LABORATORY MANUAL

FOR

QUANTITATIVE CHEMISTRY

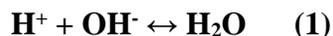# FIRST EDITION

DEPARTMENT OF CHEMISTRY

BABCOCK UNIVERSITY. ILISAN. REMO. OGUN STATE

2014

Experiment 1: ACID BASE TITRATIONS

Theory

The reaction of an acidic solution with a basic solution can be represented by the net ionic equation

$$H^+ + OH^- \leftrightarrow H_2O \qquad (1)$$

The reaction changes gradually from acidic to basic solution or vice versa and finally to a neutral solution. Therefore, the process is called **Neutralization**. This reaction proceeds to completion and is quantitative; hence, it can be used to calculate the concentration of an acid or a base. The procedure of gradually adding an acid to a base or vice versa to form a neutral solution is also called **Titration**. So, if the concentration of the acid or base is known, then the concentration of the other one can be calculated by determining the **equivalent point** or **end point** (concentration of acid= concentration of base) in the process of neutralization (titration). To determine the end point an indicator is added before titration starts. An indicator usually is a weak organic acid which changes its color at a specific pH.

At the end point, the amount of acid and the base are equal, therefore,

The number of Moles (mol) acid = the number of Moles (mol) of base   (2)

When we use **molarity (M)** to express the concentration of the acid and base**, then**

M=mol/L or M= mmol/mL   and,   (3)

# of mol= (M)(L) or # of mmol = (M)(mL)   (4)

We can then say that at equivalent or end point, equation becomes

(M $_{acid}$)(L $_{acid}$) = (M $_{base}$)(L $_{base}$) or (5a)

(M $_{acid}$)(mL $_{acid}$) = (M $_{base}$)(mL $_{base}$) (5b)

Finally,

**M $_{base}$ = (M $_{acid}$)(L $_{acid}$)/ mL $_{base}$ (6)**

Acids have replaceable hydrogen's which takes part in its chemical reaction. The number of such replaceable hydrogen's determines the **equivalent weight** of the acid. The **equivalent weight** of an acid is then defined as the weight of the acid that will react with one mole of NaOH or other monobasic substances. If an acid has one replaceable hydrogen atom, then, its equivalent weight will be the same as its molecular weight. If the acid contains two replaceable hydrogen atoms, then its equivalent weight will be half its molecular weight, and so on and so forth.

Eq. Wt = amount of acid /moles of base (7)

Experiment 1 Cont'd

When a strong acid is titrated against a strong base, a graph of changes in pH as the titrant is added can be plotted against the volume of the strong acid or base. These changes will enable us to understand what is happening during titration and we will be able to interpret an experimental titration curve.

The equation that represents the titration of strong acid with strong base is shown in equation1

$$H^+ + OH^- \leftrightarrow H_2O \quad (8)$$
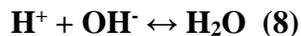
The equation can be used to calculate the composition and pH after each addition of the titrant. At the equivalent point, the equilibrium constant of this equation, $K_e = 1/K_w$ (K_w = dissociation constant of water) and one can say that the reaction has 'gone to completion'; therefore, any amount of acid or base added will consume a stoichiometric amount of base or acid respectively. It is possible to calculate the amount or acid or base needed to reach the equivalence point (V_e). For example, if 20.00 mL of 0.001000M NaOH is titrated with 0.002M HCl, the volume of HCl needed to reach the equivalence point, $(V_e)(0.01000M) = (20.00 \text{ mL})(0.001000M) = 10.00$ mL

mmol of acid at equivalence point   mmol of base at equivalence point

In the titration of any strong base or acid, there are three regions of the titration curve which represent three different types of calculations:

(i) the region before the equivalence point (pH is determined by excess base, $[OH^-]$, in the solution. For example, if 2 mL of 0.002M HCl has been added to 20 mL of 0.001000 M NaOH, then, concentration of $[OH^-]$ remaining can be calculated thus,

$[OH^-] = (10.00 - 2.00)/10.00)(0.001000)(20.00/20.00 + 2.00) = 0.0073$ M

(10.00- 2.00)/10.00)= fraction of NaOH remaining

(0.001000) = initial concentration of NaOH

(20.00/20.00 + 2.00) = dilution factor = initial volume of NaOH/total volume of solution

(ii) the region at the equivalence point where the amount of acid (H^+) is just sufficient to react with all the base in the solution to make water. The pH is determine by the dissociation of water, K_w

$$H_2O \leftrightarrow H^+ + OH^- \quad (9)$$
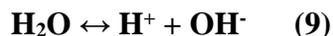
x   x

$K_w = x^2 \, 10^{-14}$ M and $x = 10^{-7}$ M. Therefore, pH = 7.00

Experiment 1 Cont'd

(iii) the region after the equivalence point where excess acid is been added. The pH is determined by the excess acid in the solution. The concentration of the excess acid, H^+ can be calculated. For example is 10.2 mL of acid has been added, the concentration of the acid $[H^+]$ can be calculated thus;

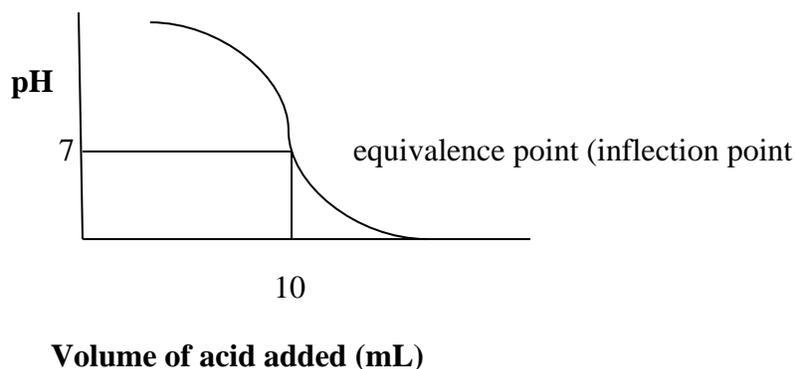$[H^+] = (0.00200)(0.2000/20.00 + 10.2) = 0.000013\ M = 1.3 \times 10^{-5}\ M$

$pH = -log[H^+] = 4.89$

(0.00200) = initial concentration of the acid, $[H^+]$

$(0.2000/20.00 + 10.2)$ dilution factor = volume of excess H^+/total volume of solution

A plot of the three regions against the volume of acid added is shown below

Volume of acid added (mL)

There is a sudden change in pH near the equivalence point and it is where the slope is the greatest $(\delta pH/\delta V_a)$. Therefore, the equivalence point is a point of inflection. The equivalence point has a pH = 7.00 which is only true for strong acid-strong base titrations. This is not true for weak acid-weak base titrations.

Apparatus: 2 Burettes, pipet, 2x 250 mL conical flask, 600 mL beaker, brush, stirring rod. Retort stand

Reagent: Sodium hydroxide (**3M** NaOH), Hydrochloric acid (0.1 M and 0.05 M HCl), methyl orange indicator, distilled water

Section A: Standardization of Strong Base (NaOH)

Procedure: In this experiment, we are going to standardize sodium hydroxide. Wash the beakers, burettes and the flask with soap and water and rinse thoroughly with distilled water. Allow the containers to dry. Use the 600 mL beaker to obtain 10 mL of 3M NaOH from the instructor and add 290 mL of distilled water. Stir the solution with the stirring rod for about 1 minute. Obtain 80 mL of 0.05 M of HCl from the instructor and use 5 mL to rinse the burettes.

Experiment 1 Cont'd

Attach the burette to the retort stand and fill it to the zero mark. Drain a little bit of the acid in the burette to remove any air bubble in the system. Stir the NaOH solution again and fill the other burette to the zero mark. Drain the NaOH burette to remove air bubbles. Record the initial volumes of the acid and the base using the lower meniscus of the liquids.

Pour 25 mL of distilled water in a 250 ml conical flask and add 3-5 drops of the methyl orange indicator. Drain 15 mL of the HCl from the burette into the flask and stir very well. Carefully and slowly drain NaOH from the burette into the acid solution while stirring at the same time. The acid solution will turn pink and disappear as you stir it. As you get nearer the end-point, the pink color will stay longer. Continue to add NaOH drop by drop at this point until the color does not fade away again. This is the end-point. If you added to much base, add 2-3 drops of acid and then neutralize with NaOH from the burette to reach a new end-point. Record the volume of the base from your burette using the lower meniscus.

Repeat the titration 2 more times.

Section B: Titration of Strong Acid with Strong Base: The Titration Curve

Procedure: Obtain 1.4 mL of the 3.00 M NaOH and dilute it to 200 mL with distilled water in a 200 mL volumetric flask. This will give a 0.21 M NaOH solution. Obtain 50.00 mL of the 0.21 00 M NaOH solution and pour it in a 250 ml conical flask and add 1-2 drops of the methyl orange indicator. Shake the mixture properly. Fill the burette to the zero mark with the 0.1M HCl solution and add to the NaOH solution to determine the equivalence point (end point) of the mixture. Clean the conical flask and obtain another 50.mL of the NaOH solution. Add 1-2 drops of the indicator and shake very well.

Region 1 of the titration curve: Add the 0.100 M HCl solution from the burette in increments of 1.00 mL and shake the mixture thoroughly. Record the total volume of the mixture after each addition and also measure and record the pH of the NaOH/HCl mixture for each addition. When getting near the end point, reduce the volume increment until the end point is reached (for example, if the volume of the HCl solution needed to reach the end point is 10.00 mL, when about 9.0 ml has been added, change the increment to 0.2 mL until the end point is reached).

Region 2 of the titration curve: After the end point is obtained, add 0.1, 0.2, 0.6, and 0.1 of 0.10 HCl from the burette. Record the total volume of solution in Table 2 after each addition and measure the pH of the acid/base mixture for each addition.

Region 3 of the titration curve: From the burette, add HCl to the acid/base mixture in increments of 1.0 mL for five more times. Record the total volume of solution and the extant pH for each addition.

Draw the titration curve for this experiment.

Clean all the containers and return them to the instructor.

Do not discard the 3M NaOH solution. It will be used in the next experiment.

