EXPERIMENT 1 : The Use of the Spectrophotometer and Beer's Law 2022-2023

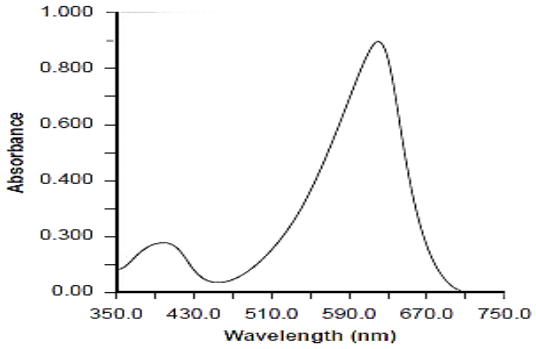
Principle

The use of a spectrophotometer to determine the extent of absorption of various wavelengths of visible light by a given solution is commonly known as colorimetry. This method is used to determine concentrations of various chemicals which can give colors either directly or after addition of some other chemicals.

Some compounds absorb light in other than the visible range of the spectrum. For example, nitrates absorb radiation of 220 nm wave length in the UV region.

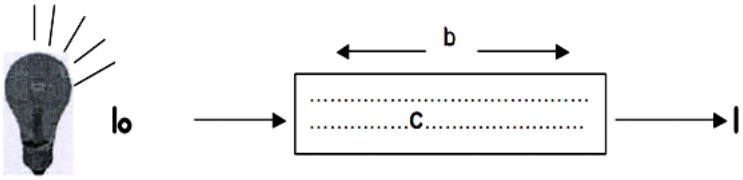
Absorption Spectroscopic methods of analysis are based upon the fact that compounds **ABSORB** light radiation of a specific wavelength. The amount of light radiation absorbed by a sample is measured. The light absorption is directly related to the concentration of the colored compound in the sample.

The wavelength (λ) of maximum Absorption is known for different compounds is shown schematically in Figure below.



The Beer-Lambert Law

The Beer-Lambert Law is illustrated in Figure below. The Absorbance (or optical density) and Transmittance of light through a sample can be calculated by measuring light intensity entering and exiting the sample,



- Light Intensity entering a sample is "**Io**"
- Light Intensity exiting a sample is "**I**"
- The concentration of analyte in sample is "**C**"
- The length of the light path in glass sample cuvette is "**b**"
- "**ε**" is a constant for a particular solution and wave length

The Beer-Lambert Law is given by the following equations:

Light Absorbance $A = \log (I_0 / I)$

$$A = \varepsilon b C$$

Light Transmittance $T = I / I_0$

$$A = -\log T$$

$$A = 2 - \log T\%$$

All spectrophotometer instruments designed to measure the absorption of radiant energy have the basic components as follows:

1. Stable source of radiant energy (Light);
2. Wavelength selector to isolate a desired wavelength from the source (filter or monochromator);
3. Transparent container (cuvette) for the sample and the blank;
4. Radiation detector (phototube) to convert the radiant energy received to a measurable signal; and a readout device that displays the signal from the detector.

The **spectrophotometers** which are used for such measurements may vary from simple and relatively inexpensive **colorimeters** to highly sophisticated and expensive instruments that automatically scan the ability of a solution to absorb radiation over a wide range of wavelengths and record the results of these measurements.

One instrument cannot be used to measure absorbance at all wavelengths because a given energy source and energy detector is suitable for use over only a limited range of wavelengths.

True linearity between absorbance and concentration according to Beer-Lambert Law requires the use of monochromatic light. In addition, a narrow band of light ensures a greater selectivity since substance with absorption peaks in other close by wavelengths are less likely to interfere. Further, it increases sensitivity as there is a greatest change in absorbance per increment of change in concentration.

Both filters and monochromators are used to restrict the radiation wavelength. Photometers make use of filters, which function by absorbing large portions of the spectrum while transmitting relatively limited wavelength regions. Spectrophotometers are instruments equipped with monochromators that permit the continuous variation and selection of wavelength. The effective bandwidth of a monochromator that is satisfactory for most applications is about from 1 to 5 nm.

