Lab Manual

Quantitative Analytical Method

M Sc Pharmaceutical Chemistry I

1. Acid-Base Titration

For Acid-Base Titration, the End Point is the condition at which the moles of acid exactly cancel out the moles of base. Hence the solution is neutral. We shall use the color changes of litmus and phenolphthalein to determine the End Point. Potassium hydrogen phthalate (KHP) or oxalic acid are primary standard used to determine the molarity of bases such as NaOH.

ACID-BASE TITRATION EXPERIMENT

Purpose: To determine the unknown concentration of a diprotic acid

Equipment: Titrant and Analyte Burettes, Buret stand, Erlenmeyer Flask

Reagents/Solutions:

NaOH (known concentration), H_2SO_4 (unknown concentration), Phenolphthalein

Procedure:

Prepare all glassware by standard laboratory procedure. Put 2 drops of phenolphthalein in the Erlenmeyer Flask. Utilizing the H_2SO_4 buret, deliver 3 mL into the flask by recording the initial volume of the buret, calculating the final volume required to deliver 3 mL of analyte and delivering that amount into the flask. It is extremely important to record the exact volume of acid delivered into the flask to **the nearest 0.05 mL**.

$$V_{Final} - V_{Initial} = V_{Delivered}$$

Fill the titrant burette (NaOH) with *approximately* 4.5 mL. Do a quick first trial run to estimate the approximate volume of NaOH required neutralizing the H_2SO_4 (turns clear to pink) then repeat with 3 runs, carefully recording volumes (initial and final) required to neutralize to the **nearest 0.05 mL**.

Name: ________________________________

Date:__________________

Analyte: H_2SO_4

S No	Initial Volume	Final Volume	Volume Delivered of the ACID (mL)
1			
2			
3			
4			

Titrant: NaOH

S. No.	Initial Volume	Final Volume	Volume Delivered of the BASE (mL)
1			
2			
3			
4			

Known Molarity of NaOH:_________

Write a Balanced Equation for this reaction:

Experiment : Standardization of a solution of sodium hydroxide using a primary standard.

Needed:

1. 125-mL or 250-mL Conical flask be sure they are clean and dry.
2. Potassium hydrogen phthalate;
3. NaOH stock solution..
4. Burette for the NaOH

Clean, rinse well (with deionized water), and dry three 125-mL or 250-mL conical flasks. Measure between 0.20 – 0.25 g of potassium hydrogen phthalate into each. conical flask. Record Clean, rinse well (with deionized water), and dry three 125-mL or 250-mL Conical flasks. Measure between 0.20 – 0.25 g of potassium hydrogen phthalate into each Conical flask. Record the exact weight added to each flask to the nearest milligram. Add about 25-mL of water to each sample. Set the samples aside. Find out from your teaching assistant which unknown you will use. Obtain about 500 mL of the stock NaOH solution in a clean, dry beaker. Make a note of its unknown code. Prepare to titrate the KHP solutions by filling a burette with NaOH solution.

Rinse the burette with de-ionized water. Then, pre-rinse it with a portion of the NaOH solution before you fill it up for the titration. Pre-rinsing is necessary to insure that *all* of the solution in the burette is the desired solution, not a diluted or contaminated solution. To do this, add about 10 mL of the NaOH solution to the clean buret. *Carefully* turn the burette on its side so the liquid slowly runs out the top. Rotate the burette on its axis during this time to make sure the solution wets the sides all the way to the top. Pour the rinse from the burette into a waste beaker. Repeat the rinsing process with a second portion of the NaOH solution. By this time, the KHP samples should all be dissolved. If not, swirl their flasks gently. When you are sure they are fully dissolved, add 2 drops of phenolphthalein indicator to each. The solution should be colorless. Begin to titrate with the NaOH until you reach the endpoint.

The first titration should be performed by adding 0.50 mL portions of the NaOH solution, then swirling the solution. The approach of the endpoint is suggested by the temporary appearance of a pink color that fades when the solution is swirled for up to 10 seconds. A pink color that persists for more than 30 seconds signals the actual endpoint. (Note that the solution may lose its color after this; all you are looking for is persistence for 30-seconds or more.) This first titration gives you a rough idea of the amount of NaOH you need to neutralize the KHP.

Repeat the titration on the second and third samples of KHP. For these you can add about 70% of the needed NaOH quickly and then, near the end, add the NaOH *one drop* at a time until you see the endpoint.

Experiment: Determination of the molarity of a solution of hydrochloric acid using the NaOH secondary standard.

Needed:

1. Approximately 125-mL sample of HCl to titrate with your NaOH solution.
2. Four clean 125-mL or 250-mL Erlenmeyer flasks for the HCl (In the absence of Erlenmeyer Flask Conical flask can use)
3. 25.00-mL pipet to transfer 25.00 mL of HCl solutions into each of the four flasks
4. Phenolphthalein indicator added to each HCl sample.

Clean and rinse well (with de-ionized water) three 125-mL or 250-mL flasks. Use a 25.00-mL pipet to transfer 25.00-mL of HCl solutions into each of the three flasks. Add 2 drops of phenolphthalein indicator to each.

Titrate the HCl solutions with the NaOH from the burette. Again, expect that you will overshoot the endpoint on the first run. Make an effort to be much more precise on the second and third trials.

Experiment : Determination of the molarity of a solution of sulfuric acid using the NaOH secondary standard.

Needed:

1. 125-mL sample of H_2SO_4 to titrate with your NaOH solution. Make a note of the unknown code of the H_2SO_4.
2. Four clean 125-mL or 250-mL Erlenmeyer flasks for the H_2SO_4
3. 25.00-mL pipet to transfer 25.00 mL of H_2SO_4solutions into each of the four flasks
4. Phenolphthalein indicator added to each H_2SO_4sample.

Use a clean dry beaker to obtain about 125-mL of your assigned solution from the stock bottle.Clean and rinse well (with de-ionized water) three 125-mL or 250-mL flasks.

Use a 25.00-mL pipet to transfer 25.00-mL of H_2SO_4 solutions into each of the three flasks.

Add 2 drops of phenolphthalein indicator to each. Titrate the H_2SO_4 solutions with the NaOH from the burette. Again, expect that you will overshoot the endpoint on the first run. Make an effort to be much more precise on the second and third trials.

Experiment: Analysis of Acetic Acid in Vinegar

Vinegar is a dilute aqueous acetic acid ($HC_2H_3O_2$) solution. It reacts with sodium hydroxide as follows:

$HC_2H_3O_2 + NaOH \text{--->} NaC_2H_3O_2 + H_2O$

In this experiment you will analyze a vinegar solution to determine the percent acetic acid. As before, phenolphthalein will be used to signal when the acetic acid has reacted completely.

1. Tare an empty 50 mL flask. The flask needs to be dry on the outside but not on the inside. Add about 2 grams of vinegar to the flask and record the mass of the solution to the nearest 0.01 g.

2. Add one drop of phenolphthalein solution. Add NaOH from the buret while you swirl the flask. Occasionally rinse the insides of the flask with a little distilled water from a wash bottle. Continue adding NaOH until one drop causes the solution to change from colorless to very light pink. **This time you cannot correct if you make the solution too pink**. Record the final volume of NaOH to the nearest 0.01 mL.

3. Calculate the volume of NaOH used.

4. From the volume and the molarity of the NaOH calculate the moles of NaOH that reacted.

5. Use the mole ratio from the chemical equation to calculate the moles of $HC_2H_3O_2$ that were in the vinegar solution.

6. Use the molar mass of $HC_2H_3O_2$ to calculate the mass of $HC_2H_3O_2$ in the vinegar solution.

7. Use the mass of $HC_2H_3O_2$ and the mass of the vinegar solution to calculate the % $HC_2H_3O_2$ in vinegar.

8. Refill the buret and repeat the experiment at least twice more.

9. Calculate the average % $HC_2H_3O_2$ in vinegar.

Molarity of NaOH: Trial #2 Trial #3

_______________M Trial #1

Mass of vinegar

Final buret reading

Initial buret reading

Volume NaOH used

Moles NaOH used

Moles $HC_2H_3O_2$ in vinegar sample

Mass $HC_2H_3O_2$ in vinegar sample

% $HC_2H_3O_2$ in vinegar

Average % $HC_2H_3O_2$ in vinegar

Experiment : To determine the acid dissociation constants (K_a) of some weak acids.

Introduction

In water solutions, weak acids react with water to establish the equilibrium:

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

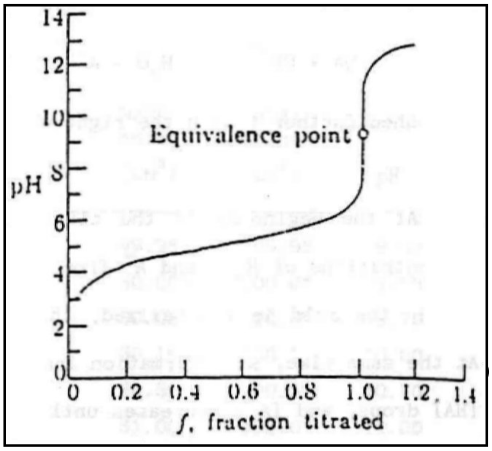
The equilibrium is represented by the *acid dissociation constant*, K_a, in which

$$K_a = \frac{[H_3O^+]_{eq} \times [A^-]_{eq}}{[HA]_{eq}} \qquad [\ ]_{eq} \text{ is the equilibrium concentration in mol/ dm}^3$$

K_a is a constant for a given acid at a given temperature. Its value can be determined by several experimental methods. Two of these that will be used in the experiment are:

1. measurement of the pH of a solution containing a known concentration of a weak acid and
2. measurement of the pH at the half-neutralization point in the titration of the weak acid with a strong base.

By following the pH during the titration of a weak acid with a strong base, you can obtain data that will enable you to calculate the K_a of the acid by both methods.



Titration Curves

In the titration of a weak acid, HA (having an ionization constant, K_a) with a strong base, a plot of pH against volume of base added will look like that in Fig. 1.

Figure 1: Titration curve for the titration of weak monoprotic acid with a strong base

That the graph should have this appearance is readily shown by the following reasoning. After each addition of base, equilibrium between the weak acid and its ion

$$K_a = \frac{[H_3O^+]_{eq} \times [A^-]_{eq}}{[HA]_{eq}}$$

is reestablished, according to the relation:

Rearranging this K_a expression gives

$$[H_3O^+]_{eq} = K_a \frac{[HA]_{eq}}{[A^-]_{eq}}$$

$$-\log[H_3O^+]_{eq} = -\log K_a + \log \frac{[A^-]_{eq}}{[HA]_{eq}}$$

Taking the logarithm of both sides of the expression and multiplying by -1, we get

or, in terms of pH and pK_a (in which $pK_a = -\log K_a$),

$$pH = pK_a + \log \frac{[A^-]_{eq}}{[HA]_{eq}}$$

This equation gives the pH at any $[A^-]/[HA]$ ratio. However, this ratio changes as base is added to the acid. The reason is that the reaction

$$HA + OH^- \rightleftharpoons H_2O + A^-$$

is pushed further toward the right with each addition of base.

At the beginning of the titration, only the acid HA and the small concentrations of H_3O^+ and A^- from its ionization are present. As base is added, the acid is neutralized, thus decreasing the concentration of HA. At the same time, salt formation increases $[A^-]$. Throughout the titration, [HA] drops, and $[A^-]$ increases until all HA is neutralized. Eq. (1) tells us that as this happens and the ratio $[A^-]$:[HA] increases from a low to a high value, the pH of the solution should change as shown in Fig.1.

It is easy to calculate the concentrations of HA and A^- at various stages during a titration. If these concentrations and the pK_a of the acid are substituted into Eq. (1), the pH at each stage also can be calculated. The results of such calculations for one titration are given in the table below.

pH CHANGES DURING THE TITRATION OF 50 cm^3 OF 0.1 M HA ($K_a = 1 \times 10^{-5}$) WITH 0.1 M NaOH

NaOH Added (cm^3)	Total Volume (cm^3)	pH	NaOH Added (cm^3)	Total Volume (cm^3)	pH

0.00	50.0	3.0
5.00	55.0	4.05
10.00	60.0	4.40
20.00	70.0	4.82
30.00	80.0	5.18
40.00	90.0	5.60
45.00	95.0	5.95
49.00	99.0	6.69
49.50	99.5	7.00
49.90	99.9	7.70
49.95	95.95	8.00
50.00*	100.0*	8.85*
50.05	100.05	9.70
50.10	100.1	10.00
50.50	100.5	10.70
51.00	101.0	11.00
55.00	105.0	11.68
60.00	110.0	11.96
70.00	120.0	12.23

* Equivalence point

Note that the first addition of base produces a significant rise in pH. This is followed by a region in which the pH changes only slightly. In this region, the solution is buffered by the presence of both weak acid and its salt. As addition of base continues, the acid concentration drops so much that the solution is no longer buffered. Now the pH rises rapidly through the neutralization or *equivalence point* and slightly beyond. Beyond this region, the acid has been neutralized and the pH of the solution changes only slightly as more base is added. The rise of pH beyond the equivalence point is due to the addition of base to a relatively large volume of solution.

