INSTRUMENTAL ANALYSIS II

Unit 2

Absorption Laws (Quantitative Analysis)

Course Instructor: Ermias Haile (MSc)

Spectroscopy

Qualitative analysis

Characteristic wavelengths for a given analyte

⇓

Identification

Quantitative analysis

Intensity of emission or absorption

⇓

Concentration of analyte

The Absorption Process

- The absorption law, also known as the Beer-Lambert law or just Beer's law, tells us quantitatively how the amount of attenuation depends on the concentration of the absorbing molecules and the pathlength over which absorption occurs.
- As light traverses a medium containing an absorbing analyte, decreases in intensity occur as the analyte becomes excited. For an analyte solution of a given concentration, the longer the length of the medium through which the light passes (pathlength of light), the more absorbers are in the path and the greater the attenuation. Also for a given pathlength of light, the higher the concentration of absorbers, the stronger the attenuation.

…..The Absorption Process

- The attenuation of a parallel beam of monochromatic radiation as it passes through an absorbing solution of thickness **b** cm and concentration **c** moles per liter.

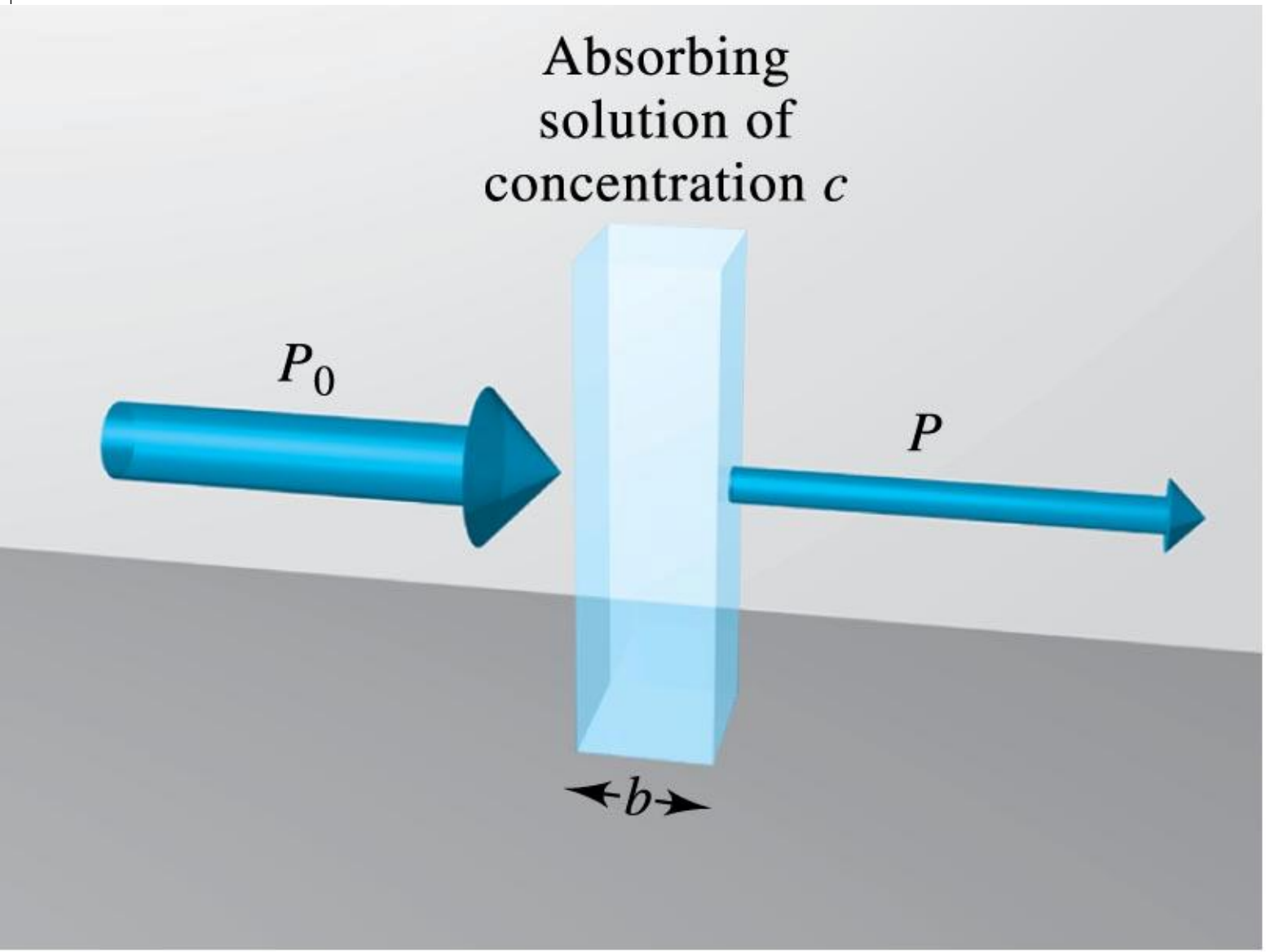
  Because of interactions between the photons and absorbing particles, the radiant power of the beam decreases from P_0 to P.

