

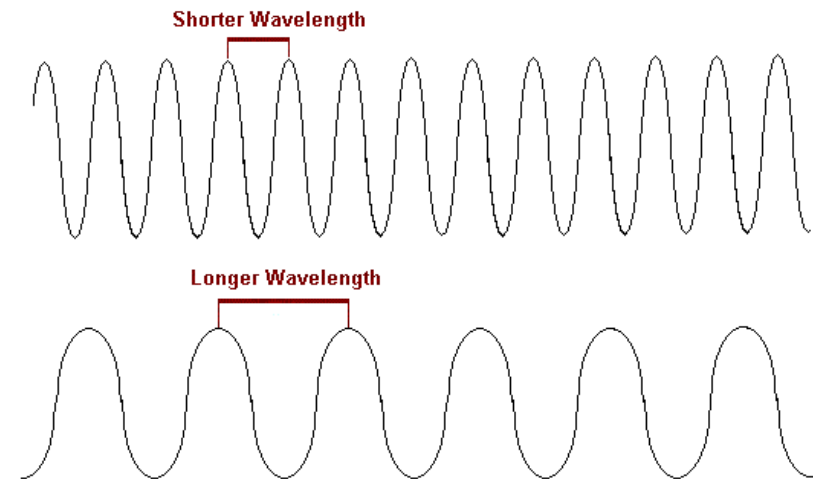
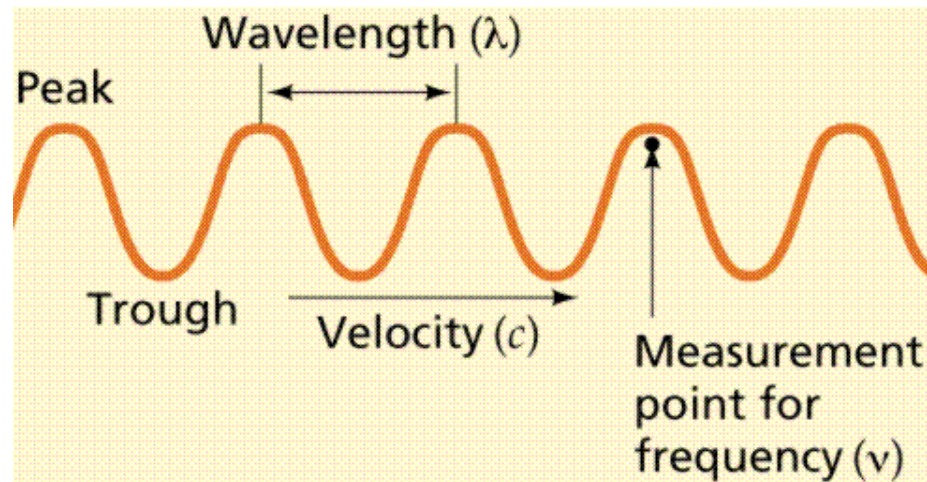
SPECTROPHOTOMETRY/BEER'S LAW

Spectroscopy will be a tool that you will use as you continue in your chemistry, biology and physics courses.

We will begin by discussing the relationships between energy, wavelength, frequency, and speed of light waves.

The wavelength, (λ) of any wave is the crest-to-crest distance between waves.

The frequency, ν (nu) is the number of complete oscillations that a wave makes each second.



The speed of a wave is the distance one wave travels in a given time period.

The relation between wavelength, frequency, and speed is

$$\text{Speed} = \text{wavelength} * \text{frequency} (1)$$

The speed of light is constant in a given medium.

For example, the speed of light in a vacuum is 2.99792458×10^8 m/s and is symbolized by the letter 'c'.

The speed of light decreases in other media such as air or water.

In water, the speed of light decreases enough to give illusions such as a spoon appearing bent when it is partially submerged in a glass of water.

The decrease in speed is negligible in air so we will consider the speed of light to be 3.00×10^8 m/s in air.

This leads to the equation

$$c = \lambda \nu$$

λ is wavelength,
 ν is frequency,
 c is speed of light

Where $c = 3.00 \times 10^8$ m/s, λ = wavelength given in meter units, and ν = frequency given in Hertz units (Hz, oscillations/second).

Each light particle, or photon, carries a discrete amount of energy.

This energy is proportional to the frequency of the light, and is therefore inversely proportional to the wavelength of light.

