CHAPTER II

MATERIALS

2.1 INTRODUCTION

This chapter describes the materials and apparatus developing solvents, preparation of ion exchange papers, procedure for spotting and detection methods used for paper chromatography.

2.2 MATERIALS AND APPARATUS

2.2.1 Apparatus

Paper chromatography was performed on 15 x 3.5 cm Whatman No.1 filter paper strips in 20 x 5 cm glass jars. They were saturated first with proper developing solvents and then used for chromatographic work.

For the measurement of the pH values of the solutions, a Philips pH meter PR 9405 L was used with Philips PV 9014 combination electrode. The pH meter was standardized by using phthalate (pH 4.0) and borax (pH 9.15) buffers.

Pear shaped 100 ml separatory funnels with interchangeable joints of corning borosilicate glass were used throughout the work.

The chromatography was performed at $25 \pm 0.5^{o}C$.

Chemicals

2.2.2 Solvents

(1) Chloroform -

Double distilled chloroform (AR-BDH) was used.

(2) Solutions of 0.1 M AR sulphuric acid, 0.1 M AR hydrochloric acid, 0.1 M AR sodium carbonate and 0.1 M AR ammonia were prepared for adjusting the pH.

(3) Glass distilled conductivity water was used throughout the work.

(4) Developing solvents -

The best possible developing solvent was selected for the separation of substances under examination. The choice of this depends upon the simple fact that R_F values should be different for different constituents present in a mixture. Generally a solvent or solvent mixture which gives a R_F value of 0.2 - 0.8 for the sample should be selected.

For the present work, various solvent systems studied are :

(i) Methanol + 10 M hydrochloric acid + acetone (M:H:A).

The system is studied by varying the compositions of solvents as (1:1:1), (1:1:2), (2:1:2), (2:1:1), (1:2:1), (1:2:2) and (2:2:1).

(ii) 10 M Hydrochloric acid + acetone + ethanol (H:A:E)

The system is studied by varying the compositions of solvents as (1:1:1), (1:2:1), (1:2:2), (1:1:2), (2:1:1), (2:2:1) and (2:1:2).

(iii) Acetone + 10 M hydrochloric acid + n-butanol (A:H:B)

The system is studied by varying the compositions of solvents as (1:1:1), (2:1:1), (2:1:2), (1:1:2), (1:2:1), (2:2:1) and (1:2:2).

(iv) 4 M Nitric acid + acetone + n-propanol (N:A:P)

The system is studied by varying the compositions of solvents as (3:3:3), (2:4:2), (4:2:2), (2:2:4), (4:3:3), (3:4:3) and (3:2:4).

(v) Ethyl methyl ketone + n-butanol + 50 % hydrochloric acid (EMK:B:H)

The system is studied by varying the compositions of solvents as (6:3:6), (3:6:1), (6:3:1), (3:6:6), (6:6:3), (3:4:3) and (1:3:6).

All the solvents were of AR grade. They were distilled twice before used for the work.

2.2.3 Preparation of metal ions

All the chemicals used were of AR(BDH) grade. The sample volume of 10-20 μ containing as many as μg of the substance was used for spotting. The metal salts were used as follows. Nickel(II) chloride, cobalt(II) chloride, copper(II) sulphate, manganese(II) chloride, cadmium(II) sulphate, zinc(II) sulphate, mercury(II) chloride, bismuth(III) nitrate and iron(III)chloride.

The solutions were prepared according to standard procedures.[118,119]

Preparation of metal dithizonates

Solid dithizone (AR-BDH) was purified according to standard procedure.[120] Solution of 1.0×10^{-4} M dithizone was prepared by dissolving 2.563 mg of it in chloroform to 100 ml. The flask was wrapped by black paper. Direct sunlight is avoided as dithizone is photochromic. Sensitive to light

Metal dithizonates were prepared by shaking excess of metal salts (aqueous) with 1.0×10^{-4} M dithizone in chloroform at desired pH for 15 minutes in a 100 ml separatory funnel. The pH values of these solutions were adjusted by using 0.1 M sulphuric acid or 0.1 M hydrochloric acid or 0.1 M sodium carbonate or 0.1 M ammonia solution. The coloured dithizonate layers were washed with little distilled water having the desired pH. The chloroform layers were then transferred through a plug of cotton wool to dry stoppered bottles (amber coloured). Many dithizonates are photochromic, hence these must not be exposed to direct sunlight.

