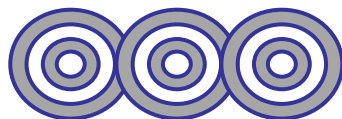




# Instrumental Methods of Analysis



## Spectroscopy Based on Absorption

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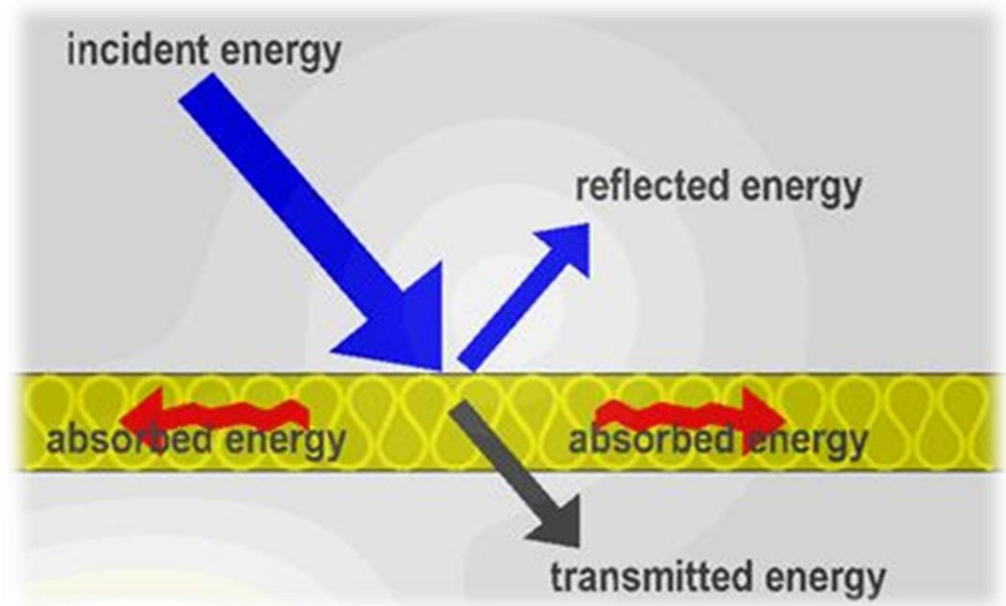
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Advanced Materials  
Research Chair



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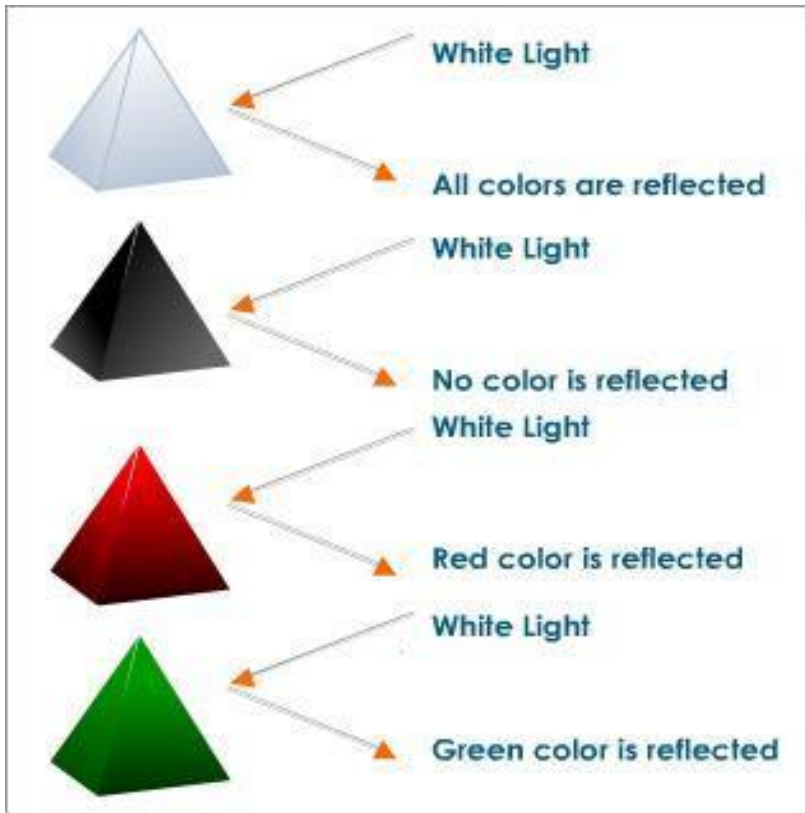


In absorption spectroscopy a beam of electromagnetic radiation passes through a sample. Much of the radiation is transmitted without a loss in intensity. At selected frequencies, however, the radiation's intensity is attenuated. This process of attenuation is called **absorption**.



**Absorbance:** the attenuation of photons as they pass through a sample.

## Absorption Phenomena



The colored appearance of most materials is due to selective absorption – the material absorbs some wavelengths very strongly and either transmits or reflects the remainder. The color is therefore produced by subtraction – some wavelength or group of wavelengths is removed from the incident light.