This relation was discovered by Max Planck in 1900 when he determined that

$$E_{\text{photon}} = h\nu$$

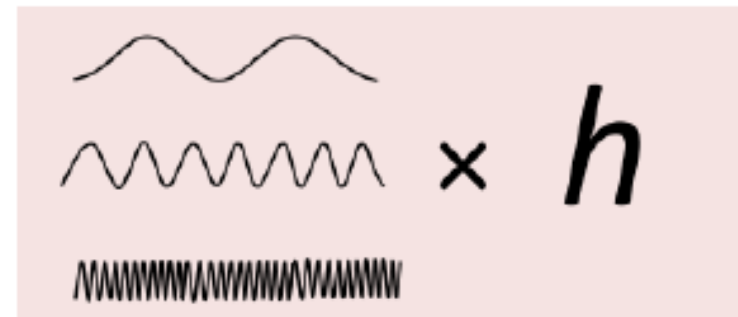
where E_{photon} = Energy of one photon (not a mole of photons!) given in Joule units and h = Planck's Constant, 6.626×10^{-34} Joule*seconds.

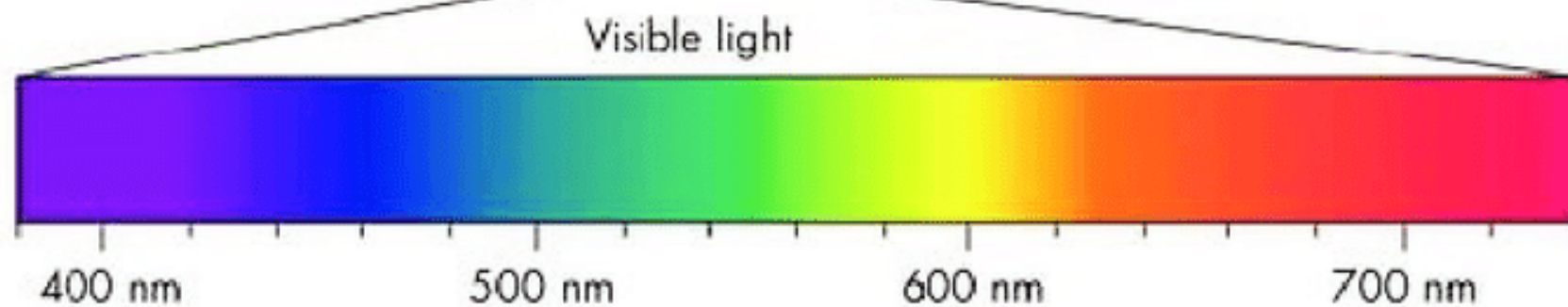
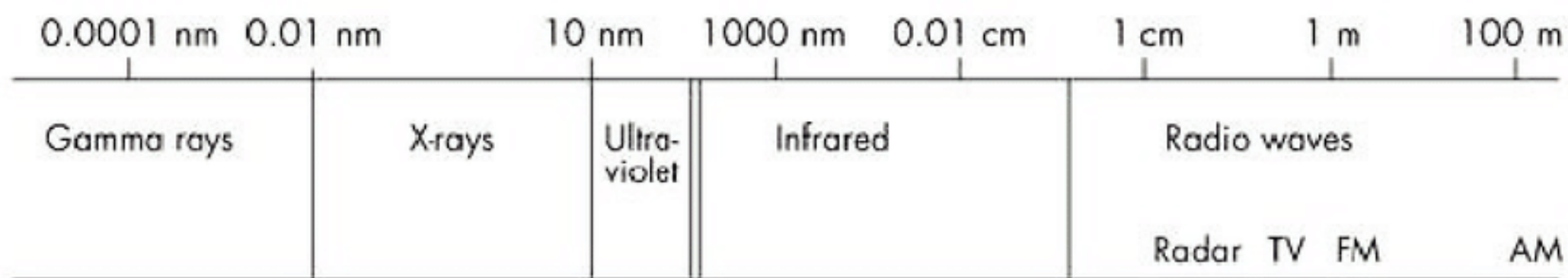
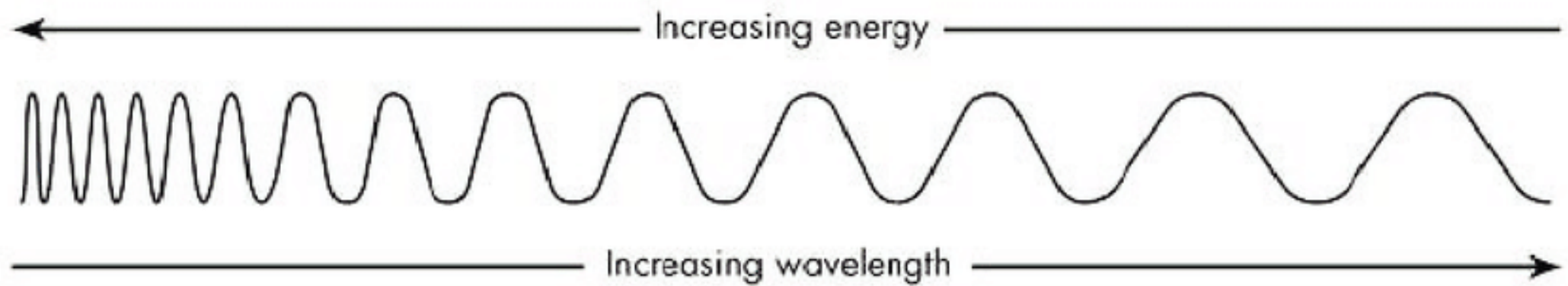
The Planck Constant: h

$$h = 6.626 \times 10^{-34} \text{ J s}$$

a proportionality between
frequency (ν) and energy

$$E = h\nu$$





Absorption and Emission of Light:

