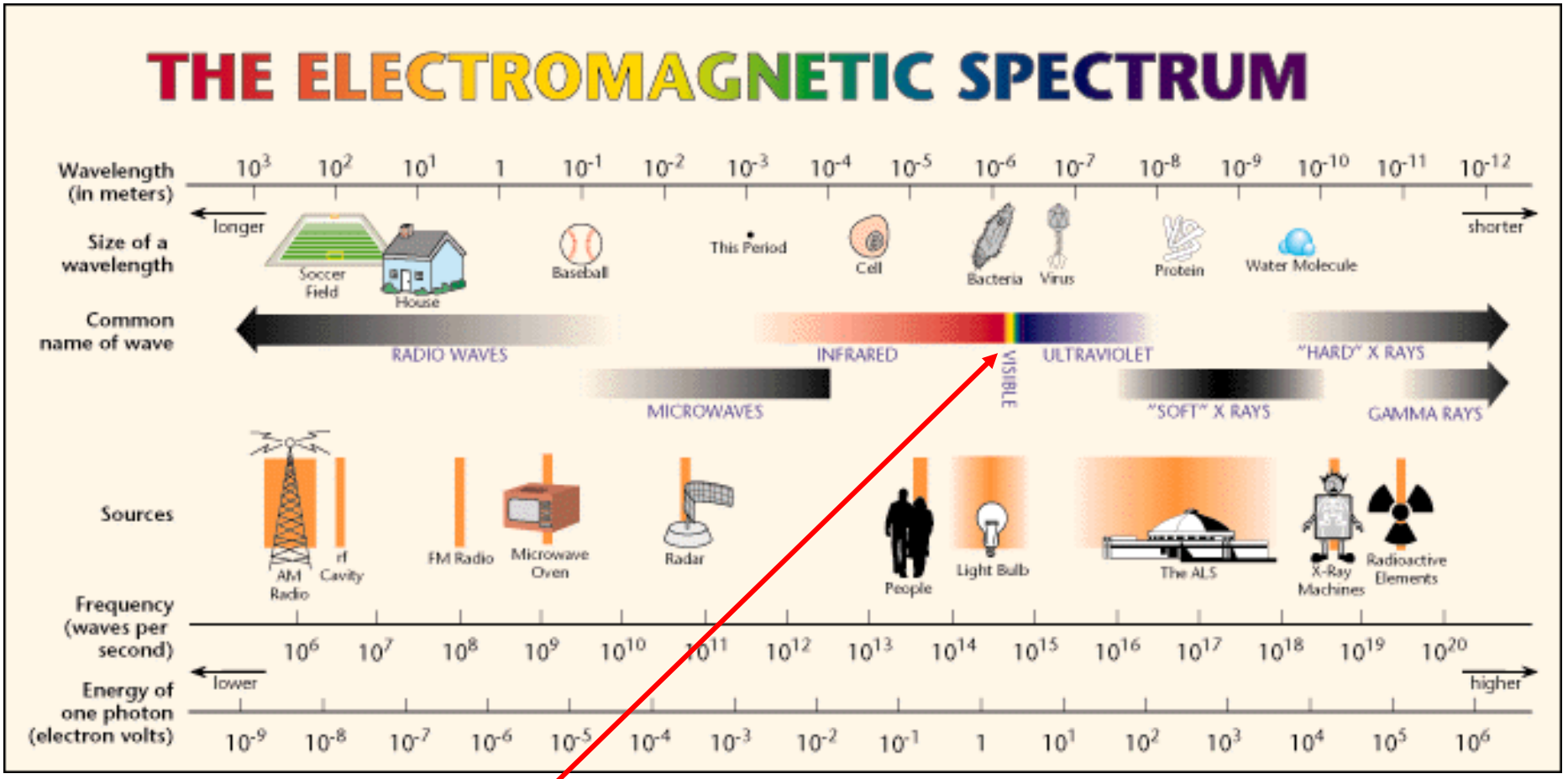
Note: This experiment will demonstrate **both techniques** on the **same set of dyes**.

