



Colorimetry

&

Spectrophotometry



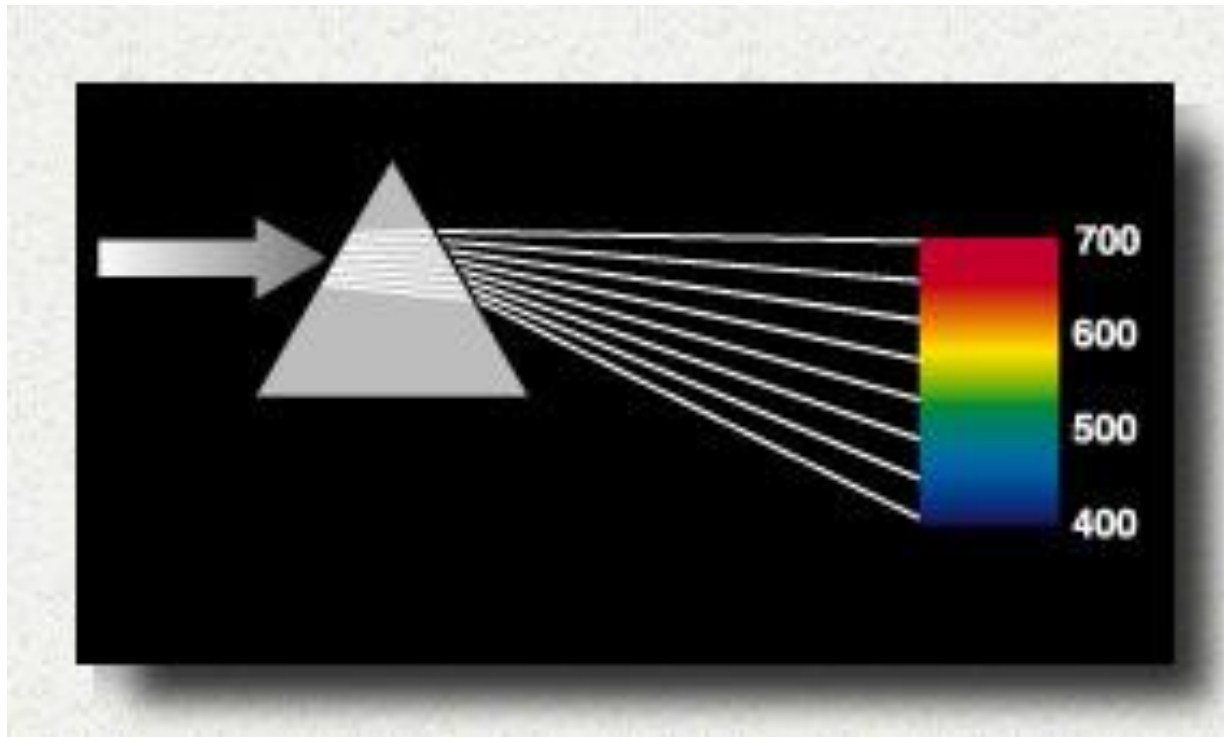
Useful Terminology

- 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.