The rapid change in pH near the equivalence point makes a quantitative titration of acid by base a feasible experiment. Note that 2 drops of base solution near the equivalence point (from 49.95 cm^3 of base to 50.05 cm^3) causes a change in pH of 1.70 units in this solution. Such a change in pH is sufficient to change the color of an indicator from its acid to its basic color, so that we can easily measure to 0.05 cm^3 in a 50 cm^3 addition, a precision of 1 part in 1000.

The rapid change near the equivalence point is the reason titrant must be added in smaller and smaller amounts as the equivalence point is approached.

Although the data in the table were obtained by calculation using Eq. (1), similar data can be obtained by measuring the pH of the solution during any similar titration. When plotted, it gives a curve similar to that in Fig. 1.

In this experiment, you will collect such data as you titrate a weak acid with a strong base. You will use the data to determine K_a for the acid and to select a suitable indicator for titrations involving this acid.

Calculation of K_a

Because the equilibrium

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

is re-established after each addition of base during a titration, a value of K_a could be obtained from the data corresponding to any point before the equivalence point on the titration curve. Using the data from two particular points - the first point on the curve and that at the half-neutralization point - makes the calculations easier.

The first point on the curve is the pH of the acid solution before any base is added. Knowing the concentration of the acid in the solution and the pH, you should be able to calculate K_a.

When the acid is exactly half neutralized, the pH of the solution is equal to the pK_a of the acid. Eq. (1)

$$pH = pK_a + \log\frac{[A^-]}{[HA]} \qquad (1)$$

shows why this is so. When half of the acid is neutralized (and present as A^-) and the other half is not neutralized (and present largely as HA), the concentrations $[A^-]$ and [HA] are equal. At this point the log ($[A^-]$ / [HA]) term in Eq. (1) becomes zero.

Eq. (1) then becomes

$pH_{1/2} = pK_a$.

There are several ways to identify the half-neutralization point. The easiest way is to select the point on the curve that corresponds to one half of the volume of base needed to reach the equivalence point. K_a of the acid can be obtained from the pH corresponding to this point by taking the antilog of -pH (the negative of the pH). Here is an example:

Suppose the pH at the half-equivalence point is 5.2.

Then $pH_{1/2} = pK_a = -\log K_a = 5.2$.

From this, $K_a = 10^{-5.2} = 10^{-6} \text{ x } 10^{0.8} = 10^{-6} \text{ x } 6.3$

or, $K_a = 6.3 \text{ x } 10^{-6}$.

Experimental Procedure

1. Calibrate the pH meter with known standard buffers, buffered at pH 4 and 7. (When not in use, always immerse the glass membrane electrode in distilled water. Never allow it to dehydrate.)

2. Pipette 25 cm^3 of acid into a 100-cm^3 beaker.

3. Titrate the sample with the pH electrode immersed in the solution. This can be done by adding an increment of NaOH, waiting for equilibrium (15 to 30 seconds), then reading and recording the pH of the solution. The size of the increment to be added will change during the course of the titration.

   Initially, rather large (3 to 4 cm^3) increments of base can be added. Near the equivalence point, rather small increments (3 to 5 drops) will be used. For each increment, the amount of base added should be that amount which will cause a change in pH of approximately 0.2 pH units. Read and record the volume of base added and the pH of the solution. You will observe sharp increases in pH as you approach this point. After the equivalence point, continue adding base, but in 3- to 4- cm^3 increments, until a pH reading of 12 is obtained.

Treatment of Data

Hand in the write-up of this experiment at the start of the next practical session. The marks are shown in brackets.

Record in the data table the volume of unknown acid used, the molarity of standard base used, and the burette reading and the pH after each addition of base.

1. Complete the data table by calculating for each increment from the burette readings the *total volume* of base added to that point in the titration. For example, if 3 cm^3 of base were added, then 3 more and then 3 more, the numbers in the second column would be 3, 6 and 9 cm^3.

   [1]

2. Prepare a graph with pH on the vertical axis (ordinate) and volume of base added on the horizontal axis (abscissa).

   [3]

3. Identify the equivalence point in the titration. This point can be approximated as the mid-point of the sharply increasing, nearly vertical, portion of the titration curve. [1]

More precisely, the equivalence point is the point at which the number of moles of base added equals the moles of acid originally present. On your titration curve, it is the point at which the slope changes from increasing to decreasing values.

4. Knowing the equivalence point, determine from your data the volume of base added to reach this point. This is the equivalence volume.
5. Using the equivalence volume and molarity of base used, calculate the concentration of acid in your unknown solution.
6. Determine K_a by the two methods described in the background section. Show your calculations. [2]
7. From your curve, select the pH color change range of indicators suitable for use in titrations of your unknown acid. From the table of indicators provided, select two or more indicators that could be used in titrating your unknown acid. 1]

Acid-Base Indicators

		Color of	
Name	**Range**	**Acidic Form**	**Basic Form**
Methyl Violet	0.0 – 1.6	Yellow	Blue
Crystal Violet	0.0 – 1.6	Yellow	Blue
Malachite Green	0.2 – 1.8	Yellow	Blue-green
Cresol Red[a]	0.4 – 1.8	Red	Yellow
Thymol Blue[a]	1.2 – 2.8	Red	Yellow
Methyl Yellow	2.8 – 4.0	Red	Yellow
Bromphenol Blue	3.0 – 4.6	Yellow	Purple
Methyl Orange	3.1 – 4.4	Red	Yellow
Bromcresol Green	3.8 – 5.4	Yellow	Blue

Methyl Red	4.4 – 6.2	Red	Yellow
Chlorophenol Red	4.8 – 6.4	Yellow	Red
Bromcresol Purple	5.2 – 6.8	Yellow	Purple
Alizarin	5.5 – 6.8	Colorless	Yellow
Bromthymol Blue	6.0 – 7.6	Yellow	Blue
Phenol Red	6.6 – 8.0	Yellow	Red
Neutral Red	6.8 – 8.0	Red	Yellow-brown
Cresol Red[b]	7.2 – 8.8	Yellow	Red
Cresol Purple	7.4 – 9.0	Yellow	Purple
Thymol Blue[b]	8.0 – 9.6	Yellow	Blue
Phenolphthalein	8.0 – 9.8	Colorless	Pink
o-Cresolphthalein	8.2 – 9.8	Colorless	Red
Thymolphthalein	9.3 – 10.5	Colorless	Blue
Alizarin Yellow R	10.2 – 12.0	Yellow	Violet

[a] The acid range of this diprotic indicator.

[b] The basic range of this diprotic indicator.

Experiment: Titration of An Antacid

Materials and Usage Directions

1. Materials:

Antacids Tables (various brands)

0.2 M HCl

0.2 M NaOH

methyl red indicator

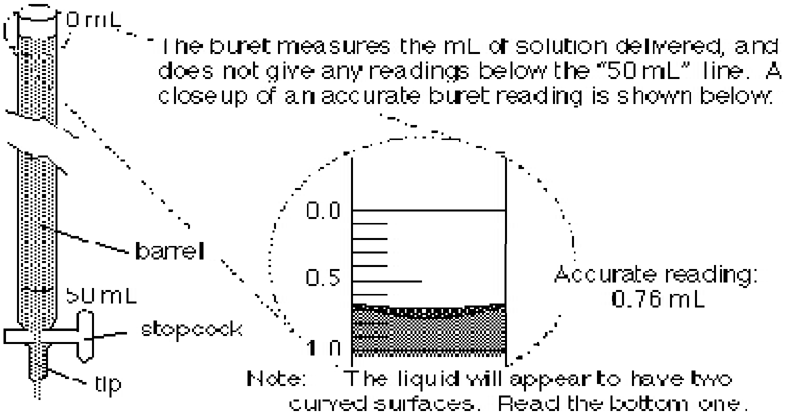
two 250-mL Erlenmeyer Flasks

two 50-mL burets

2. Buret Use and Titration Technique:

Typically, a special piece of glassware is used to measure out one or both of the solutions used in a titration. It is called a **buret**, and quickly and accurately measures the volume of the solutions delivered. A diagram of a buret, and instructions on how to use it are given below.

Before use, the buret must be rinsed with the solution it is to contain. Close the **stopcock** and use a small beaker to pour about 10 mL of solution into the buret. Tip the buret sideways and rotate it until all of the inside surfaces are coated with solution. Then open the stopcock and allow the remaining solution to run out. Again close the stopcock, and pour enough solution into the buret to fill it above the "0" mark. With the buret clamped in a vertical position, open the stopcock and allow the liquid level to drop to "0" or below. Check the buret tip. It should not contain air bubbles! If it does, see your instructor. Adjust the buret so that the liquid surface is at eye level, and take the initial buret reading as shown below:



After the buret is filled, and the initial reading taken, the solution from the buret is added to the solution to be titrated (which is normally in an Erlenmeyer flask). The solution in the flask also contains an indicator, which will change color when neutralization has occurred. At first, the solution from the buret is added rapidly and the flask swirled to mix the solutions. As the endpoint is approached (or you think it is being approached), the stopcock is partially closed to slow down the addition.

If the flask is not swirled during the addition of a few drops, the portion of solution surrounding the incoming drops will change color if the endpoint is near. If this happens, slow down the solution addition, and swirl the flask gently until the entire contents turn color. For accurate work, rinse down the inside surface of the flask with a stream of distilled. water near the endpoint. Half-drops of solution may be added by touching the side of the flask to the buret and rinsing down the flask.

Procedure

Overview:

A. Dissolving the antacid tablet. Since some brands of tablet do not dissolve in water, you will use a measured amount of "stomach acid" (actually about 0.2 M HCl) to dissolve the tablet. You will use more of this acid than can possibly be neutralized by the tablet, resulting in a mixture containing excess acid.

B. Neutralizing the excess acid. You will add base to the above mixture and measure the amount of base required to finish neutralizing the excess acid. The more base required in this step, the less effective was the antacid tablet at stomach acid neutralization.

Detailed Procedure:

A. Dissolving the antacid tablet.

1. Ground one antacid tablets with Porcelain Mortars & Pestles. Transfer all the grounded antacids into a weighed 250-mL flask. (**If tablet is of extra strength, transfer half the amount.**) Weigh the flask with the antacid and calculate the antacid weight.

2. Fill one 50 mL buret of them with 0.2 M HCl (Do follow the directions for buret use above first).

3. After recording the initial volume, allow approx. 45 mL of HCl to run into your 250 mL Erlenmeyer flask containing the grounded antacid. Do not allow the liquid level to drop below the 50 mL mark. **Leave the solution in the buret!**

4. Set the flask on a wire gauze supported by a ring, and heat until the solution boils. Continue boiling the solution for 5 minutes to remove any CO_2 gas that may be liberated from carbonates in the antacid. Cool the flask.

B. Neutralizing the excess acid.

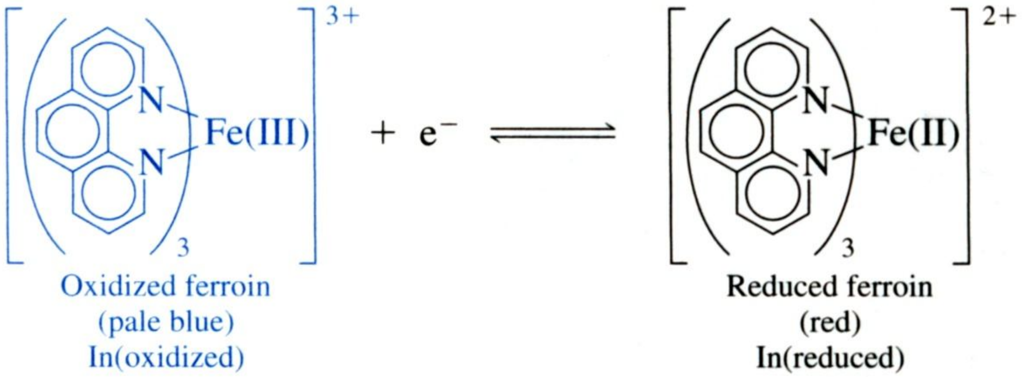
5. Fill a second buret with 0.2 M NaOH. Record the initial volume.