The particle nature of light is most often used to explain absorption and emission properties of atoms and molecules.

Molecules absorb or emit energy only in discrete amounts or packets called quanta.

Absorption or emission of light by an atom or a molecule can only occur if the energy of the photon is equal to the energy difference between two energy levels in the atom or molecule.

For the purposes of Chemistry lab, we will concern ourselves only with absorption of light for the remainder of this discussion.

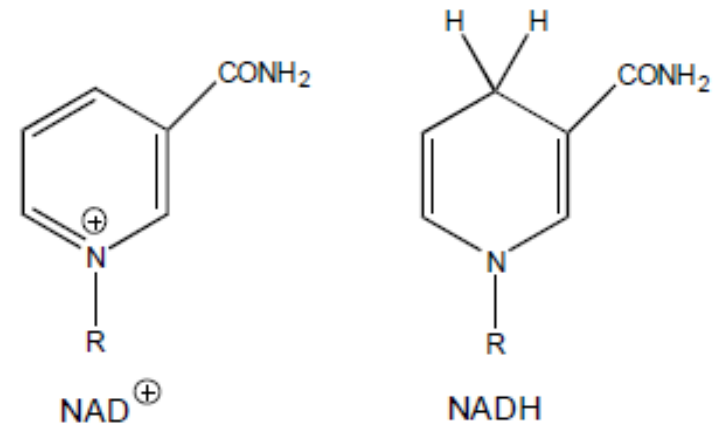
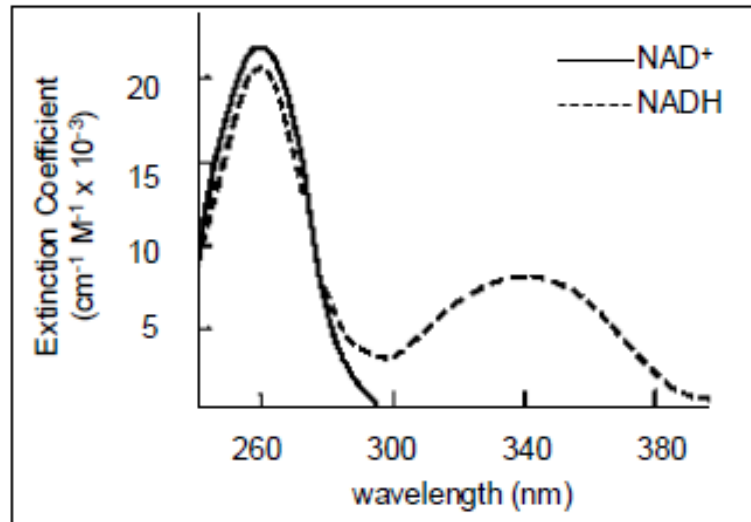
Molecules can exist only in discrete energy states.

This means that they will only absorb photons of specific energies and therefore specific frequencies and wavelengths.

This is shown graphically when the amount of light absorbed is plotted as a function of the frequency or wavelength.

This graph is called the spectrum of a particular molecule or atom.

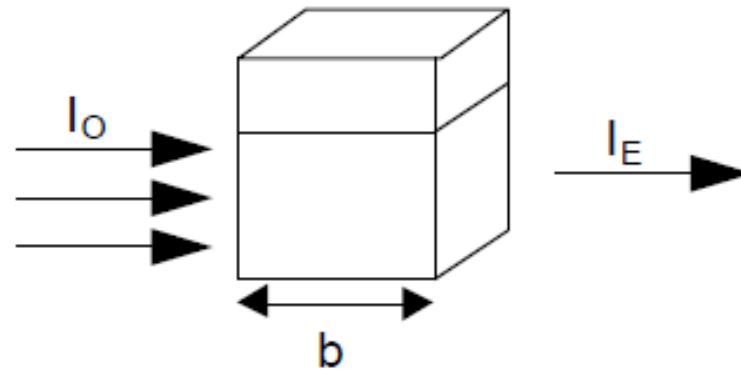
Figure 1 shows how two molecules that are very similar structurally can have different spectra.



