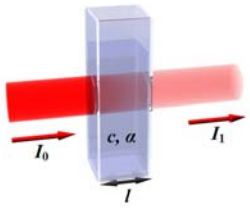
1

Beer - Lambert Law

- **Fundamental Law of Spectrophotometry**



http://en.wikipedia.org/wiki/File:Beer_lambert.png

$$\text{Transmittance} = T = \frac{P}{P_0} = \frac{S}{B}$$

$$\text{Absorbance} = -\log T$$

$$A = -\log\frac{P}{P_0} = \varepsilon bc$$

  - Assumes:
    - monochromatic radiation
    - system not saturated in light
    - absorbers behave independently an are distributed homogenously
  - The product corresponds to the number of absorbers per cm^2 area as beam passes through cell.
  - A = abc vs A = εbc

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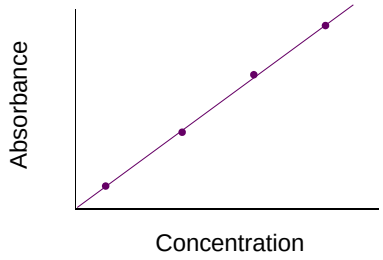
Beer - Lambert Law

- Typical analytical application: Calibration curve



- Also works for mixtures: For a given λ,

  $A_{net} = A_1 + A_2 + A_3 + \ldots$

  $A_{net} = \varepsilon_1 bc_1 + \varepsilon_2 bc_2 + \varepsilon_3 bc_3 + \ldots$

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Limitations/Deviations Affecting Linearity

A. Real Deviations: due to derivation of BL

- Law only works at low concentrations (~mM)
- At higher concentration, η of solution changes, causing ε to change.

B. Instrumental Deviations:

1. Deviations due to polychromatic radiation

- due to bandpass of measurement
- narrow features + wide bandpass $\rightarrow$ changing ε
- http://www.chem.uoa.gr/applets/AppletBeerLaw/Appl_Beer2.html

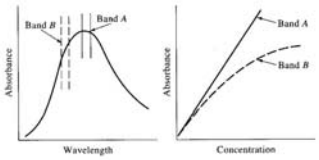

Figure 13-5 The effect of polychromatic radiation upon Beer's law. Band *A* shows little deviation because ϵ does not change greatly throughout the band. Band *B* shows marked deviation because ϵ undergoes significant changes in this region.

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Limitations/Deviations Affecting Linearity

2. Deviations due to stray light

- Increased light reaching detector
- Contributes most when P << P_0
- Causes negative deviation at high concentration (High Abs.)
- Decreasing bandpass lowers stray light
  - increased linearity

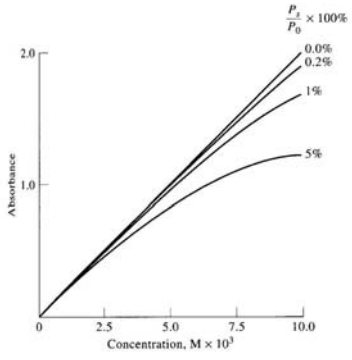


Figure 13-6 Apparent deviation from Beer's law brought about by various amounts of stray radiation.

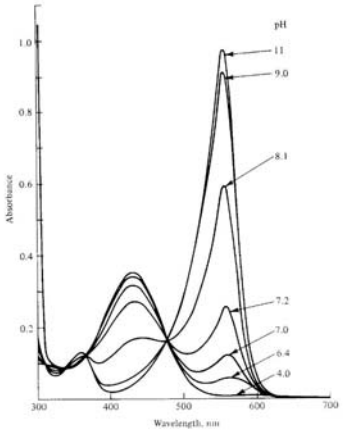
Limitations/Deviations Affecting Linearity

C. Chemical Deviation:

Shifting Equilibira

- As position of equilibrium changes, concentration of absorber changes
- Equilibrium affected by:
- Compensate by using isosbestic point: point where molar absorptivity for components of equilibria are identical.

FIGURE 7.3
Chemical equilibrium between two solution components, the conversion of phenol red ($pK_a \approx 7.9$) from the yellow (acidic) to the red (basic) form. Absorption maxima are at 433 and 558 nm, respectively, for the acidic and basic forms. Isosbestic points are recorded at 338, 367, and 480 nm.



Optimizing UV-Vis Analysis

- **Using UV-Vis for quantitative analysis:**

1. Temperature: changing temp cause shifting equilibria
2. Solvents: Transparency, Solubility, Purity
3. Photoeffects: luminescence
4. Appropriate wavelength:

   choose λ_{max} for best sensitivity and linearity
5. Appropriate sample cells: minimize scatter, etc.

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Example Applications of UV-VIS

- Determination of equilibrium constants
- Determination of reaction kinetics
- Quantitative Analysis - Calibration curves…
- Detectors for separations (HPLC)

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Example Applications of UV-VIS

- Photometric Titrations
  - Monitor absorbance of analyte (product, titrant) during titration
  - Beer's law applies!
    - away from eq. pt., observe linear regions
    - magnitude of absorbance depends on concentration
    - slope of linear portion is determined by Beer's law
    - Intersection of linear portions = eq. pt.

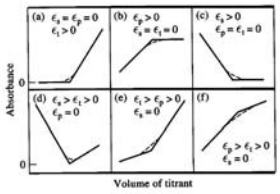

Figure 14-18 Typical photometric titration curves. Molar absorptivities of the substance titrated, the product, and the titrant are given by ϵ_s, ϵ_p, ϵ_t, respectively.

  - Need to account for effect of dilution on the absorbance
  - Endpoint is determined by data taken away from it
    - points near the endpoint aren't as critical
    - don't need sharp transition