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.

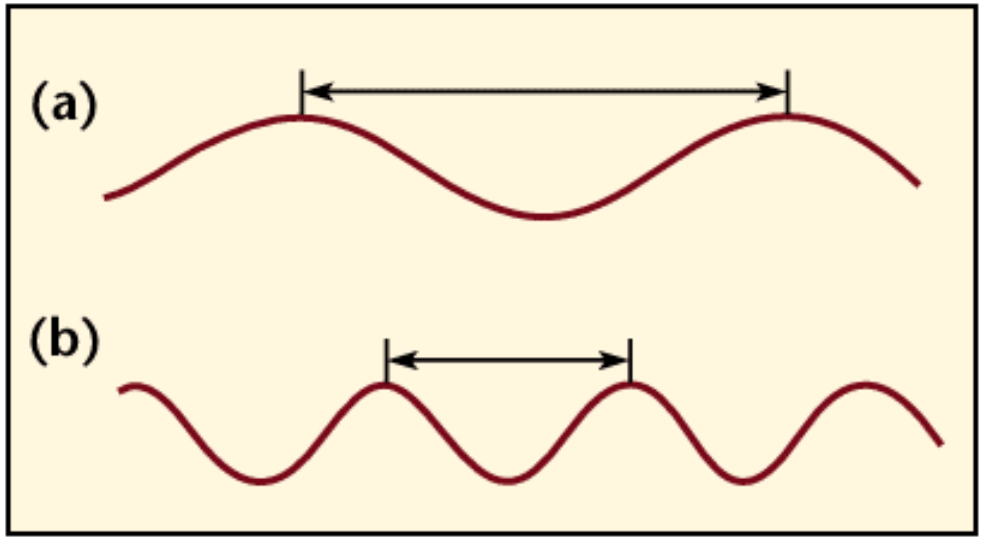
Electromagnetic Spectrum

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:

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

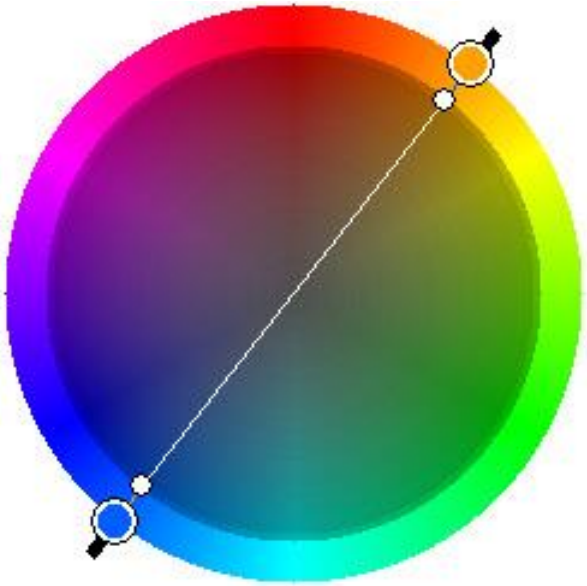
Where h = Planck's constant & c = speed of light in a vacuum.



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

Color Wheel

(ROYGBIV)