6. Add 5 drops methyl red indicator to the solution in the flask, and titrate it with the NaOH solution. Methy red is yellow if it is dissolved in a basic solution, and red in an acidic one. As the excess stomach acid is neutralized by the NaOH, the solution changes from red to yellow; the exact neutralization point is orange. If you add too much NaOH solution, you can add slightly more HCl from the first buret, and continue with NaOH addition until the endpoint is reached.

7. Read the final volumes on **both** burets. Repeat steps 1~7 one more time.

7. Redox Titration

Redox Indicator is a chemical compound that undergoes a color change as it goes from its oxidized form to its reduced form. Color Change for a Redox Indicator occurs over a potential range.Similar to acid-base indicators that change color with a change in protonation state



Color Change for a Redox Indicator occurs mostly over the range:

$$E = \left(E^{o} \pm \frac{0.05916}{n} \right) volts$$

Where E^{o} is the standard reduction potential for the indicator and *n* is the number of electrons involved in the reduction

Experiment : STANDARDISING SODIUM THIOSULPHATE SOLUTON

Iodine-thiosulphate titrations are examples of redox titrations. Iodine is reduced to the iodide ion by the thiosulphate ion. The half equations for this reaction are:

$I_2(aq) + 2e^- \longrightarrow 2I^-(aq)$ $2S_2O_3^{2-}(aq) \rightarrow S_4O_6^{2-}(aq) + 2e^-$

Overall reaction

$I_2(aq) + 2S_2O_3^{2-}(aq) \rightarrow 2I^-(aq) + S_4O_6^{2-}(aq)$

yellow colourless colourless colourless

Iodine-thiosulphate titrations are often used to determine the concentration of oxidising agents. The oxidising agent is reacted with an excess of potassium iodide, producing iodine. The iodine that is produced is then titrated against sodium thiosulphate, to find how much iodine was produced by the reaction of the oxidising agent with potassium iodide. Once the amount of iodine has been found, the amount of the original oxidising agent can be calculated.

In iodine-thiosulphate titrations, a standard solution of $Na_2S_2O_3$ is added to the iodine solution from a burette. In these circumstances the end-point would be a change from a pale yellow solution of iodine to a colourless solution of iodide ions, which does not give a clear end-point. In order to obtain a clear end-point, as it is approached (a pale yellow colour), starch solution is added. This forms an intense blue-black colour in the presence of iodine, even in low concentrations. When all the iodine has been reduced at the end-point, the solution turns colourless (although it may change back to blue due to the re-oxidation of iodide to iodine by air - the end-point is taken to be when the solution remains colourless for at least 30 seconds). The starch should not be added until the iodine solution is pale yellow, as the iodine would become strongly adsorbed onto the starch making the titration less accurate.

Method The equation for the reaction between iodate (V) and iodide is:

$$IO_3^-(aq) + 6\,H^+(aq) + 5\,I^-(aq) \text{ --> } 3\,I_2(aq) + 3\,H_2O(l)$$

1) The sodium thiosulphate has a concentration of about 0.1 M. Calculate the mass of potassium iodate needed to be dissolved in a 250 cm^3 standard solution to give a titre of around 25 cm^3. Check this value with your teacher.

2) Make up a 250 cm^3 standard solution of potassium iodate.

3) Pipette 25 cm^3 of the solution into a conical flask and add about 20 cm^3 of 1 M sulphuric acid and about 10 cm^3 of 0.5 M potassium iodide solution (both are large excesses).

4) Titrate the mixture against the sodium thiosulphate solution, using as starch as indicator near the end point.

5) Repeat until you have concordant results.

Analysis

6) Use your results to find the concentration of the sodium thiosulphate solution.

7) Calculate the maximum percentage apparatus error in the final result. Standard errors in apparatus are as follows:

balance	± 0.001 g
volumetric flask	± 0.1 cm^3
25 cm^3 pipette	± 0.1 cm^3
burette (start & end readings and end point)	± 0.15 cm^3

Evaluation

8) Comment on the reliability of your titration results.

9) Write down the mean class value for the concentration of the sodium thiosulphate (we shall assume that the class mean is the correct value).

10) Find the percentage difference between your value and the class mean value.

11) Comment on the size of your experimental error compared to the maximum apparatus error.

12) If your value is outside the apparatus error, suggest reasons why it is too high or too low (be specific, give reasons why it is too *high* or too *low*, not just why it is outside apparatus error). If your value is within

apparatus error, give reasons why somebody else's value may be too high.

13) Show that the volumes of sulphuric acid and potassium iodide added are excesses.

Iodometric Determination of Copper

INTRODUCTION

The object of this analysis is to determine the amount of copper in an ore sample (reported as the mass percentage of Cu) by an "iodometric" titration method. Like most analytical procedures based on redox chemistry, ***this experiment involves a lot of steps and some complex reactions that must be fully understood ahead of time***.

The ore sample is first dissolved in hot, concentrated HNO_3 which oxidizes Cu, yielding Cu^{2+} in solution. After some steps to make the solution less acidic, an excess of KI is added. This reduces Cu^{2+} to Cu^{+} in the form of insoluble CuI and, more importantly, produces I_3^- (which is just $I_2 + I^-$) in solution.

$$2\ Cu^{2+} + 5\ I^- \rightarrow 2\ CuI_{(s)} + I_3^-$$

The liberated I_3^- is then titrated with a standard sodium thiosulfate ($Na_2S_2O_3$) solution, reducing it back to I^-. Starch is used as an indicator because it forms an intense blue color with I_2 (actually, I_3^-). The end-point is the *disappearance* of the blue starch-iodine color.

$$I_3^- + 2\ S_2O_3^{2-} \rightarrow 3\ I^- + S_4O_6^{2-}$$

The amount of Cu in the ore sample is thus determined by the overall stoichiometry of these two reactions: moles Cu^{2+} = moles $S_2O_3^{2-}$.

The same titration procedure, with the starch-iodine indicator, is also used to standardize the $Na_2S_2O_3$. Primary standard KIO_3 is pre-treated with an excess of KI which again yields I_3^- in solution for subsequent titration with thiosulfate.

$$IO_3^- + 8\,I^- + 6\,H^+ \rightarrow 3\,I_3^- + 3\,H_2O$$

$$I_3^- + 2\,S_2O_3^{2-} \rightarrow 3\,I^- + S_4O_6^{2-}$$

Thus, the overall stoichiometry for the standardization procedure is that one mole of IO_3^- requires 6 moles of $S_2O_3^{2-}$.

UNKNOWN

The "copper ore" unknown will be a powdery solid containing Cu in various ionic forms, mainly CuO, along with other oxides that do not interfere in this Cu analysis.

REAGENTS and STANDARD SOLUTIONS

Preparation of 0.10 M sodium thiosulfate ($Na_2S_2O_3$). Boil 500 mL of DI water for 10-15 minutes. Cool to room temperature and add 12.5 g $Na_2S_2O_3 \cdot 5H_2O$ and ~ 0.05 g Na_2CO_3. Stir to dissolve the salts. Transfer to a clean bottle and store in a dark place. (Boiling the water is necessary to destroy micro-organisms which can metabolize the thiosulfate ion. A small amount of Na_2CO_3 is added in order to bring the pH to about 9.)

Preparation of standard 0.01 M KIO_3 solution. Dry ~ 1.2 g of pure KIO_3 at 110 ºC for about one hour. Weigh (to ± 0.1 mg) ~ 1.1 g of KIO_3 and quantitatively transfer it to a 500 mL volumetric flask. Dilute to the mark with DI water.

Preparation of starch indicator solution. Starch solutions should be freshly prepared and will be provided by the lab TA. Dissolve ~ 2 g of "soluble starch" per 100 mL of DI water. Boil the mixture gently, if necessary, to obtain a clear solution.

Standardization of 0.1 M sodium thiosulfate ($Na_2S_2O_3$). Pipet four 50.00 mL aliquots of the standard KIO_3 solution into 250 mL conical flasks. Proceed with each flask to completion of the titration before starting the next one. (Here the concern is to avoid error due to air oxidation of the I^- ion.) Add ~ 2 g of KI and stir to dissolve. Add ~ 2 mL of 6 M HCl and titrate at once with thiosulfate until the solution becomes pale yellow. Then add ~ 2 mL of starch indicator and titrate until the blue color disappears. (If the starch indicator is added too early, the I_3^- tends to form a kinetically stable complex with starch from which the I_3^- is released too slowly, resulting in endpoint detection problems.) Repeat the titration with each of the other KIO_3 samples. Determine the molarity of $Na_2S_2O_3$ to four significant figures, paying careful attention to the overall stoichiometry.

PROCEDURE -- Analysis of Cu Ore Unknown

1. Dry ~ 3.5 g unknown at 110 ºC for one hour. Weigh (to ± 0.1 mg) three 1-g samples and transfer each to a 250 mL beaker. Proceed separately with each sample as follows.

2. Add 5 mL of concentrated HNO_3 (***Caution***: *Handle concentrated acids carefully in the fume hood!*) and heat below boiling in the hood to dissolve the unknown. Cool and add 10 mL of concentrated H_2SO_4 and heat until yellow SO_3 gas is evolved, then cool. Cautiously add 30 mL of DI water, boil the solution for 1-2 minutes, then cool. Add concentrated ammonia dropwise until the deep blue color of the $Cu(NH_3)_4^{2+}$ complex ion appears. Then add 3 M H_2SO_4 to make the deep blue color just disappear. Finally, add 2 mL of 85% phosphoric acid and cool to room temperature. (This procedure adjusts the pH to a proper range.)

3. Each of the Cu^{2+} solutions should be completely analyzed before the next one. Add ~ 4 g of KI (to precipitate CuI) and titrate with standard $Na_2S_2O_3$ until the solution is pale yellow. Add ~ 2 mL of starch solution and continue the titration to a faint blue color. Add ~ 2 g of KSCN, stir for about 30 seconds, and then finish the titration to the disappearance of the blue color. (KSCN is used because I_2 and I_3^- tend to adsorb on the surface of solid CuI, thus becoming less available for reduction by the thiosulfate. Thus, iodometric titrations involving reduced copper tend to yield low results unless the adsorbed iodine is liberated by adding SCN^- which competes with the adsorbed iodine on the surface of CuI particles.)

4. Determine the percent Cu for each trial. Report the individual results, the mean, and the standard deviation.

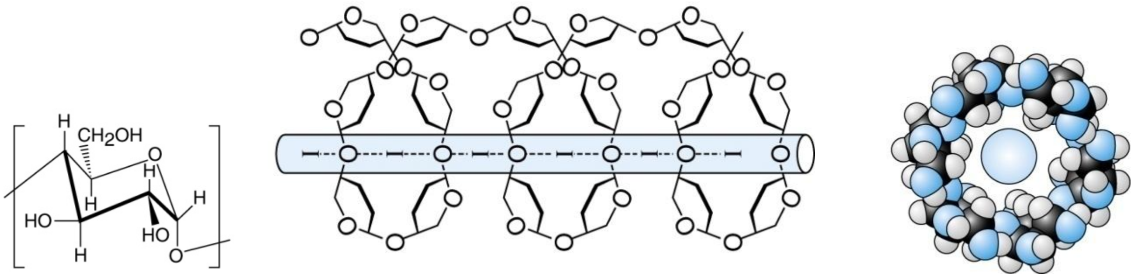
Iodometric Titrations

Molecular iodine (I_2) is only slightly soluble in water but adding iodide, I^-, produces the "triiodide" ion (I_3^-) in solution. Thus, KI is almost always added when redox reactions of I_2 are involved in quantitative analysis.

$$I_{2(s)} \quad + \quad I^-_{(aq)} \quad \rightarrow \quad I^-_{3\,(aq)}$$

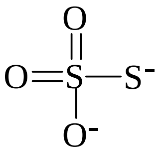
iodine ***iodide*** ***triiodide***

Starch is used as the ***indicator*** in most iodometric titrations because iodine (i.e., I_3^-) forms an intense blue colored "starch-iodine complex."