Experiment 1 Cont'd

Waste: Flush all waste through the drain

Experiment 1   **RESULT**

Name   **Department**   **Date**

Table 1

	Result 1	Result 2	Result 3
Initial burette reading, HCl			
Final burette reading, HCl			
Volume used, HCl			
Molarity of HCl			
Initial burette reading, NaOH			
Final burette reading, NaOH			
Volume used, NaOH			
Molarity of NaOH			
Average Molarity of NaOH			

Question

1. What volume of 2.0 M NaOH would be needed to make a 200mL of 0.10 M NaOH?
2. What volume of 0.10M NaOH is needed to neutralize 20 mL of 0.10 M HCl?

3. What is the concentration of formic acid solution if a 20 mL sample of the acid needs 15 mL of 0.08 M NaOH?

Experiment 1 Cont'd

Experiment 1   Table 2   RESULT

Name   Department   Date

Region	ml HCl added	Concentration of unreacted OH⁻ (M)	Concentration of excess H⁺ (M)	pH
Region 1	0.00	0.2100		
	1.00			
	2.00			
	3.00			
	4.00			
	5.00			
	6.00			
	7.00			
	8.00			
	9.00			
	9.10			
	9.30			
	9.90			
Region 2	10.0	0	0	7.00
	10.5			
	10.9			
	11.0			
Region 3	12			
	13			
	14			

Experiment 2: Standardization of an Acidic Solution (Strong Base-Weak Acid Titration)

Theory

This is a continuation of experiment 1on acids and bases.

Apparatus: 2 Burette, pipet, 2x 250 mL conical flask, 600 mL beaker, brush, stirring rod. Retort stand

Reagent: Sodium hydroxide (NaOH), Hydrochloric acid (HCl), methyl orange indicator, distilled water.

Standardization of Acetic Acid (CH₃COOH)

Procedure: In this experiment, we are going to standardize an acid solution. Wash the beakers, burettes and the flask with soap and water and rinse thoroughly with distilled water. Allow the containers to dry. Set up the burettes and rinse one with 5 mL of the NaOH solution prepared from experiment 5, then, fill it up to the zero mark with NaOH. Obtain 80 mL of acetic acid from the instructor and rinse the other burette with 5 mL of acetic acid and then fill it up with the acid to the zero mark.

Pour 25 mL of distilled water in a 250 ml conical flask and add 3-5 drops of the methyl orange indicator. Drain 15 mL of the acetic acid from the burette into the flask and stir very well. Carefully and slowly drain NaOH from the burette into the acid solution while stirring at the same time. The acid solution will turn pink and disappear as you stir it. As you get nearer the end-point, the pink color will stay longer. Continue to add NaOH drop by drop at this point until the color does not fade away again. This is the end-point. If you added to much base, add 2-3 drops of acid and then neutralize with NaOH from the burette to reach a new end-point. Record the volume of the base from your burette using the lower meniscus.

Repeat the titration 2 more times.

Clean your entire container and return them to the instructor

Waste: Flush all waste through the drain

Do not retain any solution at this time.

Experiment 2   **RESULT**

Name   **Department**   **Date**

Table 1

	Result 1	Result 2	Result 3
Initial burette reading, NaOH			
Final burette reading, NaOH			
Volume used, NaOH			
Molarity of NaOH (from Experiment 1)			
Initial burette reading, Acetic Acid			
Final burette reading, Acetic Acid			
Volume used, Acetic Acid			
Molarity of Acetic Acid			
Average Molarity of Acetic Acid			

Question

1. What volume of 1.6 M NaOH would be needed to make a 500mL of 0.10 M NaOH?
2. What volume of 0.18M NaOH is needed to neutralize 25mL of 0.15 M acetic acid?

3. If 27.66 ml of 0.12M of NaOH is needed to neutralize a sample of unknown acid that weighs 0.35 g, what is the equivalent weight of the acid?

Experiment3: Complexometric Titrations: **Ethylenediamine Tetraacetate (EDTA)**

Theory

Metal ions acts as *Lewis* acids (electron acceptor) and can bind with *ligands* (*Lewis bases; electron donors*) to form metal complexes called *chelates.* The metal ions are usually (i) p- block metal ions e.g. Mg or (ii) d-block species e.g. Co, Fe and the ligands are usually p-block species such as $[BrF_4]^-$. The ligands are either neutrally charged or anionic species are can bind metals through one atom (monodentate, e.g. CN^-) or multiple atoms (multidentate, adenosine triphosphate (ATP))

A good example of a multidentate chelating compound is ethylenediamine tetraacetate (EDTA, $((COOH)_2)H_2NCH_2CH_2NH_2 (COO^-)_2)$ which binds metal ions through two ligand atoms (bidentate) and ATP which is a physiological tetradentate compound that binds with Mg^{2+} ion.

Some metal complexes are not analytically useful because they are unstable and expensive e.g. ATP, while others such as Nitrilotriacetic acid (NTA), trans-1,2 Diaminocyclohexanetetracetic acid (DCTA) are very useful in analytical titrations are often form strong 1:1 complexes with metals. Titration based on metal-complex formation is called *complexometric titrations* and the equilibrium constant for such a reaction is called *formation or stability constant (*K_f*)*.

EDTA Complexes

EDTA is the most commonly used chelator is complexometric titrations. It can be used to analyze almost all elements in the periodic table either by direct titration or by indirect series of reactions. It has six replaceable protons (hexaprotic, H_6Y^{2+}) which give six different pK_a values.

pK_{1-4} = carboxylic protons and pK_{5-6} = ammonium protons

HO₂CCH₂   **CH₂CO₂H** $pK_1 = 0.0$ $pK_2 = 1.5$ $pK_3 = 2.0$

       HN⁺CH₂CH₂N⁺H $pK_4 = 2.66$ $pK_5 = 6.16$ $pK_6 = 10.24$

HO₂CCH₂   **CH₂CO₂H**

The neutral EDTA is tetraprotic and can be represented as H_4Y. The formation constant K_f can be represented by the following equilibrium equation

$M^{n+} + Y^{4-} \leftrightarrow MY^{n-4}$ and $K_f = [MY^{n-4}]/ [M^{n+}][Y^{4-}]$ (1)

There are seven different forms of neutral EDTA forms usually present in solution. K_f is defined for the Y^{4-} specie in equation 1. The K_f for each of the seven forms are very large and often larger for more positively charged metal ions. Metal-EDTA complexes at pH < 10.24 are not stable. Most of the EDTA at this pH's is not the neutral Y^{4-} form but exist as HY^{3-}, H_2Y^{2-} etc. The fraction of each form can be calculated: $[Y^{4-}] = \alpha_{Y4}\text{-}[EDTA]$ (2)

Experiment 3 Cont'd

(α_{Y4}- is constant and [EDTA] = total concentration of all forms of EDTA);

and the formation constant of each form (*conditional formation constant or effective formation constant*) can also be estimated.

$K_f = [MY^{n-4}]/[M^{n+}][Y^{n-4}] = [MY^{n-4}]/[M^{n+}]\ \alpha_{Y4}\text{-}[EDTA]$ (3)

If a buffer is used to make pH constant, then α_{Y4}- (Appendix A, Table 1) will also be a constant and therefore,

$K'_f = \alpha_{Y4}\text{-}K_f = [MY^{n-4}]/[M^{n+}][EDTA]$ (4)

 where K'_f is the conditional formation constant

For a titration reaction to be effective it must go to completion and its stability constant must be large. Each metal-EDTA complexes has a specific pH (fixed by a buffer) at which it can form a stable complex with large stability constant useful for complexometric titration. Therefore, selective titration of metal-EDTA can be made and used to estimate the concentration of metals in a mixture. For example, a solution containing Fe^{3+} and Ca^{2+} could be selectively titrated at pH = 4. Fe^{3+} forms a complex with EDTA at this pH while Ca^{2+} does not.

EDTA Titration Curves: EDTA titration curve is similar to the titration curve of a strong acid-weak base titration curve. The acid is replaced by the metal and EDTA is the weak base. The titration curve has three regions and can be represented by the equation:

 $M^{n+} + EDTA \leftrightarrow MY^{n-4}$ (6) where $K'_f = \alpha_{Y4}\text{-}K_f$
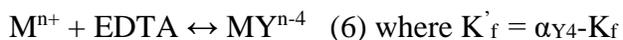
If K'_f is large, the reaction is considered to have gone to completion at each point of titration.

The titration curve natural regions are as follow:

Region 1: occurs before the equivalence point: Here, there is excess metal ion (M^{n+}) in solution after the addition of EDTA. The dissociation of the metal complex, MY^{n-4} is negligible hence; the concentration of free metal ion is equal to the concentration of the excess, unreacted M^{n+}

Region 2: this is the equivalent point: here, there is exactly the same amount of the metal ions and EDTA in solution ($[M^{n+}] = [EDTA]$). There are small amount of M^{n+} ions in solution due to slight dissociation of the MY^{n-4} ion.

 $MY^{n-4} \quad \leftrightarrow M^{n+} + EDTA$ (7)

Region 3: occurs after the equivalence point: here, there are excess EDTA in solution and all the metal ion have formed the complex MY^{n-4}. The concentration of free EDTA is equal to the concentration of excess EDTA added after the equivalence point.