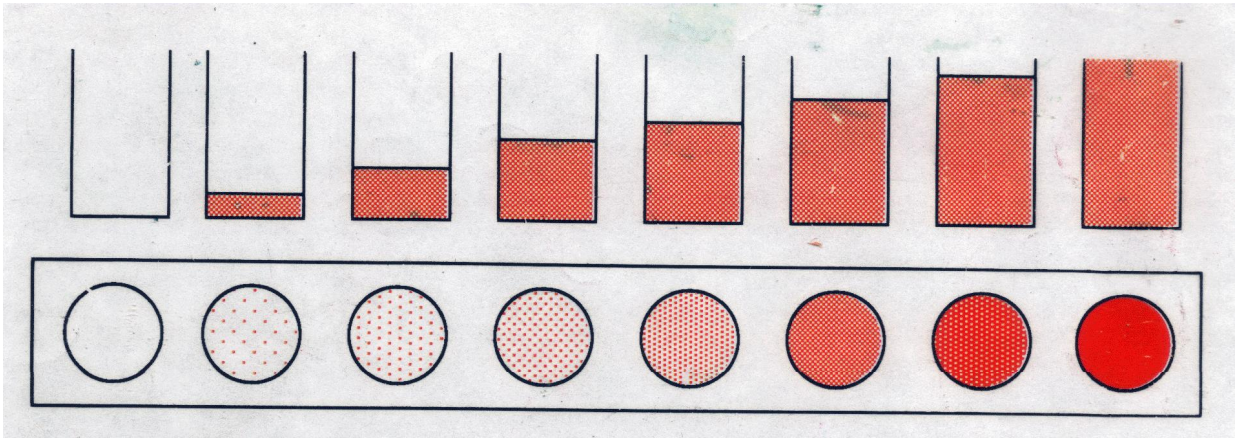
Complementary colors lie across the diameter on the color wheel and combine to form **"white light"**, so the color of a compound seen by the eye is the complement of the color of light absorbed by a colored compound; thus it **completes** the color.

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

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	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.**

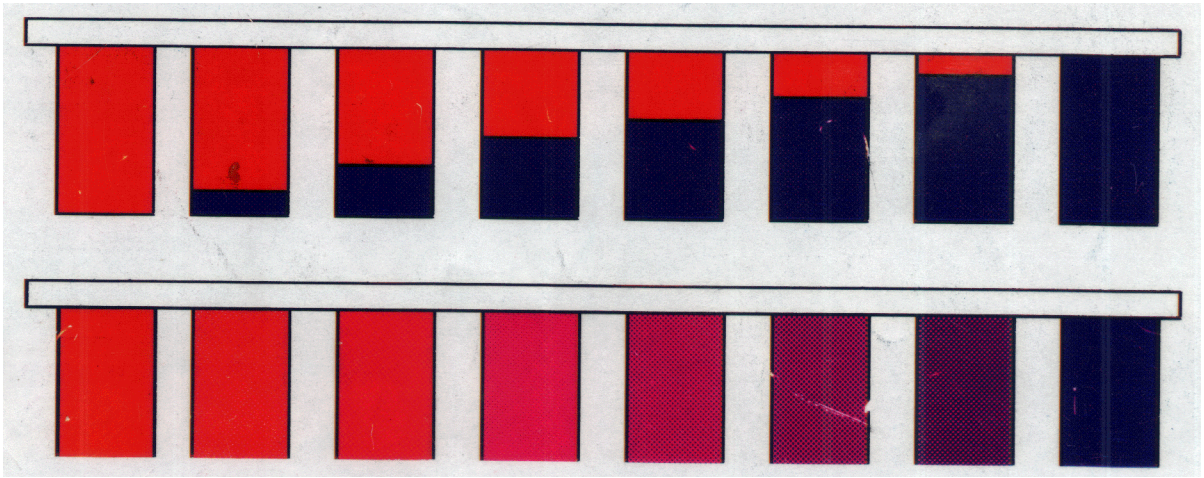


←**Side view**

←**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**

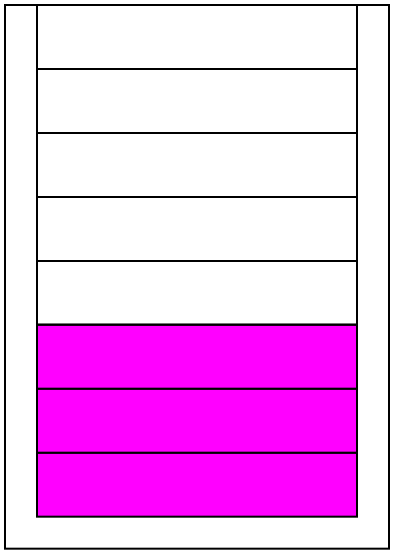
←**Purple produced**

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)

