SPECTROPHOTOMETRY

Principle:

Spectrophotometry is **a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution**. The basic principle is that e ach compound absorbs or transmits light over a certain range of wavelength.

Beer – Lamberts Law:

Statement:

The Absorbance of light by a sample solution is directly proportional to the path length and concentration of the sample.

*The Beer – Lambert's law is expressed as

$$A = \varepsilon Lc$$

Where A = Amount of light absorbed for a particular wavelength by a Sample

ε = molar extintion co-efficient

L = Diatance covered by the light throgh the sample solution

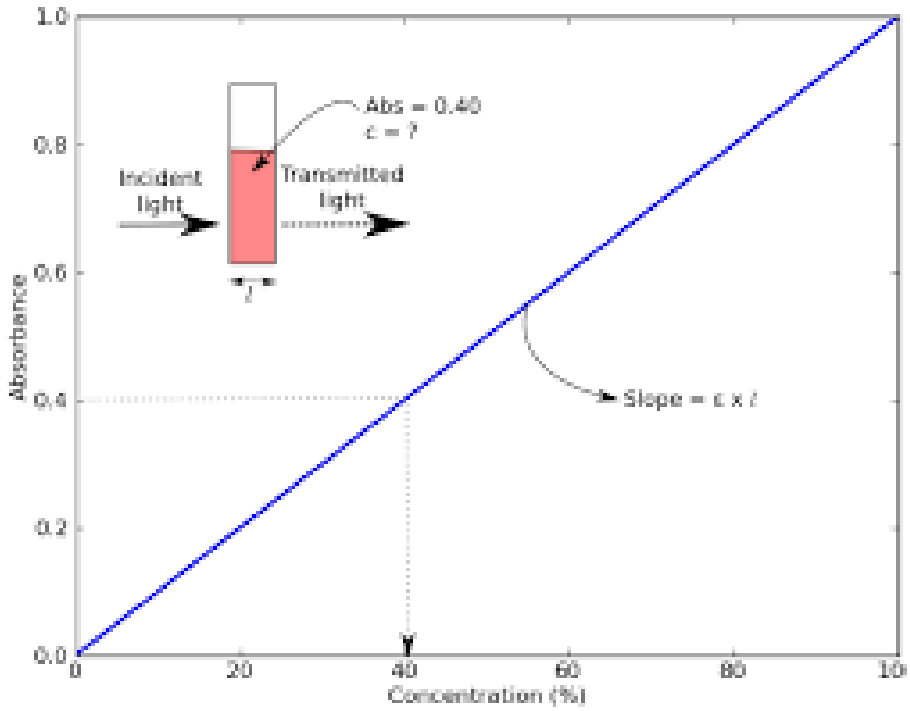
c = Concentration of the absorbing species

*Beer-Lamberts law is the combination of 2 different laws.(Beer's Law and Lamberts Law)

*Beer's law was stated by August Beer which states that concentration and absorbance are directly proportional to each other.

*Johann Heinrich Lambert stated Lambert law. It states that absorbance and path length are directly proportional.

Graph:



Limitations of Beer – Lambert's Law:

Following are the limitations of Beer-Lambert law:

1.It is applicable for dilute solutions only.

2.There shouldn't be a scattering of the light beam

3.Monochromatic electromagnetic radiation should be used

4.When different types of molecules are in equilibrium with each other.

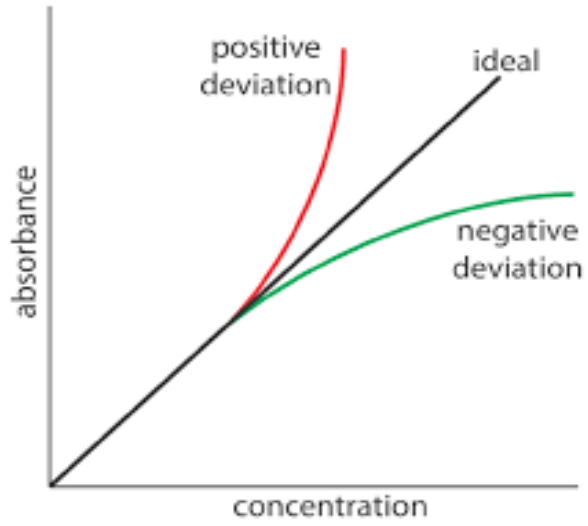
5.An association complex is formed by the solute and the solvent.

6.When fluorescent compounds are used.

7.When thermal equilibrium is attained between the excited state and the ground state.