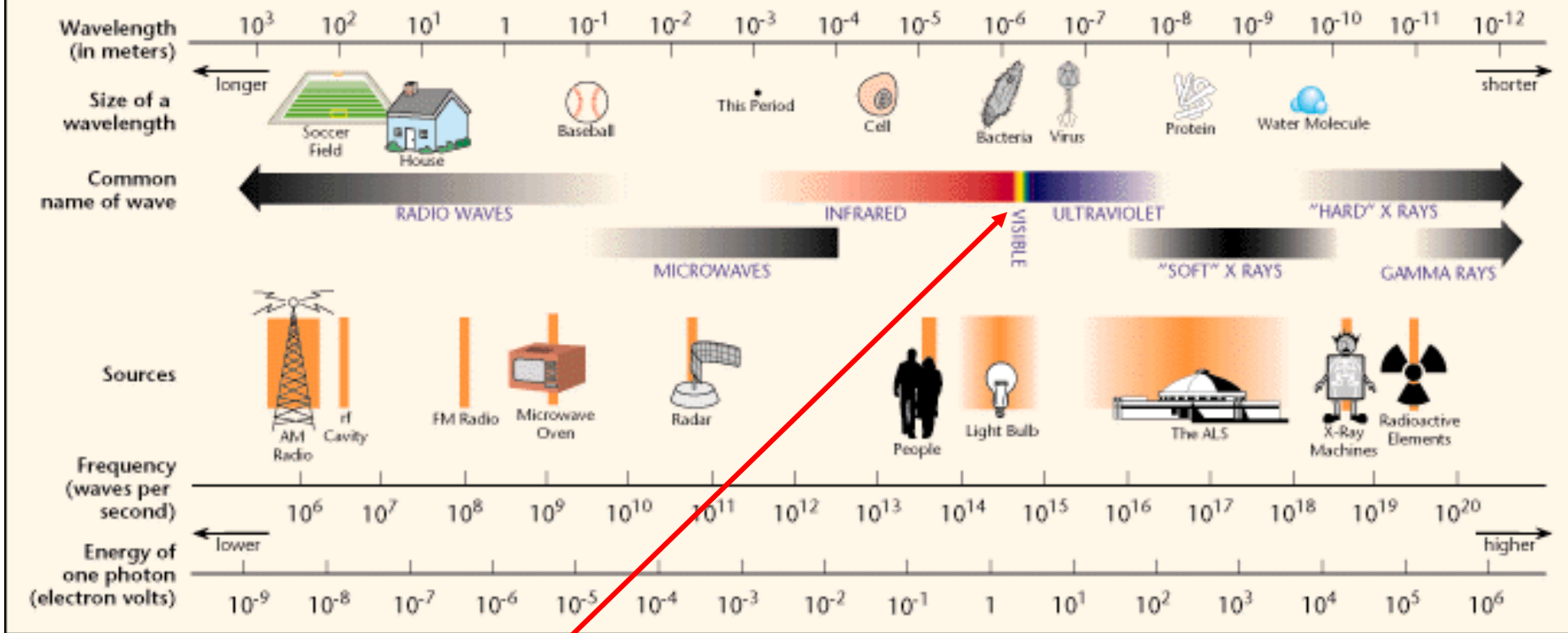
Colorimetry

- 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.

THE ELECTROMAGNETIC SPECTRUM



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

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

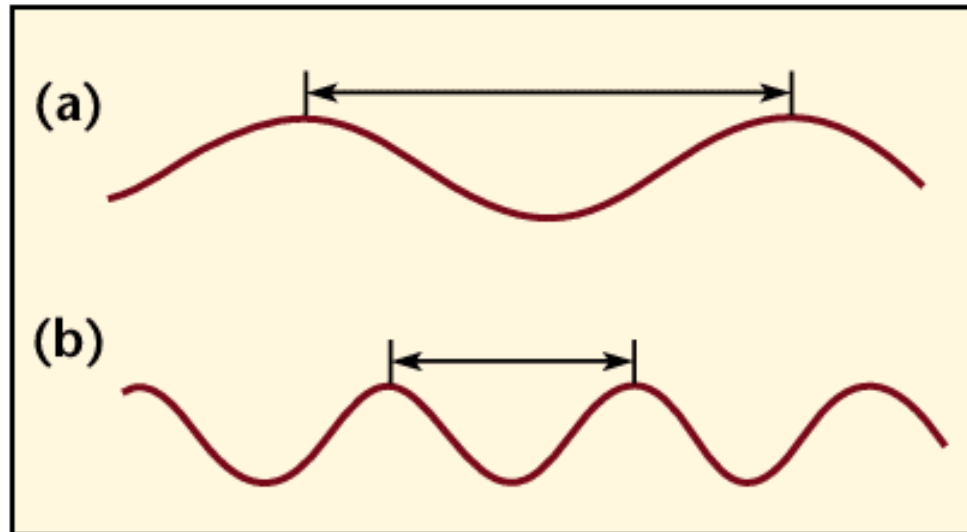
Electromagnetic Spectrum

<u>Type of Radiation</u>	<u>Frequency Range (Hz)</u>	<u>Wavelength Range</u>	<u>Type of Transition</u>
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	4-7.5×10^{14}	750 nm-400 nm	outer electron
near-infrared	1×10^{14} - 4×10^{14}	2.5 μ m-750 nm	outer electron molecular vibrations
infrared	10^{13} - 10^{14}	25 μ m-2.5 μ m	molecular vibrations
microwaves	3×10^{11} - 10^{13}	1 mm-25 μ m	molecular rotations, electron spin flips*
radio waves	< 3×10^{11}	>1 mm	nuclear spin flips*