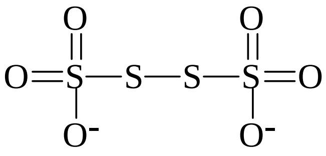
In "**iodometric**" titrations, the analyte is first reduced with an excess of I^-, producing I_2 (actually, I_3^-) which turns blue in the presence of starch. ***Back titration*** of the I_2 with a reducing agent like thiosulfate ($S_2O_3^{2-}$) permits the determination of the analyte. The end-point is the ***disappearance of the blue starch-iodine color***.

$$I_3^- \quad + \quad 2\,S_2O_3^{2-} \quad \rightarrow \quad 3\,I^- \quad + \quad S_4O_6^{2-}$$



$\mathrm{O{=}\underset{\underset{\displaystyle O^-}{|}}{\overset{\overset{\displaystyle O}{\|}}{S}}{-}S^-}$

thiosulfate



$\mathrm{O{=}\underset{\underset{\displaystyle O^-}{|}}{\overset{\overset{\displaystyle O}{\|}}{S}}{-}S{-}S{-}\underset{\underset{\displaystyle O^-}{|}}{\overset{\overset{\displaystyle O}{\|}}{S}}{=}O}$

tetrathionate

Experiment: To determine ascorbic acid (vitamin C) concentration by a redox titration with potassium iodate

Object: To determine vitamin C ($C_6H_8O_6$) by potassium iodate titration and to master iodometric titrations.

Introduction

A suitable method for the determination of vitamin C ($C_6H_8O_6$) quantities is a titration with potassium iodate (KIO_3). Potassium iodate is used as a titrant and it is added to an ascorbic acid solution that contains strong acid and potassium iodide (KI). Potassium iodate reacts with potassium iodide, liberating molecular iodine (I_2):

$$KIO_3 + 5KI + 6H^+ \rightarrow 3I_2 + 6K^+ + 3H_2O \qquad (1)$$

As long as the solution contains ascorbic acid, the iodine produced in (1) is used up in a rapid reaction with ascorbic acid, during which dehydroascorbic acid and iodide ion are formed:

$$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2I^- + 2H^+ \qquad (2)$$

Potassium iodide must be added in excess to keep iodine dissolved. Once all the ascorbic acid has been consumed, any excess iodine will remain in solution. Since aqueous iodine solutions are brown in colour, iodine can act as its own indicator. However, it is quite difficult to detect endpoints using iodine coloration alone, and it is more usual to add starch, which forms an intensely blue coloured complex with iodine but not with the iodide ion.

According to the above reactions, each mole of potassium iodate added corresponds to 3 moles of ascorbic acid dehydrogenated in the sample.

Procedure

1. Pipette 25 cm^3 of the provided ascorbic acid solution into a 250 cm^3 conical flask, add ≈ 4 cm^3 of 2M HCl, ≈ 5 cm^3 of potassium iodide (KI) solution and 3 cm^3 starch solution. Then titrate with the standard potassium iodate (KIO_3) solution (? M) until the solution changes from brown to intense blue. Write down the standard potassium iodate (KIO_3) solution volume.
2. Pipette 25 cm^3 of unknown ascorbic acid sample, a kind of juice, into a 250 cm^3 conical flask, then follow the same procedure of step 1 and write down the volume of the standard KIO_3 solution.

Repeat the steps 1 and 2 to check the precision of the experiment. Use the mean result of step 2 to determine the concentration (mol/cm^3) of ascorbic acid in the selected sample.

Note: The end point is reached when the solution turns a permanent, dark blue colour, due to the complex formed between starch and iodine. During an iodometric titration an intermediate dark blue iodine-starch complex may form momentarily, before the iodine reacts with ascorbic acid. However, if the colour disappears upon mixing, the end point has not yet been reached. Thus, magnetic stirrers or glass rod are employed in the titration to ensure proper mixing and to facilitate the reaction of iodine with ascorbic acid.

Report

1. Write up this experiment results in full (including the discussion) and hand in at the beginning of the next lab session. Marks awarded are shown in brackets.

2. Tabulate your results as follows:

	Standardization		***Unknown sample***	
	1^{st} Titration	***2^{nd} Titration***	***1^{st} Titration***	***2^{nd} Titration***
Final reading/ cm^3				
Initial reading/ cm^3				
Titre/ cm^3				
Mean titre ± / cm^3				

3. Comment upon the precision of the titrations.
4. Calculate the ascorbic acid concentration of the juice sample.
5. Briefly describe factors which influence the results from this titration.
6. What are the limitations of this method of determination of ascorbic acid?
7. Describe one other method for determining ascorbic acid in juice and decide whether it is more sensitive and more accurate than the method you have employed.

Chemicals employed in the experiment:

1). The standard KIO_3 solution with a suitable concentration of-- according to the concentration of ascorbic acid in the samples.

2). Ascorbic acid solution, 0.01136M. This concentration has been chosen since it gives you 10 mg ascorbic acid in each 5.00 cm^3 of the solution, which was taken as much of this solution as necessary to simulate the amount of ascorbic acid expected in the sample.

3). Potassium iodide is provided as a concentrated solution of KI in water. Use 5 cm^3 of KI solution for each titration.

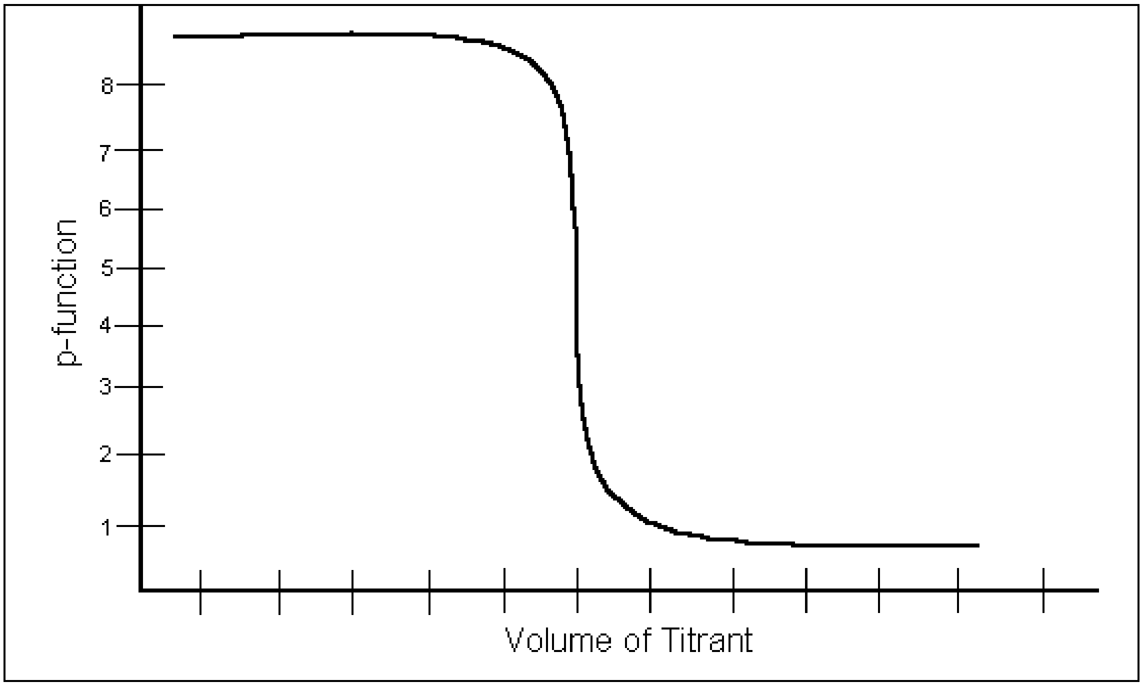
4). 2M HCl Use 4 cm^3 of the HCl solution in each titration. The addition of acid is necessary to provide the acidic conditions required in reaction (1) above.

5). Starch solution. Use 3 cm^3 of the starch solution in each titration.

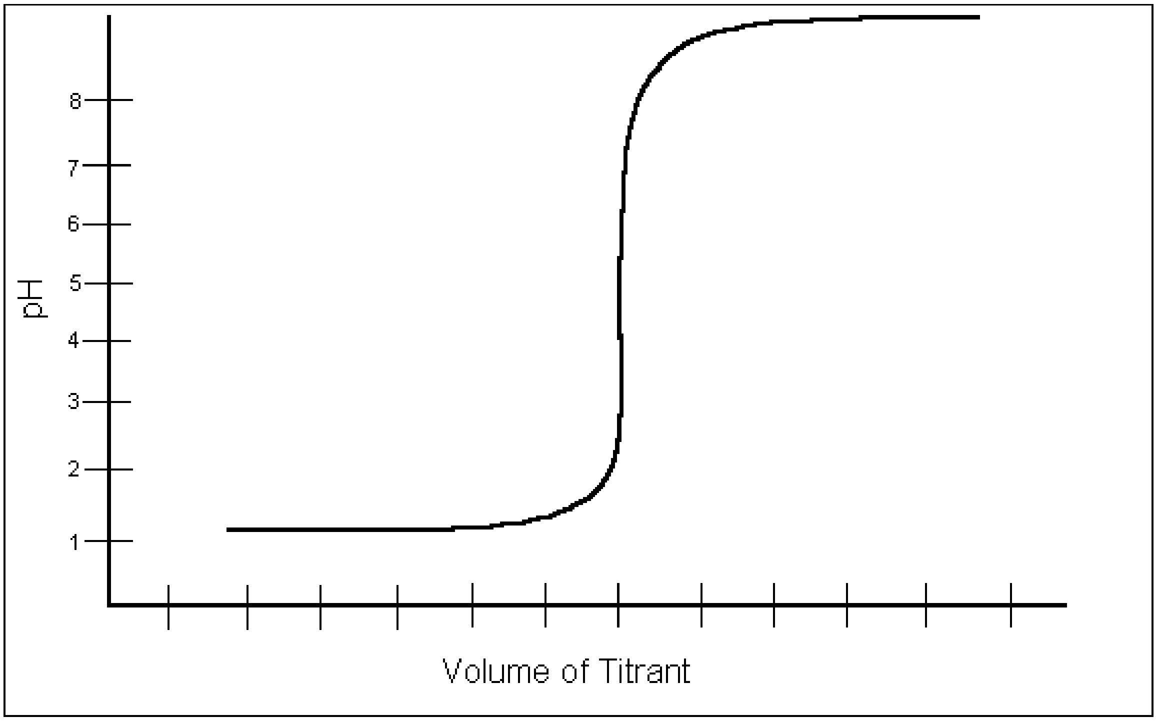
3. Precipitation Titration

Precipitation titrimetry, which is one of the oldest analytical techniques, is based on reaction that yield ionic compounds of limited solubility. The number of precipitating agents that can be used is limited because of the slow rate of formation of most precipitates. The most widely used precipitation reagent is silver nitrate, and precipitation titrimetry based on silver nitrate are known as argentometric methods.

Titration curves for precipitation reactions are very similar to those involving strong acids and strong bases. Consequently, they can be derived in the exact same way. One need only substitute the solubility product of the precipitate for the ion-product constant for the water. The following diagram illustrates such a titration curve.

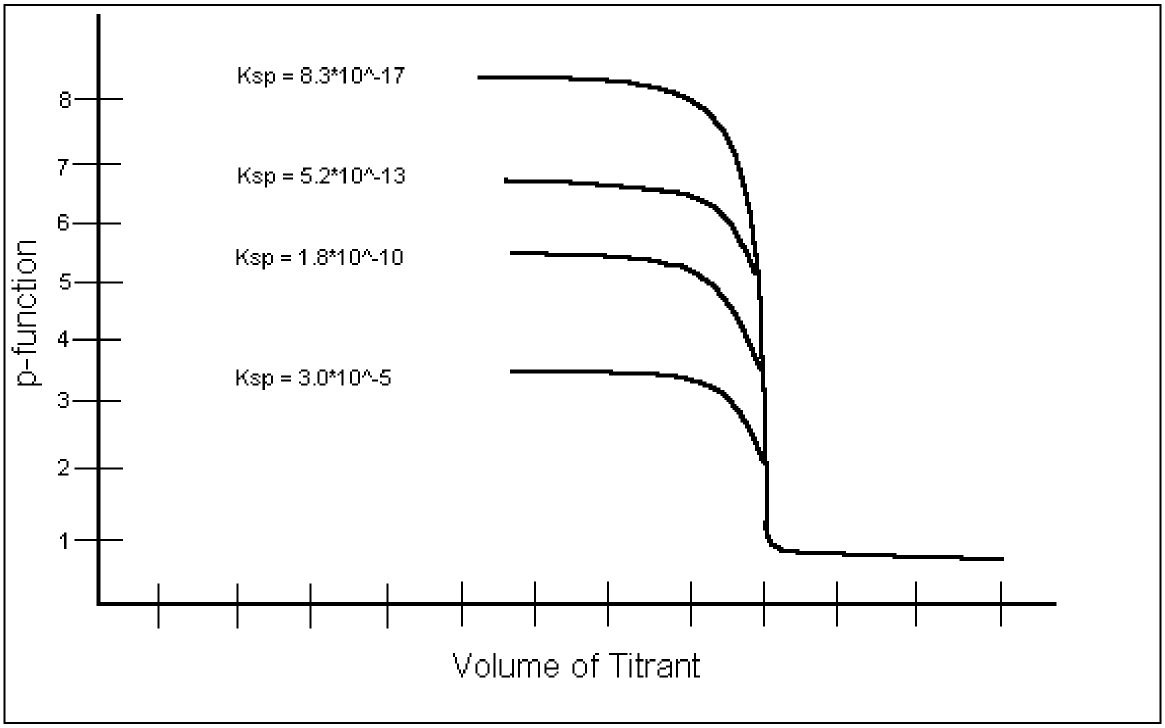


Clearly, one can see the resemblance of this curve to that of an acid/base titration shown below.