Recall: $C_1V_1 = C_2V_2$



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

Then for the dilution,

$$C_{diluted} \times V_{diluted} = C_{std} \times V_{std}$$

$$C_{diluted} = C_{std} \times (V_{std} / V_{diluted})$$

Since $V_{diluted} = V_{total}$

$$C_{diluted} = C_{std} \times (V_{std} / V_{total})$$

Substituting the volumes:

$$C_{diluted} = C_{std} \times (3 \text{ drops} / 8 \text{ drops})$$

If the **original concentration is 5.88 ppm**, then:

$$C_{diluted} = 5.88 \text{ ppm} \times (3 / 8)$$

$$C_{diluted} = 2.21 \text{ ppm}$$

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.

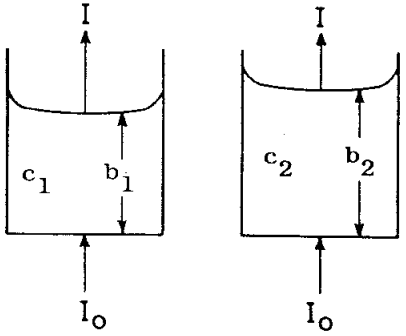


Fig. I-1. Transmission of Light Through Solutions

$$\log \frac{I_o}{I} = a_s c_1 b_1 = a_s c_2 b_2 \qquad (5)$$

$$c_1 b_1 = c_2 b_2 \qquad (6)$$

$$\frac{c_1}{c_2} = \frac{b_2}{b_1} \qquad (7)$$

This is the fundamental relationship used in color comparators.

Duboscq visual colorimeter

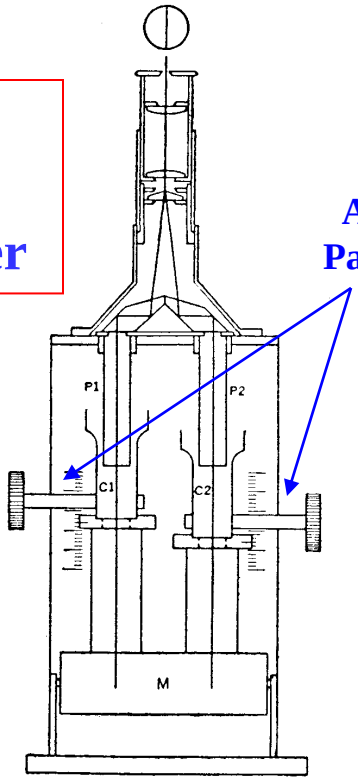


Fig. I-6. Optical Path in a Colorimeter of the Duboscq Type. P_1, P_2, plungers; C_1, C_2, cups to hold the solutions; M, mirror. The two halves of the field viewed through the ocular appear equally bright when a match has been obtained.

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**.

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)]

Also called "**extinction coefficient**" or "**ε**";
it is dependent on the material being studied.

"b" is the path length in cm

The **diameter of the cuvette** or sample holder which is the distance the light travels through the absorbing sample. "b" is a **constant** when the **same size cuvette** is used for all samples.

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

Main use of Beer's Law is to **determine the concentration** of various solutions.

