



6/28/2016



*Cryphonectria parasitica* tendrils on chestnut tree bark (Photo: Ministry of Agriculture and Regional Development Archive, Ministry of Agriculture and Regional Development, Bugwood.org)



# Spectrophotometry and Beer's Law

B3 Summer Science Camp  
at Olympic High School

Dr. Jennifer Weller

Spectroscopy measures how matter interacts with radiation.

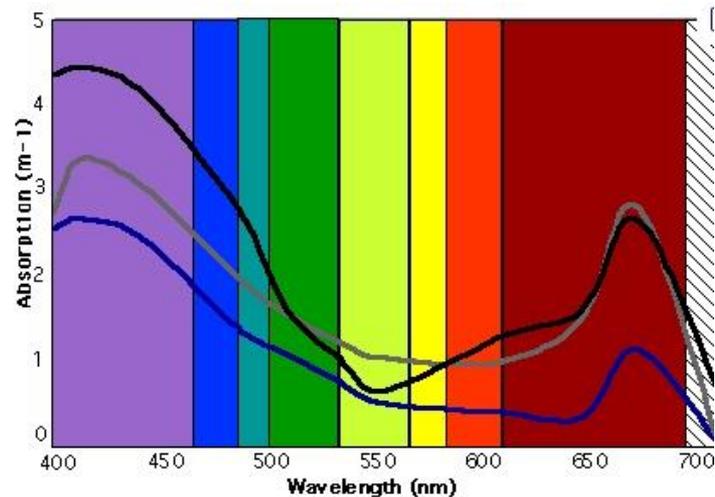
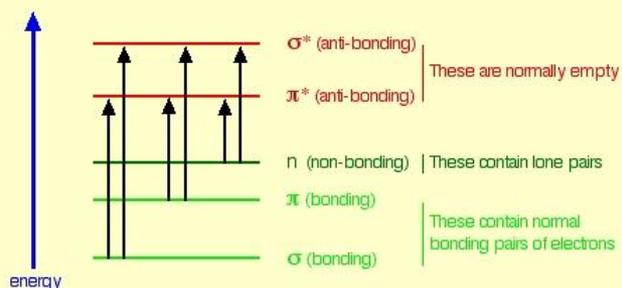
$$E = h\nu$$

$$\lambda = \frac{c}{\nu}$$

- Radiation is characterized by frequency or wavelength.
  - Radiation passes through or bounces off matter resulting in a change in the number of photons (intensity), a frequency shift or a scatter pattern
- Absorbance
  - Interactions of photons with matter are frequency/wavelength dependent so monochromatic light (single-wavelength) is used.
  - The amount of light before it gets to your material (incident intensity) is decreased by the amount the material absorbs, so exiting light intensity is less than *incident* light intensity.
- Scattering
  - Photons *reflect* from the material – coherent light will change direction
- Fluorescence
  - Interactions with molecules causes photons to shift to higher electron orbitals, energy is lost by photons as they decay back down

Absorbance: when light is *absorbed* by molecules the energy of the photon may promote an electron from a ground state to a new orbital.

- The energy provided has to match the energy difference in the orbitals, changing the state of the molecule.
  - This energy can be released by electron decay to the original state or a new state, with photon release (light is emitted at a new wavelength), or by transfer to another molecule or dissipation to the environment.
  - For proteins and nucleic acids, photons in the UV and near-UV/visible range have the right energy to be absorbed.
- Organic molecules subjected to UV-visible light have transitions from
  - pi bonding to pi antibonding orbitals
  - non-bonding to pi antibonding orbitals
  - non-bonding to sigma anti-bonding orbitals.



