Standard Curve: Food Dyes
Beer's Law

There is an inverse correlation between transmittance and absorbance. The more light that is absorbed by a sample, the less light will be transmitted. 0 absorbance = 100% transmittance. The log (1/T) = -log T = abC. By substituting absorbance (A) for transmittance, absorbance and concentration become directly proportional.

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

$$\mathbf{A = abC}$$

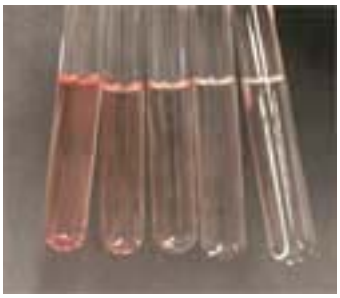
This is known as **Beer's Law.**

This relationship enables us to set up what is known as a **standard curve**. If we take absorbance readings of at least five dilutions of a standard of known concentration against a blank we can plot absorbance vs. concentration and get a straight line. By then taking an absorbance reading of a sample of the same substance of an unknown concentration and locating where that absorbance reading (Y axis) falls on the standard curve we can find the corresponding concentration on the X axis.

A. Preparation of Standard Curve

1. Make up the following solutions (A-E) from a stock solution of red food dye. These will be used to make the standard curve. The Red Food Dye Stock Solution = 0.1mL red food dye/100mL H_2O.

A = 10mL Red Stock Solution + 10mL H_2O
B = 10mL A + 10mL H_2O
C = 10mL B + 10mL H_2O
D = 10mL + 10mL H_2O
E = 10mL D + 10mL H_2O



2. Read the absorbance at 500nm for tubes A-E.

B. Drawing the Standard Curve

1. Determine the concentration of red dye in ml/100ml for tubes A-E.
   The stock solution had a concentration of 0.1 ml red dye/100 ml so the concentration of the solution in Tube A would be 0.05 ml red dye/100 ml.
   Use the equation $V_1 X C_1 = V_2 X C_2$ if necessary to determine the concentrations:
   $$(10 \text{ ml})(0.1 \text{ ml/100ml}) = (20 \text{ ml})(C_2)$$
   $$C_2 = 0.05 \text{ ml red dye/100 ml}$$

2. Convert each tube A-E to ml red dye/100ml x 10^{-3}.
   For tube A this would be 50 ml red dye/100 ml x 10^{-3}

3. Make a standard curve. Plot absorbance on the y-axis and concentration of red dye (ml/100ml x 10^{-3}) on the x-axis.

4. Determine the equation of the line.

C. Determining the Concentration of an Unknown

1. Obtain solutions of unknown concentration.

2. Take absorbance readings of the unknowns.

3. Using standard curve, determine concentration of unknowns. Plug the absorbance (y-value) into the equation and solve for x.

Solutions	Concentration of red dye (ml/100ml H_2O)	Concentration of red dye (ml/100ml H_2O x 10^{-3})	Absorbance @ 500 nm
A			
B			
C			
D			
E			
Unknown 1			
Unknown 2			