Transmittance is Related to Absorbance

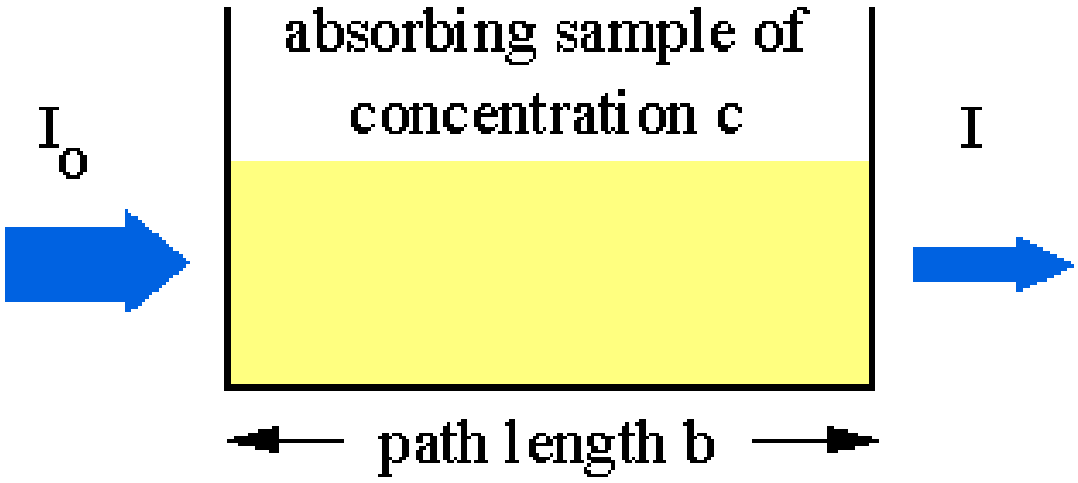
Transmittance is given by the equation:

$$T = I/I_o$$

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:

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



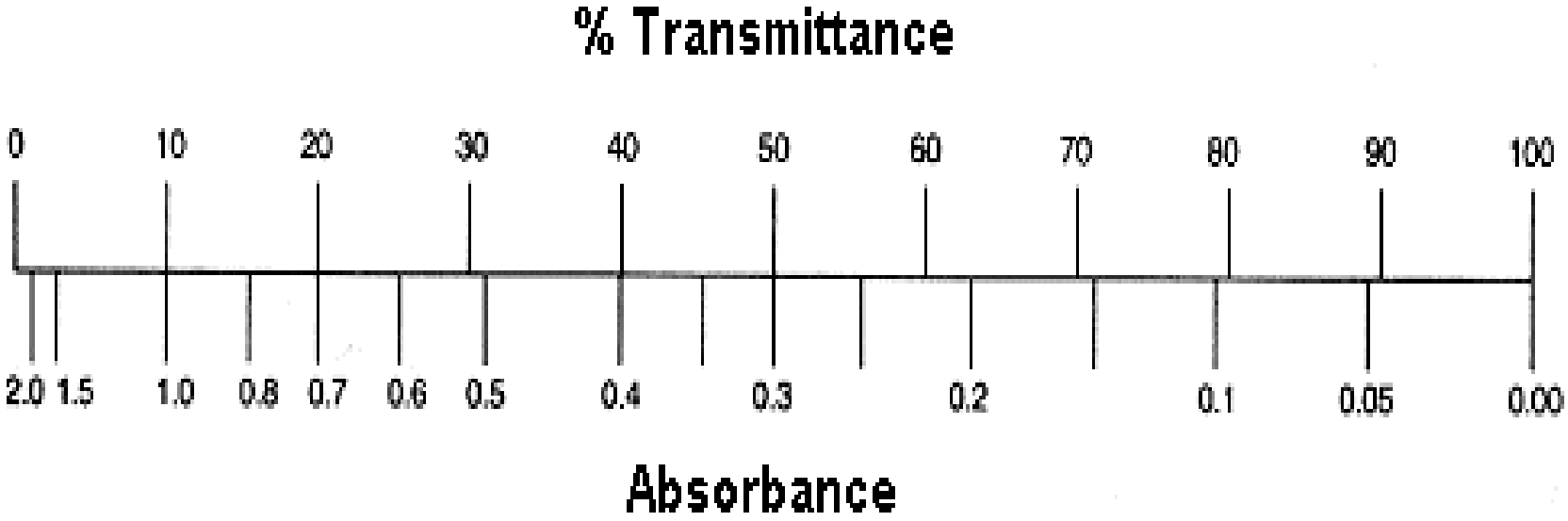
Equation Summary

$T = (I/I_o) = 10^{-A}$ $\quad$ $\%T = (I/I_o) \times 100$ $\quad$ $A = -\log T = \log(1/T)$