Experiment 3 Cont'd

Volume of EDTA added (mL)
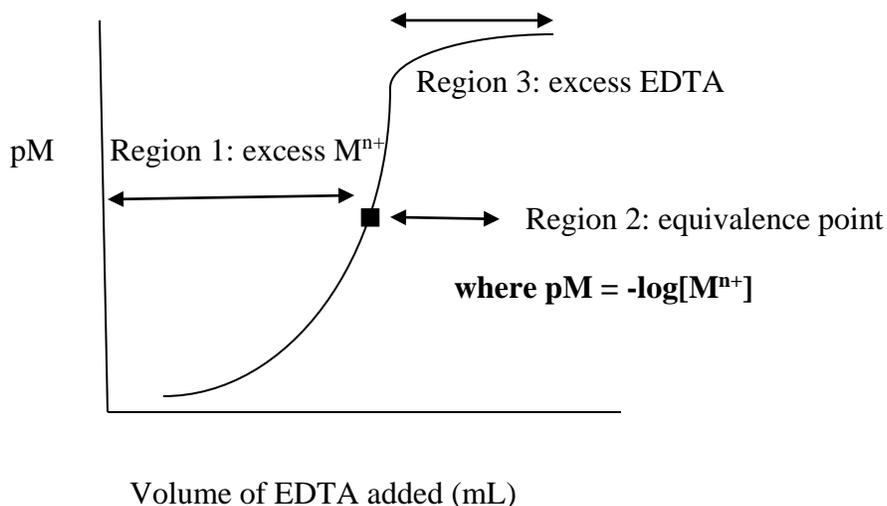
Titration Calculation: Calculating the Titration Curve

The titration reaction of 20.0 mL of 0.0200M Fe^{2+} is with 0.0200M EDTA (buffered at pH = 10), can be written as:

$$Fe^{2+} + EDTA \rightarrow MgY^{2-} \quad (8)$$
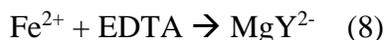
The $K'_f = \alpha_{Y4}\text{-}K_f$ for the reaction = $(0.36)(2.09 \times 10^{14}) = 7.5 \times 10^{13}$ (values of α_{Y4}- and K_f are from Apendix A)

The value of K'_f is large; hence it is safe to assume that the reaction goes to completion with the addition of titrant. The equivalence point will be 20.0 mL of EDTA. We can plot a graph of pM vs. mL of EDTA

Region 1 of the titration curve: Metal ion is in excess

If we start by adding 5.00 mL of EDTA to the Fe^{2+}, the equivalence point will be 20.0 mL of EDTA and the excess $[Fe^{2+}]$ that will remain will be:

$[Fe^{2+}]$ = (20.0 -5.0/20.0)(0.0200)(20/20.0+5.0) = 0.012 M and pM = 1.03

    ↑   ↑   ↑

Fraction

Remaining,(¾)   original Dilution factor

              concentration

           of Fe^{2+}

pM for other volumes below 20.0 mL can be calculated in a similar way

Experiment 3 Cont'd

Region 2: Equivalence Point

At the equivalence point, 20.0 mL EDTA has been added and all the metal complexes are in the form of FeY^{2-} with very little dissociation into equal amount of Fe^{2+} and EDTA:

$FeY^{2-} \leftrightarrow Fe^{2+} + EDTA$　　　(9)　　　Initial volume of Fe^{2+}

$[FeY^{2-}] = (0.0200)(20/(20.0+20)) = 0.01$ M and

Original　　　*Dilution factor*　　*total volume of solution*

concentration of Fe^{2+}
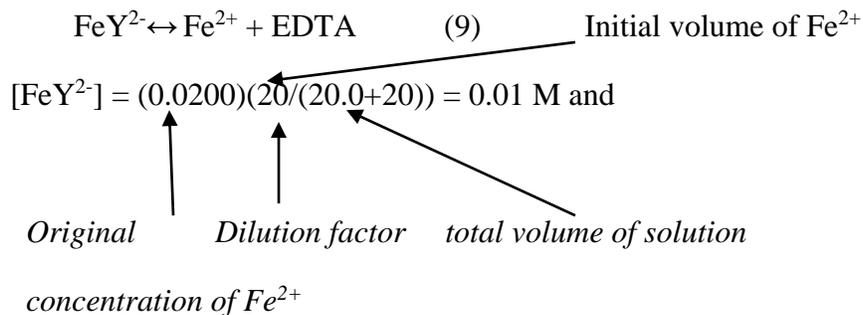
Concentration of Fe^{2+} at equivalence point is small and unknown

From equation 9, at equivalence point

	Fe^{2+}	EDTA	$[FeY^{2-}]$
Initial concentration of Fe^{2+}	0	0	0.01
Final concentration of Fe^{2+}	X	x	0.01-x

$K'_f = [FeY^{2-}]/[Fe^{2+}][EDTA] = 0.01\text{-}x/x^2 = 2.08 \times 10^{14}$ (K'_f from Table 2 in Appendix A)

$x = 1.4 \times 10^{-8}$ M

$pFe^{2+} = -\log[1.4 \times 10^{-8}] = 7.85$

Region 3: After the equivalence point

All the Fe^{2+} are binded and exist as FeY^{2-} and there are excess EDTA in this region. The concentration of the excess EDTA and FeY^{2-} can be calculated as follows:

at volume 21.0 ml of EDTA, there is an excess of 1.0 mL of EDTA

Experiment 3 Cont'd

Original concentration of EDTA   dilution factor

$[EDTA] = (0.020)(1.0/(20.0+21.0) = 4.9 \times 10^{-4}M$

Volume of excess EDTA   total volume of solution

Original concentration of Fe^{2+} dilution factor

$[FeY^{2-}] = (0.020)(20.0/20.0+21.0) = 9.8 \times 10^{-3}M$

Original volume of Fe^{2+} total volume of solution

At equivalence and past equivalence point, little amount of FeY^{2-} will dissociate into equal amount of Fe^{2+} and EDTA:
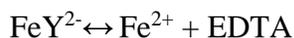
$FeY^{2-} \leftrightarrow Fe^{2+} + EDTA$

Therefore,

$K'_f = [FeY^{2-}]/[Fe^{2+}][EDTA] = 2.08 \times 10^{14}$

$= [9.8 \times 10^{-3}]/[Fe^{2+}][4.9 \times 10^{-4}] = 2.08 \times 10^{14}$

$[Fe^{2+}] = 9.8 \times 10^{-3}/[2.08 \times 10^{14}][4.9 \times 10^{-4}] = 9.62 \times 10^{-14}$

For a solution containing 2 or more metals, it is possible to use complexometric titration with EDTA to determine their concentrations in the mixture since each metal will have different conditional formation constant, $K'_f = \alpha_{Y4}\text{-}K_f$ which is pH dependent. $\alpha_{Y4}\text{-}$ decreases as pH decreases but and its value determines whether a titration is possible. However, at high pH value (high $\alpha_{Y4}\text{-}$), the end point is very sharp but one must be careful not to make the pH to high which will inadvertently precipitate the metal as an hydroxide.

Apparatus: Burette, pipet, 2x 250 mL conical flask, 600 mL beaker, brush, stirring rod. Retort stand, pH meter, 2x 1.00 L volumetric flask

Reagent: Sodium hydroxide (NaOH), Tris hydrochloride (MW = 157.597 g/mol), Magnesium Chloride, EDTA ($C_{10}H_{18}N_2O_8$), Eriochrome black T, distilled water.

Section A: Preparation of Tris Buffer

Weigh out 0.100 mol of Tris hydrochloride and dissolve it in a beaker containing 800 mL of distilled water. Prepare 1.00 M NaOH by dissolving 2.30 g of NaOH in 90 mL of distilled water and make it up with distilled water to the 100 mL mark. Place a pH meter in the TRIS buffer

Experiment 3 Cont'd

(tris(hydroxymethyl) aminoethane) solution and gradually add the NaOH solution until the pH of the buffer is 10 (pH = 10). Transfer the buffer solution into a volumetric flask and wash the beaker a few times. Add the washings to the volumetric flask. Dilute the volumetric flask to the mark.

Preparation of NH_3/NH_4^+ Buffer: Add 142 mL of 28% (wt/wt) aqueous NH_3 to 17.5 g of NH_4Cl and dilute to 250 mL with distilled water. Measure the pH of the solution (pH= 10)

Section B: Preparation of 0.05 M Magnesium Chloride Solution

Prepare a 0.05 M solution of $MgCl_2$ by weighing out 4.77 g of $MgCl_2$ and dissolving in a beaker containing 800 mL of Tris buffer. Stir properly and transfer into a 1.00 L volumetric flask and wash the beaker a few times with the buffer. Add the washings to the volumetric flask. Dilute the volumetric flask to the mark.

Section C: Preparation of 0.05 M of EDTA Solution

Dry $Na_2H_2.EDTA.2H_2O$ (F.W. = 373.25) at 80^0 for 1 hr and cool in a desiccator. Accurately weigh out ~ 1.20 g of the dry EDTA into a beaker containing 800 mL of distilled water. Stir the solution while heating. Cool the solution to room temperature and transfer into a 1.0 L volumetric flask and wash the beaker a few times. Add the washings to the volumetric flask. Dilute the volumetric flask to the mark.

Section D: Preparation of Eriochrome Black T Indicator

Dissolve 0.20 g of the solid form of the indicator in 15 mL of triethanolamine and 5.0 ml of absolute ethanol. Stir the mixture until everything dissolves to give a clear solution

Section E: Preparation of the EDTA Titration Curve

Pipette 25 mL of the $MgCl_2$ solution and put in a 250 mL conical flask, add a stirring bar and 2-3 drops of Eriochrome Black T indicator to the solution. Put the conical flask on a magnetic stirrer. Fill up a burette with the EDTA solution. Add 5.0 mL aliquots of EDTA to the $MgCl_2$ solution and stir continuously. When 20.0 mL of EDTA has been added to the solution, then add 1.0 mL aliquots of EDTA to the solution until 25.0 mL of EDTA has been added (to reach the equivalence point, red changes to blue). Continue adding 1.0 mL aliquots of EDTA to the solution until a total volume of 30.0 mL of EDTA has been added.

Record the aliquot volumes of EDTA added to the $MgCl_2$ solution and calculate the pMg^{2+} for each aliquot of EDTA in Table 3.1. Plot pMg^{2+} against the volume of EDTA added to the solution.

