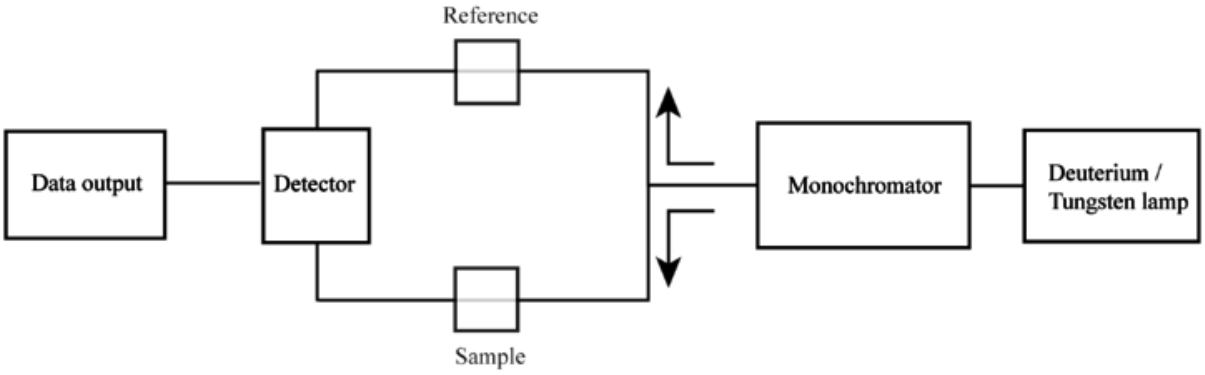
UV/VIS-spectrometer background

A non destructive quantitative and qualitative technique~ you can keep your sample after running it through the UV/VIS spectrometer.

Basic parts of UV spectrometer:

- Deuterium or tungsten lamp commonly used.
- Has diffraction grating, equally spaced, narrow, parallel source with a narrow fringe. It helps disperse light in different wavelengths. Uses the monochromator to select a wavelength.
- 2 types:
    a. Single beam: all light passes through the sample cell
    b. Double beam: light split into 2 before reaching sample. One beam is used as a reference, one passes through sample.



- Absorbance: Beer-Lambert Law states absorbance of a solution is directly proportional to the concentration. $A = \varepsilon \iota c$

A= absorbance ε= extinction coefficient c= concentration

So $A \propto C$

- Data obtained: obtain absorbance of sample at selected wavelength.
- Data analysis: create calibration curve with known concentration standards and use it to work with unknown concentration standards

Reagent preparation:

Ascorbic acid preparation:

Add Deionized water to a 50 mL volumetric flask, measure out 0.88 g ascorbic acid fill to line in a volumetric flask. Swirl until no crystals are visible. Cap and and invert a few times. This solution is good for 1 week.

Coloring Reagent preparation:

In a 25 mL volumetric flask, use 12.5 mL of 5N H_2SO_4, 1.25 mL antimony Potassium tartrate solution, 3.75 mL ammonium molybdate solution, 7.5 mL of ascorbic acid solution. Mix between each addition of reagent. Fill to the line with Deionized water. This reagent is stable for 4 hours.

Preparation of standards & samples:

Known standards are already prepared.

We will be using 0.8M, 0.6M, 0.4M, 0.2M, 0M (DeIonized Water) to create our calibration curve. We will be putting these into our test tubes, and treating with the coloring reagent.
If ascorbic acid is not already prepared, accurate preparation is crucial to obtaining accurate results.

a. Preparation of standards and samples for phosphorus reading.

I. We will be making 5 mL test tubes of our standards from 0.8M- 0M

II. These will be treated with 0.800 mL (800 microliters) of coloring reagent, after the coloring reagent addition, we will wait 10 minutes before inserting them into the UV/VIS spectrometer. Make sure to write down the time! *This will be prepared and ran first.*

III. Our unknown concentration samples will be the filtrate of the chlorophyll step, we will fill test tubes up to 5 mL and treat with 0.8 mL (800 microliters) of coloring reagent, after the coloring reagent addition, we will wait 10 minutes before inserting them into the UV/VIS spectrometer. Make sure to write down the time! *This will be prepared after the standard run.*

b. Running the samples through the UV/VIS spectrometer

I. cuvettes will be filled up with standards first. Data should be copied down on paper and can be saved as USB file if needed.
II. cuvettes will then be washed and left to dry while unknown standard is prepared.
III. Unknown standard run and data should be obtained on paper and can be saved as USB file if needed.

Analysis:
Calibration curve.
Using calibration curve to find concentration of unknown.