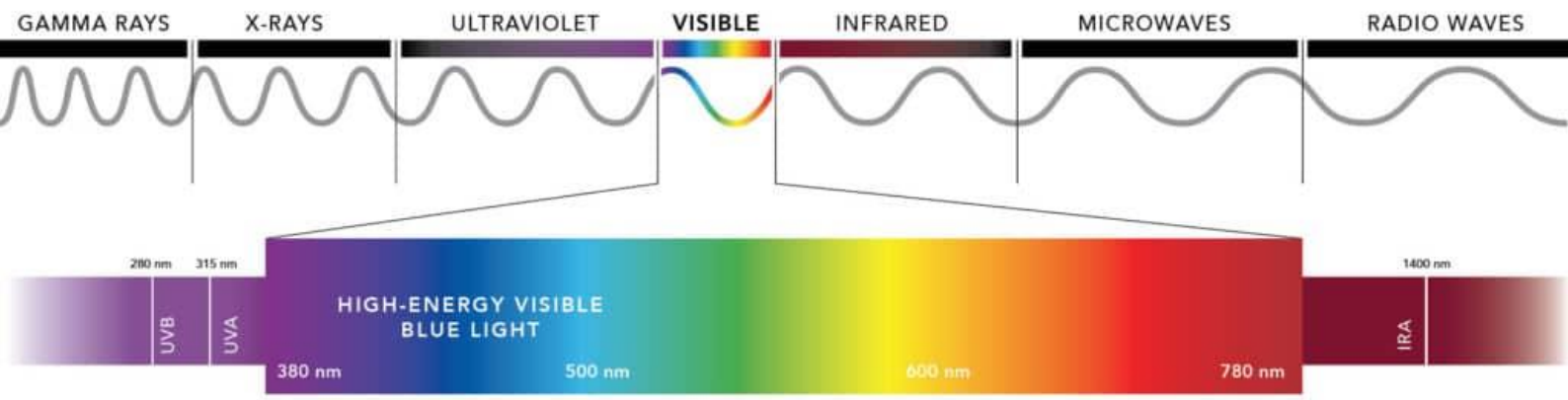
جامعة الملك سعود
King Saud University
كلية العلوم
قسم الكيمياء

Spectroscopic Analytical Methods

256 CHEM (Practical)



- The practical contents of this course have been selected carefully by some faculty members specialized in the field, and this file was written by: Dr. Wedad Alonazi

