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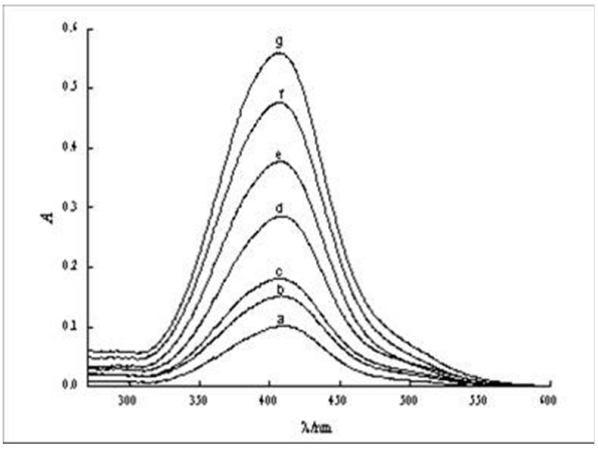
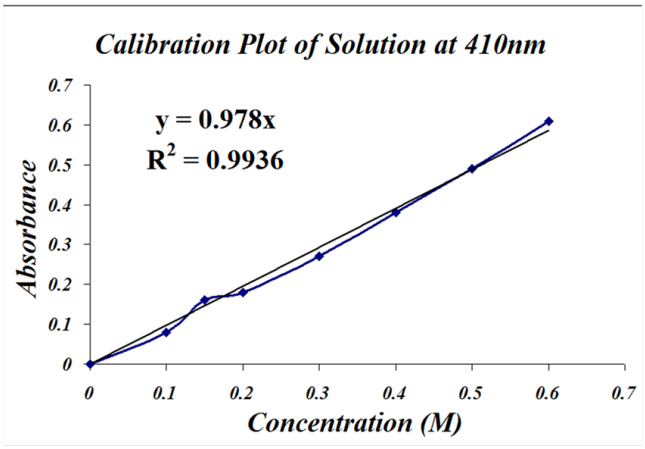
# *Generating and Using a Calibration Graph*

## *How to Work with Plots*



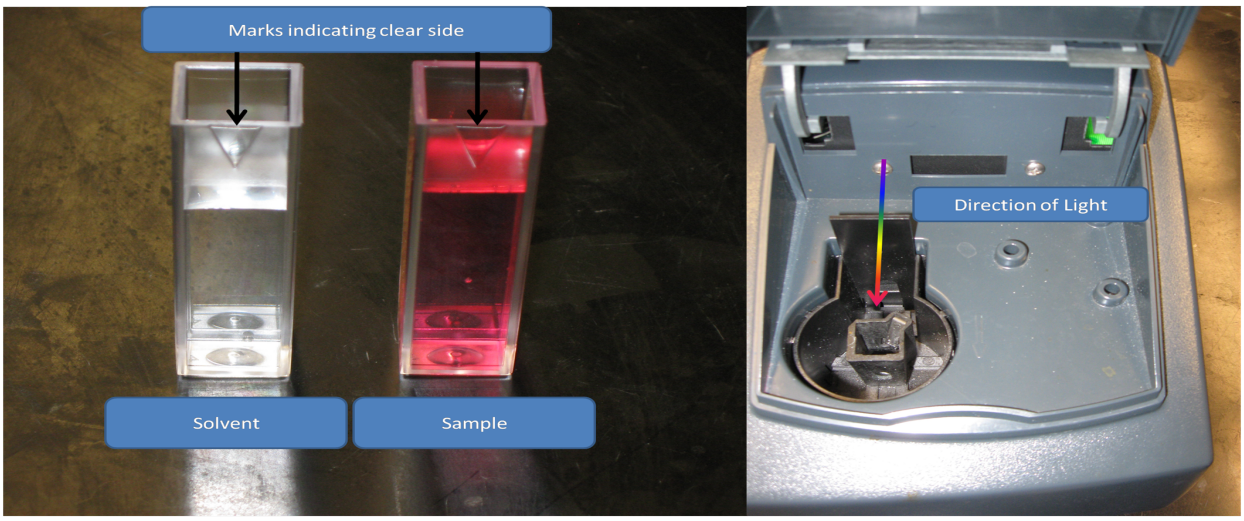






You have two different plots, you absorbance spectrum on the left, and your calibration plot on the right.

First look at the absorbance spectrum. These spectra were taken using different concentrations.

## *Choosing Your Wavelength*




PD-INEL

Look at the images above. The left is an absorbance spectrum of 0.13mM plastocyanin, while on the right is a calibration plot at two wavelengths.

**Is the slope of the calibration line at 550nm greater than, less than, or equal to the slope at 600nm?**

You can choose any wavelenght to create a calibration plot, the only differerence will be the slope of the line.

When you actually choose your wavelength to create your calibration graph, you would generally like to choose a wavelength where there is room

for the concentration to decrease. Look at the spectrum above. Do you think 450nm would be a good wavelength to use for a calibration graph? You would not choose that wavelength because when you lower the concentration, you would not be able to see much of a difference in the absorbance, and the calculations would be inaccurate. You would most likely want to choose wavelengths like 600nm or 250nm where there is a lot of room for absorbance change.