# Beer-Lambert Law

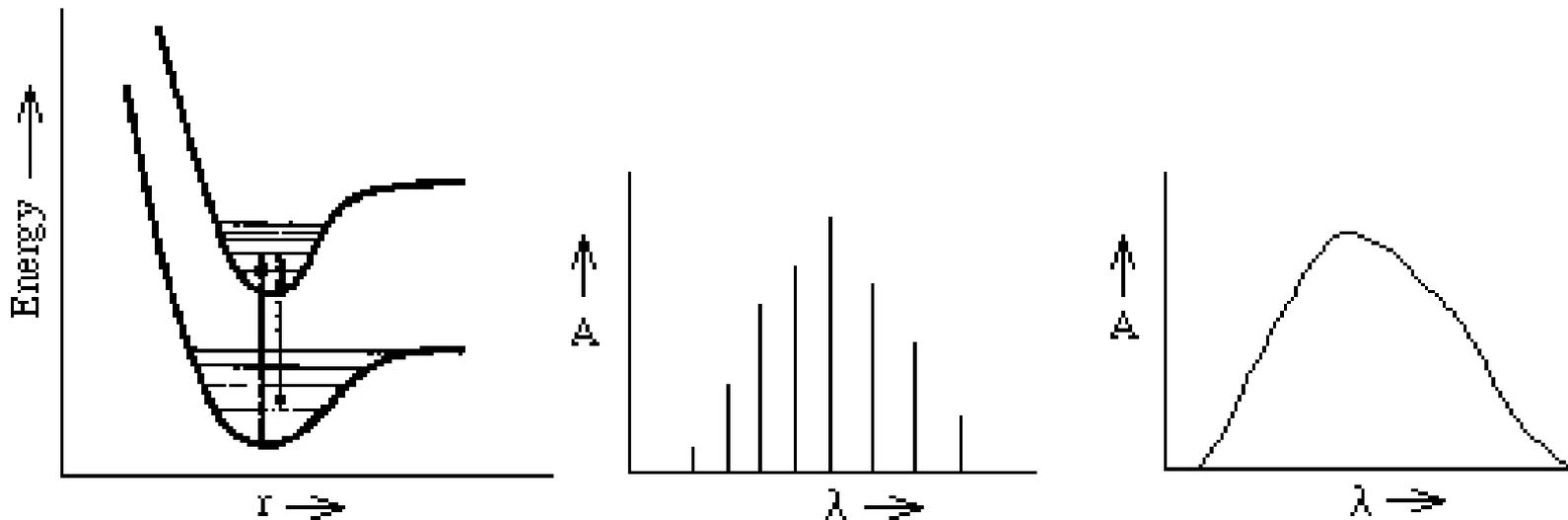
- The law is an expression of a relationship:

$$A = \epsilon C \ell$$

- A is the absorbance
- $\epsilon$  is a constant for a given substance, if it is molar units then it is {Liters per mole per centimeter, or L- mol<sup>-1</sup> cm<sup>-1</sup>}
  - For nucleic acids  $\epsilon_{260} = 50$  ml/ng-cm for dsDNA, 40 ml/ng-cm for RNA and 33 ml/ng-cm for ss DNA.
- C is the concentration (units are in moles/Liter)
- $\ell$  is the path length that the light passes through the sample (converted to centimeters for whatever spectrophotometer you used, so units of the constant cancel out properly)

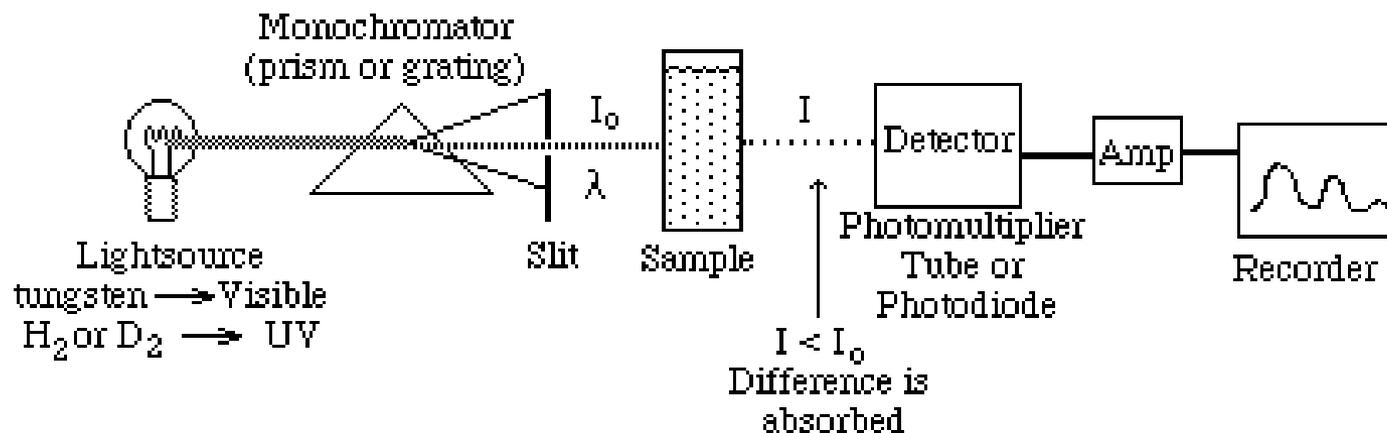
# Absorbance spectrum

- Biopolymers have many vibrational energy levels so there are many closely-spaced individual absorption peaks that sum to a broad peak.