8.It shows 2 types of deviations for non – ideal solutions.

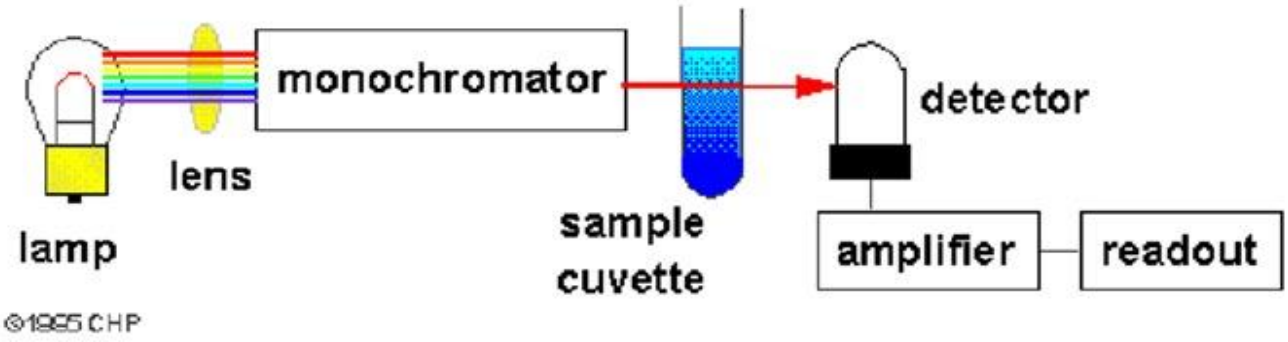
a) Positive deviation b) Negative deviation



INSTRUMENTATION:

SINGLE BEAM SPECTROMETER:

Diagram:



*Single beam spectrophotometer is an analytical instrument in which all the light waves coming from the light source passes through the sample.

Basic Components:

There are four basic components to a simple single beam UV/Vis spectrophotometer; **a light source, a monochromator, a sample, and a detector**.

1. Light Source:

Light sources are devices whose primary function is **to produce visible or near-visible radiant energy for general illumination and specialty applications**. They include incandescent, fluorescent, and high-intensity discharge (HID) lamps, as well as solid-state lighting (SSL)

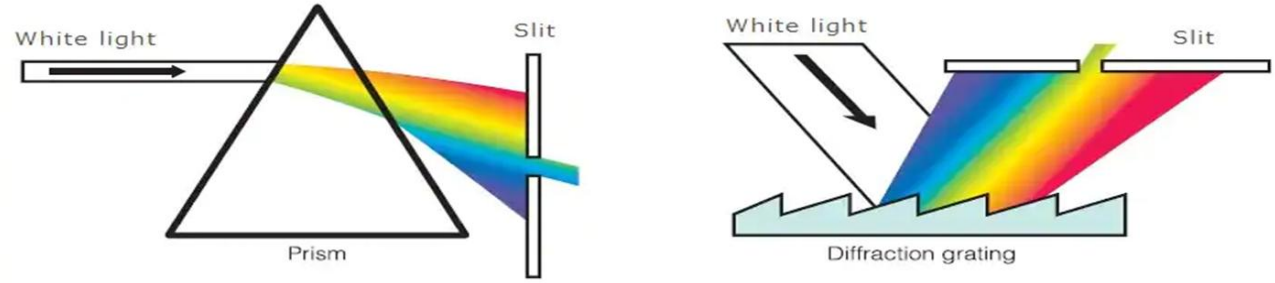
* The most common UV sources is a **high-pressure deuterium lamp**. For the visible region, the traditional source is a simple tungsten filament bulb; however improved performance can be obtained using a more modern quartz halogen bulb.

2.Monochromator:

*A monochromator is an optical instrument which measures the light spectrum.

*A monochromator is **a mechanism that emits monochromatic light from a light source**. A dispersive element, generally a prism ordiffraction grating, is used to create the monochromatic light.

*Monochromator may be a Prism or Diffraction grating.