The sample containers, cells or cuvettes, must be fabricated from material that is transparent to radiation in the spectral region of interest. The commonly used materials for different wave length regions are:

Quartz or fused silica:	uv - visible
Silicate glass:	Above 350 nm
Plastic:	visible region

Cuvettes or cells are provided in pairs that have been carefully matched to make possible the transmission through the solvent and the sample. Accurate spectrophotometric analysis requires the use of good quality, matched cells. These should be regularly checked against one another to detect differences that can arise from scratches, etching and wear. The most common cell path for UV-visible region is 1.0 cm. Care must be taken to duplicate the position of such cells with respect to the light path; otherwise, variations in path length and in reflection losses will introduce errors.

General Measurement

As explained above, the Beer-Lambert Law forms the basis of the measurement procedure. The amount of **light absorbed** by a compound is directly related to the **concentration of the compound**.

1. Prepare samples to make colored compound.
2. Make series of standard solutions of known concentrations and treat them in the same manner as the sample for making colored compounds.
3. Set spectrophotometer to one of maximum light absorption.
4. Measure light absorbance of standards.
5. Plot **standard curve** : Absorbance vs. Concentration

Once the standard plot is made, it is simple to find the concentration of an unknown sample: Measure the absorption of the unknown, and from the standard plot, read the related concentration.

In this experiment, the **absorption spectrum** of potassium permanganate will be measured between 400 and 600 nm. Based on this spectrum, λ_{max} will be determined, and a set of standard solutions will be analyzed to obtain a Beer's Law plot (calibration curve). The concentration of permanganate in an unknown will then be determined using this plot.

Procedure

Part A: Obtaining an Absorbance Spectrum to Determine λmax

1. Make sure the cuvettes are clean and dry.
2. Fill one cuvette with water, leaving a 3 mm gap at the top. This will be the "blank". It must be used to zero the spectrometer each time the wavelength is changed.
3. Fill the remaining cuvettes with each of the standard and unknown $KMnO_4$ solutions provided. Make sure the transparent sides are wiped clean of any fingerprints. If there are air bubbles in the cuvette, use a pipette to agitate them from the solution.
4. Using the cuvette filled with **$3x10^{-4}$ M KMnO4** solution, measure the absorbance between 400 and 600 nm in increments of 25 nm. Record **%T** then converts to absorbance). Be certain to re-zero the spectrometer at each wavelength using the blank solution.
5. Record these values in Table 1 of the data sheet.
6. Once the region from 400 to 600 nm has been measured, identify the wavelength with the highest absorbance.
7. In increments of 5 nm, choose four wavelengths below and four wavelengths above your highest absorbance wavelength. Record the absorbance at these new wavelengths.
8. The wavelength with the highest absorbance is **λ_{max}** and should be used in Part B for the Beer's law plot.

Part B: Preparation of a Beer's Law Plot

1. Set the spectrometer to the selected λ_{max}, and re-zero using the blank. Remember the point where absorbance and concentration both have a value of 0.00 is a data point.
2. Now obtain samples of the **$0.6x10^{-4}$ M, $1.2x10^{-4}$ M, $2.4x10^{-4}$ M and $3x10^{-4}$ M $KMnO_4$ solutions**. At the selected **λ_{max}**, measure the absorbance for each solution and record these on your data sheets. You do not need to re-zero the spectrometer between readings.

Part C: Determining the Concentration of Unknown Solutions

1. Keeping the spectrometer set at λ_{max}, measure and record the absorbance values for your unknown solutions. You do not need to re-zero the spectrometer between readings.
2. When you have completed the linear plot of Absorbance vs. Concentration, then you can determine the concentration of your unknown solutions from your graph. To determine the concentration of an unknown, use the absorbance of the unknown solution. Find that value on the absorbance scale, and then move across the graph parallel to the concentration axis until you meet the straight line. At that point move directly down to the concentration axis and read the concentration of the unknown.
3. Repeat this for each unknown solution analyzed.

