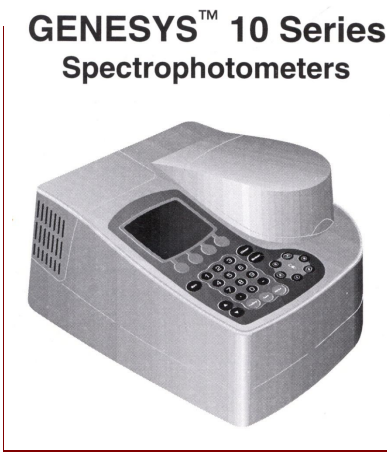
Spectrophotometry

Quantifies the transmission of light through solutions

COLORIMETRY:

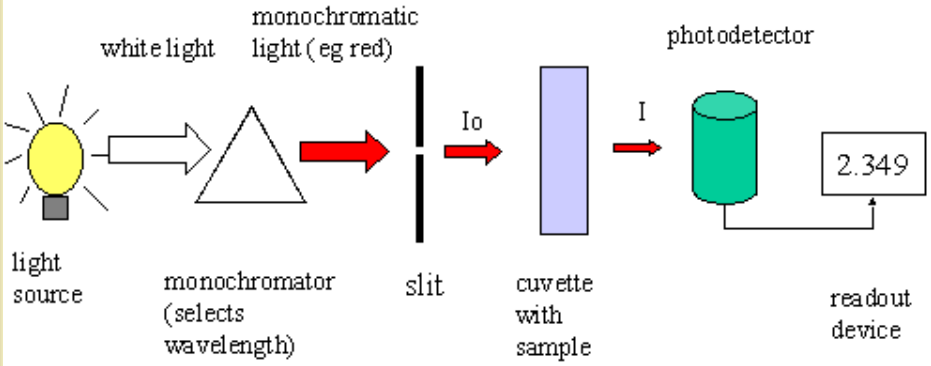
When chemical reaction occurs that changes the color of the solution, which in turn increases the absorption of light at a *specific* wavelength.



SPECTROPHOTOMETRY:

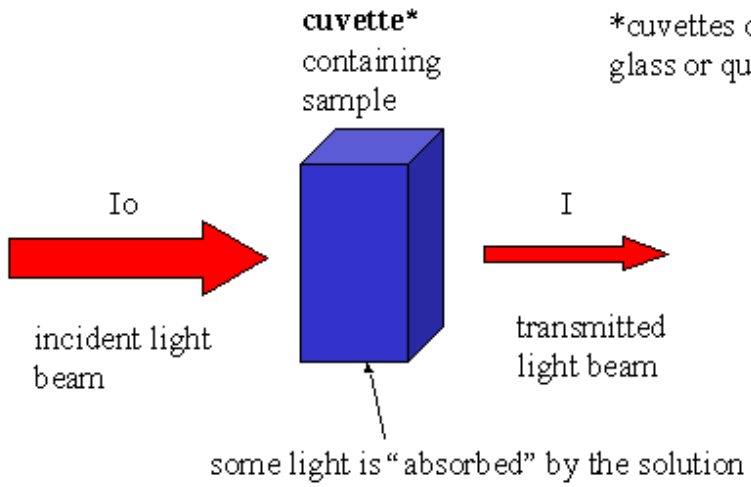
When the solution contains a *specific* molecule that absorbs light at a *specific* wavelength (*eg: NADH @ 340 nm*).

Single-Beam Spectrophotometer

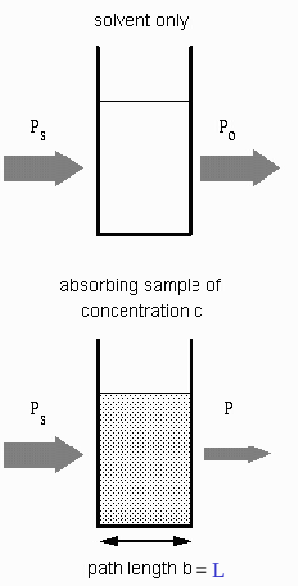


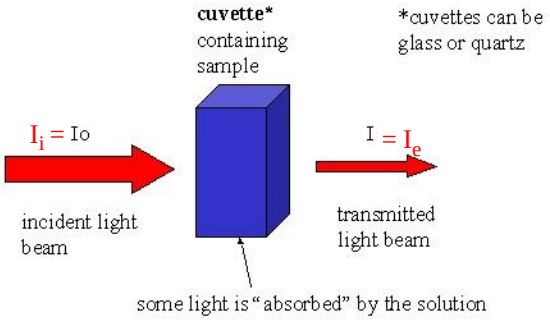
Does this arrangement measure I, I_0, or both ?

Absorption of Light



Beer-Lambert Law





where;

$$I_e = I_i \bullet e^{-acL}$$

a = absorption coefficient *(wavelength specific)*

c = concentration of dissolved molecule

L = thickness of light path length through solution

e = 2.71828

Beer-Lambert Law, cont'd.

$$I_e = I_i \bullet e^{-acL}$$

$$I_e / I_i = e^{-acL}$$

$$\text{Transmittance} = T = I_e / I_i = e^{-acL}$$

$$\ln T = \ln (e^{-acL}) = - a \bullet c \bullet L$$

$$\ln (1/T) = a \bullet c \bullet L$$

$$= \text{Absorbance}$$

Absorbance equals the natural log of the reciprocal of transmittance

Beer-Lambert Law, cont'd.

Absorbance equals the natural log of the reciprocal of transmittance

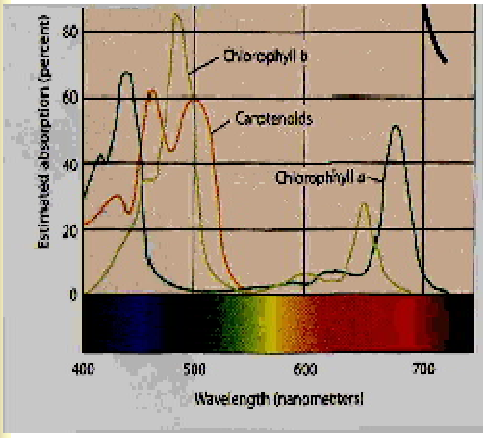
$$\ln (1/T) = a \bullet c \bullet L$$

$$= \text{Absorbance}$$

$$\text{Absorbance} = a \bullet c \bullet L$$

If you know the absorption coefficient for a given wavelength, and the thickness of the path length for light transmitted through the solution, you can calculate concentration.

Absorption Spectra



What wavelength of light best distinguishes chlorophyll a from b and carotenoids?

~ 675 nm

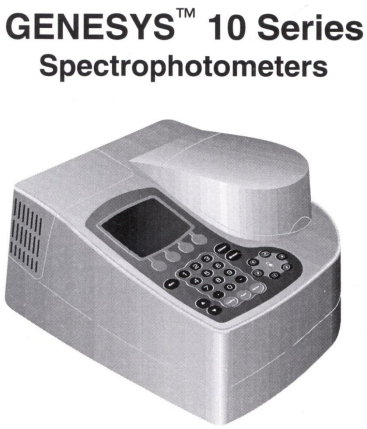
What wavelength of light best distinguishes carotenoids from chlorophyll a and b?

~ 520-530 nm

What wavelength of light best distinguishes chlorophyll b from a and carotenoids?

~ 650 nm

Spectrophotometers



Genesys™ 10 Spectrophotometer