## Complementary Colors and Color Subtraction

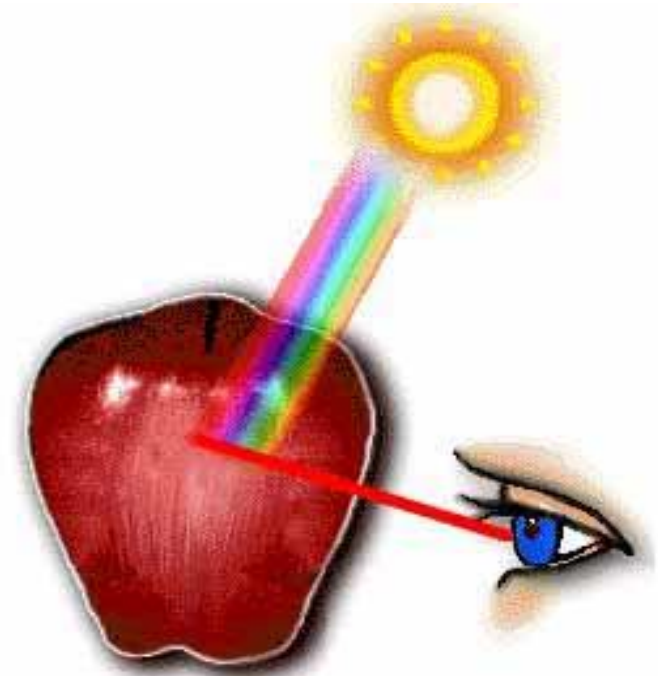
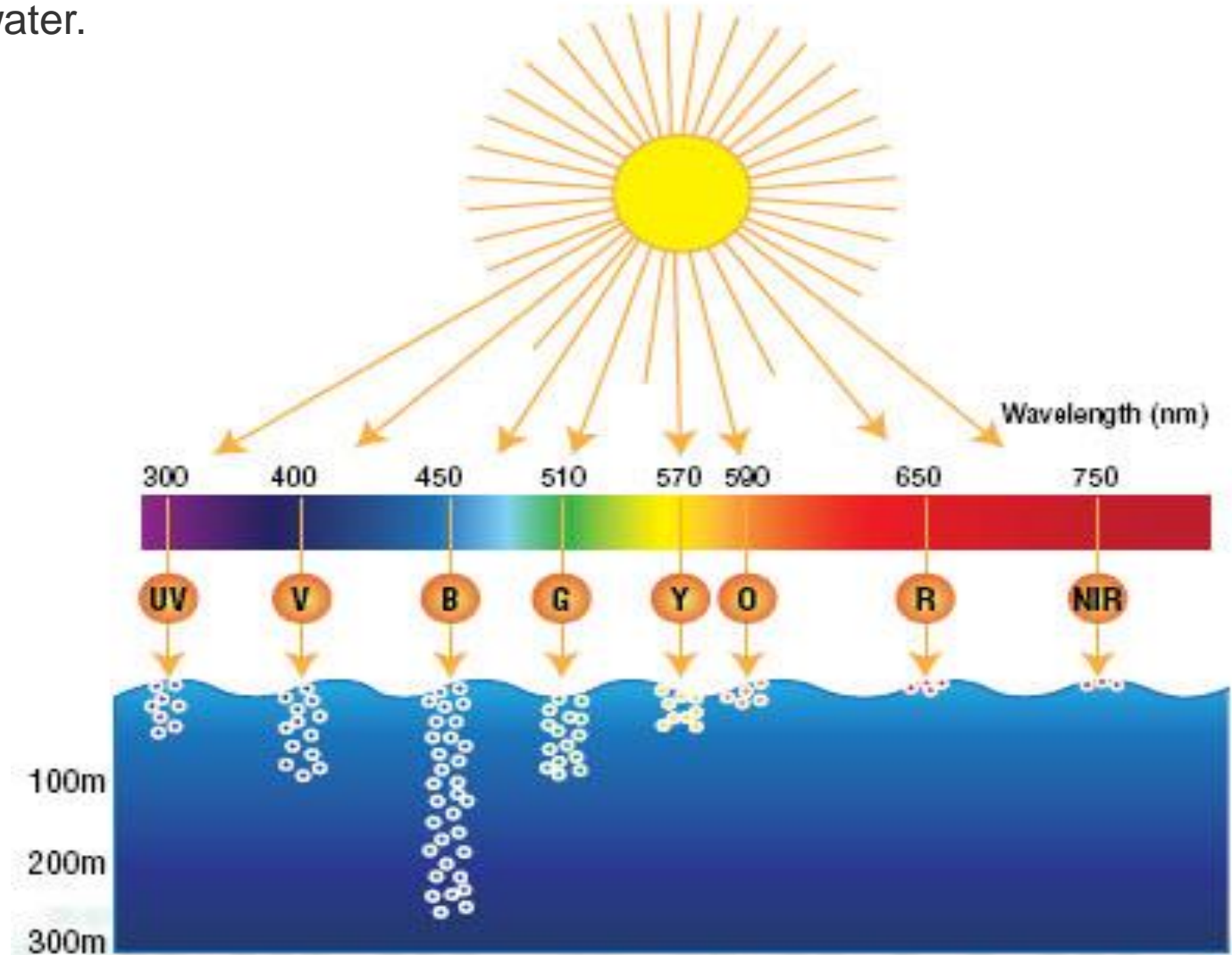
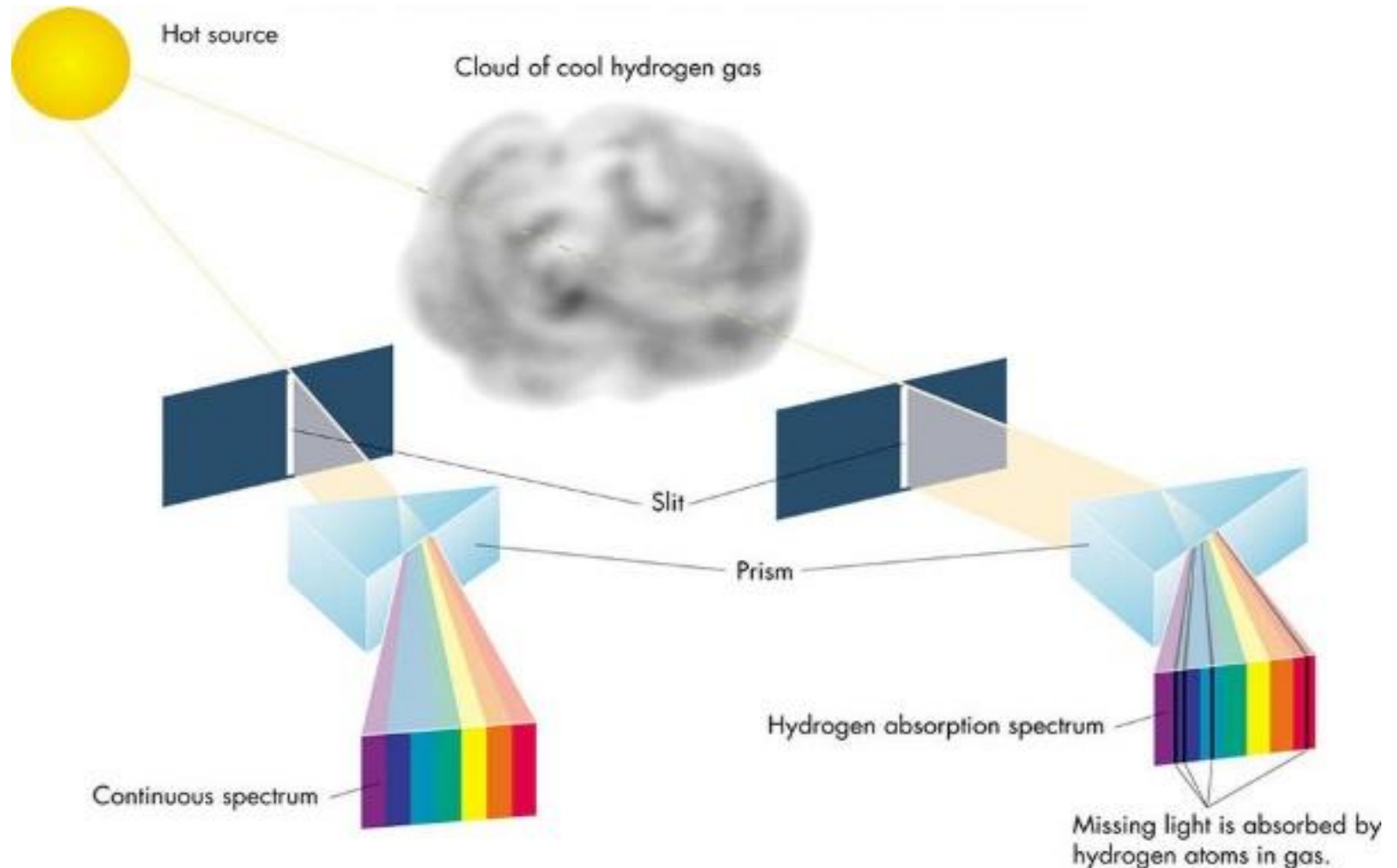


Diagram showing how different colors (wavelengths) of light are absorbed by water.



The gas ( $\text{H}_2$ ) absorbs light at specific wavelengths, creating absorption lines in the spectrum. The strength and location of these absorption lines depends on the chemical composition of the gas.