Graphs and Calculations

- Construct a graph of absorbance vs. wavelength (used to determine λmax) using all the values Listed in Tables 1 and 2. See Figure 1 below for an example. Make sure to label your axes and provide a descriptive title.
- Construct a Beer's Law Plot from the data in Table 3. See Figure 2 below for an example. Make sure to label your axes and provide a descriptive title.
- Calculate the concentration of your unknown samples by using your Beer's Law Plot. If you used the equation of the best fit line, show a sample calculation. If you just estimated from your graph, show your points on the graph.

Part A: Determination of λ_{max}

Table 1:

Wavelength (nm)	%T	A
400		
425		
450		
475		
500		
525		
550		
575		
600		

Table 2:

Wavelength (nm)	%T	A

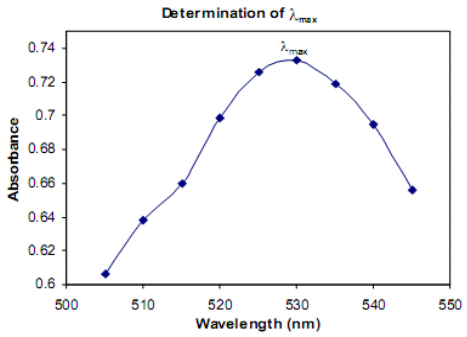


Figure 1: Sample Graph for Determination of Maximum Wavelength

Part B: Data for Beer's Law Plot

Table 3: Calibration Curve Data

Concentration (mol/L)	%T	Absorbance	ε
0.00			
0.600×10^{-4}			
1.200×10^{-4}			
2.400×10^{-4}			
3.000×10^{-4}			

Part C: Analysis of Unknowns

Table 4: Unknown Absorbance Data

Unknown #	%T	Absorbance	Concentration (mol/L)

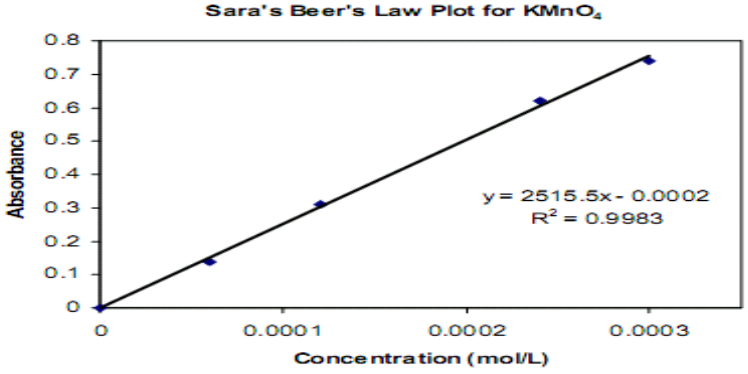
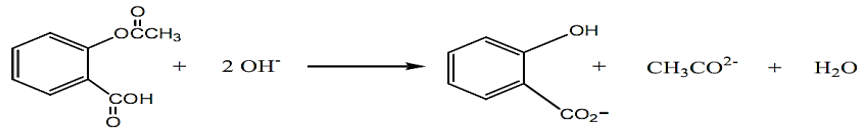


Figure 2: Sample Beer's Law Plot

EXPERIMENT 2: Visible Spectrophotometric Determination of Aspirin

The pure compound Aspirin (acetylsalicylic acid) with some binder is made into tablets, weighing slightly less than a third of a gram. When treated with basic solution the acetylsalicylic acid hydrolyzes quickly to salicylic acid and acetate ions.



acetylsalicylic acid

salicylate
$C_7H_5O_3^-$

The salicylate ions will form an intensely colored complex with the ferric ion in acidic solution.

$3C_7H_5O_3^-$ +	Fe^{3+}	→	$(C_7H_5O_3)_3Fe$
(colorless)	(faint yellow)		(deep red wine)

The wavelength of maximum absorption for this complex is **530 nm**. Thus visible spectroscopy can be used to measure the amount of aspirin present in the tablet.

1. Turn on the spectrometer.

2. Preparation of unknown. Weigh an aspirin tablet on an analytical balance. Place it in a clean 50 mL beaker and add 10 mL 0f 1.0 M NaOH. Carefully crush the tablet with a stirring rod. Dissolve as much of the tablet as possible. Heat the mixture to boiling. Boil for five minutes and avoid splattering, washing down the sides of the flask with distilled water as needed. Cool and quantitatively transfer this solution to a 500-mL volumetric flask, and dilute to the mark with D. water.

