Semester-V

16CHU513B ANALYTICAL METHODS IN CHEMISTRY PRACTICAL 4H 2C

Instruction Hours/week: L:0 T:0 P:4 Marks: Internal: 40 External: 60 Total:100

Scope

The course deals with various experiments in chromatographic separations, solvent extractions and analysis by spectrophotometric methods

Objectives

This lab course enable the students to

1. Learn the skills in paper and Thin layer chromatographic techniques
2. Learn the skills in different types of solvent extraction techniques
3. Learn the skills in spectrophotometric method of analysis

Methodology

Paper chromatography, TLC, solvent extractions methods

I. Separation Techniques

1. Chromatography:

(a) Separation of mixtures

(i) Paper chromatographic separation of $Fe^{3+,}$ $Al^{3+,}$ and $Cr^{3+.}$

(ii) Separation and identification of the monosaccharides present in the given mixture (glucose & fructose) by paper chromatography. Reporting the Rf values.

(b) Separate a mixture of Sudan yellow and Sudan Red by TLC technique and identify them on the basis of their Rf values.

(c) Chromatographic separation of the active ingredients of plants, flowers and juices by TLC

II. Solvent Extractions:

(i) To separate a mixture of Ni^{2+}, & Fe^{2+} by complexation with DMG and extracting the Ni^{2+}-DMG complex in chloroform, and determine its concentration by spectrophotometry.

(ii) Solvent extraction of zirconium with amberliti LA-1, separation from a mixture of irons and gallium.

3. Determine the pH of the given aerated drinks fruit juices, shampoos and soaps.

4. Determination of Na, Ca, Li in cola drinks and fruit juices using flame photometric Techniques.

5. Analysis of soil:

(i) Determination of pH of soil.

(ii) Total soluble salt

(iii) Estimation of calcium, magnesium, phosphate, nitrate

6. Ion exchange:

(i) Determination of exchange capacity of cation exchange resins and anion exchange resins.

(ii) Separation of metal ions from their binary mixture.

(iii) Separation of amino acids from organic acids by ion exchange chromatography.

III Spectrophotometry

1. Determination of pKa values of indicator using spectrophotometry.
2 Structural characterizations of compounds by infrared spectroscopy.
3 Determination of dissolved oxygen in water.
4 Determination of chemical oxygen demand (COD).
5 Determination of Biological oxygen demand (BOD).
6 Determine the composition of the Ferric-salicylate/ ferric-thiocyanate complex by Job's method.

Suggested Readings

Text Books:

1. Jeffery, G.H., Bassett, J., Mendham, J. & Denney, R.C. (1989). *Vogel's Textbook of Quantitative Chemical Analysis*. John Wiley & Sons.
2. Willard, H.H., Merritt, L.L., Dean, J. & Settoe, F.A. (1988). *Instrumental Methods of Analysis,* 7th Ed. Belmont, California, USA: Wadsworth Publishing Company Ltd.
3. Christian, Gary D. (2004). *Analytical Chemistry*. 6th Ed. New York: John Wiley & Sons.
4. Harris, Daniel C. (2001). *Exploring Chemical Analysis*. Ed. New York: W.H. Freeman
5. Khopkar, S.M. (2009). *Basic Concepts of Analytical Chemistry*. New Age, Internationa Publisher.

Reference Books

1. Skoog, D.A. Holler F.J. & Nieman, T.A. *Principles of Instrumental Analysis*, Cengage Learning India Ed.
2. Mikes, O. *Laboratory Hand Book of Chromatographic & Allied Methods*, Elles
3. (1979). *Harwood Series on Analytical Chemistry*. John Wiley & Sons.
4. Ditts, R.V. (1974). *Analytical Chemistry; Methods of Separation*. van Nostrand,

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KARPAGAM ACADEMY OF HIGHER EDUCATION

Faculty of Arts, Science and Humanities

(Deemed to be University Under Section 3 of UGC Act 1956)

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DEPARTMENT OF CHEMISTRY

Title of the course: Analytical Methods in Chemistry- Practical **Course code: 16CHU513B**
Class : III-B.Sc., Chemistry **Semester - V**