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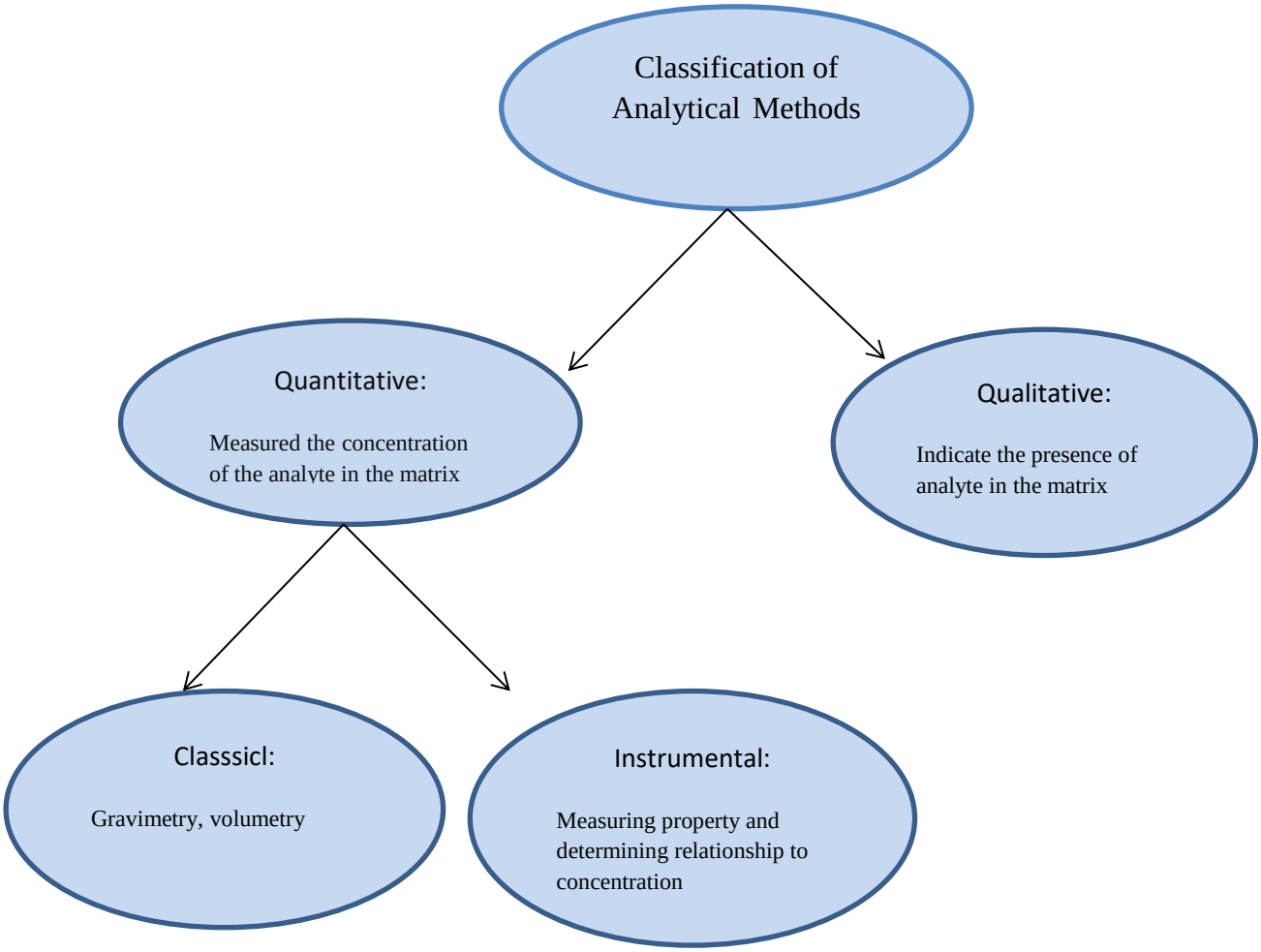
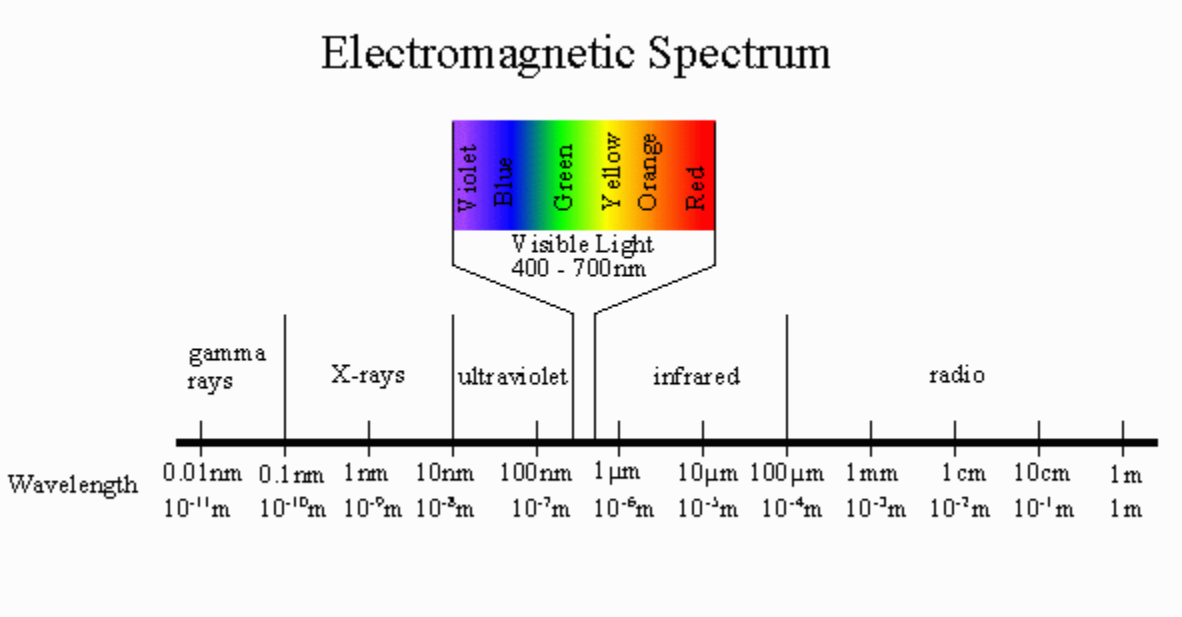
Classification of Analytical Methods