Experiment 3 Cont'd

Experiment 3   **Table 3.1**   **RESULT**

Name   **Department**   **Date**

Region	ml EDTA added	Concentration of unreacted Mg^{2+} (M)	Concentration of excess Mg^{2+} (M)
Region 1	0.00		
	1.00		
	2.00		
	3.00		
	4.00		
	5.00		
	6.00		
	7.00		
	8.00		
	9.00		
	9.10		
	9.30		
	9.90		
Region 2	10.0	0	0
	10.5		
	10.9		
	11.0		
Region 3	12		
	13		
	14		

Experiment 3 Cont'd

Question

1. Calculate the fraction of Mg^{2+} remaining when 15 mL of EDTA has been added to the $MgCl_2$ solution

2. Calculate the fraction of MgY^{2-} at the equivalence point

Experiment 4 EDTA Titration of Ca^{2+} and Mg^{2+} in Natural Waters

Theory

Ca^{2+} and Mg^{2+} are the most common metal ions in natural waters (fresh or sea water). The concentration of these metals can be determined by titration with EDTA. The assumption is that the concentration determined by titration with EDTA is equal to the concentration of the metals in the natural water. A second experiment is performed to analyze Ca^{2+} ion by first of all precipitating out Mg^{2+} ions as $Mg(OH)_2$ with a strong base.
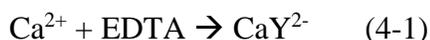
$$Ca^{2+} + EDTA \rightarrow CaY^{2-} \qquad (4\text{-}1)$$

$$Mg^{2+} + EDTA \rightarrow MgY^{2-} \qquad (4\text{-}2)$$

Apparatus: Burette, pipet, 2x 250 mL conical flask, 600 mL beaker, brush, stirring rod. Retort stand, pH meter, 2x 1.00 L volumetric flask

Reagent: Sodium hydroxide (NaOH), Tris hydrochloride (MW = 157.597 g/mol), EDTA ($C_{10}H_{18}N_2O_8$), Eriochrome black T, hydroxylnapthol, tap or well water, seawater, distilled water.

Section A Preparation of NH_3/NH_4^+ Buffer: Add 142 mL of 28% (wt/wt) aqueous NH_3 to 17.5 g of NH_4Cl and dilute to 250 mL with distilled water. Measure the pH of the solution (pH= 10)

Section B: Preparation of Eriochrome Black T Indicator

Dissolve 0.20 g of the solid form of the indicator in 15 mL of triethanolamine and 5.0 ml of absolute ethanol. Stir the mixture until everything dissolves to give a clear solution

Section C: Preparation of 0.0036 M of EDTA Solution

Dry $Na_2H_2.EDTA.2H_2O$ (F.W. = 373.25) at 80^0 for 1 hr and cool in a desiccator. Accurately weigh out ~ 1.20 g of the dry EDTA into a beaker containing 800 mL of distilled water. Stir the solution while heating. Cool the solution to room temperature and transfer into a 1.0 L volumetric flask and wash the beaker a few times. Add the washings to the volumetric flask. Dilute the volumetric flask to the mark.

Section D: Determination of Ca^{2+} and Mg^{2+} ions in Natural Waters

Pipet 50.0-mL of tap water or 1.0-mL of seawater into a 250-mLflask. If 1.0 mL seawater is used, dilute it to 50.0 mL with distilled water. To the sample, add 3-mL of pH 10 buffer and 6 drops of Eriochrome Black T indicator. Titrate with EDTA from a buret and note when the color changes from wine-red to blue. An accurate end point may be determined by adding a little tap water several times and titrating with more EDTA. Keep this solution for comparison. Repeat the titrations 3-5 times to find and accurate value of the total value Ca^{2+} and Mg^{2+} ions. Perform a

Experiment 4 Cont'd

blank titration with distilled water and subtract the value from each of the sample result. Find the average of the sample results.

Section E: Determination of Ca^{2+} from the Sample

Pipet four sample of 50 mL of tap water or 1.o mL of seawater (dilute to 50 mL with distilled water) into four separate conical flasks. Add 30 drops of 50% (wt/wt) NaOH to each flask and swirl for about 2-3 minutes to allow $Mg(OH)_2$ to precipitate. Add ~0.10 g of solid hydroxylnapthol blue indicator or Eriochrome Black T to each flask. Titrate one flask with EDTA to determine the blue end point. Allow sample to sit for 5 minutes with occasional swirling to re-dissolve any $Ca(OH)_2$ precipitate (if any). Re-titrate back to the blue end point if this happens. If the solution turns red while sitting down, re-titrate back to the blue end point. Keep the solution for comparison. Repeat titration with the other three samples.

Perform a blank titration with 50 mL of distilled water.

Calculate the concentration of the total concentration of Ca^{2+} and Mg^{2+} as well as the individual concentrations of each ion. Calculate the relative standard deviation of the replicate samples

Determine the concentration of Mg^{2+} by subtracting the result of Table 4-2 from 4-1

Calculate the amount of Ca^{2+} Mg^{2+} present in the natural water in grams

Calculation Procedure

Equivalence Point

At equivalence point,

the concentration of the EDTA consumed = Concentration of the $[CaY^{2-}]/[MgY^{2-}]$ complex

while y-mL EDTA has been added. All the metal complexes are in the form of CaY^{2-} and MgY^{2-} with very little dissociation into equal amount of Mg^{2+}/Mg^{2+} and EDTA:

$$CaY^{2-} \leftrightarrow Ca^{2+} + EDTA \qquad (4\text{-}3)$$

Initial volume of Ca^{2+}/Mg^{2+}

$[EDTA] = [CaY^{2-}]/[MgY^{2-}] = (0.0036)(y/(50.0+y)) = 0.01$ M and

Original   *Dilution factor*   *total volume of solution*

concentration of Ca^{2+}/Mg^{2+}

Experiment 4 Cont'd

Experiment 4   Table 3.1   RESULT

Name   Department   Date

Table 4.1 Concentration of $[CaY^{2-}]/[MgY^{2-}]$ Complex

Volume (mL) of EDTA Added (0.0036 M)	Concentration of $[CaY^{2-}]/[MgY^{2-}]$ complex
1	
2	
3	
4	
Average Concentration	

Table 4.2 Concentration of Ca^{2+}ion in the Analyte Solution

Volume (mL) of EDTA Added (0.0036 M)	Concentration of $[CaY^{2-}]$ complex
1	
2	
3	
4	
Average Concentration	

Experiment 5: **Spectrophotometry: Serum iron determination with UV spectrophotometer**

Theory

For a compound to be analyzed by spectrophotometry, it must absorb or transmit light. The absorption or transmittance should be distinguishable from that due to other species in the sample. Most compounds absorbs in the ultraviolet region (UV). For example, proteins absorb UV light at 280 nm because of the presence of aromatic groups in its structures.

A monochromatic beam of light with intensity P_0 passes perpendicularly through a cell of length b containing a solution with concentration c. The intensity of the beam will be attenuated to P by the absorbing specie. The *transmittance*, T, of the solution is defined as

$T = P/P_0$ which is often expressed as a percentage.

Transmittance

b (path length)

$P_0 \implies$ $\implies P$

Solution with concentration, c
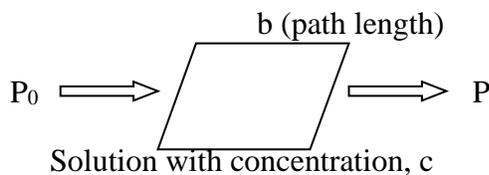
Figure 5.1 Attenuation of a beam of light by an absorbing solution

Absorbance: The amount of light absorbed by a solution, A, is defined as

$A = -\log_{10} T = \log P_0/P$

Relationship between Absorption and Concentration

Beers Law: Absorption, A = abc
- where a = absortivity constant, b = path length of the of the radiation passing through absorbing specie (which is equal to the length of the cell) and c = concentration of the specie
- Absorbance has no unit
- If c = moles/L and b is in cm, a = molar absorptivity, ε, hence,
   A= εbc
   ε = L/cm mol, Hence, A= log P_0/P = εbc

For a **mixture** where the species do not interact with each other, each specie will have its own absorbance. The absorbance for the solution will be the sum of the absorbance's for each of the specie contained in the solution: $A = \varepsilon_1 bc_1 + \varepsilon_2 bc_2 + \varepsilon_3 bc_3 + \varepsilon_4 bc_4$

Experiment 5 Cont'd

Limitations of Beers Law

Absorbance is linear with the path length (b) when concentration is constant but there is a deviation for all cases of constant path length (b) when concentrations vary. Some of the deviations are fundamental and forms real limitations to Beer's law: for example, Beer's law is only applicable to dilute solutions (10^{-2}). Other deviations are instrumental (depending on the way the absorbance is measured) or chemical (changes that occur to the analyte such as concentration changes). Beer's law is only applicable to dilute solutions (10^{-2}). For concentrations > 0.01 M, the analyte may not absorb a certain wavelength of radiation and therefore, causes deviation from the linearity between absorbance and concentration. This deviation can also occur if the analyte concentration is low while that of an interfering ion is very high. ε is dependent on the refractive index (n) of the solvent. Alterations in the refractive index affect the value of ε

Serum iron determination with UV spectrophotometer: Iron in biosystems are transported through the blood stream by attaching itself to a protein called *transferrin*. Iron in the *transferrin* can be measured with a UV spectrophotometer. This method is very sensitive with only 1.0 µg of iron needed to provide an accuracy of 2-5%. Human blood usually contains 45% (v/v) cells and 55% plasma. If the blood is collected without a coagulant, the blood will clot and the liquid that remains is called *serum*. Serum contains about 1.0 µg of Fe/mL attached to the *transferrin*

Apparatus: Centrifuge, 2x 250 conical flask, 2x 100 beaker, 6 test tubes, measuring cylinder, UV spectrophotometer, stirrer, spatula

Reagents: Blood sample with no coagulant, hydroxylamine hydrochloride ($NH_3OH^+Cl^-$) or ascorbic acid (vitamin C), trichloroacetic acid (Cl_3CCO_2H, ferrozine, tris buffer, reagent blank (consist of all reagents with distilled water replacing the analyte), 0.001 M Fe^{2+} solution, 1.0 M NaOH, , thiourea, distilled water

Section A: Preparation of the Tris Buffer

Weigh out 0.100 mol of Tris hydrochloride and dissolve it in a beaker containing 800 mL of distilled water. Prepare 1.00 M NaOH by dissolving 2.30 g of NaOH in 90 mL of distilled water and make it up with distilled water to the 100 mL mark. Place a pH meter in the TRIS buffer (tris(hydroxymethyl) aminoethane) solution and gradually add the NaOH solution until the pH of the buffer is 10 (pH = 10). Transfer the buffer solution into a volumetric flask and wash the beaker a few times. Add the washings to the volumetric flask. Dilute the volumetric flask to the mark.