3. Prepare the following solutions in volumetric flask, diluting to the mark with 0.02 M NaOH.

A- Stock salicylate solution. Weigh [on an analytical balance] 0.4 g of salicylic acid and place it into a 125-mL Erlenmeyer flask. Add 10 mL of 1.0 M NaOH and heat the mixture until it dissolve. Cool and quantitatively transfer this solution to a 500-mL volumetric flask, and dilute to the mark with water.

B- 1.00 L of 0.025 M Fe^{3+} (reagent):.

Add 6.8 g $FeCl_3.6H_2O$ to 100 mL deionized water in a 250-mL beaker. Add 3.0 mL of concentrated HCl and 12.0 g of KCl. Dissolve and dilute to 1.0 L with deionized water.

C- Unknown.

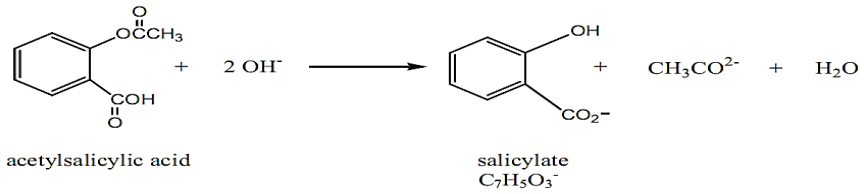
For a 25.00 mL volumetric

Solution	A	B	C
blk	0	4.00	0
Std 1	1.00	4.00	0
Std 2	2.00	4.00	0
Std 3	3.00	4.00	0
Std 4	4.00	4.00	0
Std 5	5.00	4.00	0
Unk	0	4.00	2.00

4. Set the spectrometer at 530 nm, the wavelength of maximum absorption for the iron salicylate complex. Zero the spectrometer with the blank. Measure the Absorbance of each of the standards and the unknown.

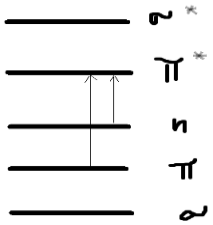
EXPERIMENT 3 : UV- Spectrophotometric Determination of Aspirin

Aspirin treated with basic solution the acetylsalicylic acid hydrolyzes quickly to salicylic acid and acetate ions.



acetylsalicylic acid

salicylate
$C_7H_5O_3^-$



The salicylate ions will **absorb UV light**. The wavelength of light which is most strongly absorbed is found by measuring the absorbance at various wavelengths between 200 - 400 nm. After the most suitable wavelength is determined, a series of salicylate standards is measured at this wavelength and a calibration plot of absorbance vs. concentration is prepared. The absorbance of the unknown sample is measured and the calibration curve is used to calculate the concentration of aspirin present in the tablet.

Reagents:

1. 1.0 M NaOH

2. Preparation of unknown. Weigh an aspirin tablet on an analytical balance. Place it in a clean 100 mL beaker and add 10 mL of 5.0 M NaOH. Heat the mixture to boiling. Boil for five minutes and avoid splattering, washing down the sides of the flask with distilled water as needed. Cool and quantitatively transfer this solution to a 500-mL volumetric flask, and dilute to the mark with water.

3. Stock salicylate solution. Weigh [on an analytical balance] 0.4 g of salicylic acid and place it into a 125-mL Erlenmeyer flask. Add 10 mL of 1.0 M NaOH and heat the mixture until it dissolve. Cool and quantitatively transfer this solution to a 500-mL volumetric flask, and dilute to the mark with water.

Procedure:

1. Turn on the spectrophotometer and allow it to warm up for at least 20 min.

2. Determine the absorption spectrum, using a standard salicylate solution.

3. Prepare series standard solutions of salicylate from the stock solution as show below: (dilute to the mark with 0.02 M NaOH) **$(0.2, 0.4, 0.6, 0.8, 1.0, 1.2) \times 10^{-4}$ M**

4. Measure the absorbance of each standard solution at (λ_{max} = 297nm) using D.W. as the blank solution.

5. Dilute the unknown solution 1 to 50 with 0.1 M NaOH and measure the absorbance of the solution at the same wavelength as your standards.