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

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

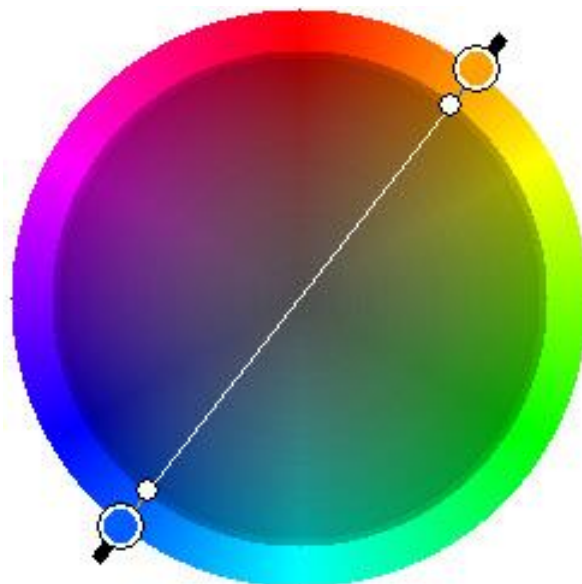
Where h = Planck's constant & c = speed of light in a vacuum.



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

Color Wheel

(ROYGBIV)



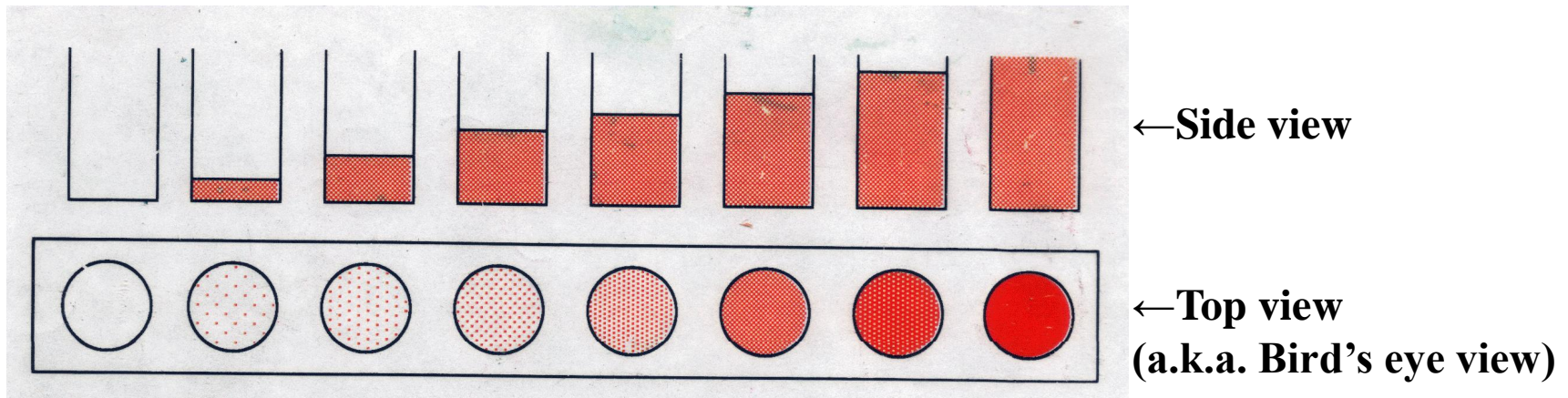
Complementary colors lie across the diameter on the color wheel and combine to form “**white light**”, so the color of a compound seen by the eye is the complement of the color of light absorbed by a colored compound; thus it completes the color.