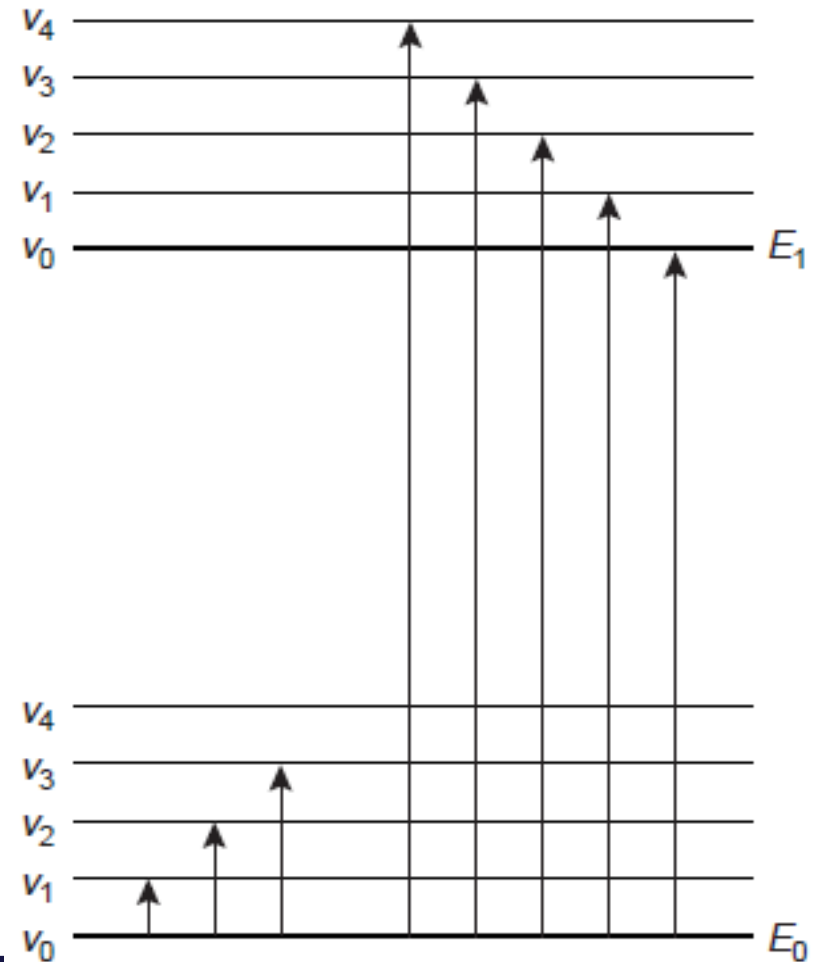


Requirements must be met if an analyte is to absorb electromagnetic radiation:

(1) Presence a mechanism by which the radiation's electric field or magnetic field interacts with the analyte. For ultraviolet and visible radiation, this interaction involves the electronic energy of valence electrons. A chemical bond's vibrational energy is altered by the absorbance of infrared radiation.

(2) The energy of the electromagnetic radiation must exactly equal the difference in energy,  $\Delta E$ , between two of the analytes quantized energy states.

$E_0$  and  $E_1$  represent the analyte's ground (lowest) electronic state and its first electronic excited state. Superimposed on each electronic energy level is a series of lines representing vibrational energy levels.



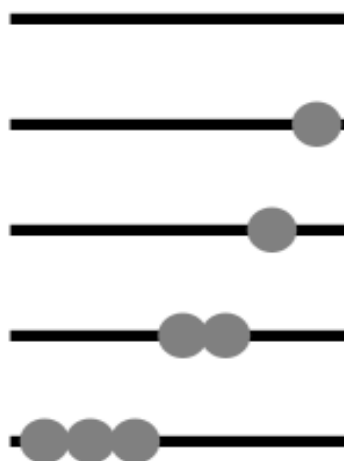
**There are three basic processes by which a molecule can absorb radiation:**

- 1- Rotational transition**
- 2- Vibrational transition**
- 3- Electronic transition**

All involve raising the molecule to a higher internal energy level, the increase in energy being equal to the energy of the absorbed radiation.