Both have a sharp inflection point. Furthermore, it is evident that at pre-equivalence and post-equivalence points, the p-function does not chance very much. At the equivalence point in contrast, a small volume of titrant corresponds to a large p-function change.

Figure 3 below shows the effect of product solubility on the sharpness of the endpoint. The change in the p-function at the equivalence point becomes greater as the solubility product becomes smaller. In other words Δ p-function increases as the reaction between the analyte and the silver nitrate becomes more complete.



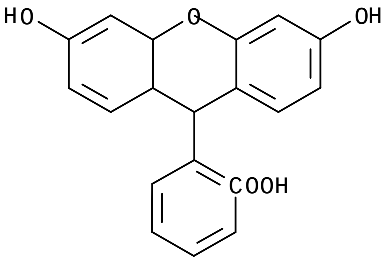
The endpoint produced by a chemical indicator usually consists of a color change. Once again, we see the relation between argentometric titrations and acid/base titrations, as the requirements for an indicator for the titrations are analogous. These requirements are namely: (1) the color change should occur over a limited range in the p-function of the reagent or the analyte, and (2) the color change should take place within the steep portion of the titration curve for the analyte. Three indicators that have widespread use in argentometric titrations are the Chromate ion, Flourescein and Iron(III) ion.

The chromate ion is employed as an indicator in the Mohr method. Sodium chromate can serve as an indicator for the argentometric titrations of chlorine, bromine and cyanide ions, by reacting with silver to form silver chromate (Ag_2CrO_4). The brick red Ag_2CrO_4 precipitates at the equivalence point region and thus is a useful indicator. The Mohr method has an inherent systematic error that is correctable. At reagent concentrations lower than .1M, the chromate ion imparts an intense yellow color to the solution that hinders the identification of the red silver chromate. As a consequence, excess silver nitrate is required before precipitation can be detected and as a result, the volume is skewed. The correction that is often made to account for this error is making a blank titration of a chloride free suspension of calcium carbonate. This volume can then be used to adjust the experimental results. The Mohr method will follow these reactions:

$$\mathbf{Ag^+ + Cl^- \rightleftharpoons AgCl_{(s)}\quad white}$$

$$\mathbf{2Ag^+ + CrO_4^{-2} \rightleftharpoons Ag_2CrO_{4\,(s)}\quad red}$$

The second popular indicator is Flourescein, an adsorption indicator used in the Fajans method. An absorption indicator is an organic compound that tends to be absorbed onto the surface of the solid in a precipitation titration. In the ideal scenario, the absorption occurs near the equivalence point and results in a color change. Flourescein is a widely used absorption indicator.



In aqueous solution partial dissociation into H_3O and the negatively charged Flourescein ion. The Flourescein forms a bright red silver salt. These types of indicators give a rapid, accurate and reliable titration endpoint.

The third indicator used is Iron(III) ion, used in the Volhard method. In this method, silver ions are titrated with a standard solution of thiocyanate ion, where Fe^{3+} serves as the indicator imparting a red color to the solution. This titration must be carried out in an acidic solution lest the Fe(III) precipitates out as a hydrated oxide. The reaction that accompany the Volhard method are:

$$\mathbf{Ag^{+} + Cl^{-} \rightleftarrows AgCl_{(s)}\ \ white}$$

$$\mathbf{SCN^{-} + Ag^{+} \rightleftarrows AgSCN_{(s)}\ \ white}$$

$$\mathbf{Fe^{3+} + SCN^- \rightleftharpoons Fe(SCN)^{2+}}\quad \textbf{red}$$

The most important application of the Volhard method is for determing the presence of halide ions. An excess of silver nitrate is added to the sample and back-titrated with a standard thiocyanate solution.

EXPERIMENT: THE VOLUMETRIC DETERMINATION OF CHLORIDE

Precipitate-forming titrations are not common. However, they are widely used for determination of the halides, chloride (Cl^-), bromide (Br^-) and iodide (I^-), using silver nitrate ($AgNO_3$) as the titrant. In this part of the experiment, chloride will be determined by titration with silver nitrate using dichlorofluorescein as an indicator. This is commonly known as Fajan's Method.

THEORY

Chloride present in a sample is quantitatively insoluble in a solution containing excess of silver ion:

$$Ag^+(aq) + Cl^- (aq) \rightleftarrows AgCl(s) \qquad K_{sp} = 1.82 \times 10^{-10}$$

The same determination may be accomplished volumetrically if a standard solution of Ag^+ is available. The end point of the reaction may be determined through the use of an adsorption indicator, dichlorofluorescein. Its function may be described as follows:

If the reaction is run in neutral or basic solution some of the indicator will dissolve to form the dichlorofluorescinate anion, which is represented as In^-. Before the equivalence point, with Ag^+ as titrant, excess Cl^- is present in solution. The excess Cl^- is adsorbed onto the precipitate particles formed and the indicator anion is repelled by the negatively-charged precipitate.

$$Ag^+(aq) + 2Cl^-(aq) \rightleftarrows AgCl{:}Cl^-(s)$$

At the equivalence point, there is little or no excess Cl^-, and just beyond the equivalence point Ag^+ is in excess and becomes the primary adsorbed ion. The charge on the precipitate changes from negative to positive and the indicator anion is adsorbed.

$AgCl{:}Cl^-(s) + Ag^+(aq) \rightleftarrows AgCl{:}Ag^+(s) + Cl^-(aq)$

$AgCl{:}Ag^+(s) + In^-(aq) \rightleftarrows AgCl{:}Ag^+In^-(s)$

yellow rose-pink

The color change is:

yellow → rose-pink

It is believed that the indicator anion (yellow) forms a complex ion with Ag^+, adsorbed on the silver chloride precipitate, which alters its light-absorbing properties, and hence its color. The indicator function is critically dependent on the availability of a large precipitate surface area to allow adsorption. The greatest surface area results from a precipitate comprised of very small particles (colloidal). Stabilization of these colloidal particles (recall that a colloid has a very high surface-to-volume ratio) is accomplished by adding a protective colloid, such as dextrin.

It is important that the titration be conducted quickly in diffuse light as photodecomposition of the silver chloride renders the solution purple, making it difficult to discern the pale pink end point signal from the purple background.

PROCEDURE

Preparation of a Standard Silver Nitrate Solution

Obtain from the instructor approximately 8.5 g of $AgNO_3$ in a **clean, dry,** weighing bottle. Grind the contents to a fine powder with an agate mortar and pestle. Return the $AgNO_3$ to the weighing bottle, dry in a oven at medium power, and accurately weigh, by difference, the contents into a 500-mL volumetric flask. Dissolve with deionized water and dilute to the mark. Store in a dark area and calculate the molarity of the solution.

Preparation of the Sample for Analysis

Using the unknown chloride sample from part 1, accurately weigh three samples of the unknown into three 250-mL Erlenmeyer flasks (the sample size should be approximately 0.3 g). Add approximately 50 mL of deionized water to each flask and swirl to dissolve. Add 10 mL of a 2% dextrin suspension, 5 drops of dichlorofluorescein indicator, and titrate each sample to the rose-pink end point.

CALCULATION OF RESULTS

Calculate your results as the % Cl^- in your sample. Be certain to report each individual value, the mean value, the absolute deviation from the mean of each value, the relative average deviation in parts per thousand, the standard deviation and the confidence interval at an appropriate confidence level.

4. Complex metric titration

The technique involves titrating metal ions with a complexing agent or chelating agent (Ligand) and is commonly referred to as complexometric titration. This method represents the analytical application of a complexation reaction. In this method, a simple ion is transformed into a complex ion and the equivalence point is determined by using metal indicators or electrometrically. Various other names such as **chilometric titrations**, chilometry, chilatometric titrations and EDTA titrations have been used to describe this method. All these terms refer to same analytical method and they have resulted from the use of EDTA (Ethylene diamine tetra acetic acid) and other chilons. These chilons react with metal ions to form a special type of complex known as **chelate**.

Objective

1. To perform water analysis regarding the presence of chloride, sulphate, and ammonium ions.
2. To measure water hardness caused by dissolved calcium and magnesium ions by complexometric titration

Equipment

- Beaker
- Test tube
- pH paper

- Erlenmeyer flask
- Burette
- Stands
- Pipette

Chemicals and Materials

- Aquadest
- Tap water
- $AgNO_3$ (silver nitrate)
- $BaCl_2$ (barium chloride)
- NaOH (sodium hydroxide)
- 1 M Ammonium buffer (1M NH_4OH)+ 1 M NH_4Cl)
- $5x10^{-3}$ M EDTA solution (ethylenediaminetetraacetic acid)
- Eriochrome Black T

Procedure

2 beakers were prepared and filled with the following fluid:

- Beaker 1: 20 ml distilled water
- Beaker 2: 20 ml tap water

2. The appearance and smell of each sample were checked and the pH values were measured using pH paper.
3. A few drops of 1 n $AgNO_3$ were put into each beaker.
4. Observation was done to see whether precipitation occurred. When precipitation occurred, chloride ions were present.

5. Some crystals of $BaCl_2$ were dissolved in 5 ml of distilled water.
6. Another set of beakers were prepared and filled with appropriate fluids
7. A few drops of $BaCl_2$ solution were put into each beaker.
8. Observation was done to see whether precipitation occurred. When precipitation occurred, sulphate ions were present.
9. 2 test tubes were prepared and filled with the following fluid:
    - Test tube 1: distilled water
    - Test tube 2: tap water
10. One flake of NaOH was put inside each test tube.
11. The appearance and smell (pungent smell of ammonia) were observed for presence of ammonium chloride.

Measurement of water hardness with EDTA titration:

1. The 1500 ml of 5 x 10^{-4} EDTA solution was prepared by mixed the 50 ml of 5 x 10^{-3} M EDTA with solution of 50 ml NH_4OH 1 M and 50 ml NH_4Cl.
2. The 25 ml tap water was put into an Erlenmeyer flask and it was added with 20 drops of ammonia buffer and 10 drops of Eriochrome Black T indicator. This solution color was red purplish.
3. The burette was clamped to the ring stand and the EDTA solution was put into the burette until it reached 50 ml.
4. Then, the solution in the Erlenmeyer flask was titrated with EDTA solution until its color reached blue color.

5. The total volume of EDTA solution that being used to titrate the solution in the Erlenmeyer flask was recorded.
6. The second procedure until fifth procedure was repeated once.

I. Observation and Measurement Result

Chemical check of water

CHARACTERISTIC	DISTILLED WATER	TAP WATER
Appearance	Transparent	Transparent
Smell	No smell	No smell
pH	5.5	6.5

Table 1: Characteristics of distilled and tap water

CONDITION	DISTILLED WATER	TAP WATER
Addition of $AgNO_3$	Colorless, no precipitation	Turbid
Addition of $BaCl_2$	Colorless, no	Colorless, no

	precipitation	precipitation
Addition of NaOH	Warm, air bubbles form a bit, no smell	Warm, air bubbles form a bit, no smell

Table 2: The reactions of each type of water after addition of compounds.

Measurement of water hardness with EDTA titration:

Distilled water

After it was added with the Eriochrome Black T Indicator, the solution color turned to dark blue.