Calculations

1. Prepare a Beer's law plot.

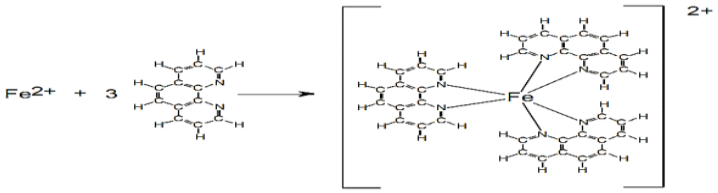
2. From the Least Squares regression, determine the concentration of aspirin in the final diluted unknown.

3- Calculate the weight of acetylsalicylic acid (ASA) in the aspirin

4. Calculate the percentage (w/w) of acetyl salicylic acid in aspirin tablet.

EXPERIMENT 4: SEQUENTIAL DETERMINATION OF IRON (II), IRON (III), AND TOTAL IRON IN WATER SAMPLE

1,10-phenanthroline ($C_{12}H_8N_2$, ortho-phenanthroline or O-Phen) is a compound that reacts with iron (II), to form a complex that absorbs in the visible portion of the electromagnetic spectrum. The wavelength of maximum absorbance intensity, λmax = 520 nm. The complex is very stable and the color intensity does not change appreciably long periods of time.



To determine the total iron in the sample, it must be completely in the ferrous state, and Fe^{2+} can readily be air-oxidized to the ferric state, Fe^{3+}. O-Phen will form a colored complex with Fe^{3+}, but its spectrum is different from that of the ferrous complex and the color is not as intense. Thus, one could not determine the total iron present by making measurements at only one wavelength. Hence, a mild reducing agent is added before the color is developed in order to provide a measure of the total Fe present in solution. Hydroxylamine, as its hydrochloride salt, can be used. The reaction is:

$$2\,Fe^{3+} + 2\,NH_2OH{\cdot}HCl + 2\,OH^- \leftrightarrows 2\,Fe^{2+} + N_2 + 4\,H_2O + H^+ + Cl^-$$

The pH is adjusted to a value between 6 and 9 by addition of an ammonia or sodium acetate buffer.

To determine the Iron (III) in the sample, it is calculated by **subtracting t**he mg/L Iron (II) from the mg/L total iron.

Solutions and Reagents

(1) Stock Iron Standard Solution, 100 ppm.

- Accurately weigh 0.7 g of pure dry ferrous ammonium sulfate onto a folded sheet of paper boat.
- Transfer the ferrous ammonium sulfate quantitatively into a 1-L volumetric flask, carefully squirting down the weighing boat and the neck of the flask to ensure a quantitative transfer. Add about 100-200 mL of distilled water. Dissolve the solid completely before diluting to volume. This is critical.
- Pipet 2.5 mL of concentrated **sulfuric acid** into the flask, rinse the neck of the flask down, and mix carefully with swirling. [Be very careful when using concentrated H_2SO_4; it is quite caustic.] Dilute the solution to the mark. Calculate the iron concentration of the solution in µg of iron per mL (ppm) and in molar (M) units.

(2) Prepare 25 mL of working standard solution **(10 ppm of Fe^{2+})** from the stock solution of Fe (II)

(3) 1,10-Phenanthroline Solution (0.1%): Dissolve approximately 100 mg 1,10-Phenanthroline in approximately 100 mL D.W water.

(4) Hydroxylammine hydrochloride Solution (1.0%) approximatlly: (please fresh prepare this solution) Dissolve approximately 0.1 g hydroxylamine hydrochloride in approximately 10 mL D.W water.

(5) Sodium Acetate Solution (10%): Dissolve approximately 10 g sodium acetate in approximately 100 mL D.W water.

(6) Unknown Solution

Procedure:

Determination of Fe (II)

(1) Pipet 1.25, 2.50, 3.75, 5.00, and 6.25 mL of the working standard iron solution into separate 25 mL volumetric flasks. Add **0.5 mL of the sodium acetate solution** to each flask, and **add 1.0 mL of the 1,10-phenanthroline solution**, Swirl the flasks and allow the color to form for 10 minutes

(2) For preparing the blank : in another 25-mL volumetric flask add approximately 10 mL D.W. Use the same above procedure .