LIST OF EXPERIMENTS

S. No.	Duration Hours	Name of the Experiment
1.	4	Writing experimental procedure and Demonstration
2.	4	Paper chromatographic separation of $Fe^{3+,}$ $Al^{3+,}$ and $Cr^{3+.}$
3.	4	Determine R_f Value Using Paper chromatography
4.	4	Separation of compounds in plants, flowers using TLC.
5.	4	Determine the pH- fruit juices, shampoos and soaps
6.	4	Determination of pH of soil
7.	4	Estimation of Ca. Mg in soil
8.	4	Characterization of Compounds by FT-IR
9.	4	Determination of chemical oxygen demand (COD).
10.	4	Determination of dissolved oxygen in water.
11.	4	Viva-voice questions
12.	4	Model practical examination

Prepared by K. Sathya KAHE

Analytical Methods In Chemistry- Manual

16CHU513B

Analytical Methods in Chemistry Practical (16CHU513B)

Ex. No: I Paper chromatographic separation of Fe^{3+}, Al^{3+}, and Cr^{3+}.

Aim

To separate Fe(III), Al(III) and Cr(III) from the given mixture by paper chromatography and to determine their RF values.

Apparatus and chemicals Required:

1. Chromatography jar with lid

2. Aqueous 1% solution of chlorides of iron(III), aluminium and chromium containing few drops of dilute HCl

3. Whatman No. 1 filter paper

4. Capillary tube for spotting

Procedure

Step:1 Take a Whatman filter paper strip (20 cm x 5 cm) and draw a line with a pencil about 3 cm above from one end.

Step:2 **Pour** the developing solvent into the chromatography jar to form a layer about 1 cm deep and replace the lid. Shake the jar lightly so that the atmosphere inside is saturated with the vapours of the solvent.

Step:3 Use a fine capillary tube (a melting point tube drawn out in the flame is suitable) to put two spots of the mixture solution on the pencil line, evenly spaced from each other. The spots should be about 0.5 cm in diameter. Let the spots dry in air; put another drop on the same spots and let them dry again. Repeat 2 to 3 times so that the spots are rich in the mixture.

Step:4 Suspend the dried filter paper carefully in the chromatography jar so that the pencil line (and the spots) is about 2 cm above the solvent level.

Step:5 Cover the jar and leave it undisturbed till the solvent has risen to about 15 cm (near the top of the paper) (Figure 1).

Step:6 Mark the position of the solvent front and allow the strip to dry, cut the paper into two halves length wise, so that the two spots are on separate strips.

Step:7 Spray one strip on both sides with 1% alizarin solution and expose it to ammonia. You may place the strip in a large covered beaker, which contains a small beaker of concentrated ammonia. Warm the paper carefully in an oven at a temperature of 60 to 70^{o}C.

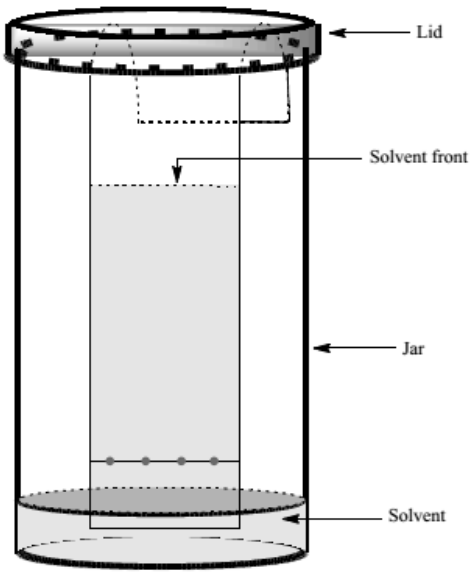
Step:8 Aluminium becomes visible as a red band while iron forms a purple band.

Step:9 Spray the other strip with 0.5 M aqueous sodium peroxide followed by 0.05% solution of benzidine in 2 M acetic acid.

Step:10 **A** bright blue band indicates chromium.

Step:11 **Determine** the R_f values of the ions.

$$R_f = \frac{\text{Distance travelled by solute from original line}}{\textit{Distance travelled by solvent from same line}}$$



Setup for ascending paper chromatography

Observations and calculations

Component	Distance travelled by component (cm)	Distance travelled by solvent (cm)	*R*F value
Fe(III) [purple]	A	X	A/X
Al(III) [red]	B	X	B/X
Cr(III) [blue]	C	X	C/X

Sequence of R_F values: Fe(III)>Al(III)>Cr(III) [If you observe that the developed chromatogram does not show a compact spot but appears as a comet and shows tailing, then in RF column write tailing.