3. Sample Holder:

Cuvettes:

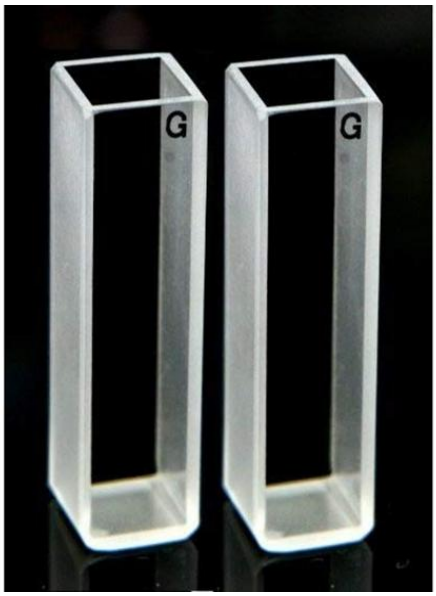
Standard cuvettes made from PMMA(Polymethylmethacrylate) polystyrene or normal glass are only transparent in the visible range. If wavelengths in the UV-range, below approximately 300 nm, are employed, **cuvettes made from quartz glass, or a special type of plastic, which provide sufficient transparency in this range, must be used.**

*The quartz material remains transparent in both visible light and UV ranges. That is why it can be easily used for UV-light spectrum sample measurements.

*Cuvettes are designed to hold liquid substances during chemical analysis.

***Plastic cuvettes** with a usable wavelength range of 380–780 nm (the visible spectrum) may be disposed of after use, preventing contamination from re-use. They are cheap to manufacture and purchase.

*Glass Cuvettes have an optimal wavelength range of 340–2500nm.



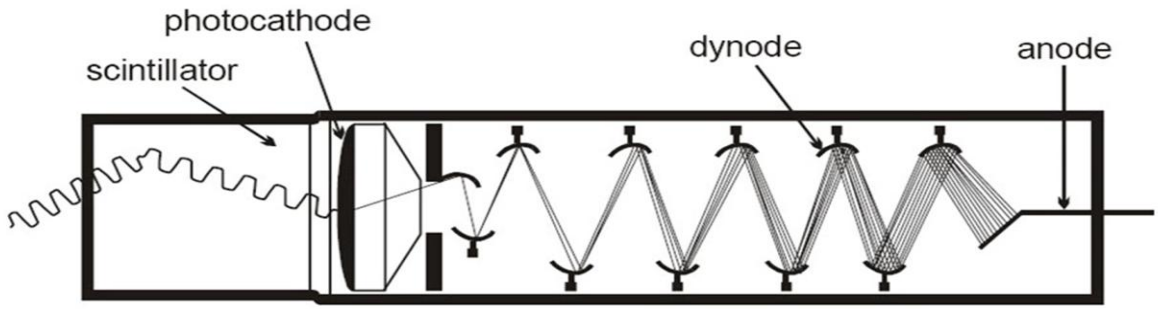
4. DETECTOR:

The term "detector" refers to a light-receiving element that absorbs the energy of light and consequently induces an electrical change.

Ex: The **photomultiplier tube** is a commonly used detector in UV-Vis spectroscopy. It consists of a photoemissive cathode (a cathode which emits electrons when struck by photons of radiation), several dynodes (which emit several electrons for each electron striking them) and an anode.

*There are two main types of detectors: **photon detectors** and **thermal detectors**.

*All detectors have similar characteristics.



AMPLIFIER:

Definition:

An amplifier boosts the electrical signal and transfers it to the internal circuit, finally converting the signal into a readable form.

*A photocell produces an electrical signal, which is directly proportional to the light reflected from the solution.

PHOTOCELL:

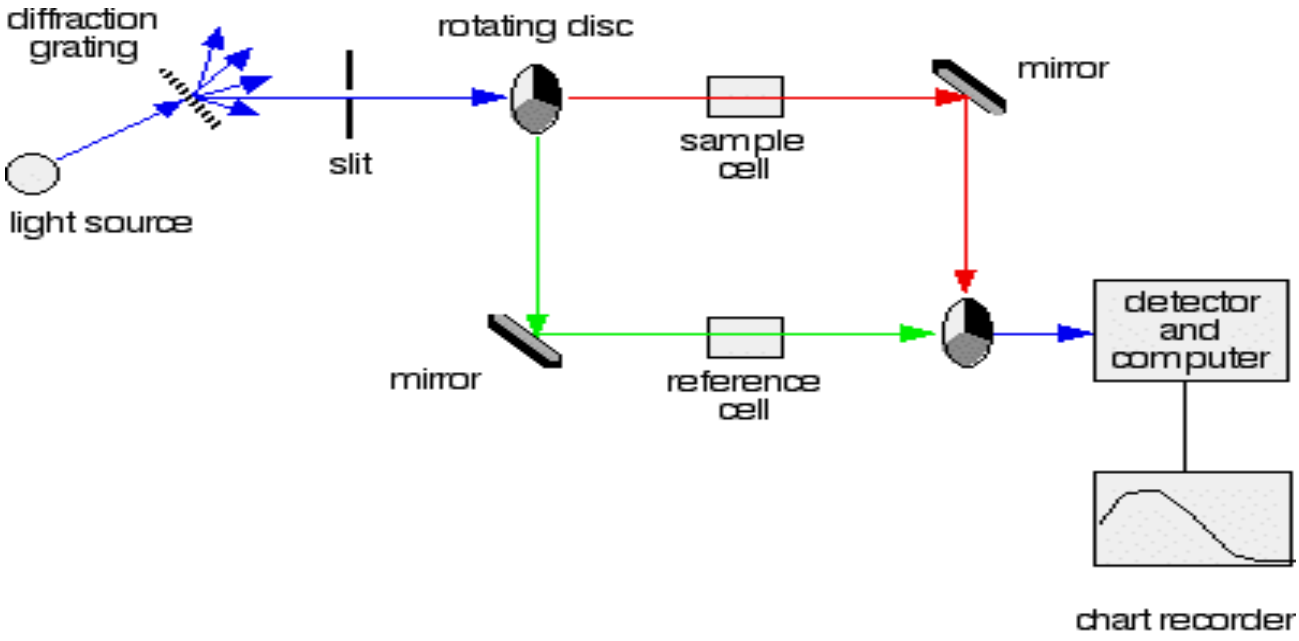
It refers to the **photoresistor** that detects the range of light transmitted from the test sample and transforms it into an electrical signal. A photocell detector shows the following properties:

- High sensitivity
- Short response time
- Long-term stability
- An electrical signal that can be easily amplified

READOUT SYSTEM:

The data from the detector are displayed by a readout device such as analog meter , digital display or liquid crystal display.
*The output can also transmitted to computer or printer.

DOUBLE BEAM SPECTROMETER:



APPLOCATIONS OF SPECTROPHOTOMETRY:

1.SPECTROPHOTOMETRIC QUANTITATIVE DETERMINATION OF Fe^{+2} IN SOLUTIONS:

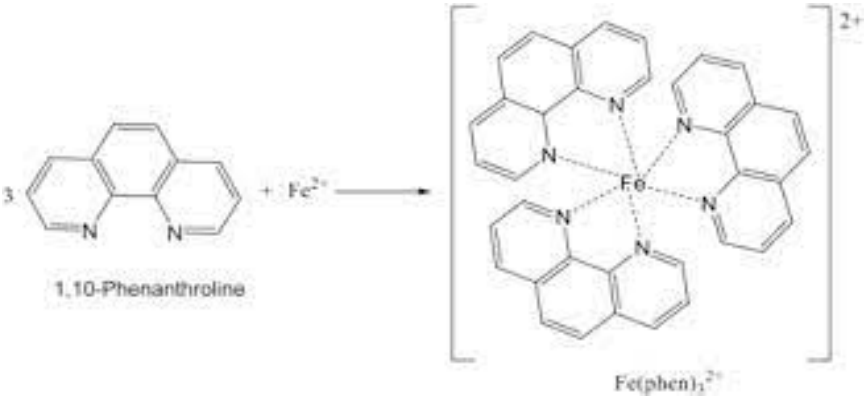
The ferrous ion combines with 1,10-phenanthroline to form a red complex serves as a sensitive method for determining iron at maximuim wavelength of 508nm at pH value of 6.0-9.0. by the addtion of Ammonia or sodium acetate.

$Fe^{2+} + 3\ phen \rightarrow Fe(phen)_3^{2+}$

Principle:

Fe(II) reduces easily in water to Fe(III). To reduce any Fe (III) back to Fe (II) we add a reducing agent to the solution. Of several reducing agents suitable for this purpose, hydroxylamine hydrochloride has been found to be very effective

$2NH_2OH + 4\ Fe^{3+} \rightarrow N_2O + 4\ Fe^{2+} + H_2O + 4\ H^+$



EXPERIMENTAL

REAGENTS:

1.1,10-phenanthroline (0.1 g of 1,10-phenanthroline monohydrate in 100 mL of distilled water, warming to effect solution if necessary).
2. Hydroxylamine hydrochloride (10 g in 100 mL of distilled water).

3.Sodium acetate (10 g in 100 mL of distilled water).