Section B: Preparation of Ferrozine Buffer Solution

Prepare 0.10 M Ferrozine solution with the tris buffer solution made in section A above

Experiment 5 Cont'd

Section C: Preparation of the Calibration Standard (FeCl$_2$)

Add 1.27g of FeCl$_2$ to a 1.0 L volumetric flask and dissolve with 500 mL of buffer solution. Shake thoroughly to dissolve. Fill the flask up to the mark with the buffer solution. This is the stock solution. Make series of dilution with the buffer solution to obtain 5.0 mL of the following concentration of iron: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μg of iron solutions. Add drops of Ferrozine to each of the solutions until a purple color is obtained.

Section D: Preparation of the blank

Mix 1.0 mL of each solution used in this experiment except the analyte.

Section E: Preparation of the Analyte

Obtain 5 mL of the blood serum sample from the laboratory instructor. Add hydroxylamine or ascorbic acid to this solution. Fe(III) in transferrin will be reduced to Fe(II) and hence, released from the protein. Add trichloroacetic acid to the Fe(II)-transferrin complex to precipitate other proteins in the blood solution. Fe(II) will be left in the solution. Centrifuge the solution to remove the protein. If there are still proteins in the solution, it will precipitate in the final solution. Transfer the Fe(II) solution into another beaker and add excess Ferrozine solution until the purple iron-ferrozine complex is obtained.

Section E: Measurement of Absorbances of the Blank, Calibration Standard and the Analyte

Put 5.0 mL of the blank solution in the cuvette, measure its absorbance and use the **zero knob** of the spectrophotometer to zero its absorbance before measuring the absorbances of the calibrating standards and the analyte (background subtraction).

Obtain the absorbances of each of the calibrating standards (iron-ferrozine complex solutions). Plot the absorbances against the concentrations of the standards to obtain a calibration curve.

Obtain the absorbance of the analyte solution and repeat the measurement 3-5 times. Use the calibration curve to find the concentration of the analyte. Find the average of these measurements.

Question

1. What is the concentration of 1.00 μg of Fe in the analyte?

2. Is the Beer's law valid in this experiment?

Experiment 5 Cont'd

Experiment 3   **Table 3.1**   **RESULT**

Name   **Department**   **Date**

Table 5.1 Absorbances of the Calibrating Standard Solutions

Concentration of Fe^{2+} (µg/L)	Absorbances
1	
2	
3	
4	
5	
6	
7	
8	
9	

Table 5.2 Concentration of Fe^{2+}ion in the Analyte Solution

Absorbances	Concentration of Fe^{2+} (µg/L)
1	
2	
3	
4	
5	
Average Concentration	

Experiment 6: **Spectrophotometric Determination of Iron in a Multivitamin Tablet**

Theory

The amount of iron (Fe^{3+}) in a vitamin supplementary tablet can be determined with an Ultra Violet (UV) spectrophotometer. Initial preparation involves reducing the iron (Fe^{3+}) to Fe^{2+} with hydroquinone and then an intensely colored complex with formed with o-Phenanthroline (color plate 19)

$$2 \, Fe^{3+} + HO\text{-}Ph\text{-}OH \leftrightarrow 2Fe^{2+} + O=Ph=O + H^+ \tag{6-1}$$
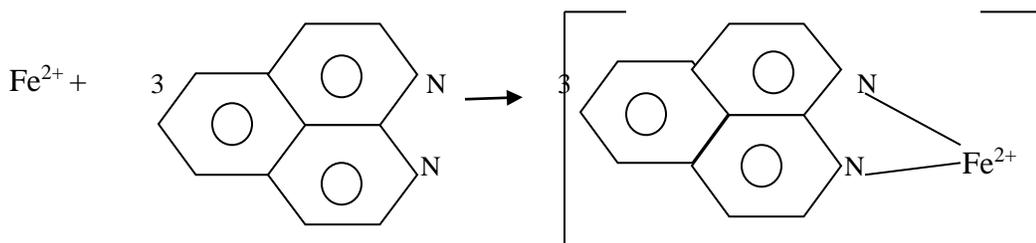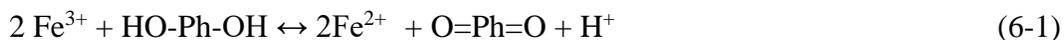
Apparatus: 4x1.0 volumetric flask, 4x 100 mL volumetric flask, 2x amber bottle, 2x100 mL beaker, filter paper, 2x10.0 mL pipet, pH meter or paper, dropper, UV spectrophotometer

Reagents: Hydroquinone, trisodium citrate, o-Phenanthroline, $Fe(NH_4)_2(S)_4)_2.6H_2O$, H_2SO_4, distilled water

Preparations

Hydroquinone: Dissolve 10 g of hydroquinone in 1.0 L of distilled water. Shake very well and keep solution in an amber bottle

O-Phenanthroline: Dissolve 2.5 g of o-Phenanthroline in 100 mL of ethanol in a 1.0 L volumetric flask. Dilute to mark with distilled water.

Stock Fe (0.04 mg/mL): Dissolve 0.281 g of reagent grade $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water in a 1.0 L volumetric flask containing 1.0 mL of 98% (wt/wt) H_2SO_4

Procedure

1. Place one tablet of the iron-vitamin tablet in a 100-mL beaker and gently boil (in a fume cupboard) with 25.0 mL of 6M HCl for 15 minutes. Filter the solution into a 100 mL flask. Wash the beaker several times to make sure that quantitative transfer occurred. If the tablet contains less than 15 mg, use 10.0 mL of the acid

2. Pipet 10.0 mL of the Fe stock solution into a beaker. Measure its pH and add sodium citrate in drops (~ 30 drops) until the pH is ~3.5. Count the number of drops of sodium citrate used.

25

3. **Calibrating Standard Solution:** Pipet another 10.0 mL of the Fe stock solution into a 100 mL volumetric flask and add the same number of drops of sodium citrate (as in step 2). Add 2.0 mL of hydroquinone and 3.0 mL of o-Phenanthroline solution. Then use distilled water to dilute the mixture to the mark and mix very well

4. Repeat step 3 with 5.0, 3.0, 2.0 and 1.0 mL Fe stock solution using the appropriate ratio of sodium citrate per volume of Fe solution (if 10 mL of Fe requires 30 drops of sodium citrate, use 15 drops for 5.0 mL of Fe solution)

5. Use distilled water as the blank solution (no Fe), measure its absorbance (508nm) and subtract from absorbances of all Fe solutions

6. **Unknown Solution**: Determine the number of sodium citrate drops that will bring 10.0 mL of the tablet solution to a pH = 3.5.

7. Pipet 10.0 ml of the unknown (tablet) solution into a 100.0 mL volumetric flask and add the required number of sodium citrate drops. Add 2.0 mL hydroquinone and 3.0 mL of o-Phenanthroline to the flask and then dilute the mixture with distilled water to the mark.

8. Allow all the solutions to sit for 10.0 minutes and then take the absorbances with a UV spectrophotometer at 508 nm.

9. Repeat step 8 with 5.0, 3.0, 2.0 and 1.0 mL of unknown solution

10. Plot the absorbances of the calibrating standards vs. the concentration to obtain a calibration curve.

11. Read the concentration of the unknowns from the calibration curve and determine the average milligrams of Fe in the vitamin tablet.

Question

1. Calculate the slope, standard deviation, average molar absorptivity (ε, calculate for each solution) of the calibrating standards. Also determine the intercept of the calibration curve.

Experiment 6 Cont'd

Name   Department   Date

Table 6.1 Calibrating Standard Solution (Fe solution)

Volume of Fe Stock Solution (mL)	Concentration (mg/L)	Absorbances
10.0		
5.0		
3.0		
2.0		
1.0		

Table 6.2 Unknown Solution (Fe from vitamin tablet)

Volume of Fe Stock Solution (mL)	Absorbances	Concentration (mg/L)
10.0		
5.0		
3.0		
2.0		
1.0		
Average Fe (mg)	**nil**	

Experiment 7 Chromatographic Separation Methods I: Introduction to Analytical Separations (Solvent Extraction Method)

The aim and objective of this chapter is to:

(i)   Teach separation of mixtures using solvent extraction method

(ii)   Understanding how analyte's can be partitioned between two liquids of different polarities to achieve separation

(iii)   At the end of the experiment, the student would have acquired the skill in solvent extraction method

Theory

Extraction Methods: Ideally, a good analytical method should be able to separate an analyte directly from many matrices. However, very few analytical methods are analyte specific because of the problem of interference. There are two methods that can be used to solve interference problems: (i) Masking the interfering ion so that it will not contribute to the measurement e.g. using complexation or chelating agents such as EDTA (ii) isolating the analyte in a different media where the interfering specie is not soluble using any of the following techniques (solvent extraction).

All separation techniques separate components of a sample by distributing them between two phases which subsequently can be separated by mechanical processes. The distribution ratio (amount of a particular component) in each phase determines if they could be separated from each other. If one component is more soluble in one phase than the other, then, there is a potential that it might be separable from the others. Distribution of solute between two immiscible liquids is an equilibrium process. Such processes can be treated by the law of mass action. The equilibrium constant for these processes differ significantly for many solutes and can be used as a basis for separation or extraction. For example, Chlorophyll is a green pigment that

is found in the chloroplast of green leaves and green stems of plants which is often accompanied by Xanthophylls ($C_{40}H_{76}$, orange red crystal) and Carotene ($C_{40}H_{56}O_2$, yellow crystal).

Nernst (Partition) Theory of Distribution:

The partition coefficient allows us to determine the conditions needed to transfer a solute from one phase to the other. For example, if one has to use V_{aq} mL of an aqueous solution containing a_0 moles of **A** and we want to extract with V_{org} mL of immiscible organic solvent, at equilibrium, a_1 moles of A will remain in the aqueous phase. Therefore we may say that:

$[A_{aq}] = a_1/ V_{aq}$ (2a) and that $[A_{org}] = (a_0-a_1)/ V_{org}$ (2b)

If we put equation 2a and 2b in equation (1) and rearrange, then

$a_1 = (V_{aq}/(V_{org}K + V_{aq}))a_0$ (3) for the first extraction. For the second extraction of the aqueous solution, a_2 moles will remain in the aqueous phase and

$a_2 = (V_{aq}/V_{org}K + V_{aq})a_1$ (4) and so on and so forth. If we put equation (4) in equation (3), we will have $a_2 = (V_{aq}/(V_{org}K + V_{aq}))^2 a_0$. So for **n** extractions, the number of moles left in the aqueous phase will be $a_n = (V_{aq}/(V_{org}K + V_{aq}))^n a$

Because this equation is exponential, it shows that extraction becomes more efficient with multiple extractions.