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	Green	500 nm
Orange	Blue	450 nm
Yellow	Violet	400 nm

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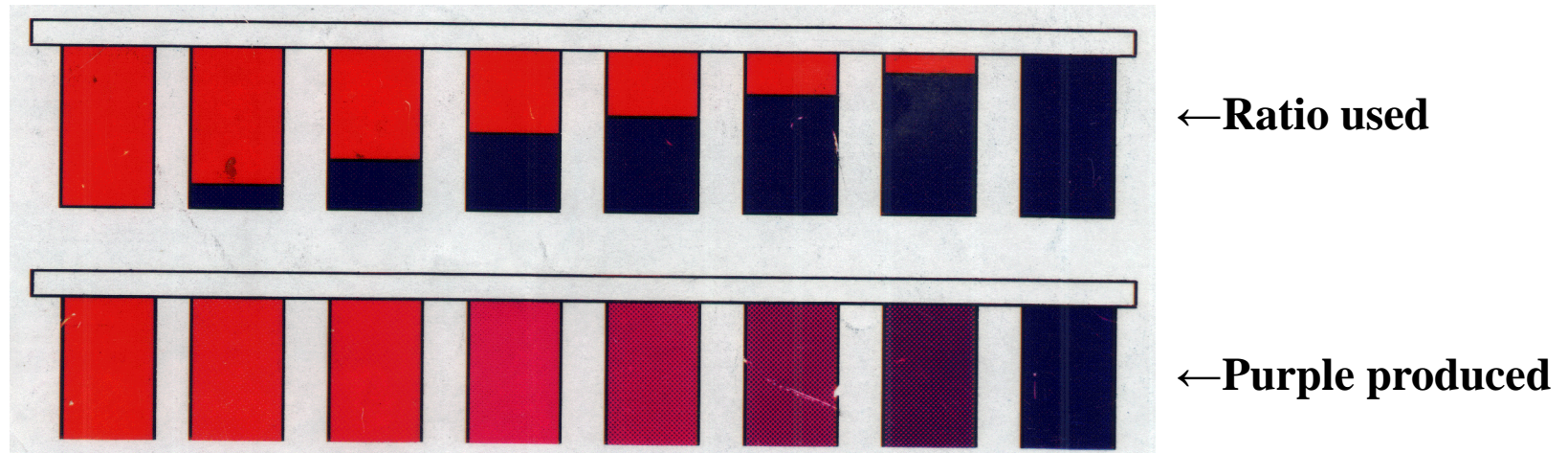
Visual Colorimetry

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.**



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



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: $C_1V_1 = C_2V_2$

Then for the dilution,

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

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

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

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

Substituting the volumes:

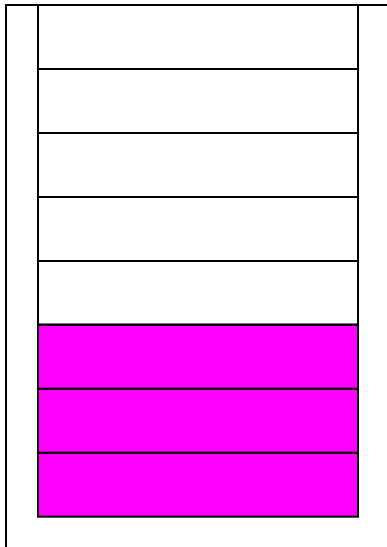
$$C_{\text{diluted}} = C_{\text{std}} \times (3 \text{ drops} / 8 \text{ drops})$$

If the original concentration is 5.88 ppm,

then:

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

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



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

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.

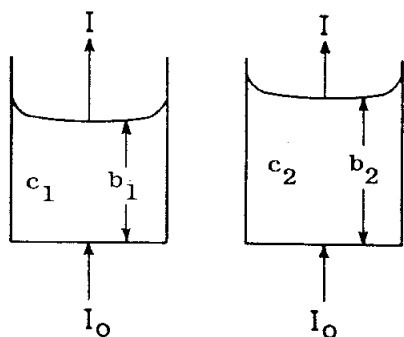


Fig. I-1. Transmission of Light Through Solutions

$$\log \frac{I_0}{I} = a_s c_1 b_1 = a_s c_2 b_2 \quad (5)$$

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

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

This is the fundamental relationship used in color comparators.