- The **transmittance** ***T*** of the solution is the fraction of incident radiation transmitted by the solution. Transmittance is often expressed as a percentage and called the ***percent transmittance***.

$$T = P / P_0$$

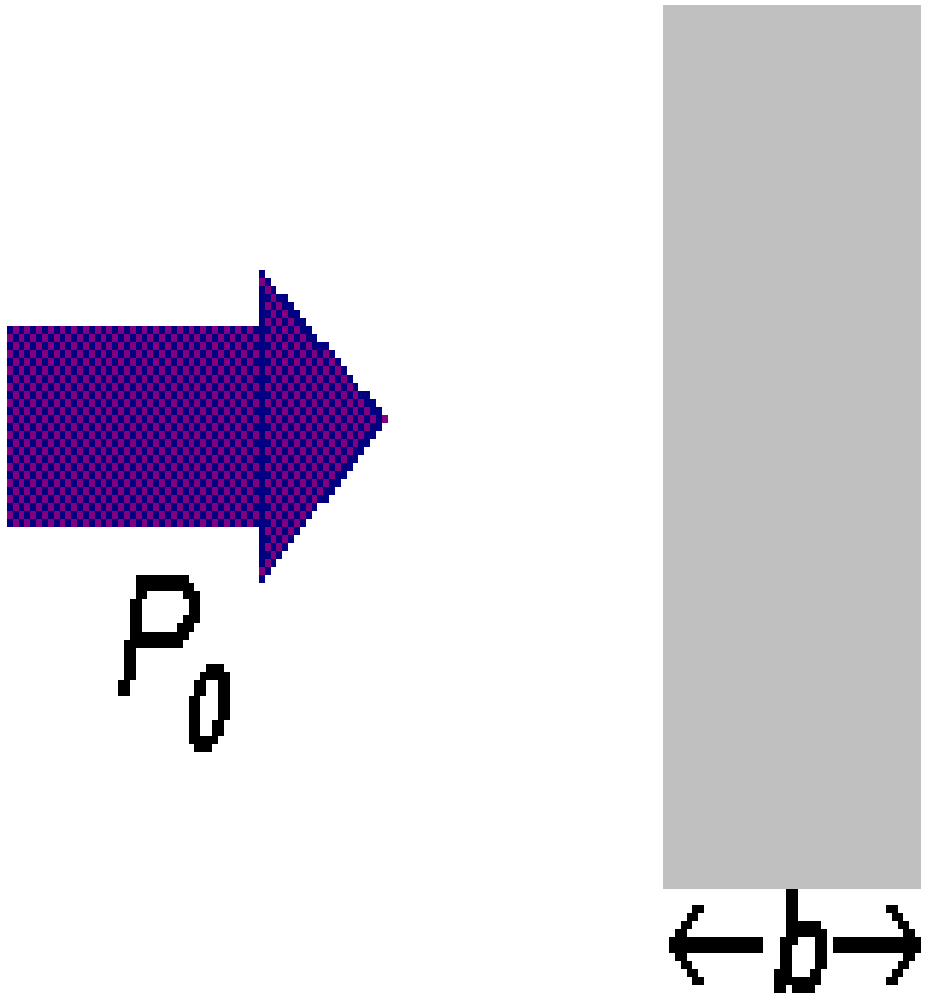
- The **absorbance A** of a solution is related to the transmittance in a logarithmic manner.

$$A = -\log T = \log(P_0/P)$$



$$T = \frac{P}{P_0}$$

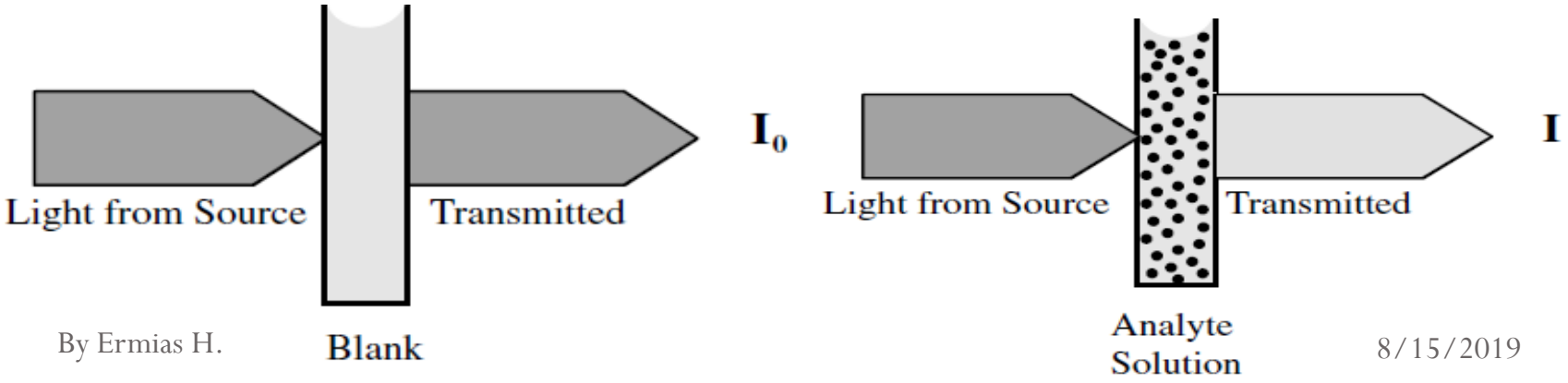
$$A = \log \frac{P_0}{P}$$


P_0
b

- Many cpds absorb radiation. The diagram below shows a beam of monochromatic radiation of radiant power $\mathbf{I_0}$ directed at a sample solution.
- Absorption takes place and the beam of radiation leaving the sample has radiant power **I**.
- Transmittance, T, is defined as the fraction of the original light that passes through the sample.
- Transmittance: **T = I/ Io**