Apparatus: Electronic balance, grinder, 2 x 500 mL conical flask, 1000 mL measuring cylinder, 2x1 Liter separation flask with its stopper, retort stand, funnel, filter paper, 4 x 500mL beakers, UV Spectrometer , UV cuvettes.

**Sample:** 100 g fresh leaves of Almond tree, Scent leaf, Bitter leaf, or grass

Reagents: Deionized water, 80% acetone, petroleum ether or hexane, methanol, 0.1 M KOH

Experiment 7 Cont'd

Theory: Chlorophyll Extraction Using Solvent Extraction (SE) Method

Chlorophyll itself is a mixture of two compounds, chlorophyll-*a* ($C_{55}H_{72}O_5N_4Mg$, MW= 893.49, blue-black crystal) and chlorophyll-*b* ($C_{55}H_{70}O_6N_4Mg$, MW= 906.51, green-black crystal) and can be separated from each other by solvent extraction method. If chlorophyll is extracted with ethanol, it gives a 'crystalline' compound after drying but if ether or aqueous acetone is used instead of ethanol, the product is 'amorphous' chlorophyll. Chlorophyll has essentially two parts:

(i) a substituted porphyrin ring and (ii) a phytol (the long carbon chain). The porphyrin ring is an excellent chelating ligand, with the four nitrogen atoms binding strongly to a coordinated metal atom (magnesium) in a square planar arrangement. Other examples of porphyrin compounds include blood heme and vitamin B_{12}. Naturally, chlorophyll-*a* and chlorophyll-*b* are always in a ratio of 3 to 1. Due to the green color of chlorophyll, it has many uses as dyes and pigments. It is used in coloring soaps, oils, waxes and confectionary. Chlorophyll's most important use, however, is in nature, in photosynthesis. It is capable of channeling the energy of sunlight into chemical energy through the process of photosynthesis
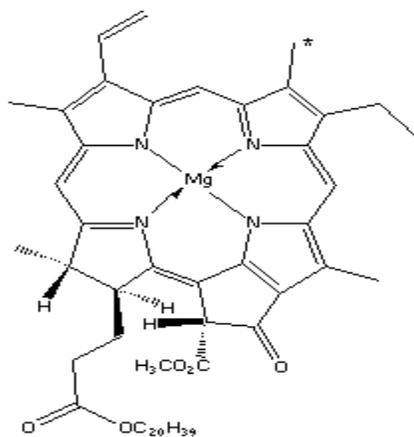
Procedure

Obtain and weigh 100 g of fresh leaves of any of the above listed leaves. Wash with water from the faucet and put in the grinder. Add 200 mL of 80% acetone to the leaves and grind to pulp. Transfer the contents of the grinder into a 500 mL flask, shake for about one minute and allowed to stand for another minute. The acetone takes up the color (deep green). Filter the acetone solution into another flask. Pour the acetone solution a 1Liter separating funnel and add 150 mL of petroleum ether and 150 mL of methanol one after the other. Shake the flask vigorously for about one minute and clamp the separating flask with the retort stand (don't tighten too much- just snuggly). Leave the mixture to settle for about 2-3 minutes. The mixture will separate into two layers. The upper layer (A), petroleum ether layer, is deep green in color and contains chlorophyll-a and carotene while the lower layer (B)- methanol layer, is light green yellow in color and contains chlorophyll-b and xanthophylls. Withdraw layer B into a 500 ml beaker while layer A is left in the separating funnel. Pour layer B into another 1Lseparating funnel and clamp to a retort stand.

Add 150 mL of methanol and 150 ml of KOH one after the other to both separating flasks and shake vigorously for one minutes. Clamp and leave both separating funnel to stand for another two minutes. Immediately, each mixture in both flasks will separate into two layers. The mixture A separates to chlorophyll-a (upper layer, deep green) and carotene (yellowish brown) as the lower layer. Mixture B also separates into Cholorphyll-b (upper layer, green) and xanthophylls (light yellow) as the lower layer

Measurement of Absorbance

Plug the UV/Visible spectrometer to the wall outlet and turn the switch to the **on-position**. Allow the spectrometer to warm up for about a minute (or turn it on at the beginning of the experiment). Obtain the cuvettes from the laboratory technologist or assistants. Fill one cuvette with 50:50 mixture of methanol and petroleum ether (blank) and the other one with chlorophyll-a. Open the cell compartment and pull out the black knob in front of the cell compartment to position the cell tray. Place the blank in the first cell hole and the sample in the second cell hole. Close the cell compartment and push back the cell tray to its **ready-position**. Press the **mode button** until the green light settles on ABS level. Adjust the wavelength to 400 nm with the wavelength knob and gently pull the cell tray forward until you hear a click sound. The first click sound puts the blank in position for measurement (each cell position should give a click sound). The spectrometer should give an absorption reading. Press the **100% adj** button to zero the blank. Then pull the cell tray forward until you hear another click sound. This positions the sample for measurement.

Read and record the absorption at this wavelength. Push the cell tray backward to its **ready-position**. Repeat the procedure between 400-700nm at wavelength intervals of 20nm. Repeat the same procedure for chlorophyll-b using methanol as the bank.
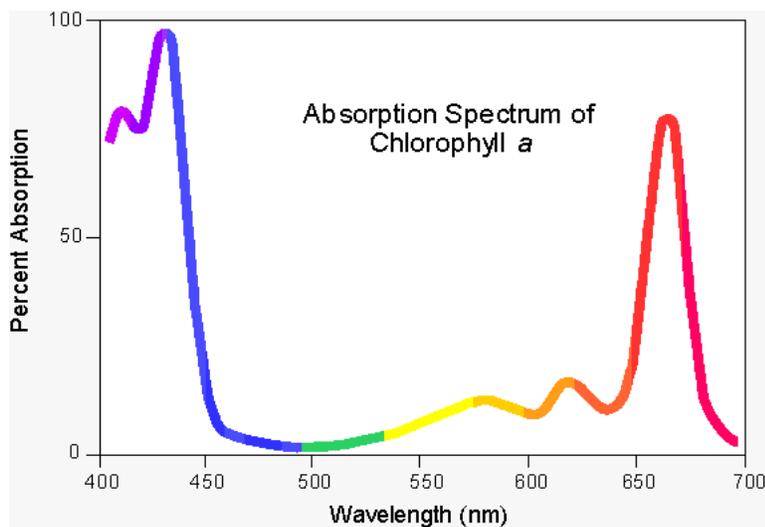
Experiment 7 Cont'd

Fig. 1 - The uv/visible adsorption spectrum for chlorophyll

Exercise

1. Plot the absorbance's against the wavelength for both chlorophylls and report the wavelength of maximum absorbance's for both chlorophylls.

2. Determine the density for each of the chlorophyll.

3. Use the density to determine the mass of the chlorophyll's obtained from extraction (multiply the density by the total volume of each extraction)

4. What is the ratio of chlorophyll-a: chlorophyll-b in this experiment?

Experiment 8: Chromatographic Separation Methods II: Paper Chromatography

Theory

Nature abounds with mixtures that need to be separated into its components for the use of man. For example, many pharmaceutical products (natural or synthetic) are initially mixtures which are subjected to separation techniques for isolation and identification of its active component and impurities. Separation techniques include solvent extraction, ion pairing or exchange, liquid-liquid extraction, gas phase, solid- liquid extraction and chromatographic extractions.

 This experiment is about chromatographic method of separation of a group of indicators. Chromatography involves partitioning mixtures between a solid phase (stationary phase) and gas or liquid phase (mobile phase). The separation process involves constant absorption and desorption of the components of the mixture between the mobile phase and the stationary phase. This is an equilibrium process that depends on the relative solubility's of the components of the mixture in the mobile phase or its affinity for the stationary phase. The more soluble a component is in the mobile phase, the faster it will be eluted or transported along the stationary phase. If the resolution is good enough, each component will appear as individual fractions that can be collected and identified by a detector.

In paper chromatography, paper is the stationary phase while different solvents such as methanol, acetonitrile etc. are used as mobile phase to elute components of a mixture. Components migrate at different rate depending on their relative affinity for the surface of the paper and the solvent. At a specific condition, the distance travelled by a components (solute) is a constant percentage of the distance travelled by the solvent (solvent front). The constant percentage is called, R_f and is good enough to identify that particular solute.

$$R_f = \text{distance traveled by the solute/distance traveled by solvent}$$

Apparatus: Chromatographic paper, depression plate, pencil, ruler, rubber band, developing chamber or clear glass jar, hood, stapler, micropipette.

Reagents: Indicators or chlorophyll extract, unknown mixture, acetone, n-butanol, ammonia

Procedure Section A: Preparing the Developing Chamber

 Place a strip of filter paper on the sides of the developing chamber. Make a 10 mL of hexane (petroleum ether): acetone mixture (70:30, v/v). Pour the mixture over the filter paper until the mixture is about 0.5 cm deep and cover the developing chamber. Covering the chamber will prevent the solvent mixture from drying off and also saturates the air in the chamber with the solvent mixture

Experiment 8 Cont'd

Section B: Preparing the Sample and the Unknown

Two Different Indicators: Add two drops of each of the known indicator and the unknown mixture into three different spots on the depression plate. Put 25 mL of each of butanol and ammonia (hexane or petroleum ether if chlorophyll is used) into the developing chamber or jar and cover with a lid. Place the developing chamber in the hood. Pour another 25 mL of ammonia into another jar or developing chamber, seal it with a lid and also place it in the hood. Handle the chromatographic paper at the edges only. Finger prints can interfere with the movement of the solvent. Draw a light pencil line 1.5 cm from and parallel to the edge of the shorter or longer side of the chromatographic paper. Spot the first known sample with a micro pipet on the pencil line and allow it to dry. Repeat the spotting three more times. Clean the micropipet with acetone and allow it to dry. Using a pencil, label the spot **A**. Repeat the process for the second unknown and the mixture leaving at least 2.5 cm in between each sample. Label the second known mixture as B and the unknown as C and allow the spots to dry.