**Duboscq
visual
colorimeter**

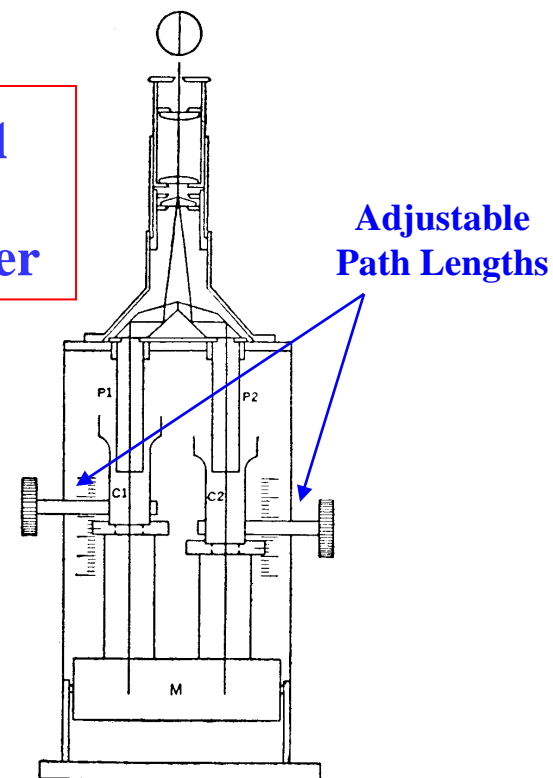


Fig. I-6. Optical Path in a Colorimeter of the Duboscq Type. P_1 , P_2 , plungers; C_1 , C_2 , cups to hold the solutions; M , mirror. The two halves of the field viewed through the ocular appear equally bright when a match has been obtained.

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

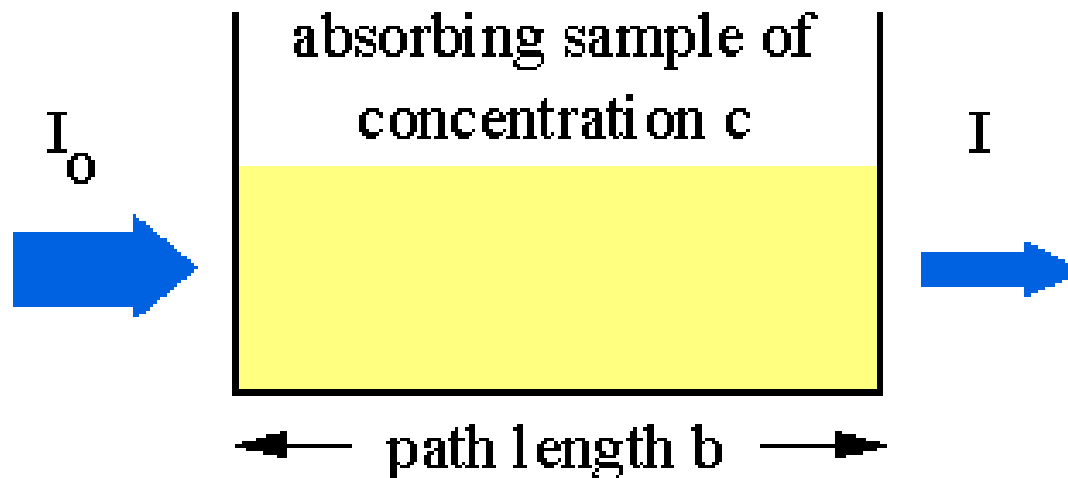
Transmittance is given by the equation:

$$T = I/I_0$$

where I is the intensity of the light after it has gone through the sample & I_0 is the initial light intensity.