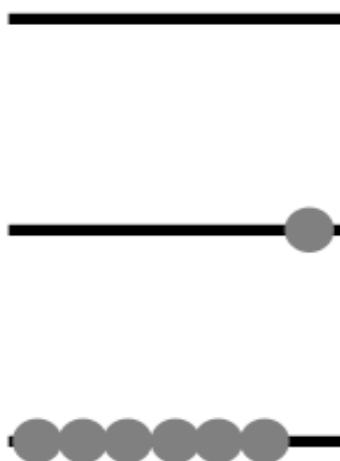
### Rotational

$$\Delta E = 0.012 \text{ kJ mol}^{-1}$$



### Vibrational

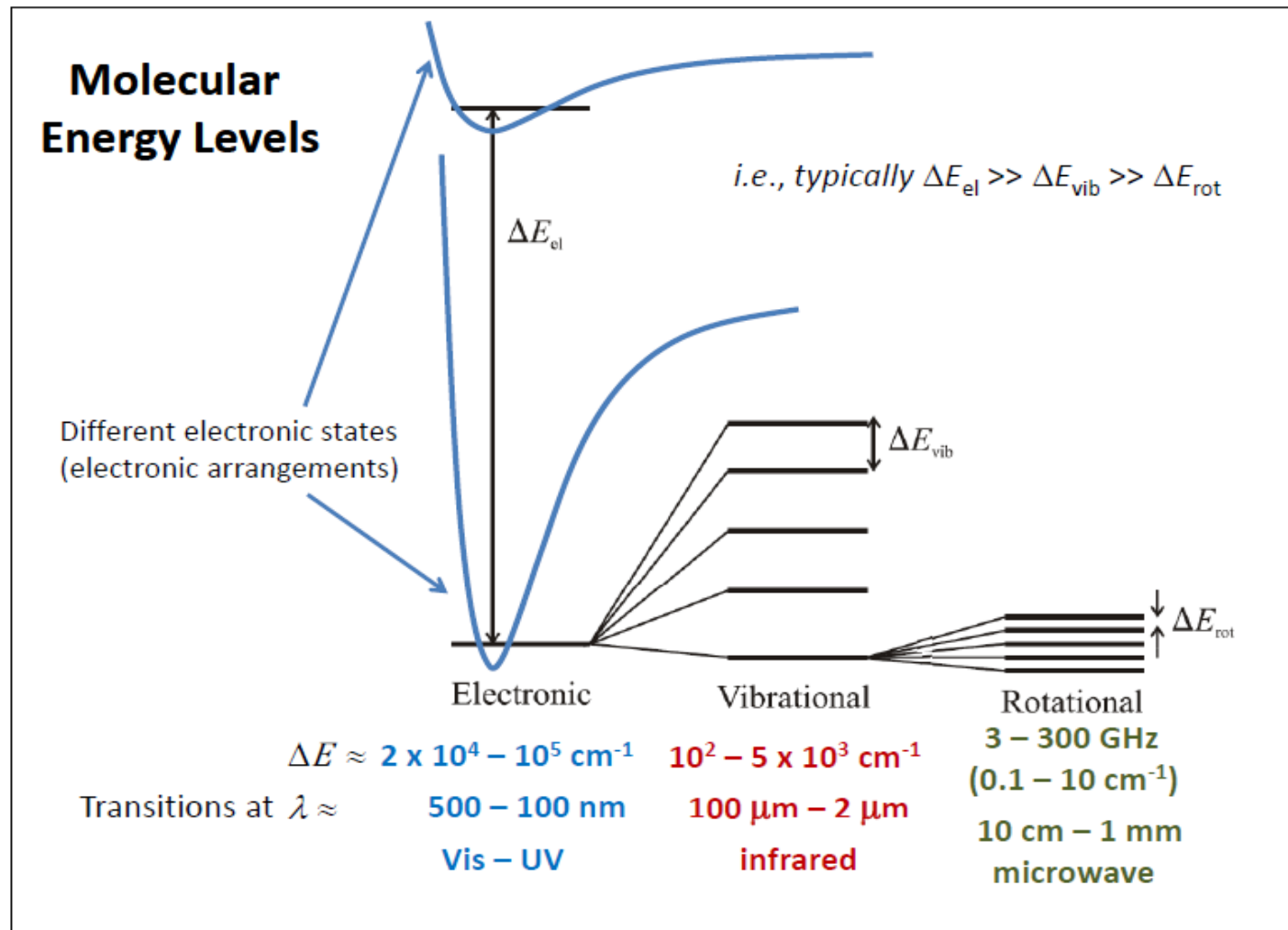
$$\Delta E = 12 \text{ kJ mol}^{-1}$$



### Electronic

$$\Delta E = 120 \text{ kJ mol}^{-1}$$



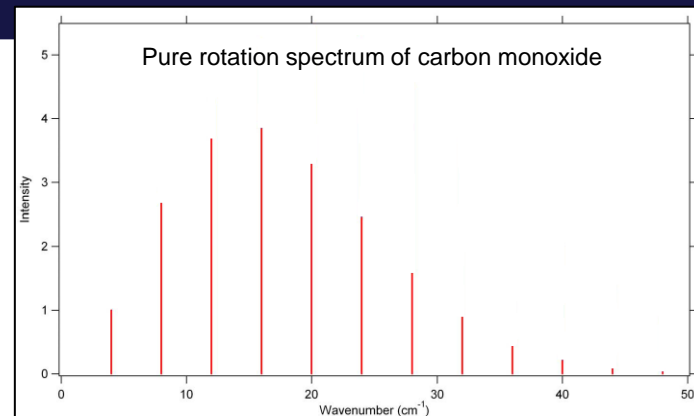


The relative energy levels of the three transition processes are in the order  
**electronic > vibrational > rotational**

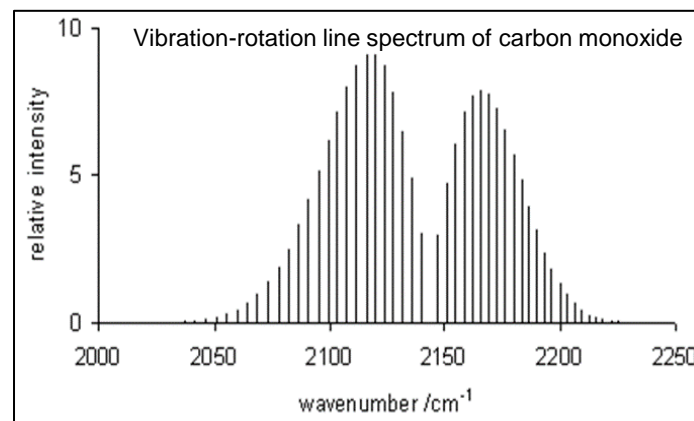
Each one being about an order of magnitude different in its energy level.



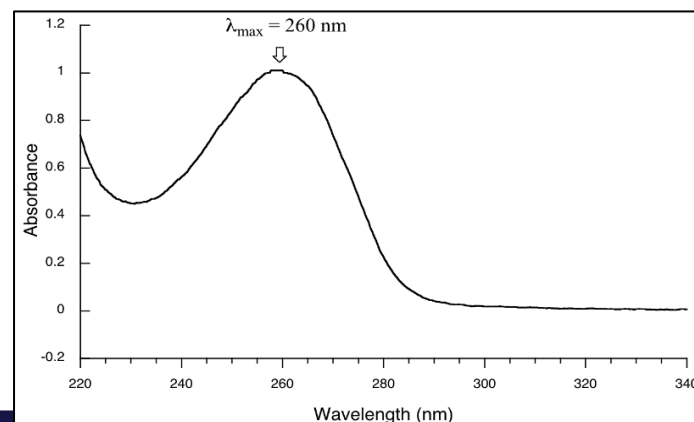
**-Rotational transitions** thus can take place at very low energies (long wavelengths). It's occur in the far-infrared and microwave regions ( $100\ \mu\text{m}$  to  $10\ \text{cm}$ ). When only rotational transition occur, discrete absorption lines will occur in the spectrum.



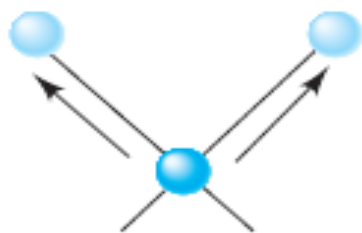
**-Vibrational transitions** require higher energies in the mid- and near-infrared regions. Vibrational transition occur in addition to rotational transition. The result is a spectrum of peaks of unresolved fine structure.



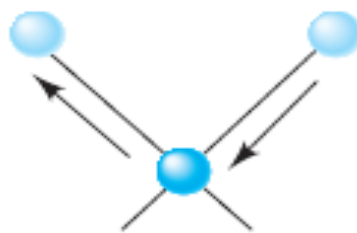
**-Electronic transitions** require still higher energies (in the visible and ultraviolet regions). Electronic transition take place, and rotational and vibrational transitions are superimposed on these. The net result is a spectrum of broad bands of absorbed wavelength.







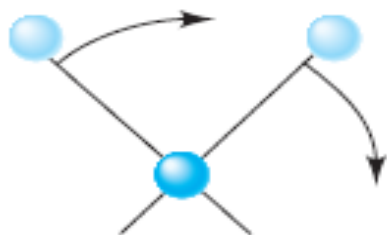
Symmetric



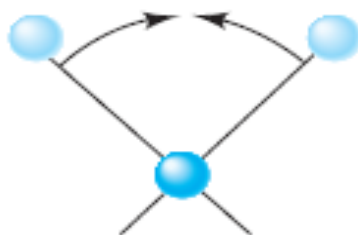
Asymmetric

Types of molecular vibrations.  
The plus sign indicates motion out of the page; the minus sign indicates motion into the page.

