Beer's Law and Spectrophotometry Assoc. Prof. Kozet YAPSAKLI



Solution Concentration Reminders

- Concentration expresses how the amount of solute and the amount of solution compare
- * Our unit of choice: Molarity = mol/L
- Conc is moles (number of ions/atoms/molecule) per volume
- * More molecules means more light is "caught"
- The color fade as concentration is lessened



Beer's Law

- Beer's Law quantifies the relationship between color (or any light absorbing species) and concentration
- Beer's Law states that the *absorbance* of light by a solution is directly proportional to
 - * Emissivity
 - Cell width
 - * concentration



Conceptual Basis of Beer's Law

Emitted radiation P_E Sample Thermal, electrical,

or chemical energy

- Light of a particular wavelength enters the 'sample'.

c. a.

I₀

 Light scatters from particles in solution reducing light transmission

- Light is *absorbed* by molecules/particles and remitted at different wavelengths, reducing light transmission

A little more In-depth:

Beer's Law is stated in a way to make certain quantities easy to compare and interpret.

Parameters:

- b sample pathlength (usually 1cm)
- c concentration (mol/vol)
- lpha molar absorption coefficient (x $1/\lambda$)
- I light intensity (W/m^2)

$$T = I / I_o \qquad \log(\frac{I_o}{I}) = \alpha bc \qquad A = \alpha bc$$
$$I = I_o 10^{-\alpha bc}$$

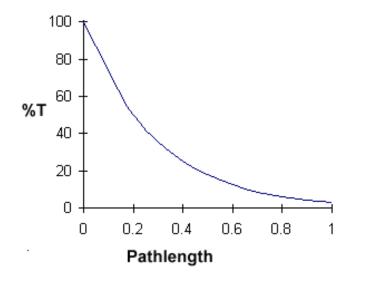
$$A = -\log T = \mathcal{E} b M$$

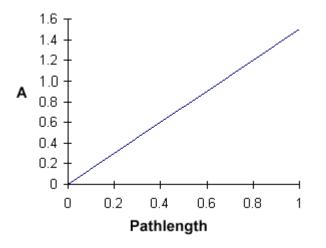
What is the absorbance when the light transmitted is 50% of the initial beam in a 2 cm path length cell for a concentration of 10⁻³ M?

Graphical Relationship

- * % transmission and % absorption are not linearly related to concentration
- * For a graph to be useful, a straight line is needed
- * ABSORBANCE = log(1/T) = -log(T)

A little more In-depth:





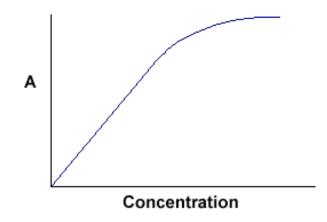
Connection between absorption and transmittance

Major application of Beers' law - determination of unknown concentration by measuring absorbance:

Α	Τ
0	1
1	0.1
2	0.01
3	0
8	0

When can I use Beer's Law?

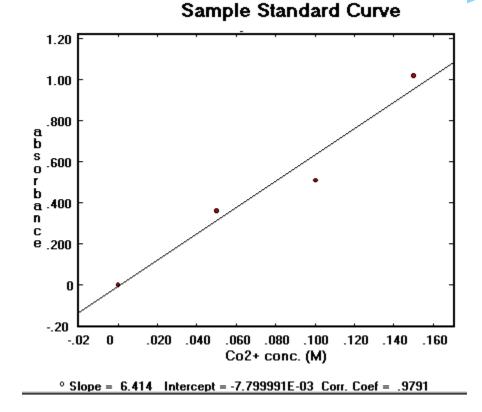
Only at low concentrations, where the absorbance is linear (single scattering event):



Rule of Thumb: A<1 for accurate results

So, What Does This Means

- It means that a graph of absorbance of light by a "kind" of solution to the concentration is linear.
- We can find concentrations by comparison



How Do We Do This?

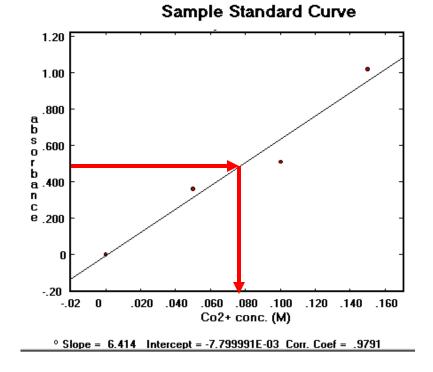
- We make several solutions with known concentrations
- We measure that transmittance and/or absorbance for the known solutions and the unknown solution
- We graph the knowns and interpolate for the unknown

A Successive Dilution Example

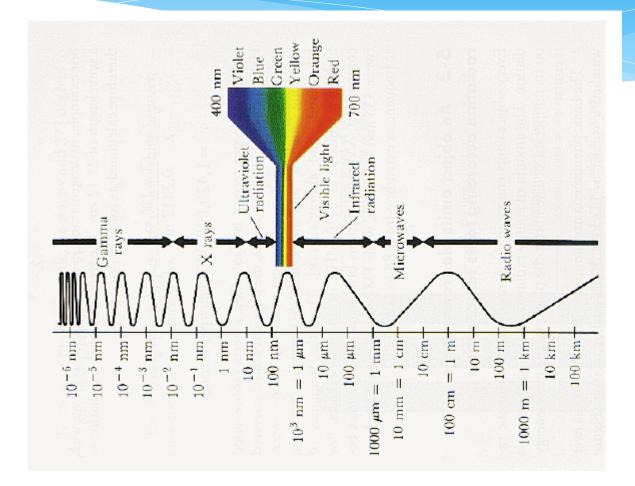
 Design a process of successive dilution to make 250.0 mls of the following solutions from a 1.00 M stock solution: 0.05 M, 0.10 M, 0.15 M.

Finding the unknown

- Suppose this graph is generated
- The unknown absorbance is 0.500
- * Find 0.500 absorbance
- * Across and down
- The concentration is
 0.775 (or so)



Spectrum of electromagnetic waves



Spectrophotometry deals with light within ~200-1000 nm