The pH values and colours of different metal dithizonates are given in following table.

Metal dithizonates	Colour	pH values
$Ni(HDz)_2$	brown-violet	12.0
$Co(HDz)_2$	reddish-violet	8.0
$Cu(HDz)_2$	brown-violet	7.0
$Mn(HDz)_2$	violet	10.0
$Zn(HDz)_2$	red	7.0
$Cd(HDz)_2$	dark red violet	8.0
$Hg(HDz)_2$	pink red	7.0
$Bi(HDz)_3$	red	12 to 14
$Fe(HDz)_3$	reddish violet	8.0

2.2.4 Spotting reagents

Visualization of the spots can be done in two ways, either by physical methods or by chemical means.

Physical methods - Some colourless spots when held under a UV lamp, fluoresce and reveal their existence.

Chemical detection - Chemical treatment can develop the colour of colourless solvents on the paper. The reagents (also known as chromogenic reagents or visualizing reagents) were applied by spraying the chromatogram by means of spray pumps. The chromatograms were then dried.

The reagents[121] used for detection are :

(i) H_2S water -

Brown black spots - Hg(II), Bi(III), Cu(II), Cd(II), Fe(III), Co(II), Ni(II), etc.

(ii) Yellow ammonium sulphide - Dip the paper in yellow ammonium sulphide solution and then expose to ammonia.

Black spots - Bi(III), Co(II), Cu(II), Fe(III), Pb(II), Hg(II) and Ni(II).

Yellow spot - Cd(II).

(iii) Rubeanic acid (0.1 % w/v in ethyl alcohol/water) - Spray the paper with reagent and then expose to ammonia.

Blue	- Nickel
Orange	- Cobalt
Yellow	- Cadmium
Olive green	- Copper
Brown	- Iron
Pale blue	- Manganese
Yellow/Brown	- Bismuth

(iv) Dithizone (0.1 % w/v in chloroform) - It is followed by ammonia

Pink	- Mercury, cadmium
Purple	- Bismuth, tin
Purplish brown	- Copper
Yellow	- Arsenic
Red	- Antimony
Weak red	- Lead

(v) Diphenyl carbazide (0.1 % w/v in ethyl alcohol) -

Pink	- Bismuth, iron, zinc
Pale pink	- Manganese
Purple/pink	- Mercury
Purple	- Lead, cobalt
Red	- Nickel

(vi) Dimethyl glyoxime (0.1 % w/v in ethyl alcohol) - It is followed by ammonia.

Pink	- Nickel
Weak orange	- Cobalt

(vii) Potassium ferrocyanide (0.1 % aqueous) -

Blue	- Iron
Brown	- Copper

(viii) Potassium iodide (0.1 % aqueous) -

Red, yellow	- Mercury
Brown, yellow	- Bismuth
Brown	- Copper, antimony

(ix) Benzidine (0.1 % in ethyl alcohol) - It is followed by alkali as sodium hydroxide.

Blue (temporary) - Manganese

(x) Cinchonine iodide -

Yellow - Bismuth

2.2.5 Preparation of impregnated papers

Whatman No.1 filter paper impregnated with potassium trioxalato aluminate and zirconium trioxalato aluminate were prepared as follows :

(i) Potassium trioxalato aluminate paper

Potassium trioxalato aluminate $\left[K_3Al(C_2O_4)_3.3H_2O\right]$ is prepared according to usual procedure.[122]

1.0 g of commercial aluminium foil is weighed into a 250 ml capacity beaker, the metal is covered with 10 ml warm water. 30.0 ml solution of potassium hydroxide (3 mol KOH) is added in portion to the solution. After subsiding the vigorous effervescence, the solution is heated to boiling temperature to dissolve aluminium foil completely. The solution is filtered. The residue which is chiefly finely divided copper is thrown away. 10.0 ml of water is added to the filtrate and again the solution is heated to boiling temperature. 14.0 g of oxalic acid is weighed and added in portion to the hot solution until the precipitate of hydrated alumina formed at first is just redissolved on continued boiling. Excess acid is avoided.