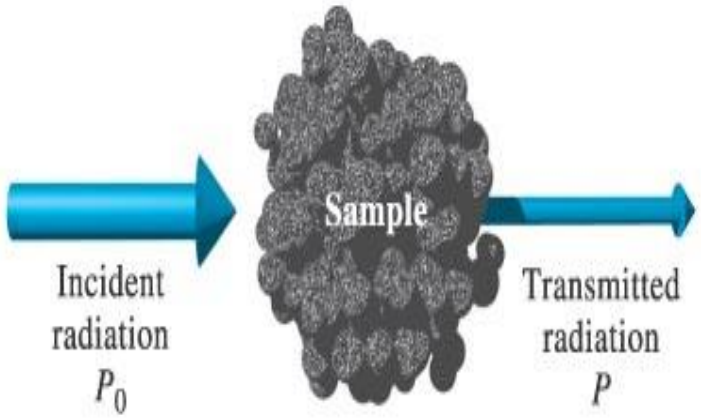
Therefore, T has the range 0 to l.

The percent transmittance is simply l00T & ranges between 0 & 100%.

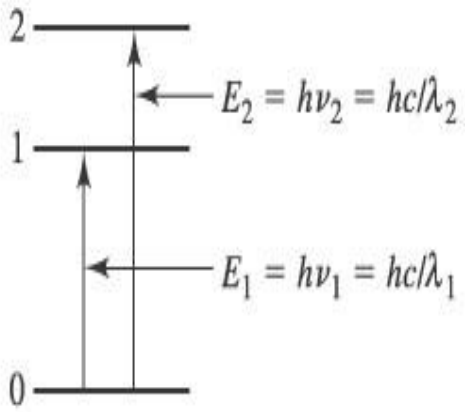


Cont…

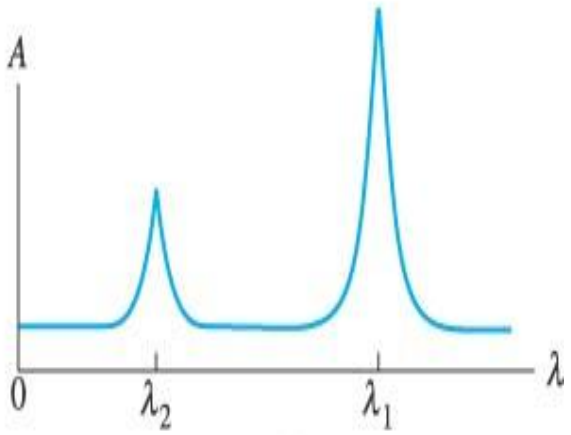
- Absorption promotes these particles from their ground state to more higher- energy excited state.



(a)