(3) Measure 5.0 mL of the sample solution into a 25 mL volumetric flask then use the same above procedure

(5) Dilute each flask to exactly 25 mL with D.W. Mix thoroughly!

(6) Place the 'blank' solution in the colorimeter and adjust absorbance to zero reading at 520nm.

(7) Measure the absorbance of each standard and the unknown

Determination of total Iron:

(1) Measure **5.0 mL of the sample** solution into a 25 mL volumetric flask. Add **0.5 mL of the hydroxylamine hydrochloride solution**(mix for 2min.), Then add 0.5 mL of the sodium acetate solution to each flask, and add 1.0 mL of the 1,10-phenanthroline solution, (including the flasks containing the blank and unknown). Swirl the flasks and allow the color to form for 10 minutes.

(5) Dilute each flask to exactly 25 mL with D.W. Mix thoroughly

(6) Place the 'blank' solution in the colorimeter and adjust absorbance to zero reading at 520nm.

(7) Measure the absorbance of each standard and the unknown.

Calculations:

A. Determination of Iron (II):

1. Show the absorbance vs. concentration graph.

2. From the Least Squares regression, determine the concentration of Fe (II) in the final diluted unknown

3. What is the concentration of Fe (II) in the sample?

B. Determination of total Fe:

1. Show the absorbance vs. concentration graph.

2. From the Least Squares regression, determine the concentration of total Fe in the final diluted unknown solution.

3. What is the concentration of total Fe in the sample?

C- Determination of Fe (III):

Fe (III) is calculated by subtracting the mg/L Fe (II) from the mg/L total Fe.

EXPERIMENT 5 : Determination of Cr (III) by UV-Vis Spectrophotometry using standard addition method.

In this experiment, Cr^{3+} in the original solution is oxidized to $Cr_2O_7^{2-}$ and the concentration is determined by the method of standard addition.

$$2Cr^{3+}_{(aq)} + S_2O_8^{2-} + 7H_2O \longrightarrow Cr_2O7^{2-}_{(aq)} + 2SO_4^{2-}_{(aq)} + 14\ H^{+}_{(aq)}$$

Chemicals and Materials:

6 M H_2SO_4 , 0.1 M $AgNO_3$, Potassium persulfate, $K_2S_2O_8$, Potassium dichromate, $K_2Cr_2O_7$, UV-Vis solution Quartz cells

standard addition

Spectrophotometric measurements are based on the absorption of radiation by a species in solution. Beer's law states

$$A = \varepsilon\ b\ c$$

where A is the absorbance, b is the solution cell length in cm, c is the molar concentration of the absorbing species, and ε is the molar absorptivity of the absorbing species. It is clear that if the concentration of an analyte is to be determined from an absorbance measurement, ε must be known. A common procedure is to prepare a series of standard solutions of the analyte measure their absorbencies, and determine ε by linear regression. Instead, we will use the standard addition procedure. In this method, measured aliquots of a standard solution of the analyte are added to a series of solutions each containing the same volume of the sample solution. The absorbance of each solution is then measured. The total concentration of analyte, c_T, in each solution is equal to the concentration of analyte from the unknown, c_o, plus the concentration of added standard, c_a

$$A = \varepsilon\ b\ c_T = (\varepsilon\ b)\ c_a + (\varepsilon\ b\ c_o)$$

A plot of absorbance vs. C_a gives a straight line with slope (ε b) and intercept ($\varepsilon b C_o$). By dividing these quantities, c_o can be determined and used to calculate the concentration of analyte in the original sample solution .

Chromium is a toxic pollutant that is sometimes present in industrial wastewater from metal plating baths or from industrial cooling towers where chromate is used to inhibit metal corrosion.