Tap water

After it was added with the Eriochrome Black T Indicator, the solution color turned to purplish red. After it was titrated with EDTA, the solution color turned to blue.

The volume of EDTA used in the first titration = 16.6 ml.

The volume of EDTA used in the second titration = 15.7 ml.

Figure: end point of titration

II. Evaluation and Calculation

Chemical check of water

In this experiment, tap water and distilled water was checked to observe the chemical compound contained in each of the water. Unfortunately, sample water from the river was not given for checking. Therefore, not much comparison could be made.

Distilled water should only contain pure water (hydrogen and oxygen molecules) with no addition of gasses, metal ions, and other compounds. This was because distilled water was made through the process of distillation which purifies the water from contamination. The appearance of distilled water is transparent and no smell was detected. This was because there were no contaminants that give out odor.

The tap water observed in our experiment was taken from the laboratory. The appearance of tap water was transparent and may contain minerals, disinfectant, and contaminants. Chloride and sulphate ions may also be present in tap water. However, the substances contained in tap water were odorless. Therefore, no smell was detected. The tap water’s pH is 6.5.

When each of the water samples were added with $AgNO_3$, the $AgNO_3$ ions dissociates:

$$AgNO_{3(aq)} \rightarrow Ag^{+}_{(aq)} + NO_3^{-}{}_{(aq)}$$

Then, the Ag^+ ions reacted in the presence of Cl^- ions:

$$Ag^{+}_{(aq)} + Cl^{-}_{(aq)} \rightarrow AgCl_{(s)}$$

AgCl is insoluble in water and it precipitated to become solid. The color of AgCl solid is white. When $AgNO_3$ was put into distilled water, no precipitate was found. However, when it was put into tap water, the water became turbid. This shows that the tap water contains chloride ions.

When each of the water samples were added with $BaCl_2$, the ions dissociates:

$$BaCl_{2(aq)} \rightarrow Ba^{2+}_{(aq)} + 2Cl^{-}_{(aq)}$$

Then, the Ba^{2+} ions reacted in the presence of SO_4^{2-} ions:

$$Ba^{2+}{}_{(aq)} + SO_4^{2-}{}_{(aq)} \rightarrow BaSO_{4(s)}$$

$BaSO_4$ is insoluble in water and it precipitated to become solid. The color of $BaSO_4$ solid is white. When $BaCl_2$ was put into distilled water and tap water, no precipitate was found. This shows that the tap water and distilled water does not contain sulphate ions.

When each of the water samples were added with NaOH, the ions dissociates:

$$NaOH_{(s)} \rightarrow Na^{+}_{(aq)} + OH^{-}_{(aq)}$$

Then, the OH^{-} ions reacted in the presence of NH_4^{+} ions:

$$NH_4^{+}{}_{(aq)} + OH^{-}{}_{(aq)} \rightarrow NH_4OH_{(aq)}$$

$$NH_4OH_{(aq)} \rightarrow NH_{3(g)} + H_2O_{(l)}$$

NH_3 is a gaseous compound and can be detected by its pungent smell. After the addition of NaOH to both samples, the test tube becomes warm. This shows that the reaction is exothermic. Also, water bubbles were observed at both test tubes. However, the smell of ammonia was not detected. This shows that ammonia gas may formed but the amount is too small to be detected. The formation of ammonia gas shows that ammonium chloride is present.

Measurement of water hardness with EDTA titration:

Water hardness is an expression for the sum of the calcium and magnesium cation concentration in a water sample. An excellent way to determine water hardness is to perform a complexometric titration using a standard ethylenediaminetetraacetic acid (EDTA) solution. EDTA is a chelating agent that can donate electrons (Lewis base) thereby forming a complex with metal ions (Lewis acid). The EDTA will complex first with the Ca^{2+} and then with the Mg^{2+} in a one-to-one molar ratio. But, in this experiment, it was assumed that only Ca ions contribute to the water hardness.

In this experiment, 20 drops of ammonia buffer and 10 drops of Eriochrome Black T indicator were added into 25 ml of tap water in Erlenmeyer flask. Then the tap water was titrated with EDTA solution two times to get the average volume of EDTA solution used. Ammonia buffer was used to adjust and keep the pH to 9.9-10.1. It is necessary to keep the pH at about 10 for two reasons. First, all reactions between metal ions and EDTA are pH dependent, and for divalent ions, solutions must be kept basic (and buffered) for the reaction to go to completion. Second, the Eriochrome black T indicator requires a pH of 8 to 10 for the desired color change.

Since both of EDTA solution and Ca ions are colorless, it is necessary to use Eriochrome black T indicator to detect the end point of titration. Eriochrome black T indicator (denoted as In) is a metal ion indicator whose color changes when it binds to a metal ion. When the indicator was added into tap water, the solution color changed from colorless into purplish red. It means the tap water which used in the experiment contained Ca ions. The reaction occurred:

$Ca^{2+} + In^{2-} \rightarrow CaIn$

(blue) (wine red)……………………equation 1

Then the solution in Erlenmeyer flask was titrated with the EDTA solution. When EDTA solution was added drops by drops into Erlenmeyer flask, the indicator must give up its metal ions, Ca^{2+} to the EDTA because EDTA can bind to the metal ions stronger than the indicator. When all metal ions bind with EDTA, the indicator is in its free form and it doesn't bind to any metal. Then the solution color will turn to blue color which meant the end point was reached. The reaction occurred:

$CaIn + EDTA^{4-} \rightarrow CaEDTA^{2-} + In^{2-}$

(wine red)

(blue)………………….equation 2

The calculation of the concentration of water hardness from tap water

The average volume of EDTA $= \frac{16.6\ ml + 15.7\ ml}{2}$

= 16.15 ml

= 16.15 x 10-3 l

n EDTA = M EDTA x V EDTA

= 5 x 10-3 M x 16.15 x 10-3 l

= 80.75 x 10-7 mol

1 mol EDTA = 1 mol Ca

80.75 x 10-7 mol EDTA = 80.75 x 10-7 mol Ca

n Ca = 80.75 x 10-7 mol

gr Ca = n Ca x Ar Ca

= 80.75 x 10-7 mol x 40.078

= 3.24 x 10-4 gr

= 0.324 mg

[Ca] $= \frac{0.324\ mg}{25.10\text{-}3\ l}$

= 12.96 mg/l

= 12.96 ppm

In this experiment, the sample water was not given so there are no much comparison could be made with the tap water. The distilled water did not need to be titrated with EDTA because when it was added with Eriochrome Black T Indicator the solution colour was dark blue. It means there were no metal ions in distilled water such as Ca2+ and Mg2+ which can bind to the indicator to give red color.

The [Ca] in tap water in this experiment were 12.96 ppm. Base on the classification of water hardness from Water Quality Association on www.ci.farmington.mn.us, it was said that between the 0 – 17.1 ppm, the water hardness can be categorized as soft so the tap water and distilled water used in this experiment could be categorized as soft.

III. Conclusion

- The distilled water sample did not contain Cl^- and SO_4^- ions and may also contain ammonium chloride.
- The tap water sample did contain Cl^- ions but did not contain SO_4^- ions. Ammonium chloride may be present.
- Distilled water did not contain any ions and can be considered as soft water.
- The concentrations of Ca ions in tap water were 12.96 ppm.
- Tap water used in this experiment is considered to be soft water.

5. Nonaqueous Titration

INTRODUCTION:

Non- aqueous titrations are those in which the titrations of too weakly acidic or basic substances are carried out using non –aqueous solvents so as to get sharp end point. Such titrations can also be used for the titration of the substances not soluble in water. The speed, precision and accuracy of the non –aqueous method are close to those of classical acidimetric and alkalimetric titrations. The apparatus involved are also same but moisture and carbon dioxide are to be avoided in non –aqueous methods because water, which is a weak base, can compete with the weak nitrogen base and the end point would not be sharp at all. It has been observed through experiments that the moisture content in non –aqueous titrations should not be more than 0.05%.Further, the temperatures during standardization in non –aqueous titrimetry should not be allowed.

TYPES OF SOLVENTS:

- Aprotic solvents
- Protogenic solvents
- Protophilic solvents
- Amphiprotic solvents
- Levelling solvents

1. Aprotic Solvents: Aprotic solvents include those substances, which may be considered chemically neutral, and virtually un-reactive under the conditions employed. Carbon tetrachloride and toluene come in this group; they possess low dielectric constants, do not cause ionization in solutes and do not undergo reactions with acids and bases. Aprotic solvents are frequently used to dilute reaction mixture.

2. Protophilic Solvents: Protophilic solvents are the substances that possess a high affinity for protons. The over all reaction can be represented as: -

$HB+S <-> SH^+ + B^-$

The equilibrium in this reversible reaction will be generally influenced by the nature of the acid and the solvent. Weak acids are normally used in the presence of strongly protophilic solvents as their acidic strengths are then enhanced and then become comparable to these of strong acids; this is known as the ***levelling effect.***

3. Protogenic Solvents: Protogenic solvents are acidic in nature and readily donate protons. Anhydrous acids such as hydrogen fluoride and sulphuric acid fall in this category, because of their strength and ability to donate protons, they enhance the strength of weak bases.

4. Amphiprotic Solvents : Amphiprotic solvents consist of liquids, such as water, alcohols and weak organic acids, which are slightly ionized and combine both protogenic and protophillic properties in being able to donate protons and accept protons ethanoic acid displays acidic properties in dissociating to produce protons:

$CH_3COOH <-> CH_3COO^- + H^+$

But in the presence of perchloric acid, a far stronger acid, it will accept a proton:

$CH_3COOH + HClO_4 \leftrightarrow CH_3COOH_2^+ + ClO_4^-$

The $CH_3COOH_2^+$ ion can very readily give up its proton to react with a base, so basic properties of a base is enhanced, so titrations between weak base and perchloric acid can often be accurately carried out using ethanoic acid as solvent.

6. **Levelling Solvents:** In general, strongly protophilic solvents are important to force equilibrium equation to the right. This effect is so powerful that, in strongly protophillic solvents, all acids act as of similar strength. The converse occurs with strongly protogenic solvents, which cause all bases to act as they were of similar strength. Solvents, which act in this way, are known as ***Levelling Solvents.***

INTERFERENCE DUE TO WATER IN NON –AQUEOUS TITRATIONS:

1. When a weakly basic drug is present, water (OH^-) acts as stronger base as compared to the former one and preferentially accepts proton from an acid. Thus there is interference in the reaction of weak base with an acid.
2. Similarly when a weakly acidic drug is present, water (H^+) behaves like a strong acid as compared to the former one and preferentially donates proton to the base. Thus there is interference in the reaction of weak acid with a base.
3. Hence in the presence of water, titration of either weakly acidic substances with stronger base or weakly basic substances with stronger acid is not possible.

BASIC CONCEPT OF NON –AQUEOUS TITRATIONS: The Bronsted Lowery theory of acid and bases can be applied equally well to reactions occurring during acid base titrations in non-aqueous solvents. This is because this approach considers an acid as any substance, which will tend to donate a proton, and a base as any substance, which will accept a proton. Substances which give poor end points due to being weak acids or bases in aqueous solution will frequently give far more satisfactory end point when titrations are carried out in non-aqueous media. An additional advantage is that many substances, which are insoluble in water, are sufficiently soluble in organic solvents to permit their titrations in these non-aqueous media. In the Bronsted Lowery theory, any acid, (HB) is considered to dissociate in solution to give a proton

(H^+) and a conjugate base (B^-):- where as any base (B) will combine with a proton to produce a conjugate acid (HB^+):

$HB \leftrightarrow H^+ + B^-$

$B + H^+ \leftrightarrow HB^+$

The ability of substances to act as acids or bases will very much depend on the choice of solvent system.

ADVANTAGES OF NON AQUEOUS SOLVENT OVER AQUEOUS SOLVENT:

1) Organic acids and bases that are insoluble in water are soluble in non-aqueous solvent.

2) Organic acid, which is of comparable strength to water, can be titrated easily in non-aqueous solvent. Bases also follow the same rules.

3) A non-aqueous solvent may help two are more acids in mixture. The individual acid can give separate end point in different solvent.

4) By the proper choice of the solvents or indicator, the biological ingredients of a substance whether acidic or basic can be selectively titrated.

5) Non aqueous titrations are simple and accurate, examples of non aqueous titration are: Ephedrine preparations, codeine phosphate in APC, tetracycline, teramycin, Antihistamines and various piprazine preparations.