Types of some Instrumental Methods:

Property	Example Method
Radiation emission	Emission spectroscopy - fluorescence, phosphorescence, luminescence
Radiation absorption	Absorption spectroscopy - spectrophotometry, photometry, nuclear magnetic resonance, electron spin resonance
Radiation scattering	Turbidity, Raman
Radiation refraction	Refractometry, interferometry
Radiation diffraction	X-ray, electron

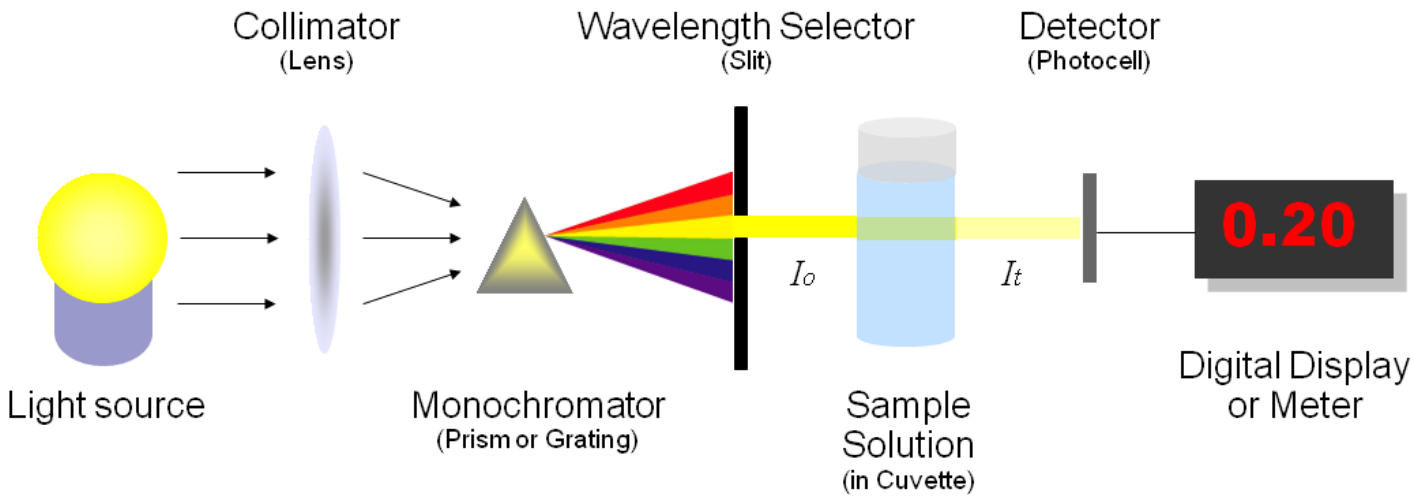
Spectroscopy is the study of the interaction between matter and radiated energy.



Ultraviolet Spectroscopy

UV/Vis spectroscopy is routinely used in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions, highly conjugated organic compounds, and biological macromolecules. the UV spectrum ranges from 100 to 400 nm.

A UV-Vis spectrophotometer measures the amount of light absorbed at each wavelength of the UV and visible regions of the electromagnetic spectrum.



In a standard UV-Vis spectrophotometer, a beam of light is split; one half of the beam (the sample beam) is directed through a transparent cell containing a solution of the compound being analyzed, and one half (the reference beam) is directed through an identical cell that does not contain the compound but contains the solvent. Intensities of the two beams as it scans over the desired region of the wavelengths.

If the compound absorbs light at a particular wavelength, the intensity of the sample beam *(IS)*will be less than that of the reference beam *(IR)* .The intensity of the absorption band is measured by the percent of the incident light that passes through the sample:

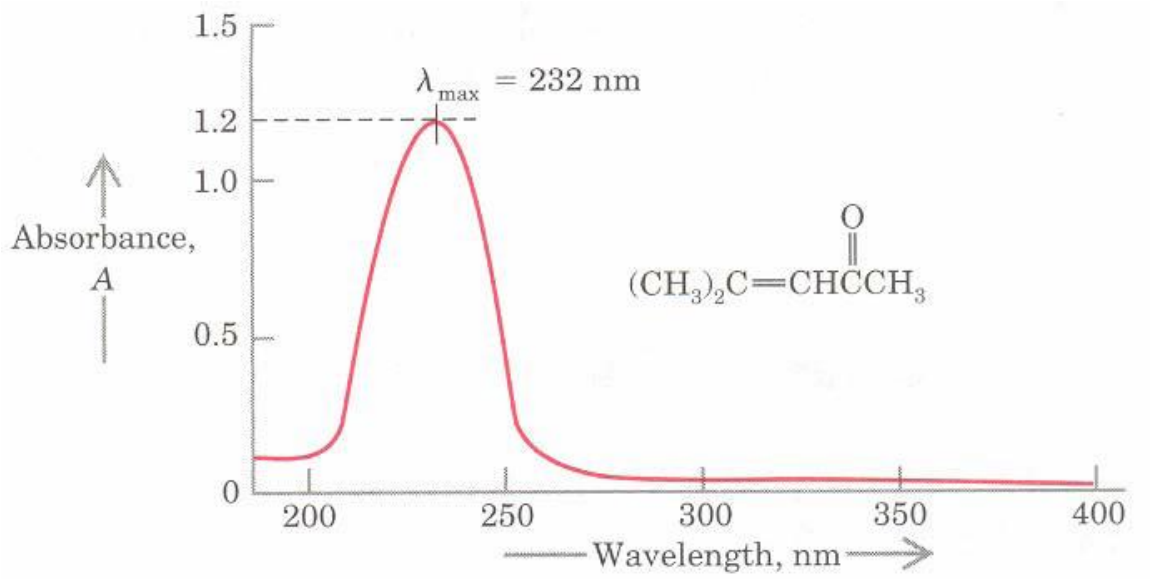
$$\% \text{ Transmittance} = (I / I_0) * 100\%$$

where:

I = intensity of transmitted light

I_0 = intensity of incident light

Absorption of radiation by a sample is measured at various wavelengths and plotted by a recorder to give the spectrum which is a plot of the wavelength of the entire region versus the absorption (A) of light at each wavelength.



Beer-Lambert Law

The wavelength of absorption is usually reported as λ max which represents the wavelength at the highest point of the curve. The absorption of energy is reported as absorbance. The absorbance at a particular wavelength is defined by the equation:

$$A = \log \frac{I_0}{I}$$

where A = absorbance

I_0 = intensity of the reference beam

I = intensity of the sample beam

The absorbance by a compound at a particular wavelength increases with an increasing number of molecules undergoing transitions. Therefore, the absorbance depends on the electronic structure of the compound and also upon the concentration of the sample and the length of the sample cell.

$A = \epsilon\, cl$ where ϵ = molar absorptivity

A = absorbance

c = concentration, in M

l = cell length, in cm

The relationship ***A*** **=** Cl indicates that the amount of absorption by a sample at a certain wavelength is depe ndent on its concentration. ***A*** **=** ε***Cl***

The calibration curve:

a **calibration curve** is a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration. The calibration curve is a plot of how the instrumental response, the so-called **analytical signal**, changes with the concentration of the analyte (the substance to be measured).

SPECTROPHOTOMETRIC DETERMINATION OF IRON

I N T R O D U C T I O N:

In this procedure, iron is, firstly, reduced to Fe^{2+} with, Hydroxylamine and then complexed with o-phenanthroline to form an intensely colored complex.

$2\ Fe^{3+} + 2\ NH_2OH + 2\ OH^- \rightarrow 2\ Fe^{2+} + N_2 + 4\ H_2O$

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

2+

The intensity of the color is independent of pH in the range 2 to 9. The complex is very stable and the color intensity does not change appreciably over long periods of time. Beer's law is obeyed.

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

R E A G E N T S:

Hydroxylamine: Freshly prepared solution containing 10 g/L in water.

Soduim acetat: 10 g/L in water.

o-Phenanthroline

Standard Fe: Prepare by dissolving a Fe^{+3} salt of reagent grade in water so that the concentration calculated is for 10ppm.

Part 1: Obtain absorption spectrum of complex and determine λmax

P R O C E D U R E:

- 1-Prepare 1.5 ppm of standard Fe solution(10 ppm) into 25 mL volumetric flask,
  2- Add 0.25ml of Hydroxylamine ,And 1.25 ml of 1,10 –Phenanthroline, Then waite till the complex produced.
- 3- Add 2ml of Soduim acetate As a puffer, to adjust the pH.