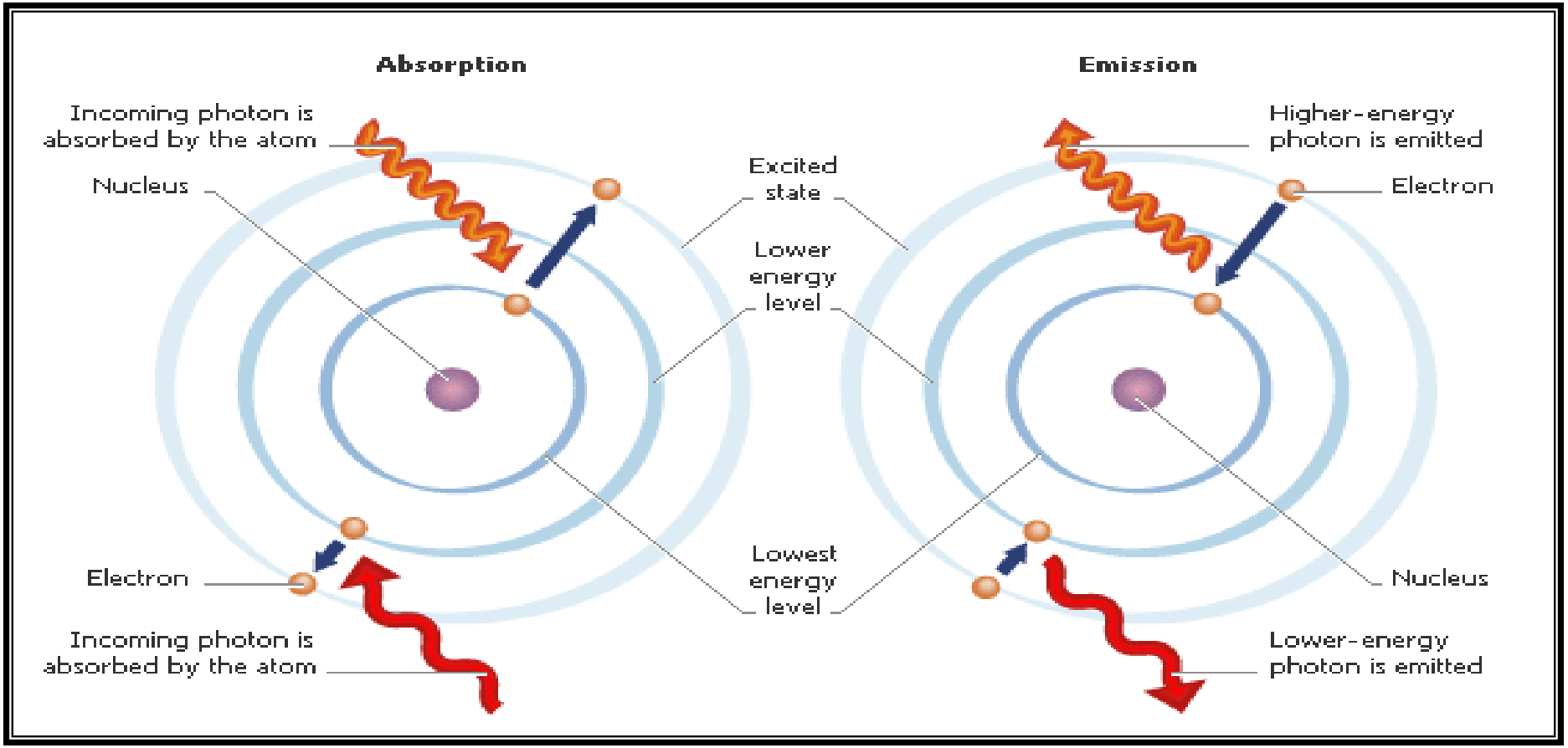


(b)



(c)

Cont…



The amount of radiation absorbed may be measured in a number ofways:

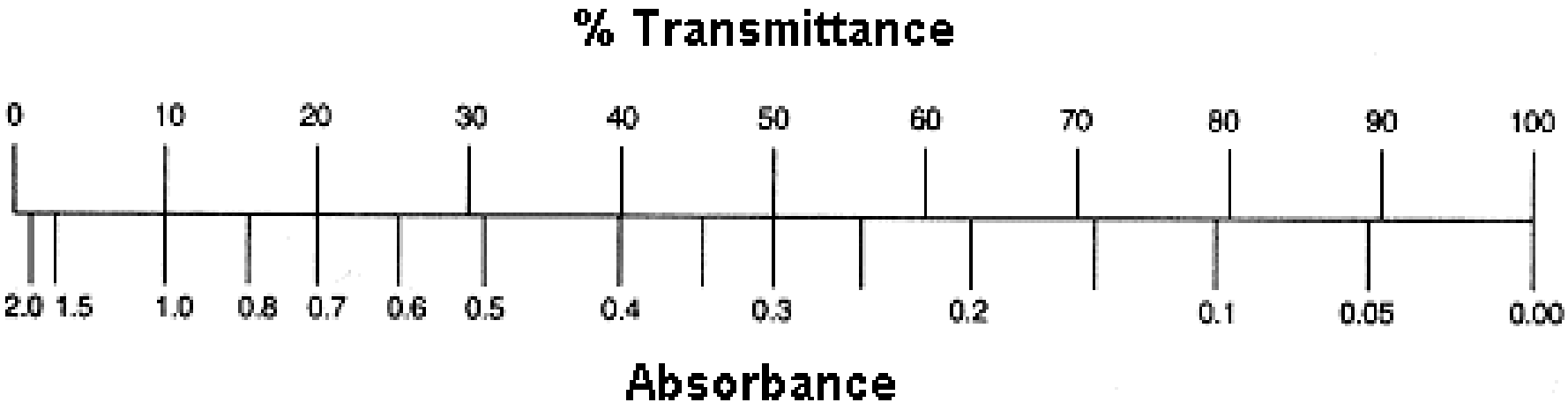
Transmittance, $T = I / I_0$ **% Transmittance**, $\%T = 100\ T$