Roll the paper to form a cylinder with a little gap between the edges and staple. Gently lower the paper into the developing chamber with the side containing the spots at the bottom of the jar or developing chamber. Close the developing chamber (jar) and allow the chromatogram to develop for one hour.

After one hour, remove the paper from the chamber and immediately use your ruler and pencil to mark the solvent front. Expose the paper to the ammonia or iodine chamber. Identify the spot(s) for each sample and mark the center of each spot. Measure the distance travelled by each spot and the solvent front. Spray the paper with water at the solvent front and expose to ammonia again. Mark and measure the distance travelled if any new spot(s) is found.

Calculate the R_f value for each component found in the chromatogram.

Chlorophyll mixture: or chlorophyll extract

Experiment 8 Cont'd

Experiment 8   **RESULT**

Name   **Department**   **Date**

Table 8-1

Indicator	Color of Spot in Ammonia	Distance from origin to solvent front	Distance from Origin to center of Spot	R_f
Unknown				

Question

1. A spot and its solvent front migrated 6.80 cm and 10.50 cm from the origin respectively. Calculate the spot's **R_f** value**?**

2. What is the physical property applicable to separations with paper chromatography?

3. If ink is used to mark the origin, how would the chromatogram look?

Experiment 9   Protein Analysis: Determination as Nitrogen in Organic Compounds Using Kjeldahl Procedure (pronounced Kel-dall)

Theory

Kjeldahl procedure is used for analysis of protein by determination of the nitrogen content of an organic compound. The method is applicable to a lot of organic compounds. Kjeldahl process is a four step procedure, viz;

(i) *Digestion*: this is the first step- chemical digestion with boiling H_2SO_4 and a catalyst that converts all organic nitrogen to $NH4^+$ - N(in protein) $\rightarrow NH_4^+$ (7)

(ii) *Conversion of NH_4^+ to NH_3* (solution is made basic) – NH_4^+ + OH^- $\rightarrow$ $NH_{3(g)}$ + H_2O (8)

(iii) *Distillation*- NH_3 is distilled into excess HCl – NH_3 + H^+ $\rightarrow$ NH_4^+ (9)

(iv) *Back Titration:* Excess HCl is back titrated with NaOH to determine how much of NH_3 was collected – H^+ + OH^- $\rightarrow$ H_2O (10)

Apparatus: Kjeldahl apparatus (if available), 2x 200 ml beaker, 4x250 conical flask, 2x 50 mL buret, magnet stirrer, 2x 100 mL volumetric flask, weighing balance, heating mantle, 2x 10 ml pipette

Reagent: 0.50 mL protein solution (containing 15.5% (wt/wt) nitrogen), 0.02 M HCl, 0.0190M NaOH, concentrated H_2SO_4, distilled water

Procedure: Weigh 5 mg of an unknown protein and add it to a 250 mL beaker. Gently add 10 mL of concentrated H_2SO_4 and a magnetic stirrer. Put beaker on a heating mantle and heat the mixture to boil or until all protein dissolves. Pipette 10 mL of dissolved protein into the Kjeldahl apparatus; add 10 mL of 0.015M NaOH and. Add 20 mL of 0.0195 M HCl to the Kjeldahl apparatus and ru the process for 1 hr. Titrate the mixture with 0.021M NaOH to determine the excess NaOH used in the process.

Find the concentration of protein (mg)/L of protein) in the original protein solution?

Example: 0.500 mL aliquot of a protein solution was analyzed by Kjeldahl process. The protein is known to contain 16.2% (wt/wt) nitrogen. The liberated ammonia was collected in 10.00 mL of 0.02140 M HCl and the unreacted acid required 3.26 mL of 0.0198 M NaOH for complete titration. Find the concentration of protein (mg)/L of protein) in the original solution?

   Answer:

   The total moles of HCl in the flask receiving NH_3:

   (10.00 mL)(0.0214 mmol/mL) = 0.2140 mmol HCl

Experiment 9 Cont'd

The # of moles of NaOH required for back titration of the excess HCl:

(3.26 mL)(0.0198 mmol/L) = 0.0646 mmol NaOH

Since mole ratio of HCl/NaOH in eqn. 10 = 1/1, therefore,

# of moles of excess HCl = 0.0646 mmol

The # of moles of NH_3 distilled into flask of HCl is, therefore, equal to the difference between the # of moles of HCl:

(0.2140- 0.0646) = 0.149 mmol of NH_3

The mole ratio of N/NH_3 = 1/1, therefore,

The # of moles of N in the protein solution = 0.149 mmol

The weight of Nitrogen is therefore:

(0.149 mmol)(14.0067 mg/mmol of N) = 2.093 mg

If the protein contains 16.2% (wt/wt) of nitrogen, then the weight of nitrogen in the protein will be:

2.093 mg N/0.162 mg N/mg protein = 12.9 mg protein

Therefore in 0.500 mL of protein solution, the amount of protein in it (mg/mL) will be:

12.9 mg protein/0.500 mL = 25.8 mg/mL

Dispose all reagents down the drain. Be careful when using concentrated H_2SO_4. It is very CORROSSIVE. Wear gloves when using H_2SO_4.

Question

In the Kjeldahl titration

   A. What acid is used to dissolve or digest the sample?
   B. What substance (be specific) is added to neutralize this acid?
   C. What substance (be specific) is distilled into the receiving flask?
   D. What kind of substance (acid or base) is in the receiving flask before the distillation?
   E. What kind of substance (acid or base) is the titrant

Experiment 9 Cont'd

Experiment 9   **RESULT**

Name   **Department**   **Date**

Table 9-1

| Steps	Process	Result
1	Total # of moles of HCl in the receiving flask	
2	Total # of moles of NaOH needed for back titration of excess HCl	
3	# of moles of excess HCl (same as 2)	
4	Total # of moles of NH_3 distilled into flask (1-3)	
5	The # of moles of N in the protein solution	
6	Weight of Nitrogen	
7	Weight of Protein (weight of Nitrogen/% of protein (wt/wt)	
8	Weight of protein mg/mL	

Experiment 10 Atomic Absorption Spectrometry

Theory

Atomic absorption spectrophotometry (AAS) is one of the principal instrumental techniques for elemental analysis in agricultural and environmental laboratories.

In AAS, a solution containing a metallic salt is aspirated into a flame (oxyacetylene flame) form vapor which contains atoms of the metal(s) (*atomize*). Some of these gaseous metal atoms may be excited to higher energy levels that allow emission of radiation characteristic of the particular metal which is imparted to the flame (yellow color for sodium atoms). *This is the basis for flame emission spectroscopy (FES) or Atomic Emission Spectroscopy (AES).* AES does not need a resonance line source for its detection. However, a larger percentage of gaseous atoms remain in an unexcited state (ground state). These atoms are capable of absorbing radiant energy of their own specific resonance wavelength which is the wavelength they will emit if excited from the ground state. If light of the resonance wavelength (cathode tube; at wavelengths characteristic of each element) is passed through the flame, atoms in the oxyacetylene flame will absorb some of the light. The extent of the light absorption is proportional to the concentration of atoms in the light path (in the flame). *This is the underlying principle of Atomic Absorption spectroscopy (AAS).* If the absorbed light is reemitted with different wavelength from the absorbed wavelength and monitored, then it will be reported as *Atomic Fluorescence Spectroscopy (AFS).*

AAS requires uses standard curve techniques for its quantitative analysis. The technique requires obtaining signal of known concentration of the element of interest (standard solution). The signal is plotted against the concentration of the standards. The composition of the standard solution must be similar to that of the analyte. The standard curve is then used to quantify the analyte.

Absorbance
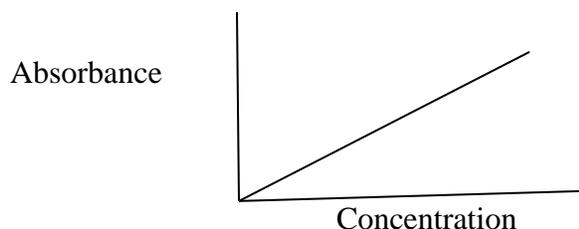
Concentration

There are two other methods that can be used to obtain the concentration of the analyte:

(i)   *Standard Addition Method*: Known quantities of the analyte (standard solution) of interest are added to the analyte and the increase in signal is measured. Each solution is diluted to the same total volume with the same final composition (except the analyte concentration). If the analyte concentration is [X] and that of the added standard is [S], then,

$$[X]/[X] + [S] = A_x/A_{x+s} \qquad 7\text{-}1$$

A_x = absorbance (emission) of analyte and A_{x+s}= absorbance (emission) of analyte + standard. Equation 7-1 can be solved for X

Equation 7-1 is true when the concentration and the absorbance are linearly related. Each element has a range where this relationship is true.

(a) The other way the standard addition method can be used is to ***prepare a series of the standard addition solution and plot their signal against the concentration of the added analyte after it has been mixed with the analyte and diluted to the final volume***. The graph is extrapolated to have two intercepts. ***The y-intercept represent the signal of the analyte (unknown) without the added standard and the x-intercept is the concentration of the analyte (unknown) after it has been mixed with the standard solution and diluted to the final volume.***

Conc. of unknown   Abs   Signal of unknown without added standard

Concentration of added analyte (ppm)
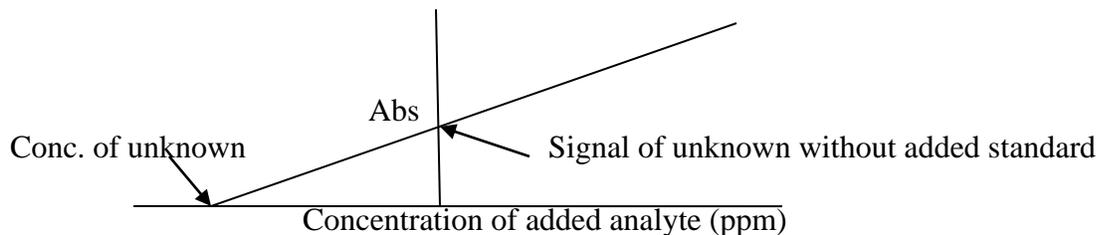
The most useful range of standard addition should increase the concentration of the analyte to between 1.5 – 3 times its original values. The *main advantage of standard addition method is that the matrix remains the same for all samples.*

(ii) **Internal Standard Method**: An element that is different from the analyte of interest is used as a standard. Series of known concentration of the standard [S] and element of interest (analyte, [X]) is prepared and used to make a calibration curve. A plot of the ratio of the signal of analyte and the standard ([X]/[S]) is plotted against the ratio of the concentration of the analyte and the standard (C_X/C_S) is used as the calibration curve. Usually the concentration of the standard (C_S) is held constant. The standard is then added to the unknown and its signal obtained. The signal is then used to determine the concentration from the calibration curve. Since C_S is known, then C_A can be obtained.