Absorbance is related to the %T:

$$A = -\log T = -\log(I/I_0)$$



Equation Summary

$$T = (I/I_0) = 10^{-A}$$

$$\%T = (I/I_0) \times 100$$

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

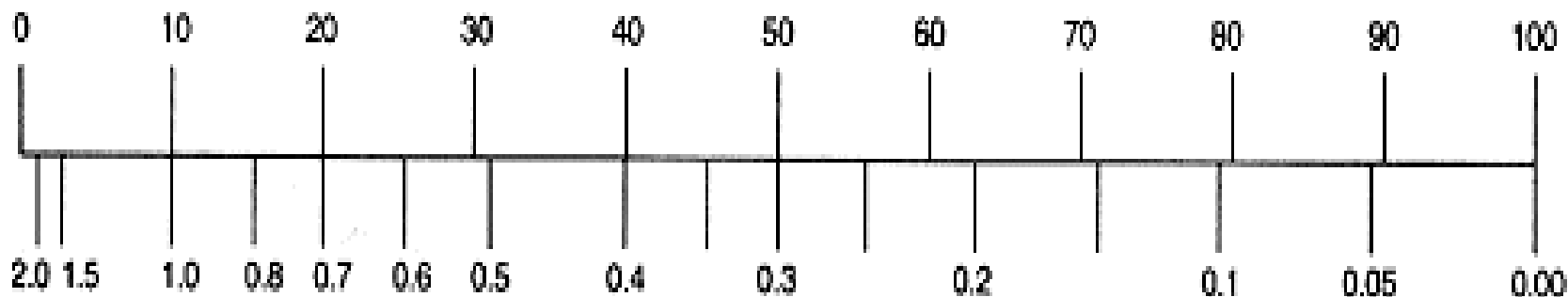
Sample Calculation

If $\%T = 95\%$, then

$$A = \log(100/95) = \log(1/.95) = -\log(.95)$$

$$A = 0.02227$$

% Transmittance



Absorbance

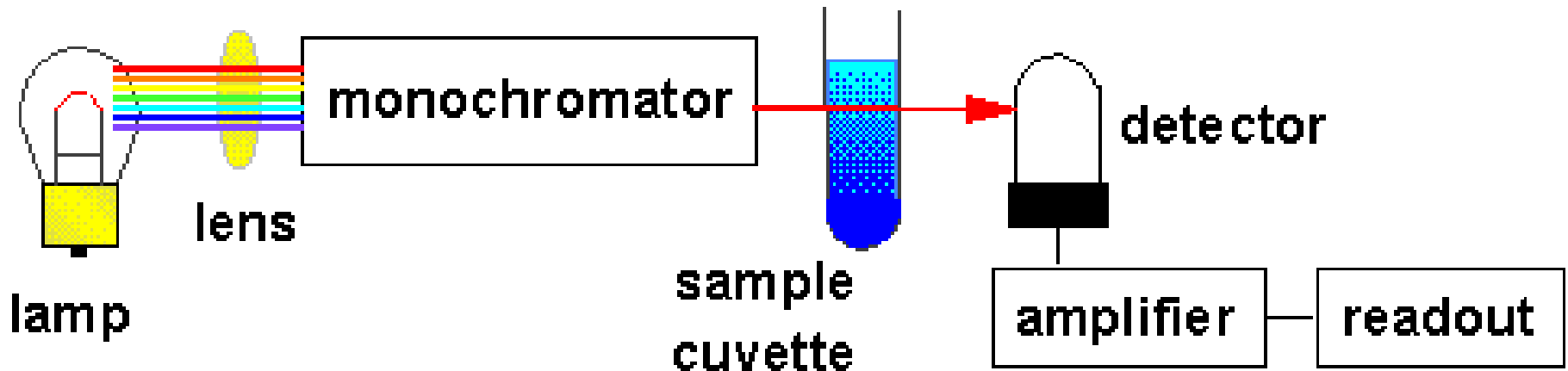
Note the scale for Absorbance: 9/10th of the scale is from 0-1 and 1/10th is from 1-2. For this reason, the spectrometers have been calibrated in % Transmittance and all readings will be taken in % Transmittance.