SOME EXAMPLES OF NON-AQUEOUS SOLVENTS:

***Glacial Ethanoic Acid*:** Glacial ethanoic acid is the most frequently used non-aqueous solvent. Before it is used it is advisable to check the water content. This may be between 0.1% and 1.0%.

Dimethylformamide: Dimethylformamide (DMF) is a protophillic solvent, which is frequently employed for titrations between, for instance, benzoic acid and amides, although end points may sometimes be difficult to obtain.

Acetonitrile: Acetonitrile (methyl cyanide, cyanomethane) is frequently used with other solvents such as chloroform and phenol and especially with ethanoic acid. It enables very sharp end points to be obtained in the titration of metal ethanoates when titrated with perchloric acid.

***Dioxane*:** Dioxane is another popular solvent, which is often used in place of glacial ethanoic acid when mixtures of substances are to be quantified. Unlike ethanoic acid, dioxane is not a levelling solvent and separate end points are normally possible, corresponding to the individual components in the mixtures.

Alcohol: Salts of organic acids, especially of soaps are best determined in mixtures of glycols and alcohols or mixtures of glycols and hydrocarbons. The most common combinations are ethylene glycol (dihydroxyethane) with propan-2-ol or butan-1-ol. The combinations provide admirable solvents for both the polar and non-polar ends of the molecules.

APPLICATIONS OF NON –AQUEOUS TITRATIONS

Although indicators may be used to establish individual end points, as in traditional acid-base titrations, potentiometric methods of end point detection are also used extensively, especially for highly coloured solutions. Non aqueous titration have been used to quantify the mixtures of primary, secondary and tertiary amines, for studying sulphonamides, mixture of purines and for many other organic amino compounds and salts of organic acid. And also it is used for the titration of Halogen acid salts of weak bases.

EXPERIMENT: TITRATIONS OF WEAK BASES WITH PERCHLORIC ACID

***Principle*:** weak bases are dissolved in acetic acid and are titrated with acetous perchloric acid. The various reactions which occur are given as follow: Acetic acid alone behaves as a weak acid, because of **poor dissociation** into H^+

$CH_3COOH \leftrightarrow CH_3COO^- + H^+$

But when a strong acid (Perchloric acid) is added to acetic acid, there is formation of **Onium ions,** which has more tendency to donate protons.

$HClO_4 <-> H^+ + ClO_4^-$

$CH_3COOH + H^+ <-> CH_3COOH_2^+$

(Onium ion)

When weak bases like pyridine are dissolved in acetic acid, equivalent amount of acetates ions are produced which have more tendency to accept protons.

$C_5H_5N + CH_3COOH <-> C_5H_5NH^+ + CH_3COO^-$

Ultimately, the titration of weakly basic drug in acetic acid against acetous perchloric acid yields accurate end point. The series of reactions are given as follows:

$HClO_4 + CH_3COOH -> CH_3COOH_2^+ + ClO_4^-$

$C_5H_5N + CH_3COOH -> C_5H_5NH^+ + CH_3COO^-$

(Acetate ions)

$CH_3COOH_2 + CH_3COO^- -> 2CH_3COOH$

(Burette) (Conical flask)

The net reaction is given as

$\mathbf{HClO_4 + C_5H_5N \rightarrow C_5H_5NH^+ + ClO_4^-}$

Thus we have seen that, on one hand ***the tendency of acid to donate proton is increased*** and on the other hand, ***the tendency of base to accept proton is increased*****.** These leads to the sharp end point in non –aqueous titrations.

Table: Acidimetric Assays: Non-aqueous Titrations with Perchloric Acid using Mercuric Acetate and different Indicators:

S.No.	Name of Substance	Indicator Employed
1.	Amantadine hydrochloride	Crystal violet
2.	Chlorpromazine hydrochloride	Methyl orange
3.	Clonidine hydrochloride	α -Naphthol benzein
4.	Cyproheptadiene.HCl	Crystal violet
5.	Dehydroemetine.HCl	-do-
6.	Ephedrine hydrochloride	-do-
7.	Imipramine hydrochloride	-do-
8.	Isoprenaline hydrochloride	Crystal violet
9.	Lignocaine hydrochloride	-do-
10.	Morphine hydrochloride	-do-
11.	Morphine sulphate	-do-
12.	Phenylephrine hydrochloride	-do-
13.	Phenytoin sodium	α -Naphthol benzein
14.	Promethazine hydrochloride	Methyl orange
15.	Thiabendazole	Crystal violet

REQUIREMENTS FOR THE TITRATION OF WEAK BASES WITH PERCHLORIC ACID Following points should be considered:

1. Titrant used.
2. Preparation of 0.1N ($HClO_4$) and it standardization.
3. Solvent used.
4. Practical examples of Indicators.

***Titrant used*:** Solution of $HClO_4$ in either glacial acetic acid or dioxane solution is used for titration of weak bases. Generally $HClO_4$ with a normality of 0.1N to 0.05N is used.

***Preparation of 0.1N solution of $HClO_4$ and its standardization*:** Dissolve 8.5 ml of 72% $HClO_4$ in about 900 ml glacial acetic acid with constant stirring, add about 30 ml acetic anhydride and make up the volume (1000 ml) with glacial acetic acid and keep the mixture for 24 hour. Acetic anhydride absorbed all the water from $HClO_4$ and glacial acetic acid and renders the solution virtually anhydrous. $HClO_4$ must be well diluted with glacial acetic acid before adding acetic anhydride because reaction between $HClO_4$ and acetic anhydride results in the formation of acetyl perchlorate which is an explosive.

Standardization of 0.1N Perchloric acid: Weigh accurately 0.7g of potassium hydrogen phthalate (primary standard), previously powdered light and dried for 2 hours and dissolve it in 50 ml of glacial acetic acid. Add few drops of crystal violet solution as indicator and titrate with perchloric acid solution until the violet colour changes to emerald green. Perform a blank titration by using 50 ml of glacial acetic acid and subtract the volume of perchloric acid consumed (crystal violet -0.5% w/v in acetic acid).

Each ml of 0.1N Perchloric acid ≡ 0.02042 g of Potassium hydrogen Phthalate

Strength of 0.1N Perchloric acid = wt. of potassium hydrogen phthalate taken / Vol. of Perchloric acid X 0.02042

204.2g of $C_8H_5O_4K$ ≡ 1N 1000 ml $HClO_4$

0.02042g of $C_8H_5O_4K$ ≡ 1 ml of 0.1 N $HClO_4$

Solvent used*:** Glacial acetic acid alone or sometimes in combination with some aprotic solvents is often used. The other solvents are $CHCl_3$, benzene, chloro benzene, acetic anhydride and various combinations of these sometime glycohydrocarbon mixtures are also used. ***Indicators used: Crystal violet 0.05% w/v in glacial acetic acid, methyl red 0.1% w/v in anhydrous methanol, oracet blue 0.5% w/v in glacial acetic acid.

EXPERIMENT TITRATION OF HALOGEN ACID SALTS OF BASES WITH PERCHLORIC ACID

In general, the halide ions, namely: chloride, bromide and iodide are very weakly basic in character so much so that they cannot react quantitatively with acetous perchloric acid. In order to overcome this problem, mercuric acetate is usually added (it remains undissociated in acetic acid solution) to a halide salt thereby causing the replacement of halide ion by an equivalent amount of acetate ion, which serves as a strong base in acetic acid as shown below:

$$2R.NH_2.HCl \leftrightarrow 2RNH_3^+ + 2Cl^-$$

$$CH_3COO)_2Hg + 2Cl^- \rightarrow HgCl_2 + 2CH_3COO^-$$

(Undissociated) (Undissociated)

$$2CH_3COOH_2^+ + 2CH_3COO^- \leftrightarrow 4\,CH_3COOH$$

NON –AQUEOUS TITRATIONS OF WEAKLY ACIDIC SUBSTANCES

There are several drugs which are weakly acidic. Such substances can be titrated against strong bases like potassium methoxide, sodium methoxide, lithium methoxide, tetra butyl ammonium hydroxide, etc in solvents like toluene- methanol. The principle is similar to the titration of weak bases against perchloric acid.

Table: Alkalimetric Assays: Non-Aqueous Titrations using Lithium Methoxide/Sodium Methoxide either Potentiometrically or Titrimetrically

S.No.	**Name of Substance**	**Indicator Employed**
1.	Acetazolamide	Potentiometric determination
2.	Bendrofluazide	Azo violet
3.	Allopurinol	Thymol blue
4.	Mercaptopurine	-do-
5.	Amylobarbitone	Quinaldine Red
6.	Nalidixic acid	Thymolphthalein

Preparation of 0.1 N Potassium Methoxide in Toluene-Methanol:

***Material Required*:** Absolute methanol, dry toluene, Potassium metal.

***Preparation of 0.1 N Sodium Methoxide*:** It is prepared exactly in a similar manner as for

Procedure: Add into a dry flask, a mixture of methanol (40 ml) and dry toluene (50 ml) and cover it loosely. Carefully add freshly cut pieces of potassium metal (5.6 gm) to the above mixture gradually with constant shaking. After complete dissolution of potassium metal, add enough absolute methanol to yield a clear solution. Toluene 50 ml is added with constant shaking until the mixture turns hazy in appearance. The process is repeated by the alternate addition of methanol and benzene until 1 litre of solution is obtained, taking care to add a minimum volume of methanol to give a visible clear solution. 0.1 N Potassium Methoxide, using 2.3g of freshly cut sodium in place of potassium.

***Preparation of 0.1 N Lithium Methoxide*:** It is prepared as for 0.1 N Potassium Methoxide, but using 0.7 g of lithium in place of potassium.

Standardization of 0.1 N Methoxide Solution

***Material Required*:** Dimethylformamide (DMF): 10 ml; thymol blue (0.3% in MeOH); 0.1 N lithium methoxide in toluene methanol; benzoic acid: 0.6 g.

Procedure: Transfer 10 ml of DMF in a conical flask and add to it 3 to 4 drops of thymol blue and first neutralize the acidic impurities present in DMF by titrating with 0.1 N lithium methoxide in toluene-methanol. Quickly introduce 0.06g of benzoic acid and titrate immediately with methoxide in toluene-methanol.

Equations: The various equations involved in the above operations are summarized as stated below:

i) **$Na + CH_3OH \rightarrow CH_3ONa + H\uparrow$**

Interaction between sodium metal and methanol is an exothermic reaction and hence, special care must be taken while adding the metal into the dry solvent in small lots at intervals with adequate cooling so as to keep the reaction well under control.

(ii) **$H_2O + CH_3ONa \rightarrow CH_3OH + NaOH$**

$H_2CO_3 + 2CH_3ONa \rightarrow 2CH_3OH + Na_2CO_3$

The clear solution of sodium methoxide must be kept away from moisture and atmospheric CO_2 as far as possible so as to avoid the above two chemical reactions that might ultimately result into the formation of
turbidity.

(iii) **$C_6H_5COOH + H—CON(CH_3)_2 \leftrightarrow HCON^+H(CH_3)_2 + C_6H_5COO^-$ ------1**

(DMF)

$CH_3ONa \leftrightarrow CH_3O^- + Na^+$ --2

$HCON^+H(CH_3)_2 + CH_3O^- \rightarrow HCON(CH_3)_2 + CH_3OH$ -------------------3

Summing up: **$C_6H_5COOH + CH_3ONa \rightarrow C_6H_5COONa + CH_3OH$**

Step 1**:** It shows the solution of benzoic acid (primary standard) in DMF,

Step 2: It depicts ionization of sodium methoxide,

Step 3**:** It illustrates the interaction between the solvated proton and the methylated ion.

summing up, the net reaction between the water in the solvent (DMF) and the titrant is equivalent to the volume of sodium methoxide consumed by DMF or may be considered as a blank determination.

N/10 KOH in Methanol Dissolve 5.6 gm of anhydrous KOH in 1000 ml of anhydrous methanol. This titrant is not as powerful as others. Its main disadvantage is that it reacts with acidic functional groups and produces a molecule of water, which would affect the sensitivity of titration.

Standardisation:

All these titrants are usually standardized against standard benzoic acid AR-Grade. A sufficient amount of benzoic acid which would give a titrate value of 20-30 ml is transferred in a dry flask and dissolved in 25 ml dimethylformamide, 2-3 drops of 0.5% thymol blue indicator in dry methanol is added to the solution. A blank titration is also per formed in the solvent to account acidic impurity in dimethylformamide and the correction is made accordingly.