Absorbance, $A = \log_{10} 1 / T$ ⟶ $A = \log_{10} I_0 / I$

$A = \log_{10} 100 / \%T$ ⟶ $A = 2 - \log_{10} \%T$

Cont…

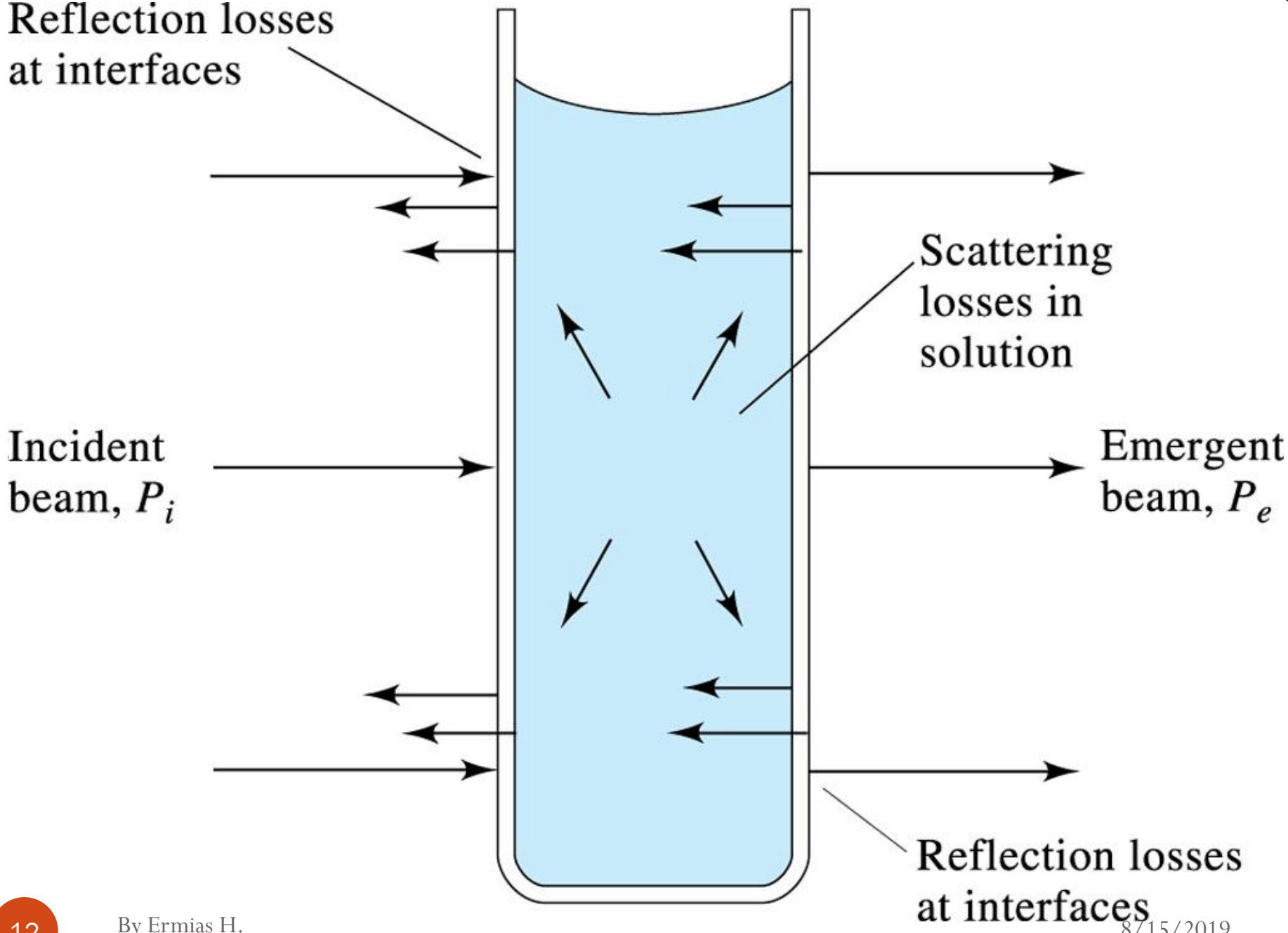
- The relationship between absorbance and transmittance is illustrated in the following diagram:



A is absorbance (no units, $A = log_{10} I_0 / I$)

Measuring Transmittance and Absorbance

- Ordinarily, transmittance and absorbance, cannot be measured as shown because the solution to be studied must be held in some sort of container (cell or cuvette).
- Reflection and scattering losses can occur at the cell walls. These losses can be substantial.
- Light can also be scattered in all directions from the surface of large molecules or particles, such as dust, in the solvent, and this can also cause further attenuation of the beam as it passes through the solution.



By Ermias H.

Cont…

- To compensate for these effects, the power of the beam transmitted through a cell containing the analyte solution is compared with one that traverses an identical cell containing only the solvent or a reagent blank.

  An experimental absorbance that closely approximates the true absorbance for the solution is thus obtained; that is

$$A = \log P_0 / P \approx \log P_{solvent} / P_{solution}$$

Beer's Law

- According to Beer's law, absorbance **A** is directly proportional to the concentration of the absorbing species **c** and the pathlength **b** of the absorbing medium

$$A = \log P_0 / P = abc$$

- Here, **a** is a proportionality constant called the **absorptivity**. Because absorbance is a unitless quantity, the absorptivity must have units that cancel the units of b and c.
- If, for example,

  c has the units of grams per liter ($g\ L^{-1}$) and

  b has the units of centimeters (cm),

  a absorptivity has the units of liters per gram centimeter ($L\ g^{-1}\ cm^{-1}$).