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

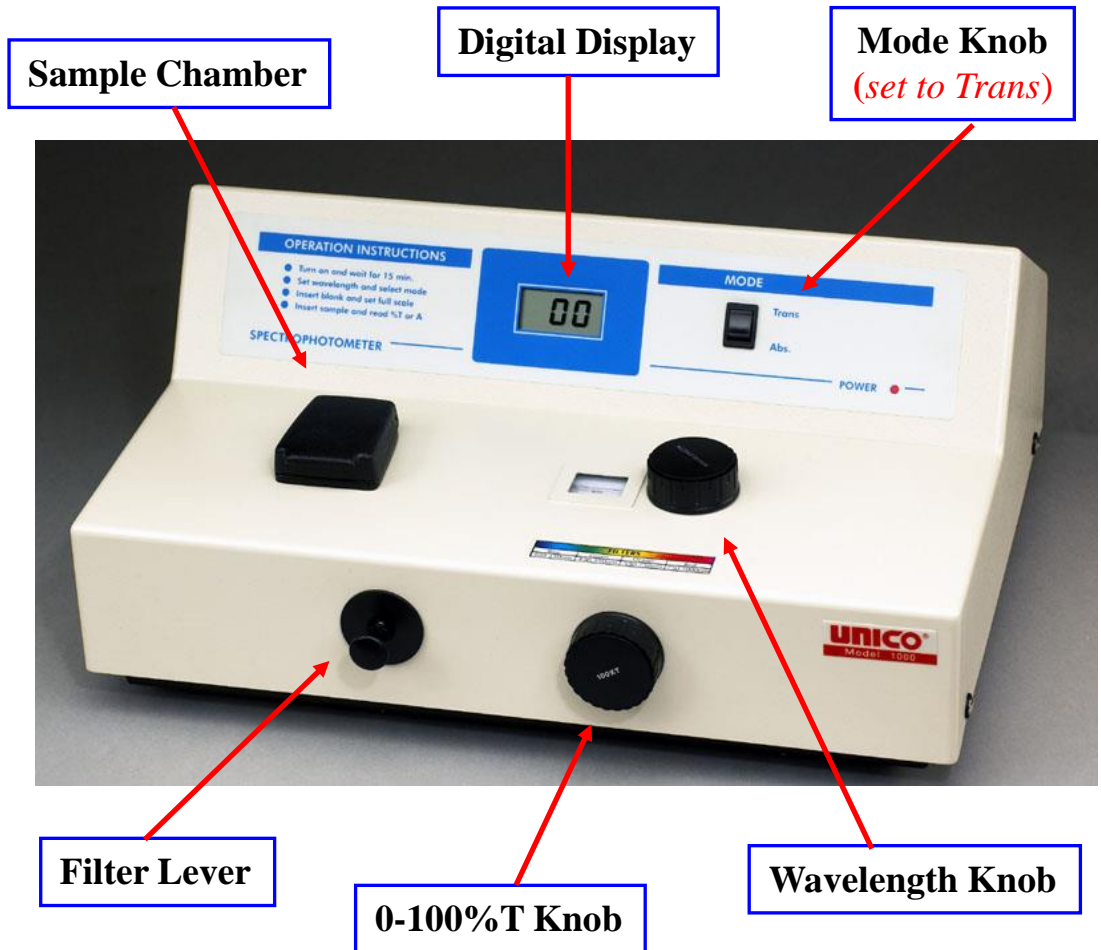


- **The lamp** emits all colors of light (i.e., white light).
- **The monochromator** selects **one wavelength** and that wavelength is sent through the sample.
- **The detector** detects the wavelength of light that has passed through the sample.
- **The amplifier** increases the signal so that it is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

Spectronic 20 Instructions

(Directions below will be available next to each instrument)