In this experiment, the sample to be analyzed contains chromium (III), but we will add the oxidizing agent potassium persulfate to convert the chromium (III) ions to dichromate ions. The reaction equation is:

A small amount of Ag^+ is also added to catalyze the reaction, and sulfuric acid is added to prevent conversion of the dichromate to chromate $CrO4^{2-}$. There are several reasons for the conversion to dichromate. First, chromium can exist in several oxidation states such as Cr^{2+}, Cr^{3+}, and $CrO4^{2-}$. The oxidation step ensures that all chromium species are converted to the highest oxidation state $Cr_2O_7^{2-}$. Also, the preparation of a chromium (III) standard solution is difficult due to the fact that chromium (III) salts are hydrates and are hygroscopic making them hard to weigh accurately. Potassium dichromate, $K_2Cr_2O_7$, on the other hand, is not a hydrate and is available in a pure form excellent for preparing standard solutions. Another practical advantage of the oxidation step is that the molar absorptivity for dichromate is many times greater than for chromium (III). So for the same absorbance, a much lower concentration of dicromate is detected.

Procedure:

Standard Solution

Carefully weigh out about 0.2 g of dry $K_2Cr_2O_7$ on an analytical balance and record the exact mass.
Transfer the solid to a 500 mL volumetric flask and dilute to the mark with 0.05 M H_2SO_4.

Sample Solution:

Carefully pipet 1mL of the unknown Cr^{3+} solution into a 150 mL beaker. Add 1 mL of 6 M H_2SO_4, 4 drops of 0.1 M $AgNO_3$, and enough water to bring the total volume to about 50 mL. Weigh out 0.1 g of $K_2S_2O_8$ and add to the beaker. Bring the solution to a boil on a hot plate and boil gently for 5 minutes. Be careful not to lose a single drop of the solution. Cool the solution for a few minutes and then transfer quantitatively to a 100 mL volumetric flask. Fill the volumetric flask to the mark and invert several times to mix.

Standard Addition method

To a series of four 25 mL volumetric flasks, pipet in the following volumes of sample and standard solutions :

Flask	Sample sol. (mL)	Standard sol. (mL)
1	5.00	0
2	5.00	5.00
3	5.00	10.00
4	5.00	15.00

Dilute each flask to the mark with **0.05 M H_2SO_4**.

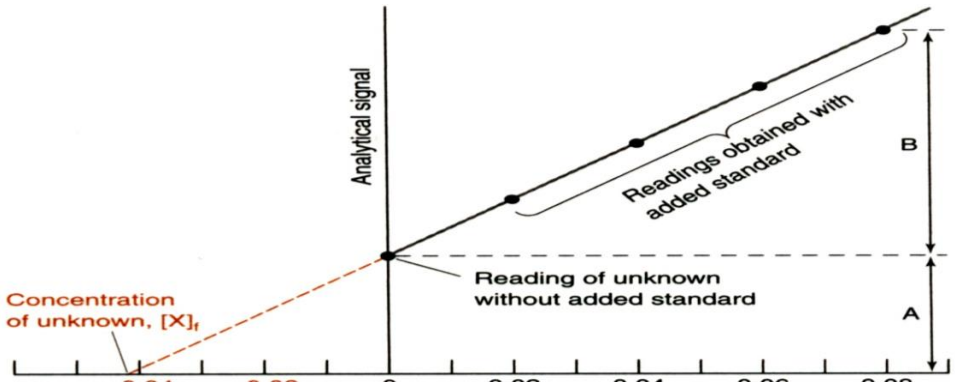
Measurements

1- Adjust the spectrophotometer to zero Abs. with 0.05 M H_2SO_3 (blank).

2- Measure the absorbance of each of the four standard addition solutions at **450 nm (λ_{max} of $K_2Cr_2O_7$)**.

Data Analysis

Plot absorbance vs. concentration of added (C_a) dichromate and determine the concentration of dichromate from the unknown solution (C_o). .



$$\mathbf{Abs. = \varepsilon\ b\ C_T}$$
$$= \mathbf{(\varepsilon\ b)\ C_a} + \mathbf{(\varepsilon\ b\ C_o)}$$
$$= \mathbf{(}\text{slope}\mathbf{)\ C_a} + \text{intercept}$$

C_o can be determined by dividing these quantities, and used to calculate the concentration of analyte in the original sample

$$\left(\frac{\text{intercept}}{\text{slope}}\right)\ \frac{\varepsilon b c_o}{\varepsilon\ b} = \mathbf{C_o}$$