Cont…

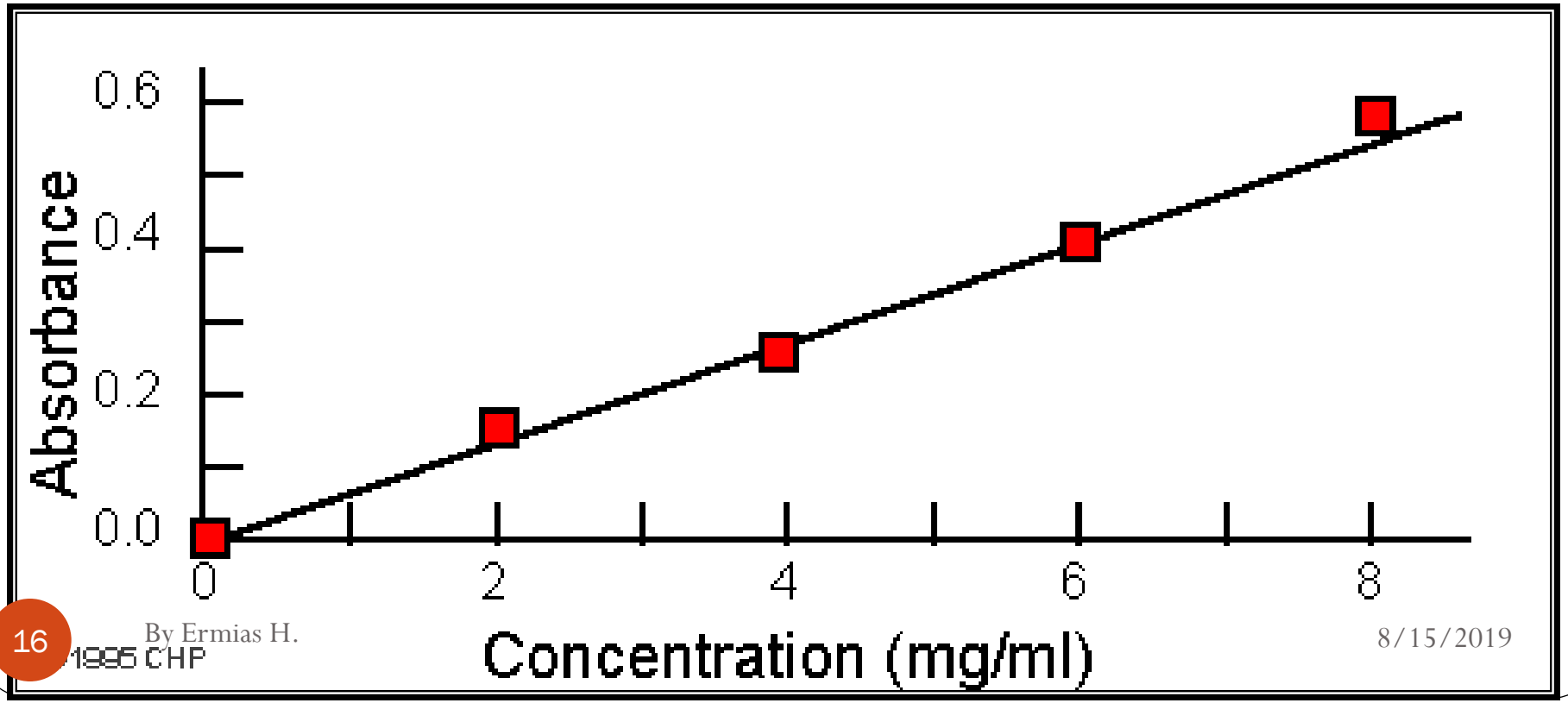
- When we express the concentration in moles per liter and b in centimeters, the proportionality constant, called the **molar absorptivity**, is given the special symbol **ε**. Thus,

$$A = \varepsilon bc$$

where, **ε** has the units of liters per mole centimeter ($L\ mol^{-1}\ cm^{-1}$).

Cont…

- We will express this measurement in cm and c is the conc. of the cpd in solution, expressed in mol L^{-1}.
- Beer's law tells us that absorbance depends on the total quantity of the absorbing cpd in the light path through the cuvette.
- If we plot absorbance against concentration, we get a straight line passing through the origin (0,0).



Applying Beer's Law to Mixture

- Beer's law also applies to solutions containing more than one kind of absorbing substance.
- Provided that there are no interactions among the various species, the total absorbance for a multi component system is the sum of the individual absorbances.
- In other words,

$$A_{total} = A_1 + A_2 + \ldots A_n$$
$$= \varepsilon_1 bc_1 + \varepsilon_2 bc_2 + \ldots + \varepsilon_n bc_n$$

where the subscripts refer to absorbing componets 1, 2, …, n.

TABLE

Important Terms and Symbols Used in Absorption Measurements

Term and Symbol*	Definition	Alternative Name and Symbol
Incident radiant power, P_0	Radiant power in watts incident on sample	Incident intensity, I_0
Transmitted radiant power, P	Radiant power transmitted by sample	Transmitted intensity, I
Absorbance, A	$\log(P_0/P)$	Optical density, D; extinction, E
Transmittance, T	P/P_0	Transmission, T
Path length of sample, b	Length over which attenuation occurs	l, d
Absorptivity,† a	$A/(bc)$	Extinction coefficient, k
Molar absorptivity,‡ ε	$A/(bc)$	Molar extinction coefficient

*Terminology recommended by the American Chemical Society (*Anal. Chem.*, **1990,** *62,* 91).
†c may be expressed in g L^{-1} or in other specified concentration units; b may be expressed in cm or other units of length.
‡c is expressed in mol L^{-1}; b is expressed in cm.

Limitation to the Applicability of Beer's Law

- There are few exception to the linear relationship between absorbance and pathlength at a fixed concentration.

- We frequently observe deviations from the direct proportionality between absorbance and concentration where b is a constant. Some of these deviations, called ***real deviations***, are fundamental and represent real limitations to the law.

- Others occur as a consequence of the manner in which the absorbance measurements are made or as a result of chemical changes associated with concentration changes. These deviations are called ***instrumental deviations*** and ***chemical deviation*** respectively.

i. Real Limitations to Beer's Law

- Beer's law describes the absorption behavior of dilute solutions only and is a limiting law.

- At concentrations exceeding about 0.01 M, the average distances between ions or molecules are diminished to the point where each particle affects the charge distribution, and thus the extent of absorption of its neighbors.