- 4- Dilute the solution to the 25mL marks with D water and allow them to stand for 10 minutes.
- 5- Using the blank as a reference and one of the iron solutions prepared above , measure the absorbance at different wavelengths in the interval from 450 to 550 nm. Take reading about 10 nm apart.
- 6-. Plot the absorbance vs. wavelength and connect the points to from a smooth curve. Select the proper wavelength to use for the determination of iron with 1,10-phenanthroline.

Part II- Construct Beer's Law Plot (A vs. C) and calculate Fe concentration in sample .

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- Prepare the following iron calibration solutions by pipetting the indicated amounts of the Iron standard solution and from the sample into labeled 25 mL volumetric flasks. The first flask is a blank containing no iron.

Concentration of Fe	Volume to pipet
0.00 ppm	-
0. 5 ppm	-
1.00 ppm	-
1.5 ppm	-
2.00 ppm	-
-	3ml(from the sample)

- . Using a graduated cylinder, add 0.25 mL of hydroxylamine And 1.25 mL of 0.3% o-phenanthroline solution to each volumetric flask.
- Swirl and allow the mixture to stand for 10 minutes.
- Add 2ml of Soduim Acetate
- 7. Dilute each flask to the mark with distilled water and mix well by inverting and shaking the capped volumetric flasks several times.
- . Measure the absorbance of all four standard solutions and the unknown solutions versus the blank solution(5ml water then add all the reagents axcept the iron) at the selected wavelength.
- Calculate the Concentration of Iron in the Sample.

Determination Of Manganese In Steel

Introduction

Manganese is a minor constituent found in many steels.

You will first dissolve the sample in an acidic solution and then oxidize the colorless Mn^{2+} to form the purple permanganate ion (MnO4-). Once this has been done, it is possible to measure the absorbance of this solution and use Beer´s Law (A = εbc) to determine the concentration of the permanganate ion. You will be using potassium periodate to oxidize the Mn2+.

The reaction equations are as follows:

$$\mathbf{3\ Mn^{2+} + 2\ NO^{3-} + 8\ H^{+} \rightarrow 3\ Mn^{+2} + 2\ NO + 4H_2O}$$

$$\mathbf{2\ Mn^{2+} + 5\ IO^{4-} + 3\ H_2O \rightarrow 2\ MnO_4^{-} + 5\ IO^{3-} + 6\ H+}$$

During dissulotion NO_2 and NO are produced these may interfare later in expermint by reducing Perodic acid , hence they are removed by boiling

Procedure:

1-IN Six Conical flask transfer the following volume from the standard solution and the sample solution*:*

N0.of Flask	Volume(ml)
1	0.6 (from the Standard solution500 ppm)
2	0.8
3	1
4	1.2
5	0.9 (from the Sample solution)
6	1 0ml of water(as blank)

2- Add 10 ml of water to the first five flasks

3- Add 0.5 ml of H_2SO_4 to all the Flasks

4-Add 0.25 g of potassium periodate, the heating all the flasks for short period of time

5-Transfer the solutions to the 25 volumetric flasks then diluted to the mark by distilled water

6- by the 1ml of standard solution and the blank solution measure the absorbance at different wavelengths in the interval from 510 to 550 nm. Take reading about 5nm.

7-Plot the absorbance vs. wavelength and connect the points to form a smooth curve. Select the proper wavelength .

8- Measure the absorbance of all four standard solutions and the unknown solutions versus the blank solution(5ml water then add all the reagents except the iron) at the selected wavelength.

9-Calculate the Concentration of manganese in the Sample.

SPECTROPHOTOMETRIC ANALYSIS OF A PERMANGANATE - DIACHROMATE MIXTURE

In this experiment, you will quantitatively determine the concentrations of permanganate and dichromate ions in a mixture. Because of the overlap of spectra, it is not feasible to generate a calibration curve of one ion and then the other and use a straight forward Beer`s Law approach to determine each concentration. The problem is that each ion has significant absorbance at the other`s .. max. Diachromate is normally quantified at a wavelength of 440 nm. Permanganate is measured at 545 nm. These wavelengths correspond, more or less, to wavelengths where the absorbance is not changing much over a range of wavelengths. Hence, deviations in measurement wavelength do not affect the measured absorbance.

$$A_{\lambda 1} = \epsilon_{\lambda 1}\, b\, C_{(Cr_2O_7^{-2})} + \epsilon_{\lambda 2}\, b\, C_{(MnO_4^-)}$$

$$A_{\lambda 2} = \epsilon_{\lambda 2}\, b\, C_{(Cr_2O_7^{-2})} + \epsilon_{\lambda 2}\, b\, C_{(MnO_4^-)}$$

Part I: Construction the calibration curve of $KMnO_4$ at 440 and 545 nm , and determination the slope of the curves at two wavelengths.