Result

The components were separated and the RF values were found to be ____, _____

and ___ for Fe(III), Al(III) and Cr(III) respectively.

Ex. No: II DETERMINATION OF CALCIUM AND MAGNESIUM:

(E.D.T.A. TITRIMETRIC METHOD BY EL MAHI, et.al. (1987)

Aim:

Calcium and Magnesium ions serve as plant nutrients in cation exchange capacity of soils calcium and magnesium forms the predominant exchangeable base, constituting 60 to 80% of total exchangeable cations. Calcium clay and magnesium clay possesses excellent physical conditions. It develops good crumb structure by virtue of the flocculation and aggregation of primary particles allow free movement of water without stagnation and contains sufficient air for the proper aeration of plant roots. Such a soil is highly productive it supplies necessary plant nutrients.

Principle:

The method is based on the fact that ca, Mg and number of other ions from stable complexes with versene (Ethylene diamine tetra acetic acid disodium salt) at different pH and Sn, Cu, Mn, Zn may interfere in the determination of calcium and Magnesium if present in appropriate amounts. Their interference is prevented by use of 2% Nac N solution.

Apparatus:

1) 1 litre flaks

2) Porcelain dishes 3" to 4" diameter

3) Burette of 50 ml capacity

4) Pipette of 5 ml and 10 ml.

5) Beaker of 100 ml.

6) Centrifuge tubes / polythene shaking bottle.

Reagents:

1) Standard Ca solution, 0.01 N : Weigh 0.05 gm CaCo3 and dissolve in 10 ml of 2N HCl. Heat till the solution boils and

CO2 is completely driven off. Cool and make the volume accurately to 1 litre.

2) EDTA Solution : Dissolve 2 gm disodium EDTA in distilled water and make the volume 1 litre. Standardize against standard Ca Solution.

3) NH4Cl – NH4OH buffer solution : Dissolve 67.5 gm NH4Cl in 570 ml of concentrated NH4OH solution and make 1 litre volume.

4) NaOH 10% : Add 10 gm NaOH to 90 ml distilled water.

5) NH2OH.HCL : Dissolve 5 gm hydroxylamine hydrochloride in 100 ml of distilled water.

6) K4Fe(CN)6 : Dissolve 4 gm potassium ferrocyanide in 100 ml of distilled water.

7) Triethanolamine: TEA.

8) EBT: Dissolve 0.2 gm Erichrome black T in 50 ml of methanol.

9) Calcon indicator: Dissolve 20 mg calcon in 50 ml methanol.

10) Neutral normal ammonium acetate solution – Take 58 ml of glacial acetic acid in 500 ml volumetric flask. Also take 71 ml of concentrated ammonium hydroxide solution in another 500 ml volumetric flask. Dilute both the solutions with distilled

Water upto the 2/3 volume and mix both in 1 lit. Flask then adjusts pH to 7.0 and finally makes up the volume to 1 lit. For bringing pH of solution to 7, add dilute acetic acid or ammonium hydroxide, or dissolve 77 gm/lit. NH4OAC and

Adjust pH to 7 by acetic acid or ammonium hydroxide.

Procedure:

Extraction:

1) Weigh 2 – 4 gm of soil sample (2mm sieved) in conical flask or polythene shaking bottle or 100 ml centrifuge tubes.

2) Add 30 ml of NH4OAc and shake for 5 min and decant.

3) Then add 30 ml of 0.5N HCl to each sample and agitate the contents, shake for 5 min. in a upright loosened position.

4) Then filter the solution using whatman No. 1 filter paper. Collect the filtrate.

Estimation of Ca & Mg (determination of ca & Mg together) :

1) Pipette out 20 ml of the filtrate into a 150 ml conical flask.

2) Add 50 ml distilled water.

3) Add 10-15 ml NH4Cl – NH4OH buffer solution and add 10

drops each of NH2OH.HCL, K4Fe(CN)6, TEA and EBT indicator.

4) Titrate with standard EDTA to permanent blue colour.

Determination of Calcium alone :

1) Pipette out 5 or 10 ml extract, add 10 drops each of NH2OH.HCL, K4Fe(CN)6 and TEA and enough of 10% NaOH to raise pH to 12.

2) Add 5 drops of calcon indicator.

3) Titrate against standard EDTA. The end point is the change of colour from red to blue.

The value obtained from Ca plus Mg and Ca alone are used to calculate the Ca and Mg in the soil samples respectively.