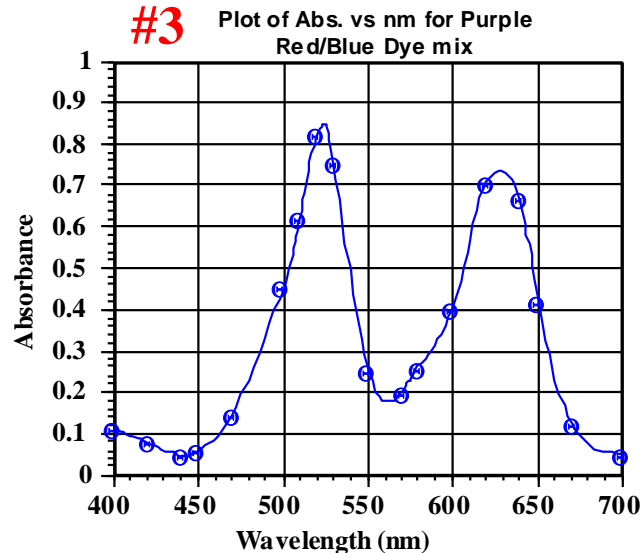
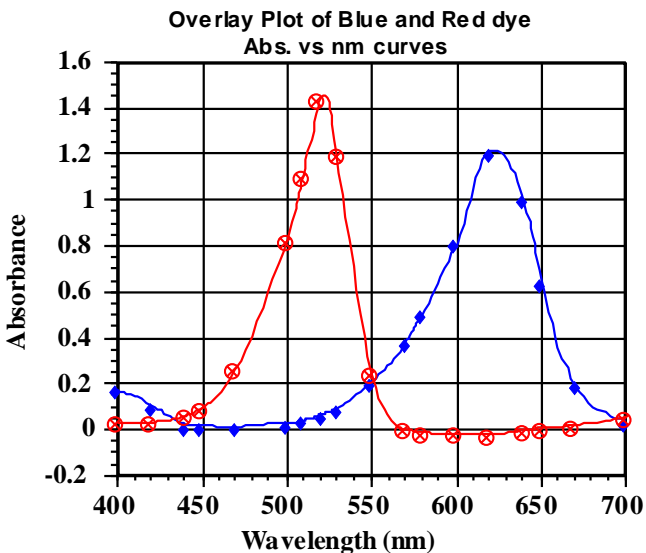
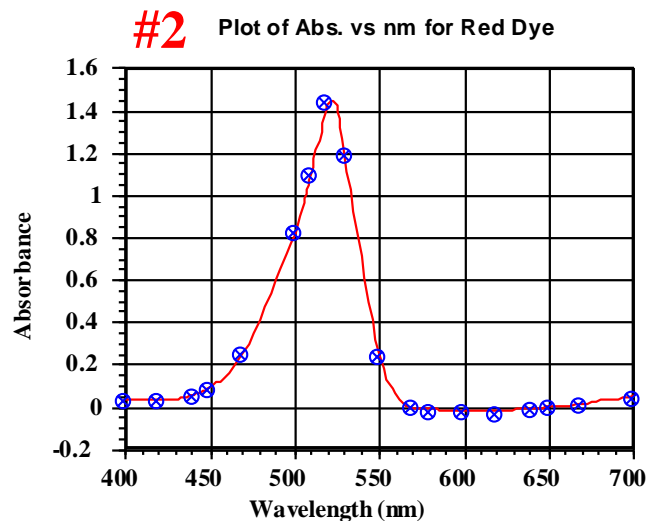
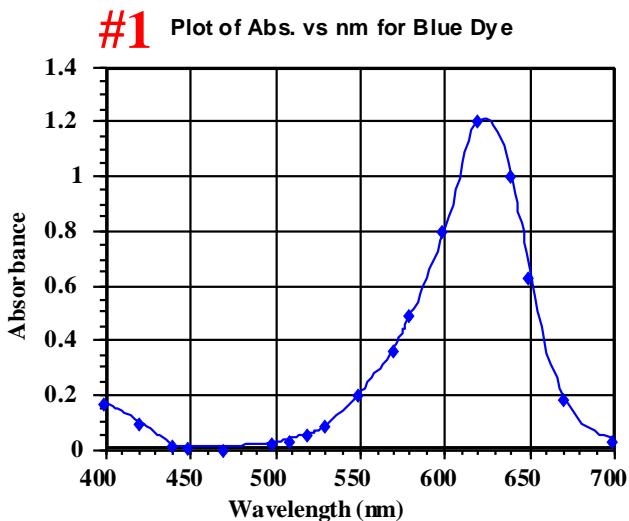
1. With sample **chamber empty**, set desired wavelength then adjust to **0%T** with right knob on front panel.
2. **Insert blank solution**, close lid and adjust **100%T** with right knob on front panel.
3. **Insert dye solutions**, read and record **%T** values.
4. **Change wavelength***, repeat **steps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



Checkout

Visual Portion

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

*2 of which need to be at least 9 wells long.

****Don't have to be returned.**

Spec-20s

- 5 - cuvettes in a test tube rack

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).**