- Prepare a set of solutions (0.0002 , 0.0004 , 0.0006 , 0.0008 M) of (0.004) KMnO4 in 25 ml volumetric flasks.
- Add in each flasks 5ml of dil. H2SO4 , then complete to the marks by distilled water
- Measure the absorbance for these solutions at 440 and 545 nm against the blank solution .
- Form the slope , calculate the molar absorbtivities for each wavelength.

Part II.: Construction the calibration curve of $K2Cr2O_7$ at 440 and 545 nm , and determination the slope of the curves at two wavelengths.

- Prepare a set of solutions (0.0015, 0.003, 0.0045 , 0.006 M) of (0.02) $K_2Cr_2O_7$ in 25 ml volumetric flasks.
- Add in each flasks 5ml of dil. H2SO4 , then complete to the marks by distilled water
- Measure the absorbance for these solutions at 440 and 545 nm against the blank solution .
- Form the slope , calculate the molar absorbtivities for each wavelength.

Determination of Concentration of $KmnO_4$ and $K_2Cr_2O_7$ in mixture

Procedure:

1-Pippete 6.5 ml of mixture in 25 ml volumetric flask

2- Add 5ml of H_2SO_4 by sylinder then diluted the solution to the mark by distilled water

3- measure the absorbance of the mixture solution at 440 and 525 nm versus blank solution (5ml H_2SO_4 in 25ml Volumetric flask then diluted to the mark by distilled water)

4- repate the steps 1-3 and take the Average of the Absorbance value at the two wave length

5- Calculate the Concentration of $KmnO_4$ and **$K_2Cr_2O_7$**

Spectral study for Determination of the composition of iron complex

Materials:

1- standard solution of ferric ammonium sulphate 0.0005 M
2- spectrophotometricaly reagent 1,10-phenanothroline 0.0005 M
3- Sodium acetate 10g/100 ml.
4- Hydroxyl amine hydrochloride 10g/100 ml.

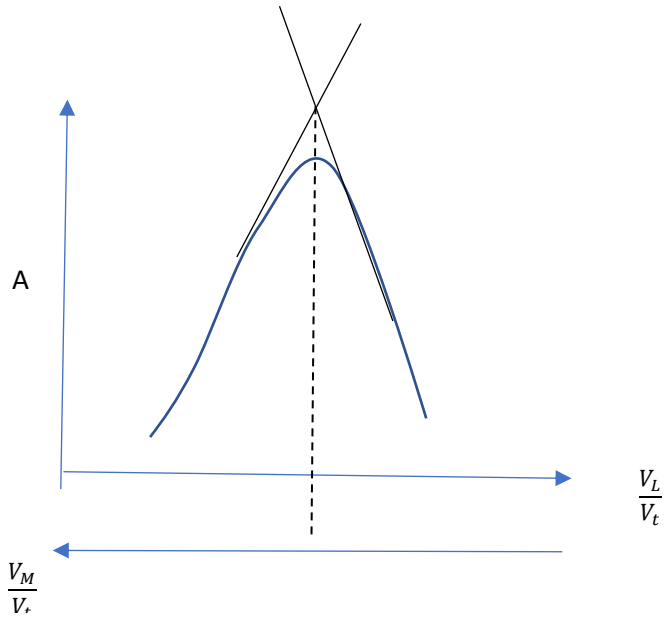
Theory:

Two different analytical methods are used spectrophotometrically to study the composition of complexes:

1- continuous variations method:
In this method the sum of the molar concentrations of the two reactants is kept constant as their ratio is varied. The abscissa of the extrapolated peak will correspond to the ratio present in the complex.