Transmittance and Absorbance:

It is difficult, if not impossible, to measure actual absorbance of light. Instead, we measure transmittance or the fraction of light that is able to pass through a solution of molecules.

A spectrophotometer measures the intensity of light entering a sample and compares this to the intensity of light emerging from the sample (Figure 2).



I_0 = Intensity of incident light

I_E = Intensity of exiting light

b = path length of sample

Figure 2: Transmittance of light in a spectrophotometer. Transmittance = I_0/I_E .

There are three things that will effect the amount of light emerging from the sample.

First, the concentration of molecules in the solution affects the transmittance.

Each molecule can absorb light. As you increase the number of molecules in the solution, you also increase the photons absorbed. Therefore, as you increase the concentration of a sample, you decrease the transmittance.

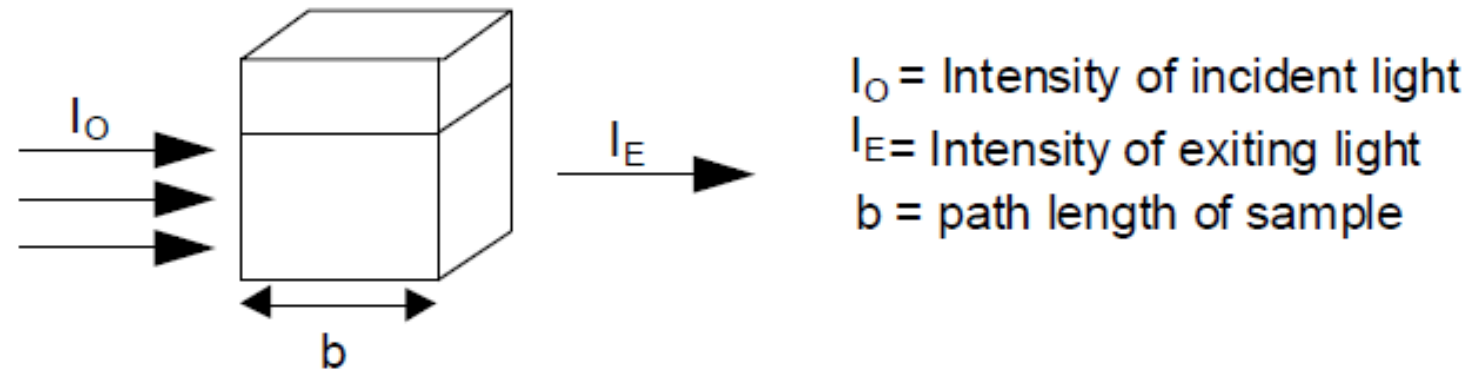
Second, the length of the sample path will affect the transmittance.

By increasing the pathway that the light must travel through your sample, you are increasing the number of molecules that will interact with the light; in effect, you are increasing the apparent concentration.

Third, the transmittance will be affected by specific properties of the molecules. Molecules absorb light at different efficiencies and at different energies.

Therefore, the transmittance will be dependent upon the specific molecule in solution and the wavelength of light being passed through the sample.

In 1729 Pierre Bouguer discovered that the relationship between transmittance and concentration or sample path length is a logarithmic one.



The molecules nearest the source of light will experience I_o the same as that which was measured by the spectrophotometer.

However, molecules nearest the exit point of the sample will experience I_o that is less than the original because molecules have absorbed light throughout the sample.

In other words, as light travels through the sample, there is a drop in I_o in each succeeding layer.

The definition of transmittance tells us that $T = I_E/I_o$. Because I_o changes throughout the sample, we have a logarithmic, rather than linear, relationship between transmittance and concentration or sample path length.

The Lambert-Beer Law:

The fact that transmittance of light varies exponentially as it passes through an absorbing medium was rediscovered by Johann Heinrich Lambert in 1760.

Later, in 1852, August Beer defined absorbance:

$$A = -\log_{10}(T) \quad (4)$$

When no light is absorbed, $I_E = I_O$, $T = 1.00$ and $A = 0$. When 90% of the light is absorbed, $T = 0.1$ and $A = 1$.

Beer then showed that absorbance was linearly related to concentration.

These two men are given credit for the Lambert-Beer Law:

$$A = \epsilon c l \quad (5)$$

Where ϵ = a constant which takes into account the specific properties of the molecules which are absorbing photons, l = sample path length, usually 1 cm, and c = concentration of solution, M.

Note that Absorbance is unitless.

Beer's Law is only true for dilute solutions--the exact range of solutions must be determined experimentally.

Beyond this range, measurements and calculations using Beer's Law will be erroneous.

Other common sources of error include the use of dirty cuvettes, poorly mixed solutions, poor pipetting techniques, and incorrect light source or wavelength.

Because you have control over these errors, you must make sure to minimize these problems in your laboratory exercises.