- The occurrence of this phenomenon causes deviations from the linear relationship between absorbance and concentration. When ions are in close proximity, the molar absorptivity of the analyte can be altered because of electrostatic interactions, which can lead to departures from Beer's law.

ii. Chemical Deviations

- Deviations from Beer's law appear when the absorbing species undergoes association, dissociation, or reaction with the solvent to give products that absorb differently from the analyte.
- The extent of such departures can be predicted from the molar absorptivities of the absorbing species and the equilibrium constants for the equilibria involved. Unfortunately, we are usually unaware that such processes are affecting the analyte, so compensation is often impossible. Typical equilibria that give rise to this effect include monomer dimer equilibria, metal complexation equilibria where more than one complex is present, acid/base equilibria, and solvent-analyte association equilibria.

iii. Instrumental Deviations

- The need for monochromatic radiation and the absence of **stray** (outside the nominal wavelength band chosen) radiation are practical factors that limit the applicability of Beer's law.
- Beer's law strictly applies only when measurements are made with monochromatic source radiation. If the band selected corresponds to a region in which the absorptivity of the analyte is essentially constant, departures from Beer's law will be minimal.

Many molecular bands in the UV/visible region fit this description. To avoid deviation, it is advisable to select a wavelength band near the wavelength of maximum absorption where the analyte absorptivity changes little with wavelength.

...continued...

- ***Stray light(radiation)***, is defined as radiation from the instrument that is outside the nominal wavelength band chosen for the determination.
- This stray radiation is often the result of scattering and reflection off the surfaces of gratings, lenses or mirrors, filters, and windows. When measurements are made in the presence of stray light, the observed absorbance is given by ;

$$A' = \log \frac{P_0 + P_s}{P + P_s}$$

where P_s is the radiant power of the stray light.

- Stray light always causes the apparent absorbance to be lower than the true absorbance.

The deviations due to stray light are most significant at high absorbance values. Because stray radiation levels can be as high as 0.5% in modern instruments, absorbance levels above 2.0 are rarely measured unless special precautions are taken or special instruments with extremely low stray light levels are used.

- Another deviation is caused by mismatched cells.
- If the cells holding the analyte and blank solutions are not of equal pathlength and equivalent in optical characteristics, and intercept will occur in the calibration curve. This error can be avoided either by using matched cells or by using a linear regression procedure to calculate both the slope and intercept of the calibration curve.

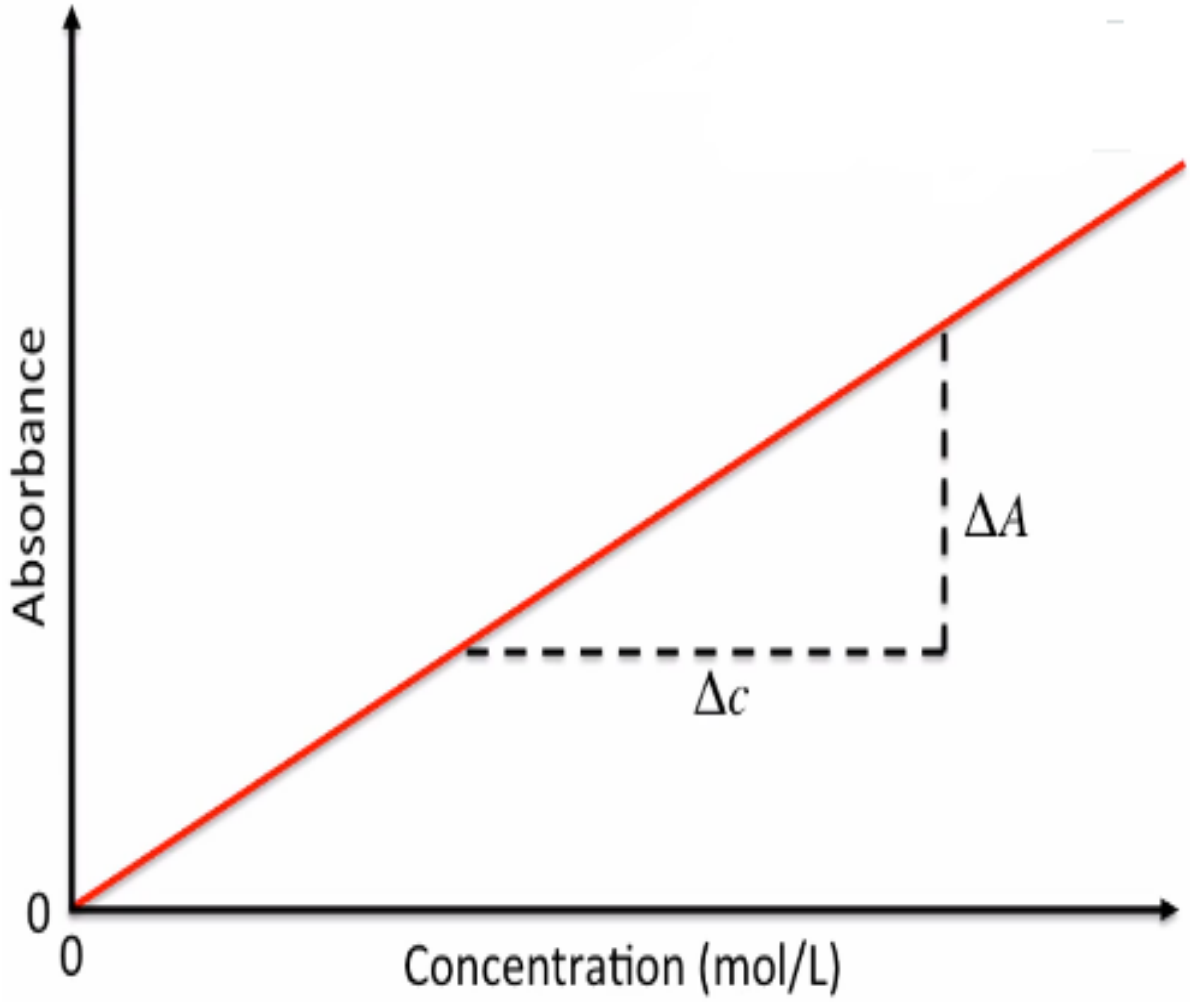
Beer–Lambert law:

The ***Beer–Lambert law****:*

$$A = \varepsilon \times l \times c$$

$$y = ax$$

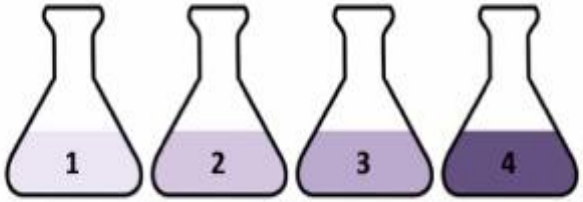
slope $= \varepsilon \times l$



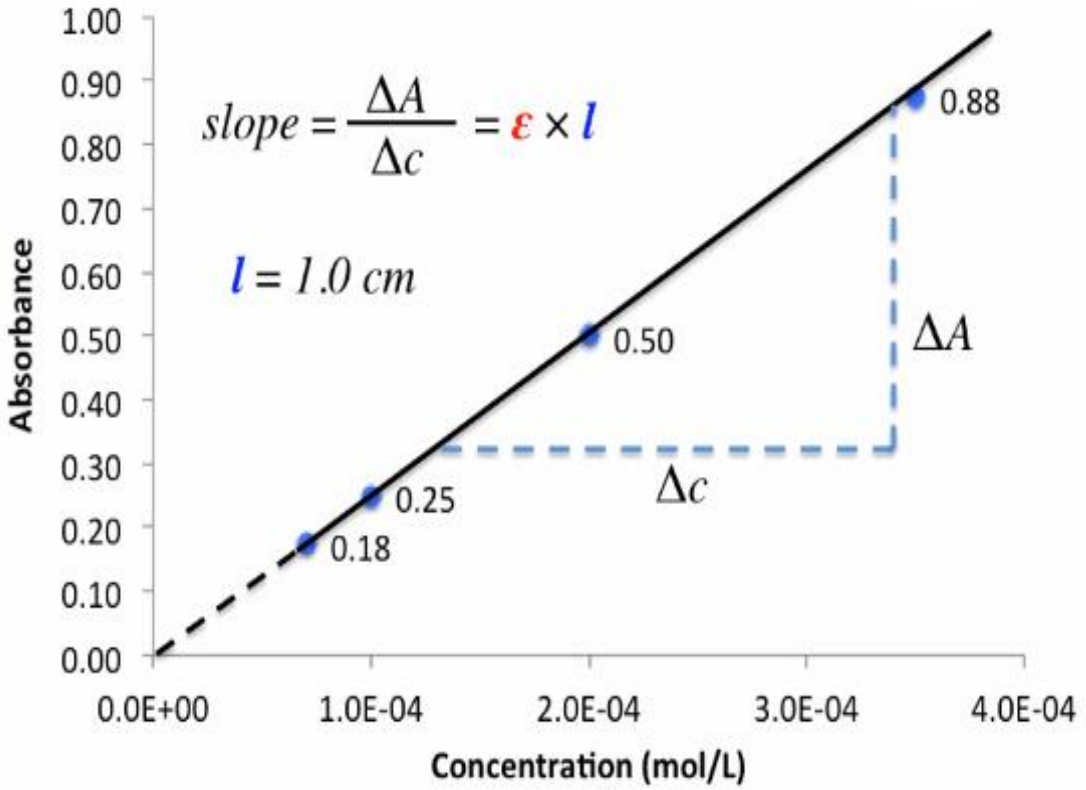
Beer–Lambert law: *Finding ε Experimentally.*

Determine the molar absorptivity of a $KMnO_4$ solution.

Measure the **absorbance values** for **at least four** solutions with **known $KMnO_4$ concentrations** to obtain the following data:



Solution	$[MnO_4^-]$ (mol/L)	Absorbance
1	7.00×10^{-5}	0.175
2	1.00×10^{-4}	0.250
3	2.00×10^{-4}	0.500
4	3.00×10^{-4}	0.875



- next

Example:

Calculate the slope and intercept of the regression line for the data given in the previous example. we calculated that, for this calibration curve:

$$\sum_i (x_i - \bar{x})(y_i - \bar{y}) = 216.2; \qquad \sum_i (x_i - \bar{x})^2 = 112; \qquad \bar{x} = 6; \qquad \bar{y} = 13.1$$

$$b = \frac{\sum_{i=1}^{i=n} [(x_i - \bar{x})(y_i - \bar{y})]}{\sum_{i=1}^{i=n} (x_i - \bar{x})^2}$$

$$a = \bar{y} - b\bar{x}$$

we calculate that

$$b = 216.2/112 = 1.93$$

$$a = 13.1 - (1.93 \times 6) = 13.1 - 11.58 = 1.52$$

The equation for the regression line is thus $y = 1.93x + 1.52$.

.

Spectrophotometry - Beer-Lambert Law._2.MP4