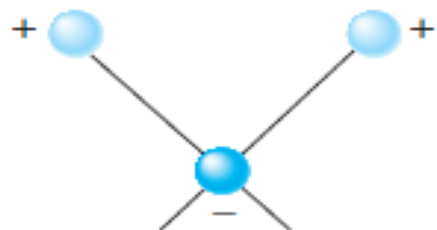
(a) Stretching vibrations



In-plane rocking



In-plane scissoring



Out-of-plane wagging

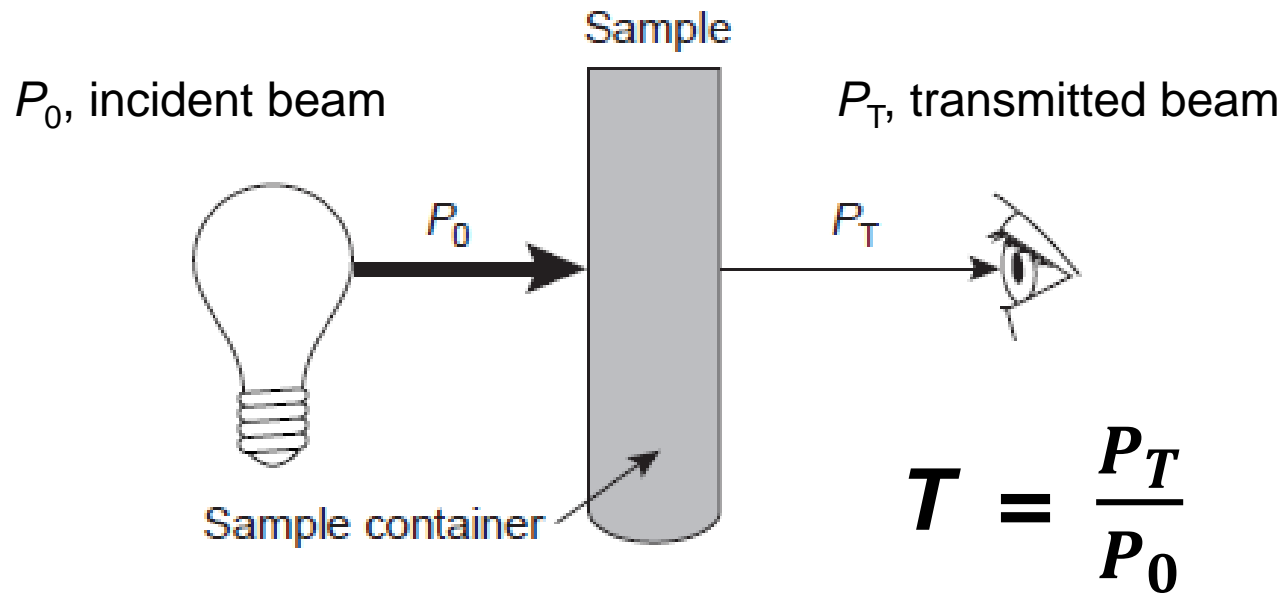


Out-of-plane twisting

(b) Bending vibrations

# Absorbance and Transmittance

**Transmittance (T):** the ratio of the radiant power passing through a sample to that from the radiation's source.



Multiplying the transmittance by 100 gives the percent transmittance ( $\%T$ ), which varies between 100% (no absorption) and 0% (complete absorption).

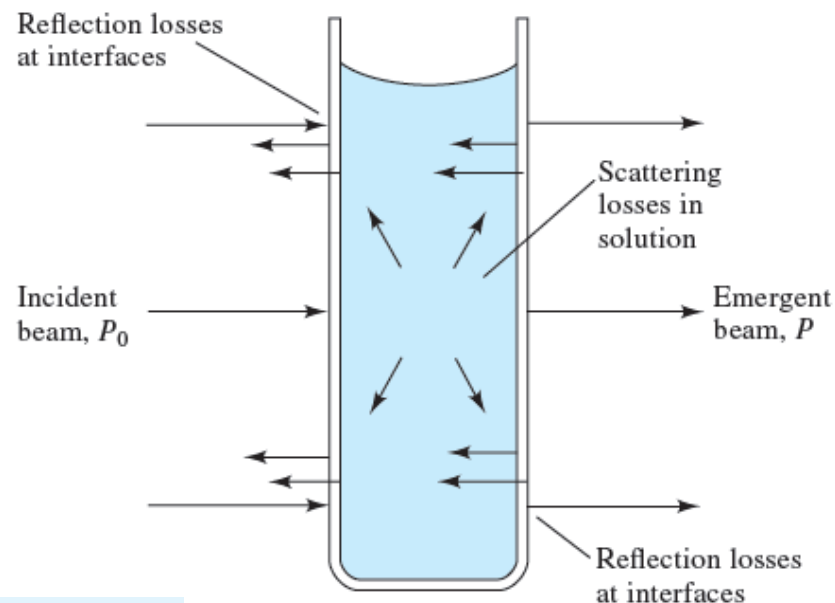
An alternative method for expressing the attenuation of electromagnetic radiation is **absorbance**, **A**, which is defined as

$$A = -\log T = -\log \frac{P_T}{P_0} = \log \frac{P_0}{P_T}$$

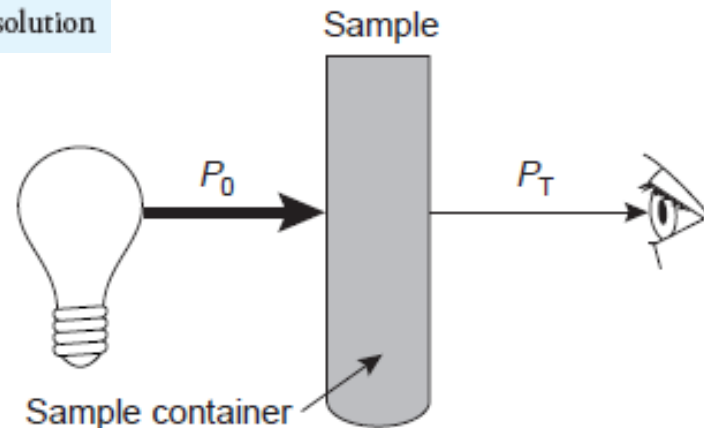
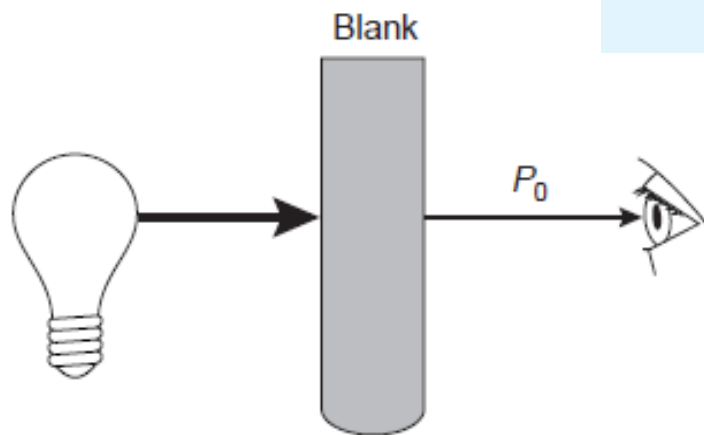
**Absorbance (A):** the attenuation of photons as they pass through a sample.

Absorbance is the more common unit for expressing the attenuation of radiation because, it is a linear function of the analyte's concentration.

Besides absorption by the analyte, several additional phenomena contribute to the net attenuation of radiation, including reflection and absorption by the sample container, absorption by components of the sample matrix other than the analyte, and the scattering of radiation. To compensate for this loss of the electromagnetic radiation's power, we use a method blank. The radiation's power exiting from the method blank is taken to be  $P_0$ .



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



**Blank:** a sample that contains all components of the matrix except the analyte. Measuring blank correcting the measured signal for contributions from sources other than the analyte.

### EXAMPLE:

- (a) A sample has a percent transmittance of 50.0%. What is its absorbance?
- (b) A sample has a percent transmittance of 34.0%. What is its absorbance?

### SOLUTION:

- (a) With a percent transmittance of 50.0%, the transmittance of the sample is 0.50.

$$A = -\log T = -\log(0.500) = 0.301$$

- (b) With a percent transmittance of 34.0%, the transmittance of the sample is 0.34.

$$A = -\log (0.34) = 0.469$$

As  $T$  increase,  $A$  decrease

# Absorbance and Concentration

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 path length over which absorption occurs.

**According to Beer's law, absorbance is directly proportional to**

- concentration, ***c***, of the absorbing species.
- path length, ***b***, of the absorbing medium.
- proportionality constant, ***a***, called the absorptivity.

$$A = \log \frac{P_0}{P_T} = abc$$



## Absorptivity and Molar Absorptivity

Because absorbance is a unitless quantity, the absorptivity unit depend on the units of ***b*** and ***c*** (must have units that cancel the units of ***b*** and ***c***).