Tetrabutylammonium Hydroxide The alkalimetry in non-aqueous titrations may also be carried out efficiently by using tetrabutylammonium hydroxide along with an appropriate indicator.

Preparation of 0.1 N Tetrabutylammonium Hydroxide in Toluene-Methanol

Materials Required**:** Tetrabutylammonium iodide: 40 g; absolute methanol: 90 ml; silver oxide: 25 g; dry toluene: 150 ml.

Procedure: Carefully dissolve 40 g of tetrabutylammonium iodide (Bu_4 NI) in 90 ml of absolute methanol, add to it 20 g of finely powdered purified silver oxide and finally shake the mixture thoroughly for 1 hour. Centrifuge about 2-3 ml of the resultant mixture and test for iodide in the supernatant liquid. In case, it gives a positive test, add about 2 g more of silver oxide and shake for an additional period of 30 minutes. The said method may be repeated until the supernatant liquid obtained is completely free from iodide. The mixture thus obtained is filtered through a fine sintered glass filter and finally rinse the container with 3 portions, each of 50 ml of dry toluene. These washings may be added to the filtrate and the final volume is made up to 1 litre with dry toluene. The clear solution should be kept duly protected from both CO_2 and moisture during storage.

Equation:

$$2Bu_4NI + Ag_2O + H_2O \rightarrow 2Bu_4NOH + 2AgI$$

Tetra butyl- *Tetra butyl-*

Ammonium Iodide *ammonium hydroxide*

***Precautions*:**

Following points should be considered:- Moisture and CO_2 have to be excluded, water being weakly basic would compete with perchloric acid and sharpness of end point would be lost, therefore, moisture contents should be less that 0.05%. The presence of CO_2 affects basic solvent like Dimethyleformamide, ethylene diamine and pyridine as they adsorb CO_2 from air.

- During preparation of Perchloric acid it must be well diluted with acetic acid before adding the acetic anhydride to prevent the formation of explosive acetyl per chlorate.
- Do not use a solvent until fully acquainted with its hazards and how to use it safely.

REFERENCE:

1. K A Conners, A Text Book of Pharmaceutical Analysis, Wiley-Intersciences, New York.

2. A H Backett and J B Stenlake, Practical Pharmaceutical Chemistry, Vol.I and II, The Athlone Press of The University of London. **3.** Pharmacopoeia of India, Govt.of India, Ministry of Health, Delhi.

4. J Bassett, R C Denney, G H Jeffery, J Mendham, Vogel's Textbook of Quantitative Inorganic Analysis, The ELBS and Longman, London.

6. Chromatography

Introduction

Chromatography in all its variations is one of the most widely used and most potent of all the laboratory purification methods in the chemist's armamentarium. First demonstrated by Michael Tswett, a Russian botanist who report the separation of plant pigments (coloring agents) by this method in 1903, chromatography has since been applied to every conceivable type of compound in a wide variety of uses. The name chromatography stems from the early application to mixtures of colored compounds; the separation amounted to graphing the color (Greek: chroma = color).

Chromatography involves an analogous principle. A mixture of two or more compounds is placed on a section of stationary material over which a fluid is slowly passed. The various compounds will be attracted to (adsorbed on or dissolved in) the stationary material, but will also have a competing tendency to dissolve in the fluid and pass slowly through the stationary material and out. This competition will cause some compounds of mixture to move through relatively quickly (they will be very soluble in the fluid and only weakly absorbed – the thin people) and some to move through very slowly (much less soluble in the fluid, strongly absorbed –the wider people). By collecting and analyzing the fluids leaving the stationary material in small batches, one can separate compounds that can never be purified of each other by other by any other technique. Alternatively, for analytical purposes, one could stop the fluid flow at some point before the compounds leave the stationary material and inspect their relative positions to see how many compounds are present and what their chromatographic properties are. Both approaches have been widely used, Numerous forms of chromatography have been developed:

a) **Column chromatography--**The stationary material is often crushed mineral (silica gel, for instance) and the moving phase a solvent (acetone, benzene, etc.). The stationary material is packed in a vertical glass tube (column) and a sample to be separated is placed at the top of a column. Then solvent is added to the top of the column and passed through the sample and column of stationary material. The emerging liquid from the bottom of the column is collected in portions to isolate the individual compounds composing the sample. The method is useful as an analytical (identification) and preparative technique (10 mg – 1 kg of sample). A variation, high speed liquid chromatography (HSLC), has recently been developed where the above process is automated. The solvent is rapidly forced through the column under high pressure, the emerging liquid is analyzed continuously, and its content is indicated in a strip of chart paper. This method is used both for analytical (1 ug-100 mg) and preparative work.

b) **Gas chromatography**- (variously known as GC, GLC – gas liquid chromatography or VPC – vapor phase chromatography) – The stationary material is an oily liquid coated thinly on a crushed mineral and the moving liquid is a gas (He, N_2). The sample is injected into the column at an elevated temperature (50^0 – 250^0C) and the compounds in the gas emerging from the end of the column are continuously analyzed and record on a chart as in HSLC above. This method is used predominantly for analytical work and is capable of difficult separations (e.g. mixtures of structural alkali isomers).

c)**Thin layer chromatography** – (TLC) – both stationary and moving materials are as in column chromatography. However, the adsorbent is coated on a glass plate in a thin layer, the mixture is deposited neat one edge of the layer as a spot or a streak and the plate then dipped in a shallow bath of a solvent. The solvent rises up the layer through capillary action (this is called developing the plate). When it reaches to the top, the positions of the spots of the various compounds in the mixture are observed, or, in the preparative mode, the adsorbent holding the spot of each is scraped off separately for recovery of the substance. This technique is useful for analytical or preparative (up to 1 g) applications.

d) **Paper chromatography** – This is a variation of (TLC) where the stationary substance is a strip of paper. It is useful for analytical purposes only, in situations where the compounds to be identified are suitably adsorbed not on silica gel but on paper.

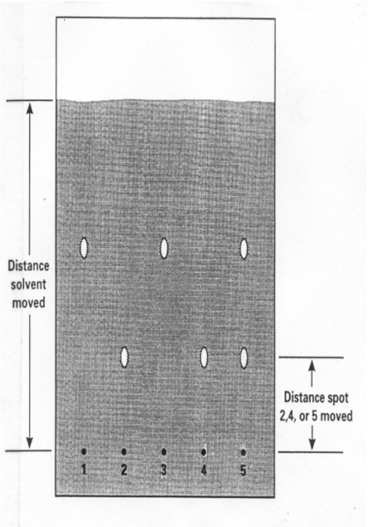
In this experiment paper chromatography will be applied to different samples and the components of the mixture characterized. The experimenter controls three variables in paper chromatography: solvent, paper, and distance solvent moves. The latter is difficult to repeat precisely and to compare experiments the ratio called the representative fraction, R_f, is calculated. The representative fraction, R_f, is defined by the equation:

Rf = Distance from center of spot to starting point/ Distance from solvent front to starting point.

Figure 1 is a sample chromatogram and shows how the quantities in the equation are determined from the experiment. The R_f is independent of the distance the solvent was allowed to move and can be easily repeated.

Figure 1 also shows how paper chromatography can be used to determine whether a sample is a mixture and to identify the components of a sample. For example, the chromatogram in Figure supports the idea that spot 2 and spot 4 are identical because both have the same R_f. For spot 5, we see the result of chromatographing a mixture of compounds. This mixture is clearly separated into two components and the identify of each component established. Sometimes when a mixture consisting of two compounds of similar R_f is chromatographed the components of the mixture will not be clearly separated or "resolved". The resolution of a chromatograph can be changed by using a different solvent, using a different stationary phase or by letting the solvent move farther along the chromatogram.

Figure 1—Sample Chromatogram



Procedure

A. Procedure for Amino Acid mixture

The following procedures can be used to identify an unknown amino acid, separate plant pigments, and separate and identify the amino acids present in orange juice and lemon juice.

1. Preparation of development tank
2. Preparation of the paper
3. Development of chromatogram
4. Location of the amino acids after development of the chromatogram

Preparation of the developing tank

1. Pour enough "Eluting solution" into an 800 or 1000 ml empty beaker until the bottom of the beaker is completely covered with liquid. Cover the beaker with a piece of aluminum foil. Note, the eluting solution is a 75:25:30 mixture of methyl ethyl ketone, propionic acid, and water.

Preparation of paper

2. Wear gloves when handling the paper as to avoid depositing amino acids from your fingers.

3.. With a lead pencil, draw a line, 1.5cm from a parallel to one of the narrow edges of the 12cm x 14 cm filter paper sheet provided.

4. Along this line, mark off tick marks 1 cm apart. Number the tick marks.

5. Distributed around the laboratory you will find watch glasses with a few milliters of 5% amino acid solution in them. Using a glass capillary tube, draw up a small volume of amino acid solution into the capillary and then deposit the amino acid onto the paper by touching the capillary to one of the tick marks. Record the tick mark number and sample identity in your data record.. Do not apply samples to the tick marks in the center of the paper as the paper will be folded for developing the chromatogram.

6. Allow the spots to dry before developing the chromatogram..

Development of the Chromatogram

7. When the spots on the paper are dry, fold (wear gloves) the paper at the center and so the spots are on one edge.

8. Uncover the development tank and stand the folded chromatogram with the spots at the bottom in the eluting solution. Recover the development tank.

9. Let the solvent run up the chromatogram until the solvent front is about 1 cm from the top of the chromatogram. At this point remove (wear gloves) the chromatogram from the development tank and mark with a pencil the location of the solvent front (See Figure 1). Hang the chromatogram to dry in the hood.

Location of the amino acids after development of the chromatogram

10. Clip a test-tube holder (large clothes pin) to the bottom of the dry chromatogram and then spray the chromatogram with 1% ninhydrin solution. DO THIS IN THE HOOD TO AVOID INHALING THE VAPORS.

11. Let the sprayed chromatogram dry and then place in the oven for about 3 minutes, or until spots are visible.

12. Circle spots with a pencil, as the ninhydrin color will slowly fade.

13. Mark the center of each spot, as best you can judge and record color of the spot.

C. Procedure for amino acids in orange and lemon juice.

The chromatography procedure is identical to that for amino acid identification, except the unknown amino acids will come from the orange and lemon juice. The procedure for obtaining the amino acids is outlined below.

Record the appropriate information from this chromatogram on the report sheet.

1. Squeeze fresh juice from an orange and lemon slice into separate beakers.
2. Pour a few ml of each juice into separate centrifuge tubes. The levels of the liquid in each tube should be matched.
3. Centrifuge then pour off solution into separate labeled test tubes.
4. Clean centrifuged tubes.

5. Prepare a chromatogram with two spots using these solutions.
6. Report stationary phase, moving phase, R_f for each amino present and the identity of as many amino acids as possible.

D. Procedure for pigments in green plants.

The chromatography procedure for this experiment is similar to that for amino acid identification except the ‘composition’ of the eluting solution is changed and the pigments to be separated must be extracted from their normal location (the leaf itself). The extraction procedure is outlined below:

1. Place “green pigment” eluting solution in the 1000ml beaker. (see preparation of the development tank in part a A).
2. cut away the stems and midribs from fresh spinach and chop into small pieces.
3. place 2 to 3 grams of the spinach into a small beaker and add 10 ml of acetone.
4. Stir the mixture with a glass rod and crush against the side of the beaker.
5. Pour off the acetone into a clean beaker and then add 10 ml of ethyl alcohol to the spinach.
6. Repeat step four with the alcohol.
7. Pour off the alcohol into the beaker with the acetone. This solution will be your sample for the chromatography of green plant pigments.

E. Procedure for a mixture of indicator dyes

A mixture of synthetic dyes may be separated by paper chromatography in the same manner similar to that for natural pigments except the eluting solution will have a different composition. A solution that can be used to separate the acid base indicators below is n-butanol saturated with 1.5 ammonia solution. Spot the strips as follows:

1. Bromthymol blue
2. Alizarian yellow
3. Bromcersol purple
4. Phenolphthalein
5. Phenol red
6. Unknown mixture

Report sheet for paper chromatography

Name ______________________Lab section ______Date_________________

Staple chromatograms to report

. Amino- Acid Chromatography

Initial Spot No.	Material Applied	Distance moved by spot (s)	Developed color of spots	Distance Moved solvent	Rf of spot (s)
1					
2					
3					
4					
5					
6					
7					
8					
9					

Unknown Letter_________ Amino acids Identified in Unknown___________________