Abs ratio in unknown/Abs ratio in standard= Abs ratio in unknown/abs ratio in standard

$$[X]/[S]_{unk}/\ [X]/[S]_{std} = [C_X]/[C_S]_{unk}/\ [C_X]/[C_S]_{std}$$

Apparatus: Thermo Elemental Atomic Absorption Spectrophotometer, 1x 100.0 mL volumetric flask, 5 x 10 volumetric flask, blender, 2 x 200 mL beaker, knife, filter paper, Buchner funnel, Vacuum pump, 10.0 mL pipet

Reagent: Deionized water, Standard Nitric acid (1000 ppm), concentrated Nitric acid, ethanol,

Sample: Telfairia occidentalis (Ugu) leaf, banana, plantain peels

Procedure

Section A: Preparation of 10.0 ppm Iron (Fe) Stock Solution: Pipet 1.0 ml of the 1000 ppm standard solution of iron (Fe) into a 100.0 mL volumetric flask and dilute to mark with 1:4 concentrated HNO_3: Ethanol solution.

Section B: Preparation of the Calibration Standard Solutions

Pipet 1.0, 2.0, 3.0, 4.0 and 5.0 ml of the stock solution (from section A) into five different 10.0ml volumetric flask and dilute to mark with 1:4 concentrated HNO_3: Ethanol solution. This solution will give 1.0, 2.0, 3.0, 4.0 and 5.0 ppm solutions of the calibration standard.

Section C: Preparation of the Sample

Weigh 50g of Ugu leaf. Rinse the leaf and put in a blender. Add 30 mL of a 1:4 concentrated HNO_3: Ethanol solution to the Ugu leaf and grind with the blender. Attach the vacuum pump to the Buchner funnel and pour the leaf pulp from the blender into it. Turn on the vacuum pump to filter the leaf pulp. Rinse the blender with 20 ml HCl: Ethanol solution and pour it into the Buchner funnels for filtration. Repeat the same procedure for the same amount of banana and plantain peels.

Section D: Analysis

Follow the operational procedure of the Atomic Absorption Spectrometer (AAS) for analysis shown in Appendix C. Program the AAS to use the Least Square Method for the calibration curve. Use the same amount of solution (10.0 mL) to run the blank, standards and the sample. Run the blank (1:4 concentrate HNO_3: Ethanol solution), calibration standard and the sample as recorded in the Method section of the AAS procedure. Print out the Result sheet. If the sample needs dilution, remember to use the dilution factor to multiply the result to get the actual amount of the Fe in the sample.

APPENDIX A

Table 1 Values of α_{Y4-} for EDTA at 20^0C and $\mu= 0.10$ M

pH	α_{Y4-}
0	1.3×10^{-23}
0	1.9×10^{-18}
2	3.3×10^{-14}
3	2.6×10^{-11}
4	3.8×10^{-9}
5	3.7×10^{-7}
6	2.3×10^{-5}
7	5.0×10^{-4}
8	5.6×10^{-3}
9	5.4×10^{-2}
10	0.36
11	0.85
12	0.98
13	1.00
14	1.00

APPENDIX B

Table 2 Formation constants for EDTA Complexes

Ion	Log K_f	Ion	Log K_f	Ion	Log K_f
Li^+	2.79	Mn^{3+}	25.3 (25ºC)	Ce^{3+}	15.98
Na^+	1.66	Fe^{3+}	25.1	Pr^{3+}	16.4
K+	0.8	Co^{3+}	41.4 (25ºC)	Nd^{3+}	16.61
Be^{2+}	9.2	Zr^{4+}	29.5	Pm^{3+}	17.0
Mg^{2+}	8.79	Hf^{4+}	29.5 (μ = 0.2)	Sm^{3+}	17.14
Ca^{2+}	10.69	VO^{2+}	18.8	Eu^{3+}	17.35
Sr^{2+}	8.73	VO_2^+	15.55	Gd^{3+}	17.37
Ba^{2+}	7.86	Ag^+	7.32	Tb^{3+}	17.918.303
Ra^{2+}	7.1	Tl^+	6.54	Dy^{3+}	18.62
Sc^{2+}	23.1	Pd^{2+}	18.5(25ºC, μ = 0.2)	Ho^{3+}	18.85
Y^{3+}	18.09			Er^{3+}	19.32
La^{3+}	15.5			Tm^{3+}	19.51
V^{2+}	12.7	Zn^{2+}	16.50	Yb^{3+}	19.83
Cr^{2+}	13.6	Cd^{2+}	16.46	Lu^{3+}	17.8 (25ºC)
Mn^{2+}	13.87	Hg^{2+}	21.7	Am^{3+}	18.1(25ºC)

Source: A.E Martell and R.M Smith, Critical Stability Constants, Vol. 1(NY: Plenum Press, 1974) pp. 204-211

Appendix B Cont'd

Table 2 Cont'd   Formation Constants for EDTA Complexes

Ion	Log K_f	Ion	Log K_f	Ion	Log K_f
Fe^{2+}	14.32	Sn^{2+}	18.3 ($\mu = 0$)	Cm^{3+}	18.1 (25°C)
CO^{2+}	16.31	Pb^{2+}	18.04	Bk^{3+}	18.5(25°C)
Ni^{2+}	18.62	Al^{3+}	16.3	Cf^{3+}	18.7(25°C)
Cu^{2+}	18.80	Ga^{3+}	20.3	Th^{4+}	23.2
Ti^{3+}	21.3(25°C)	In^{3+}	25.0	U^{4+}	25.8
V^{3+}	26.0	Tl^{3+}	37.8 ($\mu = 1.0$)	Np^{4+}	24.6(25°C, $\mu = 1.0$)
Cr^{3+}	23.4	Bi^{3+}	27.8		

Source: A.E Martell and R.M Smith, Critical Stability Constants, Vol. 1(NY: Plenum Press, 1974) pp. 204-211

APPENDIX C   Atomic Absorption Operation Procedure

Thermo Elemental Atomic Absorption Spectrophotometer

Set-Up Procedure

1. Open the tray compartment and install the cathode tubes for each element (maximum of 6 tubes at a time)
2. Connect the Atomic Absorption spectrophotometer (AAS) to the CPU and the CPU to the monitor
3. Connect the compressor pump and the acetylene gas tank to the AAS
4. Connect the AAS, compressor pump and the CPU to a UPS or a voltage regulator
5. Connect the UPS or voltage regulator to the electrical wall outlet.
6. Make sure the AAS burner is in line with the gas outlet (Exhaust pipe on the wall) and turn the Exhaust **ON**
7. Turn the electrical wall outlet, acetylene gas tank, compressor pump, and then the AAS **ON**
8. Turn the knob on the acetylene regulator clock wisely until the pointer reaches the **'out'** (**blue**) region (achieve the required gas pressure)
9. Allow the gas to flow for about 2 minutes (burners button will be blinking) and then press the burners "**ON"** button (left panel of the burner) and hold until the burner's flame comes up
10. Turn the CPU **ON**

Running the AAS: Method Development

1. Allow complete booting of the CPU
2. Double click the AAS icon (Solaar) on the desktop and allow to LOAD software
3. The **Wizard** window appears. Click on "Method of Analysis and fill how many analysis that can be run within a method
4. May use to the **Wizard** to complete the Method set or **Exit**
5. The operating window will appear with the Analysis ICON's after exiting **Wizard**
6. Left click the **Action** Icon on the menu bar of the Analysis window and click on **Communication** radio button. **Click on 'Connect'** radio button to connect the AAS to the Solaar software on the CPU (if the CPU was on before the AAS is turned on)

Lamp

1. Double Click the LAMP icon on the menu bar and turn on all the lamps in the tray (the second ICON to the Right on the menu bar)
2. lamps needs to warm up to give a steady flame
3. Suck a dilute acid (HCl or HNO_3) through the sample inlet to clear the line of any debris

Method Development

1. Double click the Method Icon on the menu bar (1ˢᵗ ICON to the right on the Menu bar) and fill all the windows: Method name, Description (describe the analysis), Operator's name, etc. Flame dilution for Air: Acetylene – 0.7 :2.07 bar
2. Click on the **Sequence** window on Method menu bar and fill all the required windows: Element, flame etc
3. Click on the **Calibration** window on Method menu bar and fill all the required windows: blank, calibration standards concentrations, number of standards etc. ("Cook book" shows the standard condition for each element)
4. Click on "Spectrophotometer" and fill the windows
5. Click on Flame: fill all windows: Air- Acetylene
6. Click on "Calibration" ICON. Chose method of calibration , fill in calibration standards (from the lowest to the highest concentration) etc
7. Confirm that all accessories are in good condition, Click ACTION button on the Menu bar and choose SET OPTICS options
8. Make sure all blanks, standards and samples are ready before clicking on the **Run** button on the **Menu bar** (Green arrow on the menu bar pointing to the Right)
9. Monitor results on the **Result and Calibration** window
10. Repeat the same procedure for each element
11. Shut Down Procedure: (a) Turn the burner off   (red button on the left side of the AAS
    (b) Turn the AAS off
    (c) Turn the Acetylene tank off
    (d) Turn the Compressor off
    (e) Turn the PC off
    (f) Turn the Gas Exhaust outlet off

Reference Readings

1. Instrumental Analysis by Skoog and West. 3ʳᵈ Edition. USA

2. Instrumental Analysis Manual. Delaware University Newark Delaware. USA

3. Quantitative Chemical Analysis by Harris Daniel