FAQ'S

1. **Define: Beers Law**

2. **Beer's law states that the absorbance is directly proportional to the concentration of a solution. If you plot absorbance versus concentration, the resulting graph yields a straight line. The equation for the straight line (termed regression line) can be used to determine the concentration of an unknown solution once the %T has measured**

3. **What are Beers law components from the formula?**
   A=Absorbance T= transmission (or transmissivity) A = 2.00 -[log (%T)] If T = 85%, then Absorbance = 2 - log[85] = 0.071

4. **In absortion spectrophotometry: Absorbance in directly porportional to concentration and path length.**

   Beer's law states that A= axbxc, where a is the absorbtivity coefficient (a constant) b is the path length, and c is the concentration. Absornance is directly proportional to both b and c. DDoubling the path length results in incident light contacting twice the number of molecules in solution. This causes absorbance to double, the same effect as doubling the concentration of molecules.

5. **Which wavelength would be absorbed strongly by a RED colored solution: 450nm...**
   A solution TRANSMITS light corresponding in wavelength to its color and usually ABSORBS light of wavelenghts complementary to its color. A RED solution TRANSMITS light of 600-650 nm and strongly ABSORBES 400-500nm light.
   A green-colored solution would show higest transmittance at: 525 nm.
   Green light consist of wavelengths from 500-550nm. A green colored solution with transmittance maximum of 525 nm and a 50 nm band pass TRANSMITS light of 525 nm and ABSORBES light below 475 and above 575 nm . A sloution that is green would be quantitated using a wavelength that it absorbes strongly, such as 450 nm.

6. **Which type of monochromator produces the PUREST monochromatic light in the ultra violet (UV) range?**
   A prism and a variable exit slit.

   Differaction gratings and prisms both produce a continuos range of wavelengths. A diffraction grating produces a UNIFORM separation of wavelengths. A prism produces a MUCH BETTER SEPARATION of HIGH FREQUENCY LIGHT because refraction is greater for higher energy wavelengths. Instrument using a prism and a variable exit slit can produce UV light of a very NARROW BANDPASS. The adjustable slit is required in order to allow sufficient light to reach the detector to set 100 %T.

7. **Which photodector is most sensitive to low levels of light?**
   Photomultiplier tube.

   The photomultiplier tube uses dynodes of increasing voltage to amplify the current produced by the photosensitive cathode. It is 10,000 times as sensitive as a barrier layer cell, which has

no amplification. A photomultplier tube requires a DC- regulated lamp because it responds to light fluctuations caused by an AC cycle.

8. **Which of the following materials is best suited for verifying the wavelength calibration of a spectrophotometer?**
   Holmium oxide glass.
   Wavelength accuracy is verified by determining the wave length reading that gives the highest absorbance (or transmittance) when a substance with a narrow bandpass (sharp absorbance or transmittance peak) is scanned . For example didymium glass has a sharp peak at 585 nm. Therefore an instrument sholud give its higest absorbance reading when the wavelength dial is set at 585nm. Holmiun oxide produces a very narrow absorbance peak at 361 nm. likewise, the hydrogen lamp of a UV spectrophotometer produces a 656 nm emission line that can be used to verify wave length. Neuteral density filters and dichromate solutions are used to verify absorbance accuracy or linearity. A wratten filter is a wide bandpass filter made by placing a this layer of colored gelatin between two glass plates and is unsuitable for spectrophotometric calibration.
9. **Which component is required in a spectrophotometer in order to produce a spectral absorbance curve?**
   Photodioide array.

There are two ways to perform spectral scanning for compound identification. One is to se a stepping motor that continuously turns a monochromater so that the wavelenght aligned with the exit slit changes at a constant rate. A more practical method is to use a diode array detector. This consist of a chip embedded with as many as several hundred photodiodes each photodiode is aligned with a narrow part of the spectrum produced by a diffraction grating and produces current proportional to the intensity of the band of light striking it.(usually 1-5 nm in range) The diode signals are processed by a computer to create a spectral absorbance or transmittance curve.

10. **Which instrument requires a highly regulated DC power supply?**

A spectrophotometer with a photomultiplier tube. When AC vltage regulaters are used to isolate source lamp power, liht ouput flucuates as the voltage changes. Because this occurs at 60 Hz, it is not detected by the naked eye or slow responding detectors. Photomultiplier tubes are sensitive enough to respond to the AC frequency an d require a DC power supply.