4. **Ferrous ammonium sulfate Solution:**0.07 g of pure ferrous ammonium sulfate hexahydrate, dissolve it in water, and transfer the solution to a 1-liter volumetric flask. Add 2.5 mL of concentrated sulfuric acid and dilute the solution to the mark.

5. **Preparation of Unknown sample solution:**Add about 0.12 g of the solid unknown and approximately 0.25 mL concentrated sulfuric acid into a 100 mL volumetric flask and dilute to the mark. Now transfer a 1 mL aliquot of this solution to another 100 mL volumetric flask.

6. Preparation of complex solution: To 1,5,10,25 and 50ml portions of the standard solutions, add 1ml of hydroxylamine solution, 10ml of the 1,10 – Phenanthroline solution and 8 ml of the sodium acetate solution. Alloe them to stand for 10 min with occassional shaking.

Preparation of the blank solution: To the test tube add 50ml of distilled water, 1ml of hydroxylamine solution, 10ml of 1,10-Phenanthroline solution and 8 ml of sodium acetate solutn.

Absorption Spectra:

The maximum absorbance for the complex1,10-Phenanthroline – Ferrous (II) is obtained at 508 nm at pH 6.0 - 9.0.

Diagram:

Procedure:

1. Measure the absorbance values of all the prepared sample solutions using reagent blank solution as the reference at 508nm.
2. The calibration curve is plotted by which the conentration of unknown substance present in the sample can be determined.

Calibration curve:

ACalibration curve is plotted by taking concentrations of standard & unknown samples of the complex on X-axis and their absorbance values on Y – axis at wavelength of 508nm.

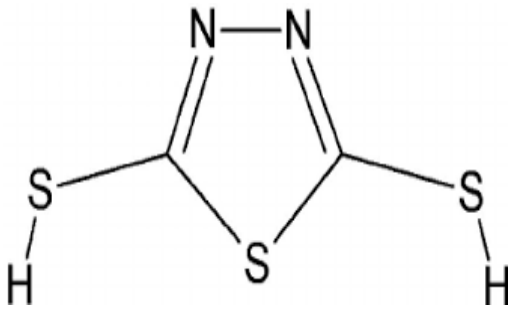
Diagram:

*The concentration of unknown lead nitrate solution can be calculated from its absorbance value by the formula mentioned below.

$$\text{Conc. of Unknown Lead Nitrate solution} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Standard solution}} \times \text{Conc. of standard solution}$$

2.SPECTROPHOTOMETRIC DETRMINATION OF LEAD

A very simple, ultra-sensitive and fairly selective non-extractive spectrophotometric method for the determination of trace amounts of lead with 2,5-dimercapto-1,3,4-thiadiazole (DMTD) has been developed. DMTD reacts in slightly acidic (0.0015–0.01 M HCl) aquatic media with lead(II) to give a greenish-yellow chelate, which has an absorption maximum at **375 nm.**



Structure of 2,5-dimercapto-1,3,4-thiadiazole (DMTD)

Reagents:

DMTD solution : 4.42×10^{-3} M This solution was prepared by dissolving the requisite amount of DMTD, dipotassium salt in a known volume of distilled de-ionized water. More dilute solutions of the reagent were prepared as required.

Lead(II) standard solutions :4.83×10^{-3} M A 100-ml amount of stock solution (1 mg ml^{-1}) of divalent lead was prepared by dissolving 159.9 mg of lead nitratein doubly distilled de-ionized water. One ml of dilute nitric acid was added to the stock solution to prevent hydrolysis.

More dilute standard solutions were prepared by appropriate dilutions of aliquots from the stock solution with de-ionized water as and when required. Concentrations were checked using the dithizone method.

Potassium permanganate solution : A 1% potassium permanganatesolution was prepared by dissolving in de-ionized water. Aliquots of this solution were standardized with oxalic acid.

Tartrate solution : A 100-ml stock solution of tartrate (0.01% w/v) was prepared by dissolving 10 mg of ACS grade (99%) potassium sodium tartrate tetrahydrate in (100 ml) de-ionized water. 2.8. Aqueous ammonia solution A 100-ml solution of aqueous ammonia was prepared by diluting 10 ml concentrated NH4OH to 100 ml with de-ionized water. The solution was stored in a polypropylene bottle.

Absorption Spectra:

The maximum absorbance for the complex2,5-dimercapto-1,3,4-thiadiazole – lead (II) is obtained at 375 nm at pH 6.0 - 9.0.