Sample Calculation

If %T = 95%, then $\quad$ $A = \log(100/95) = \log(1/.95) = -\log(.95)$

A = 0.02227

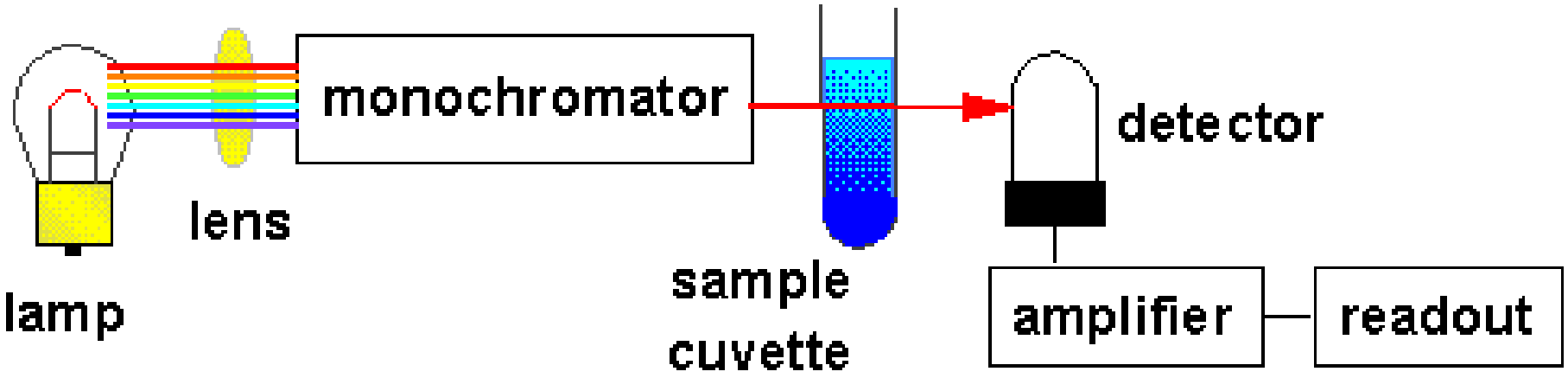


Note the scale for Absorbance: 9/10th of the scale is from 0-1 and 1/10th is from 1-2. For this reason, the spectrometers have been calibrated in % Transmittance and all readings will be taken in %Transmittance.