Observation Table:

A) For Ca determination:

S.NO	Sample No	Burette reading	Ca meq/lit

B) For Ca + Mg determination:

Calculations :

Meq (Ca + Mg) or Ca/100 gm

- $\frac{\text{ml. of EDTA required x Normality of EDTA (0.01) x vol. made 100 ml x 100}}{\text{sample taken for titration (ml) x Weight of soil in gm.}}$

Meq (Mg) = Meq (Ca + Mg) – Meq (Ca)

Result:

The Amount of calcium and Magnesium present in the given solution_____.

Ex No: III Determination of pH of soil

Aim:

The determination of pH in soil is important as it plays a great role in availability of nutrients to plants. This determination can be done more accurately in the laboratory by electrometric method. pH determination is useful for soil classification on the basis of acidity or alkalinity.

Principle:

The electrometric determination of pH by a pH meter is based on measuring the e.m.f. (milivolts) of a pH cell both a reference buffer and then with a test solution. The change in the potential difference at 25oC for 1 pH unit is 59.1 mV. The pH of a soil is a measure of the hydrogen or hydroxyl ion activity of the soil – water system. It indicated whether the soil is acidic, neutral or alkaline in reaction. By shaking a certain amount of soil with a certain amount of liquid, soil suspension is brought in equilibrium with a supernatant solution. In the supernatant solution the pH is measured potentiometrically on a direct reading pH meter using a glass electrode with a saturated KCl – calomel reference electrode.

Reagents:

i) Standard buffer solutions: Dissolve one commercially available buffer tablet each of pH 4.0, 7.0 and 9.2 in freshly prepared distilled water separately and make up the volume to 100 ml. Prepare the fresh solution every week as these solutions are unstable.

Alternatively the buffer solutions can be prepared in the laboratory as given below.

ii). **0.05 M Potassium hydrogen phthalate ($KHC_3H_4O_4$, Mol. Wt. 204.22):** Dissolve 10.21 gm AR grade potassium hydrogen phthalate in warm water and making volume to 1 L. This gives a pH of 4.00 at 25^oC and can be used as standard buffer.

iii) Buffer solution pH 6.86 : Potassium dihydrogen phosphate + Disodium hydrogen phosphate, each 0.025 M – Dissolve 3.40 gm of potassium dihydrogen orthophosphate and 4.45 gm disodium hydrogen orthophosphate dihydrate (Sorenson's salt – $Na_2HPO_4.2H_2O$) to 1 L in distilled water.

iv) Buffer solution pH 9.2: Dissolve 3.81 gm sodium tetraborate (A.R.) in water and dilute to 1000 ml.

Apparatus:

i) pH meter with glass electrodes, ii) Thermometer, iii) Glass beaker (100 ml), iv) Glass rod

Procedure:

1) Weigh 20 gm of 2.0 mm air dry soil into a beaker. Add 50 ml of distilled water and stir with a glass rod thoroughly for about 5 minutes and keep for half an hour.

2) In the meantime turn the pH meter ON, allow it to warm up for 15 minutes. Standardize the glass electrode using standard buffer of pH = 7 and calibrate with the buffer pH = 4 or pH = 9.2.

3) Dip the electrodes in the beakers containing the soil water suspension with constant stirring.

4) While recording pH, switch the pH meter to pH reading, wait 30 seconds and record the pH value to the nearest 0.1 unit. Put the pH meter in standby mode immediately after recording.

5) Remove the electrodes from soil suspension and clean the electrodes with distilled water.

6) Rinse the electrodes after each determination and carefully blot them dry with filter paper before the next determination. Standardize the glass electrodes after every 10 determinations.

7) Dip the electrodes in distilled water, when not in use and ensure that the reference electrode always contains saturated potassium chloride solution in contact with solid potassium chloride crystals.

8) Three to four drops of toluene are added in standard buffer solutions to prevent growth of mould.

Ratings :

< 4.5 Extremely Acidic

4.6 to 5.2 Strongly Acidic

5.3 to 6.0 Moderately Acidic

6.1 to 6.5 Slightly Acidic

6.6 to 7.0 Neutral

7.1 to 7.5 Slightly Alkaline

7.6 to 8.3 Moderately Alkaline

8.4 to 9 Strongly Alkaline

> 9 Extremely Alkaline

Result

The pH of the given soil_____