When we express the concentration in moles per liter (molar, *M*) and ***b*** in cm, the proportionality constant is called the **molar absorptivity** (or molar absorption coefficient) and is given the symbol  **$\epsilon$** . Thus,

$$A = \epsilon bc$$

where  **$\epsilon$**  has the units of (L mol<sup>-1</sup> cm<sup>-1</sup>).

The molar absorptivity of a species at an absorption maximum is characteristic of that species. High molar absorptivities are desirable for quantitative analysis because they lead to high analytical sensitivity.

The absorptivity and molar absorptivity give, in effect, the probability that the analyte will absorb a photon of given energy. As a result, values for both ***a*** or  **$\epsilon$**  depend on the wavelength of electromagnetic radiation.

Substance	Absorption Peak	Molar Absorptivity
Benzene	255nm	180
Naphthalene	286nm	360
Anthracene	375nm	7100



Benzene

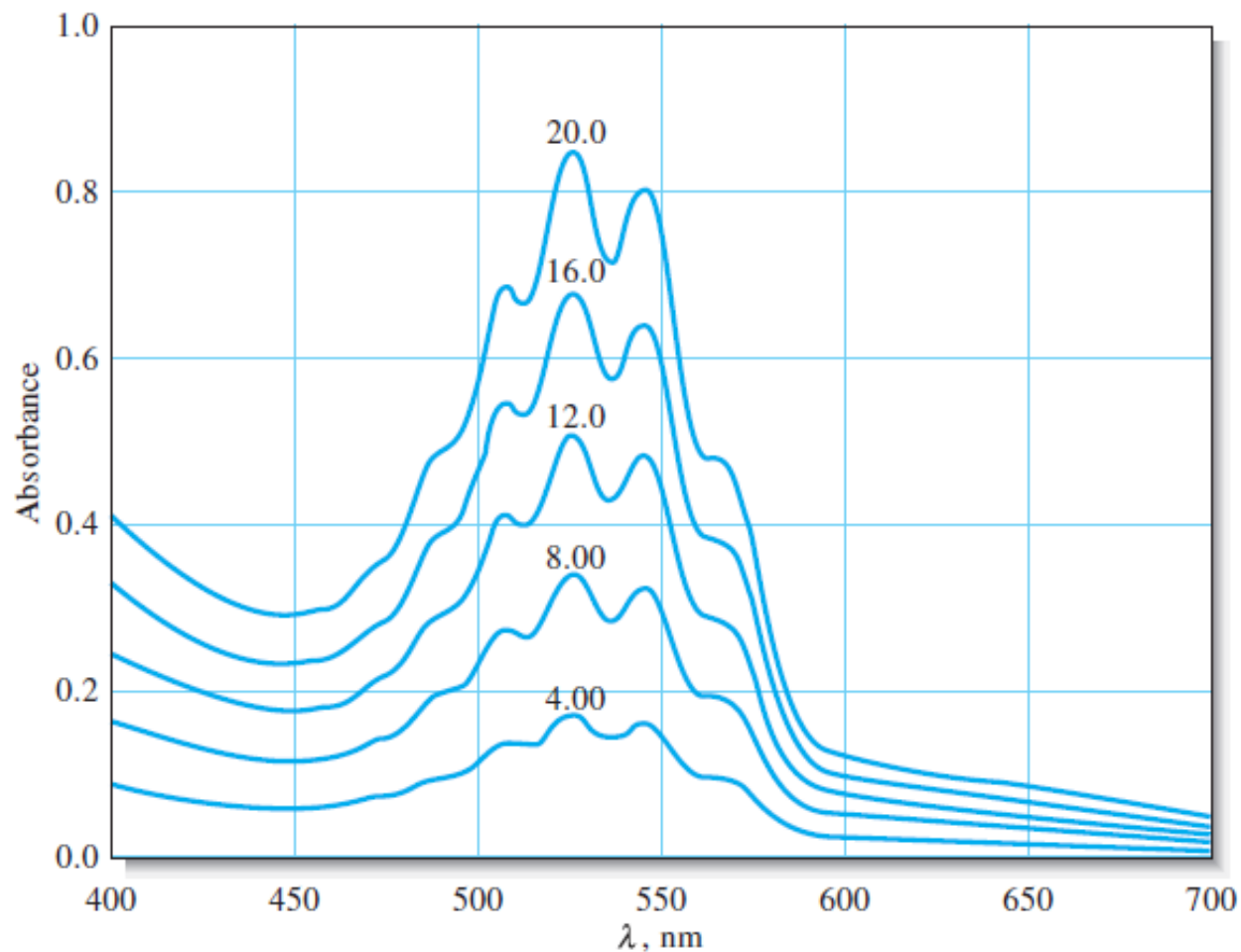


Naphthalene



Anthracene

## Absorbance and Concentration



Typical absorption spectra of potassium permanganate at five different concentrations. The numbers adjacent to the curves indicate concentration of manganese in ppm, and the absorbing species is permanganate ion,  $\text{MnO}_4^-$ .

## Deriving Beer's Law

When monochromatic electromagnetic radiation passes through an infinitesimally thin layer of sample, of thickness  $dx$ , it experiences a decrease in power of  $dP$ . The fractional decrease in power is proportional to the sample's thickness and the analyte's concentration,  $C$ ; thus

$$\frac{-dP}{P} = \alpha C dx$$

where  $P$  is the power incident on the thin layer of sample, and  $\alpha$  is a proportionality constant. Integrating the left side of above equation from  $P = P_0$  to  $P = P_T$ , and the right side from  $x = 0$  to  $x = b$ , where  $b$  is the sample's overall thickness