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

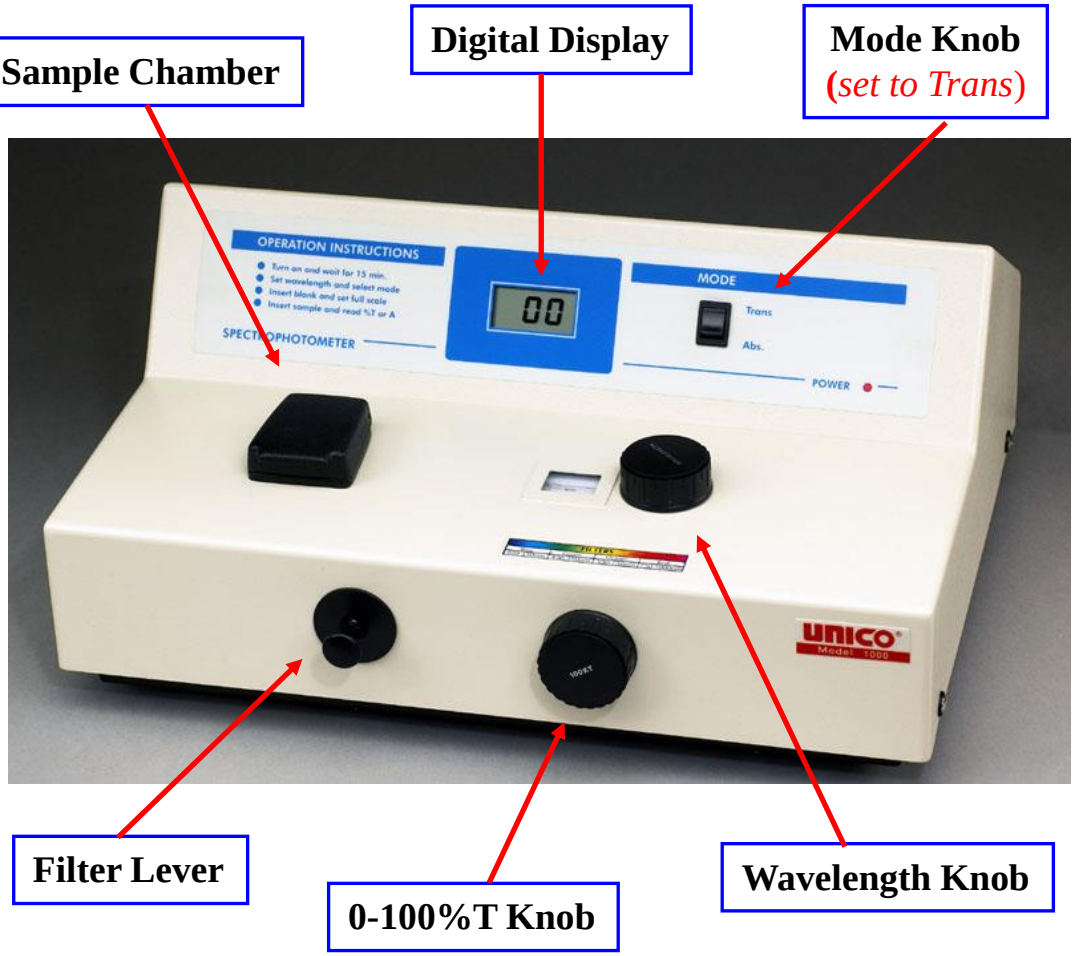


- **The lamp** emits all colors of light (i.e., white light).
- **The monochromator** selects **one wavelength** and that wavelength is sent through the sample.
- **The detector** detects the wavelength of light that has passed through the sample.
- **The amplifier** increases the signal so that it is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

Spectronic 20 Instructions

(Directions below will be available next to each instrument)

1. With sample **chamber empty**, set desired wavelength then adjust to **0%T** with right 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.**

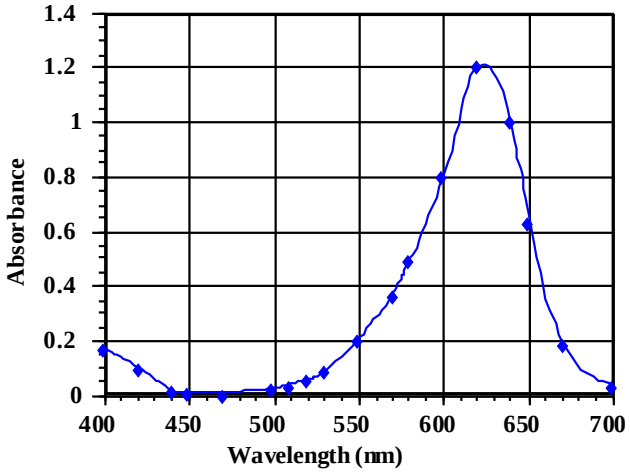


***NOTE:** The **filter** must be **changed** periodically **to coordinate with the wavelength** range studied: **blue (400-449), green (450-549)** and **orange (550-749).**

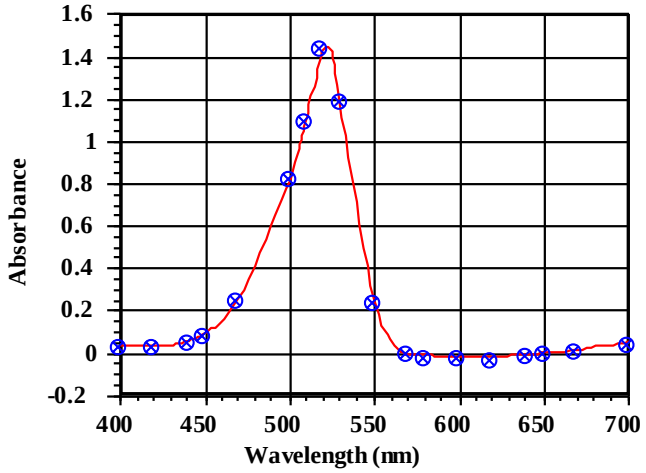
Post Lab: 4 Plots of Absorption Data

Plots similar to the 3 below will need to be generated using a computer program such as Excel. You will also need to make a plot of your unknown blue or red which will look similar to #1 or #2.