# Measuring the changes in light as it interacts with matter is called spectrophotometry

- A spectrophotometer selects light of a determined wavelength from a source, passes it through a sample, and detects the number of photons (intensity) and/or frequency of the wavelength that reaches the detector.



# Absorption of light by DNA and Proteins

- DNA and proteins absorb light (photons) whose frequency is in the ultraviolet range (240-300nm).
- If some photons are absorbed going through a sample, fewer will emerge from the far side of a sample
- The intensity of the light (number of photons) will decrease on the far side.

**Transmittance,  $T = P / P_0$**

**% Transmittance,  $\%T = 100 T$**

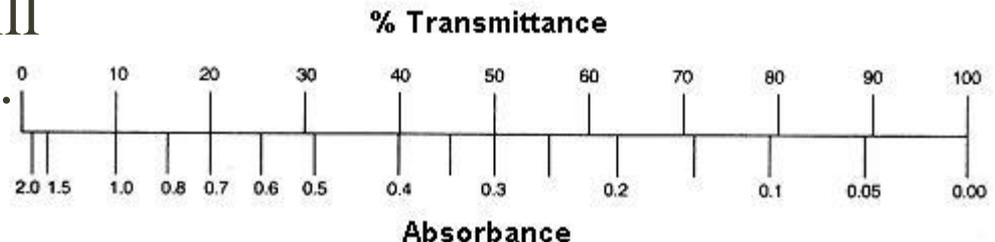
**Absorbance,**

$$A = \log_{10} P_0 / P$$

$$A = \log_{10} I / T$$

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

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



Beer's law says that the fraction of light absorbed by each layer of solution is the same.

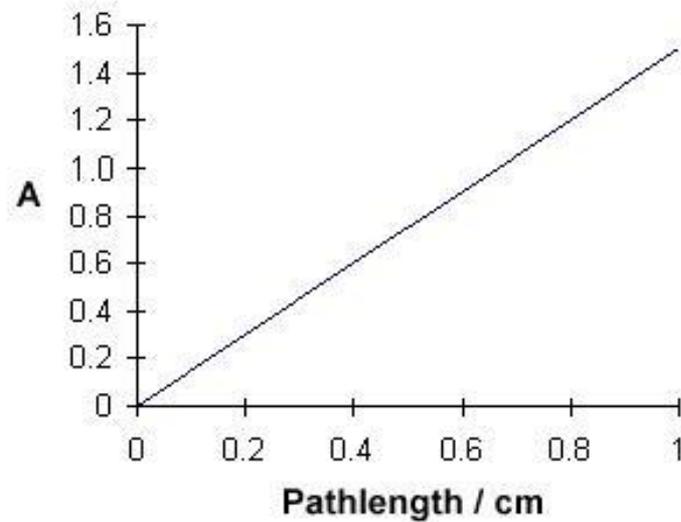
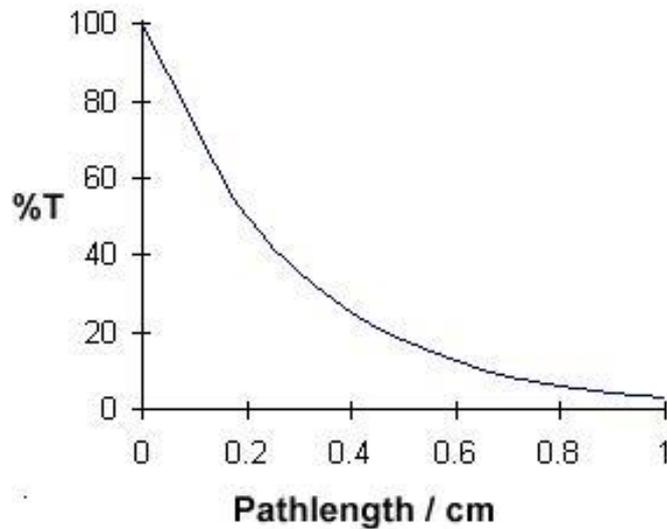
- Say that the fraction is 0.5 for each 0.2cm layer.
- Here is the data:

Path length / cm	0	0.2	0.4	0.6	0.8	1.0
%T	100	50	25	12.5	6.25	3.125
Absorbance	0	0.3	0.6	0.9	1.2	1.5

# Graphing the relationship.

$$A = \epsilon C \ell$$

$$\%T = 100(P/P_0) = e^{-\epsilon c \ell}$$



# Is the law true everywhere?

- When the solution is too concentrated no light at all passes through.