Diagram:

Procedure:

A volume of 0.1–1.0 ml of a neutral aqueous solution containing 1–400 g of lead in a 10-ml calibrated flask was mixed DMTD reagent solution (preferably 1.0 ml of 4.42×10^{-3} M) followed by the addition of 0.3–2 ml (preferably 1 ml of 0.005 M) of hydrochloric acid. The mixture was diluted to the mark with de-ionized water. After 1 min, the absorbance was measured at **375 nm** against a corresponding reagent blank. The lead content in an unknown sample was determined using a concurrently prepared calibration graph.

Calibration Curve:

ACalibration curve is plotted by taking concentrations of standard & unknown samples of the complex on X-axis and their absorbance values on Y – axis at wavelength of 375nm.

Diagram:

*The concentration of unknown lead nitrate solution can be calculated from its absorbance value by the formula mentioned below.

$$\text{Conc. of Unknown Lead Nitrate solution} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Standard solution}} \times \text{Conc. of standard solution}$$

3.SPECTROPHOTOMETRIC DETERMINATION OF MANGANESE:

Manganese ion in aqueous solutions quantitatively determined by the making comples with reagents o-hydroxyhydroquinonepthalein and zephiramine at maximum wavelengthof **535nm.**

Principle:

The solutions given contain manganese ions, $Mn^{2+}(aq)$, and is an almost colorless solution. The manganese ions are easily oxidized in acidic solution to form permanganate ions, $MnO^{4-}(aq)$, an intensely purple species. The very intense color means that the analysis can be very sensitive because the light absorption will be relatively large, even with small amounts of manganese in the sample.

Potassium periodate, $KIO_4(s)$, will be used to oxidize $Mn^{2+}(aq)$ to the purple $MnO^{4-}(aq)$ ion, according to the following balanced chemical equation (all species aqueous):

$$2Mn^{2+}(\text{Colorless}) + 5KIO_4 + 3H_2O \longrightarrow 2MnO^{4-}(\text{Purple}) + 5KIO_3 + 6H^+$$

Experimental:

1.Standard Manganese(II) Solution:

A Stock solution of manganese (II) was prepared by dissolving 55mg of metallic manganese in 2ml of 20 % of hydrochloric acid and 1ml of 50% sulphuric acid by heating and diluted to 100ml with water.

2.O-hydroxyhydroquinonepthalein solution:

This solution was prepared in $1X10^{-3}$ M methanol solution.

3. Zephiramine solution:

A 5.0 X 10^{-2} M Zephiramine solution is prepared by dissolving zephiramine in water.

4. Buffer solution:

A0.05M Sodium tetraborate – 0.1 N hydrochloric acid buffer solution is prepared.

Absorption Spectra:

The maximum absorbance for the complexo-hydroxyhydroquinonepthalein – Manganese (II) in Zephiramine solution is obtained at 535 nm at pH 9.0.

Diagram :

Materials Required:

- 1 100.0-mL volumetric flask
- 3 25.00-mL volumetric flasks
- 2 cuvettes for the spectrophotometer
- 1 1.00 mL volumetric pipette
- 1 5.00 mL volumetric pipette
- 1 10.00 mL volumetric pipette
- 1 rubber pipette bulb
- 1 beaker tongs

Procedure:

1. Pipette 5.00 mL of Mn^{2+} (*aq*) Stock Solution into a clean 400 mL beaker. Record the exact concentration.

2. Add 30 mL deionized water + 10 mL 9M H_3PO_4 + KIO_4 (*s*) .Add the acid IN THE FUME HOOD *before* you add the KIO_4(*s*).
3. Use a glass stirring rod to mix. Place IN THE FUME HOOD and Boil **gently** for 1-2 minutes after a the solution turns purple. After heating, Remove from hood and allow to cool. If the solution does not turn purple add some additional KIO_4 (*s*).

4. Transfer the cool solution and rinse to a 100.0-mL volumetric flask using a funnel. Add deionized water to the mark and mix so the solution volume is 100.0 mL.
5. Calculate the molarity (M) of MnO^{-4} (*aq*) in this new solution. This will be your "standard" solution.Finallycalculate the molarity of each diluted solution, measure absorbances, and make a calibration curve.

Calibration Curve:

A Calibration curve is plotted by taking concentrations of standard & unknown samples of the complex on X-axis and their absorbance values on Y – axis at wavelength of 535nm.

Diagram:

*The concentration of unknown lead nitrate solution can be calculated from its absorbance value by the formula mentioned below.

$$\text{Conc. of Unknown Lead Nitrate solution} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Standard solution}} \times \text{Conc. of standard solution}$$