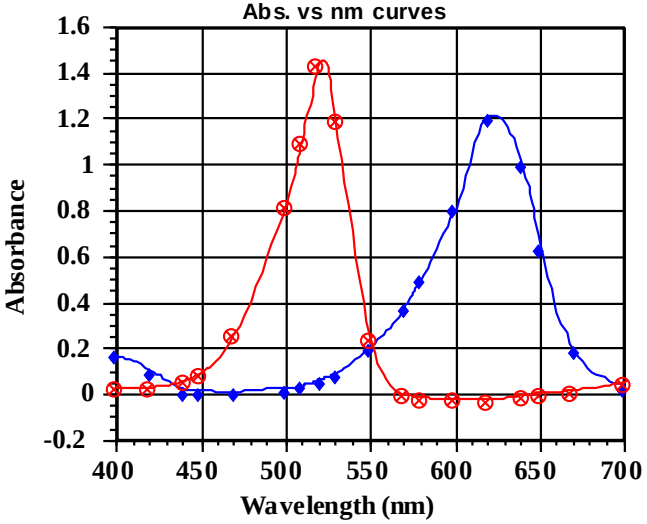
#1 Plot of Abs. vs nm for Blue Dye



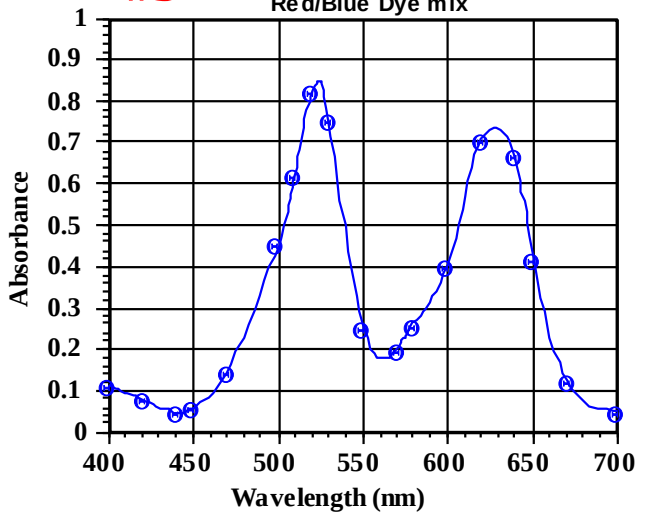
#2 Plot of Abs. vs nm for Red Dye



Overlay Plot of Blue and Red dye
Abs. vs nm curves



#3 Plot of Abs. vs nm for Purple
Red/Blue Dye mix



Checkout

Visual Portion

1 - 12 well plate
3 - 12 well strips*
5 - Beral pipets**

Spec-20s

5 - cuvettes in a test tube rack

***2 of which need to be at least 9 wells long.**
****Don't have to be returned.**

**There aren't enough Spec-20s for all groups.
So 1/2 will start with the Spec-20s
and 1/2 will start with the visual portion.**

Dyes - Located in Lab: Record Concentrations

Blue std. = _____ ppm
Red std. = _____ ppm

Waste

We are using FDA food dyes and distilled water.


For April 9-12

Turn In:

Colorimetry & Spectrophotometry pp 51-58
+ 4 Graphs

Read Over:

Antacid Analysis (pp 33-34) in Green Book
& **Dimensional Analysis #4-5**
(pp 28-34 in the first book).