$$-\int_{P=P_0}^{P=P_T} \frac{dP}{P} = \alpha C \int_{x=0}^{x=b} dx$$

gives

$$\ln\left(\frac{P_0}{P_T}\right) = \alpha b C$$

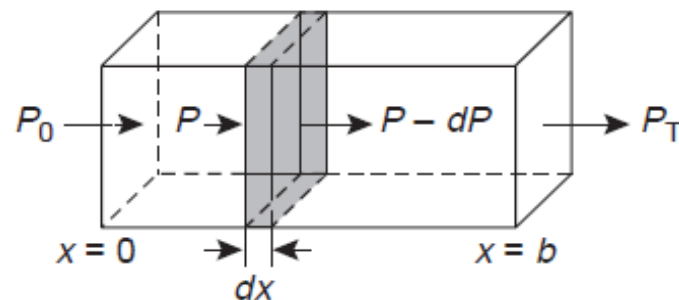
$$\ln x = 2.303 \log x$$

Converting from  $\ln$  to  $\log$ , and substituting into equation,

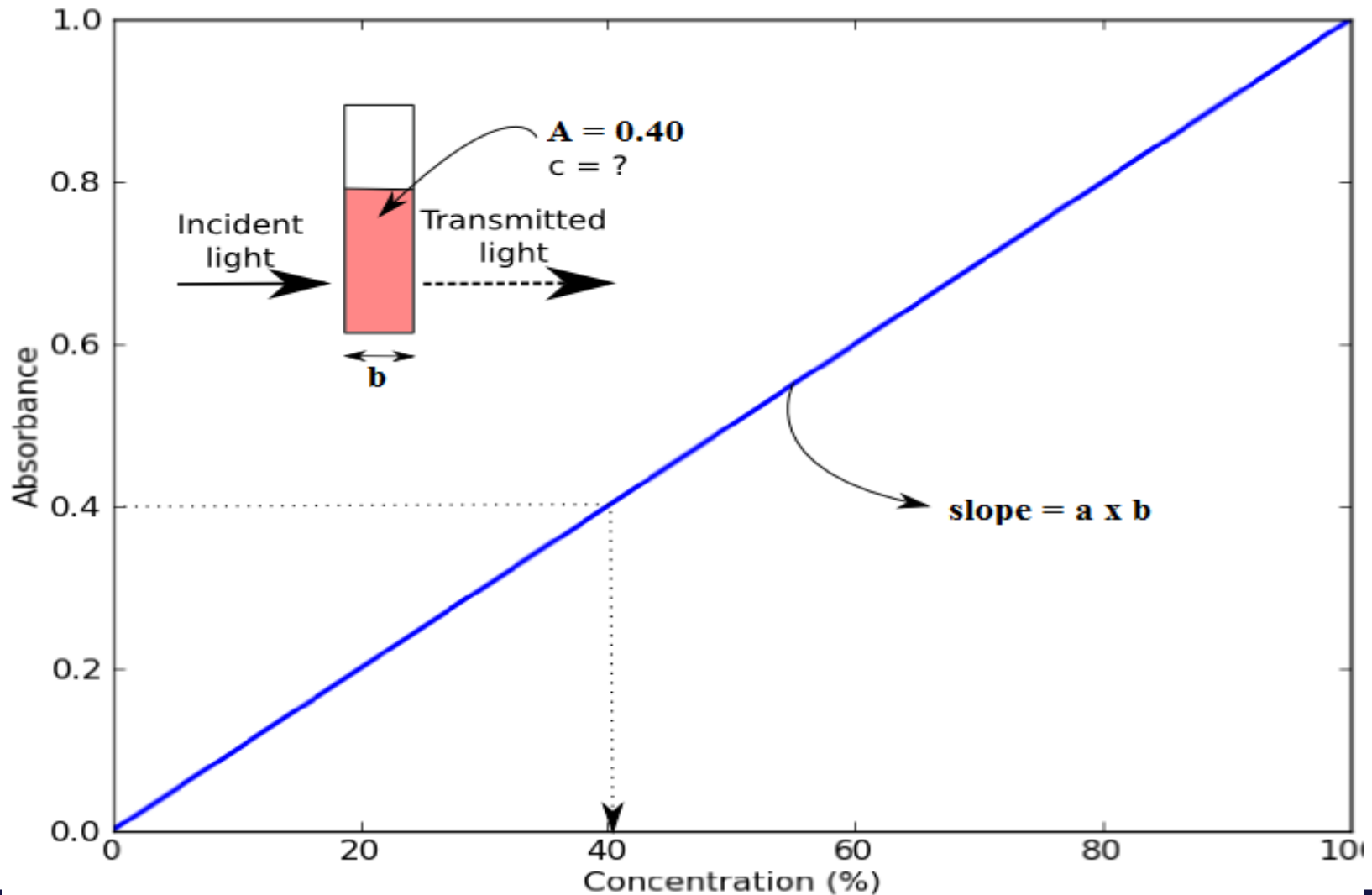
$$A = -\log T = -\log \frac{P_T}{P_0} = \log \frac{P_0}{P_T}$$

gives

$$A = abc$$



**Beer's law equations** establish the linear relationship between absorbance and concentration. Calibration curves based on Beer's law are used routinely in quantitative analysis.



## EXAMPLE:

A  $5.00 \times 10^{-4}$  M solution of an analyte is placed in a sample cell that has a path length of 1.00 cm. When measured at a wavelength of 490 nm, the absorbance of the solution is found to be 0.338. What is the analyte's molar absorptivity at this wavelength?

## SOLUTION:

$$\epsilon = \frac{A}{bC} = \frac{0.338}{(1.00 \text{ cm})(5.00 \times 10^{-4} \text{ M})} = 676 \text{ cm}^{-1} \text{ M}^{-1}$$

## EXAMPLE:

A  $7.25 \times 10^{-5}$  M solution of potassium permanganate has a transmittance of 44.1% when measured in a 2.10 cm cell at a wavelength of 525 nm. Calculate (a) the absorbance of this solution and (b) the molar absorptivity of  $\text{KMnO}_4$ .

## SOLUTION:

$$(a) A = -\log T = -\log 0.441 = -(-0.356) = 0.356$$

(b)

$$\epsilon = \frac{A}{bc}$$

$$\begin{aligned} & 0.356 / (2.10 \text{ cm} \times 7.25 \times 10^{-5} \text{ mol L}^{-1}) \\ & = 2.34 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1} \end{aligned}$$

## Beer's Law and Multicomponent Samples

*(Applying Beer's Law to Mixtures)*

Beer's law can be extended to samples containing several absorbing components provided that there are no interactions between the components.

The total absorbance for a multi-component system at a single wavelength is the sum of the individual absorbances.

$$\begin{aligned} A_{\text{total}} &= A_1 + A_2 + \cdots + A_n \\ &= \epsilon_1 b c_1 + \epsilon_2 b c_2 + \cdots + \epsilon_n b c_n \end{aligned}$$

where the subscripts refer to absorbing components 1, 2, . . . ,  $n$ .

## Limitations to Beer's Law

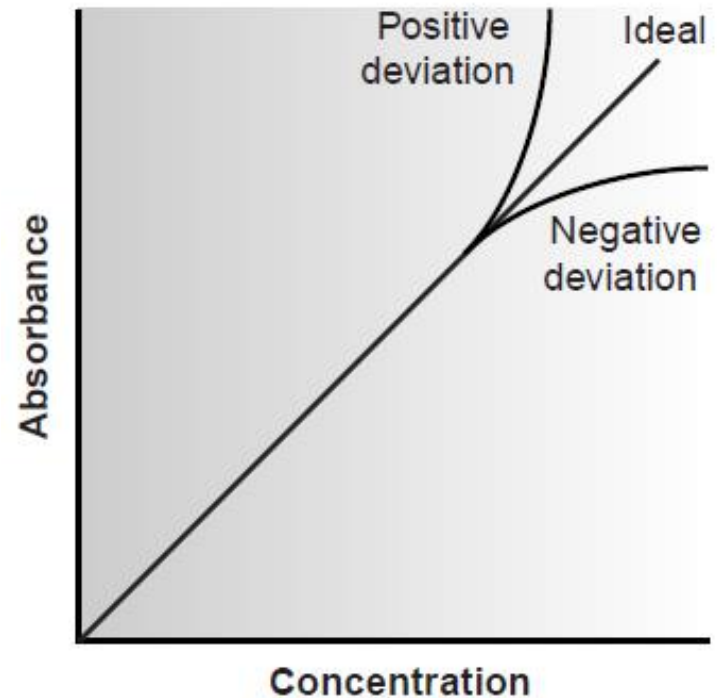
According to Beer's law, a calibration curve of absorbance versus the concentration of analyte in a series of standard solutions should be a straight line with an intercept of 0 and a slope of  **$\epsilon b$**  or  **$\epsilon b$** . In many cases, however, calibration curves are found to be nonlinear.

$$A = \epsilon b c + 0$$

slope                      intercept

Deviations from linearity are divided into three categories:

- **Fundamental or real,**
- **Chemical,**
- **Instrumental.**

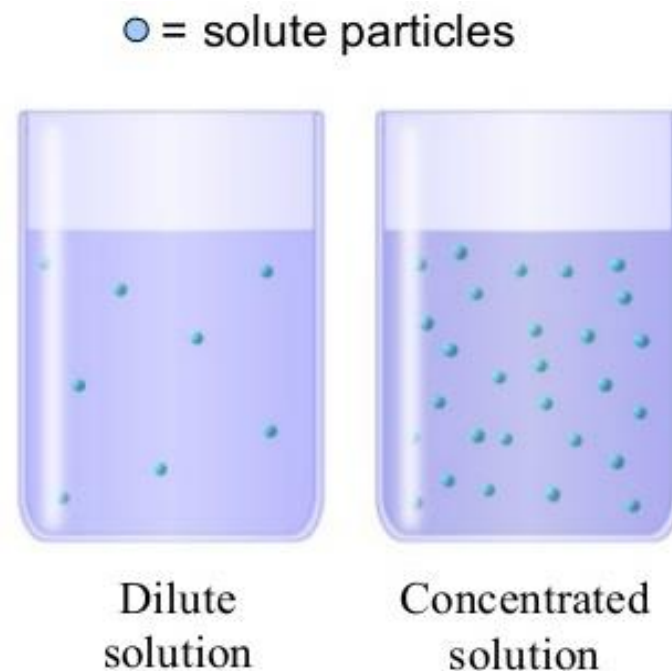




## Fundamental or Real Limitations to Beer's Law:

Beer's law is a limiting law that is valid only for low concentrations of analyte. At high concentrations (exceeding about 0.01 M), the individual particles of analyte no longer behave independently of one another. The resulting **interaction (attractive and repulsive forces)** between particles of analyte may change the value of the molar absorptivity.

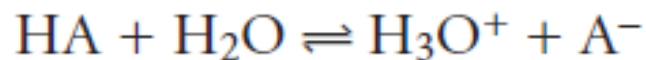
A second contribution is that the molar absorptivity depends on the sample's **refractive index**. Since the refractive index varies with the analyte's concentration, the values of molar absorptivity will change. For sufficiently low concentrations of analyte, the refractive index remains essentially constant, and the calibration curve is linear.



## Chemical Limitations to Beer's Law:

Occur when the absorbing species undergoes association, dissociation, or reaction with the solvent to give products that absorb differently from the analyte. Chemical deviations from Beer's law can occur when the absorbing species is involved in an **equilibrium reaction**.

Consider, as an example, an analysis for the weak acid, HA. Since HA is a weak acid, it exists in equilibrium with its conjugate weak base, A<sup>-</sup>.



If both HA and A<sup>-</sup> absorb at the selected wavelength, then Beer's law is written as

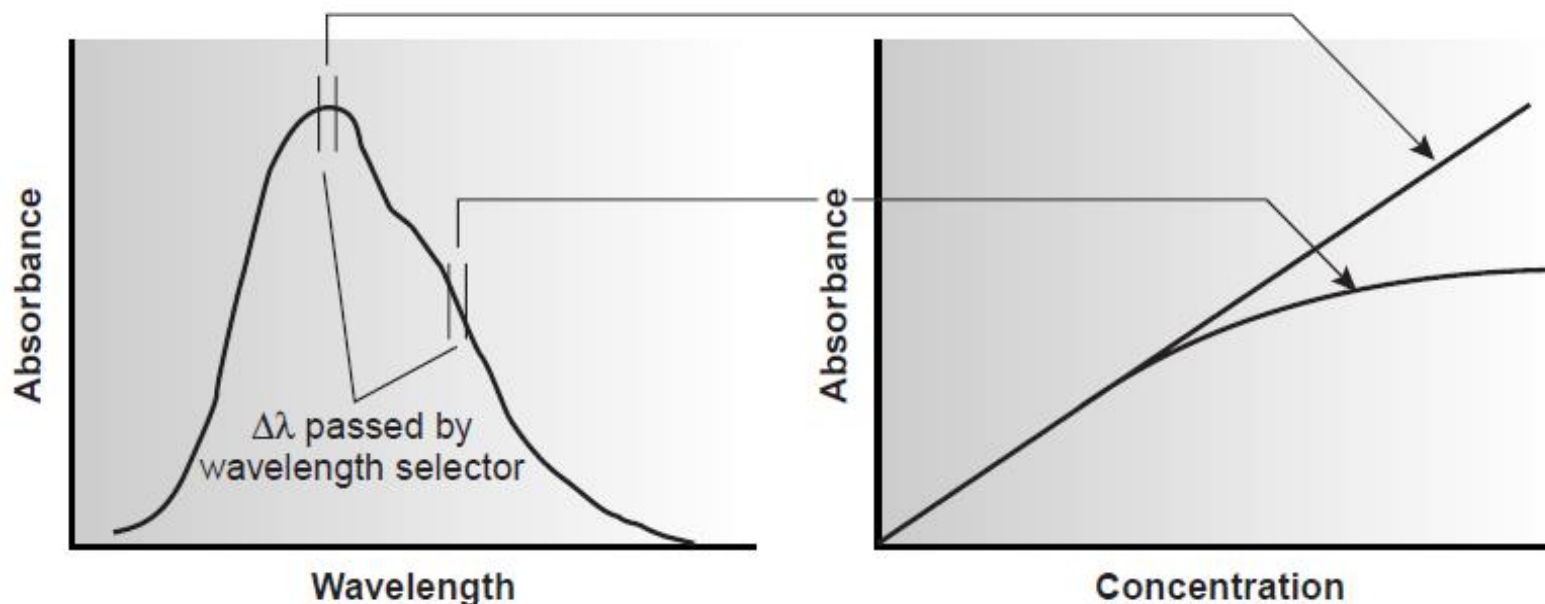
$$A = \epsilon_{\text{HA}} b C_{\text{HA}} + \epsilon_{\text{A}} b C_{\text{A}}$$

where  $C_{\text{HA}}$  and  $C_{\text{A}}$  are the equilibrium concentrations of HA and A<sup>-</sup>. Depending on the relative values of  $\epsilon_{\text{HA}}$  and  $\epsilon_{\text{A}}$ , the calibration curve will show a positive or negative deviation from Beer's law if the standards are not buffered to the same pH.

## Instrumental Limitations to Beer's Law:

### - *Polychromatic Radiation*

Beer's law is strictly valid for purely monochromatic radiation. The value of  $\epsilon$  is wavelength dependent. However, even the best wavelength selector passes radiation with a small, but finite effective bandwidth. Using polychromatic radiation always gives a negative deviation from Beer's law, but is minimized if the value of  $\epsilon$  is essentially constant over the wavelength range passed by the wavelength selector.



To avoid such deviations, it is best to select a wavelength band near the wavelength of maximum absorption where the analyte absorptivity changes little with wavelength.

## - *Stray Light*

Stray radiation arises from imperfections within the wavelength selector that allows extraneous light to “leak” into the instrument. Stray radiation adds an additional contribution,  $P_{\text{stray}}$ , to the radiant power reaching the detector; thus

$$A = \log \frac{P_0 + P_{\text{stray}}}{P_T + P_{\text{stray}}}$$

**Stray radiation** is any radiation reaching the detector that does not follow the optical path from the source of radiation to the detector.

Stray radiation is often the result of scattering and reflection off the surfaces of gratings, lenses or mirrors, filters, and windows. It leads to negative deviation from Beer’s law.