Procedure:

1- transfer 0,1,2,3,4,5,6,7,8 ml of iron solution (V_m) to 9 volumetric flasks 25 ml, then add to each 0.5 ml of hydroxyl amine hydrochloride solution.
2- Add to all flasks in order 10, 9, 8,7,6,5,4,3,2 ml of spectrophotometric reagent (V_L) (always total -10), then wait for 10 min.
3- Add to all flasks 4 ml of sodium acetate solution and complete to the mark by distilled water.
4- Measure the absorbance of all solutions at 508 nm –
5- * blank?
6- Plot the relationship between Absorbance and the ratio [L/M] as shown in figure (1), and then calculate the ratio [L/M]

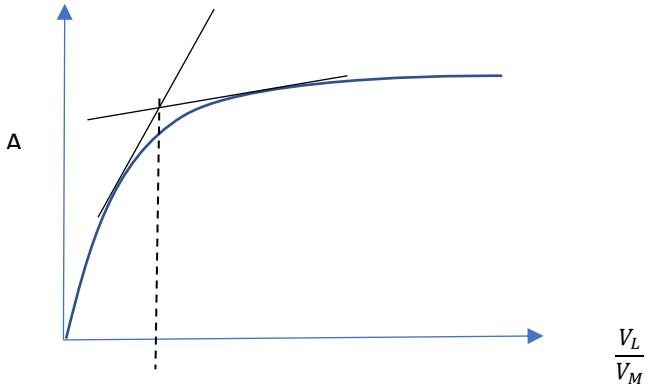


2- molar ratio method:

In this method the concentration of metal ion is held fixed and the concentration of the reagent is increased stepwise. On the graph the absorbance versus moles of reagent added, the intersection of the extrapolated linear segments determines the ratio: moles of reagent / moles of metal.

Procedure:

1- Transfer 2 ml of iron solution (V_m) to 8 volumétric flasks 25 ml, and then add to each 0.5 ml of hydroxyl amine hydrochloride solution.
2- Add to all flasks in order 2,3,4,5 6,7,8 ml of spectrophotometricaly reagent (V_L), then wait for 10 min.
3- Add to all flasks 4 ml of sodium acetate solution and complete to the mark by distilled water.
4- Blank is 0.5 ml of hydroxyl amine hydrochloride, 5 ml of spectrophotometricaly reagent and 4 ml of sodium acetate, then complete to the mark by distilled water.
5- Measure the absorbance of all solutions at 508 nm.
6- Plot the relationship between Absorbance and the percentage of reagent to metal as shown in figure (2), and then calculate the percentage of reagent to metal.



PHOTOMETRIC TITRATION OF COPPER (II) WITH EDTA

The reaction for this experiment is:

$H_2Y^{2-} + Cu^{2+} === CuY^{2-} + 2H^+$

Where $H2Y^{2-}$ is Na_2H_2Y

The titration is performed at 625 nm; both the copper-EDTA chelate and the copper (I) ion absorb at this wavelength, but the molar absorptivity of the chelate is much higher.

Figure 1a is a plot of absorbance (uncorrected for dilution) vs. milliliters of titrant. The points fall below the extrapolated lines in the end-point region, because the reaction is incomplete near the equivalence point. After the equivalence point, the added excess EDTA titrant forces the reaction to completion. The further addition of titrant leads to dilution; therefore, the absorbance will then decrease slightly.

The pH is critical for this titration, because a large change in pH changes the effective binding constant. An acetate buffer is used to maintain the pH between 2.4 and 2.8 to avoid this problem. This low pH also permits the copper to be titrated in the presence of metal ions that form weaker complexes with EDTA. Photometric titrations offer additional advantages. s.

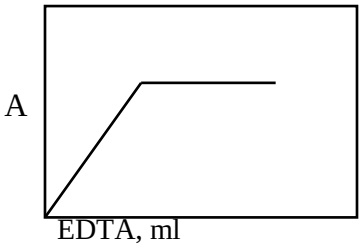


Figure 1. Photometric titration of copper (II) with EDTA titrant at pH 2.4

Procedure

1. Turn on the spectrometer to allow it to warm up. Set the wavelength to 625 nm and zero the meter with distilled water.

2. Add 10 ml of Cu^{2+} solution and 10ml of the acetate buffer solution to all 9 the beakers. At this point, the pH of the solution should be 2.4 to 2.8.

3. Add 0.2M EDTA to all 9 the beakers with these volume (0 ,2 , 3 , 4 , 4.5 , 5 , 5.5 , 6 , 7).

4. Record the absorbance of all solution at 625 nm

5. Plot the absorbance of the solution against the volume of the EDTA, determine the endpoint by extrapolating the two linear portions of the curve to an intersection point.