The neutralised solution is filtered. The filtrate is cooled to room temperature. Then 50 ml ethanol is added to the filtrate and cooling is continued. The complex oxalate is separated as small colourless prisms. The crystals are washed with a mixture of ethanol and water and finally with ethanol. The product is then dried in air at room temperature.

3.0 g of potassium trioxalato aluminate is dissolved in 100 ml conductivity water. The solution is stirred to obtain the clear solution. Whatman No.1 filter paper strips are impregnated in this solution for 10 minutes. Excess reagent is removed by placing the strips on filter paper sheets and are dried partly. The strips are then washed with distilled water three times. Finally they dried at room temperature and used for chromatography.

(ii) Zirconium trioxalato aluminate paper

5.0 g zirconium oxychloride ($ZrOCl_2$ - AR/BDH) is dissolved in sufficient water. To clear the solution a few drops of dilute hydrochloric acid is added. Whatman No. 1 filter paper strips are impregnated in zirconium oxychloride solution for ten minutes. Excess reagent is removed by placing the strips on filter paper sheets and dried partly. Paper strips are then dipped in potassium trioxalato aluminate complex solution for two minutes. Thus zirconium trioxalato aluminate precipitate is formed on the filter paper strips. They are dried at room temperature and then washed with conductivity water thrice to remove excess of reagent. Finally the paper strips are dried at room temperature and used for chromatography.

2.3 PROCEDURE FOR SPOTTING

For ascending technique, a strip of Whatman filter paper No.1 of the size 15.0 cm x 3.5 cm is used. A horizontal line is drawn on the filter paper by a lead pencil (original line). On the origin line, cross marks (X) are made with a pencil. With the help of thin glass capillaries, the mixture of test solutions are applied on cross marks. The spots are dried cautiously by a stream of hot or cold air. The filter paper is then conditioned for 15 minutes and then dipped into the developing solvent until the solvent ascent is 11.0 cm. As soon as the filter paper gets the liquid through its capillary axis and when it reaches the spot of the test solution, (a mixture of two or more substances), the various substances are moved by solvent system at various speeds. When the solvent has moved these cations to a suitable height, the chromatogram is dried cautiously.

2.3.1 Metal ions on Whatman No.1 filter paper

The chromatograms are prepared by applying the mixture of metal ions as [Ni(II) + Cu(II) + Cd(II)] , [Co(II) + Zn(II) + Bi(III)] and [Mn(II) + Hg(II) + Fe(III)] on Whatman No.1 filter paper using above procedure.

2.3.2 Metal dithizonates on Whatman No.1 filter paper

The chromatograms are prepared by applying the mixture of metal dithizonates as $[Ni(HDz)_2 + Cu(HDz)_2 + Cd(HDz)_2]$, $[Co(HDz)_2 + Zn(HDz)_2 + Bi(HDz)_3]$ and $[Mn(HDz)_2 + Hg(HDz)_2 + Fe(HDz)_3]$ on Whatman No.1 filter paper by using above procedure.

2.3.3 Metal ions on Whatman No.1 filter paper impregnated with potassium trioxalato aluminate

The chromatograms are prepared by applying the mixture of metal ions, [Ni(II) + Cu(II) + Cd(II)], [Co(II) + Zn(II) + Bi(III)] and [Mn(II) + Hg(II) + Fe(III)] on the Whatman No.1 filter paper impregnated with potassium trioxalato aluminate as above.

2.3.4 Metal dithizonates on Whatman No.1 filter paper impregnated with potassium trioxalato aluminate

The chromatograms are prepared by applying the mixture of metal dithizonates $[Ni(HDz)_2 + Cu(HDz)_2 + Cd(HDz)_2]$, $[Co(HDz)_2 + Zn(HDz)_2 + Bi(HDz)_3]$ and $[Mn(HDz)_2 + Hg(HDz)_2 + Fe(HDz)_3]$ on Whatman No.1 filter paper impregnated with potassium trioxalato aluminate as above.

2.3.5 Metal ions on Whatman No.1 filter paper impregnated with zirconium trioxalato aluminate

The chromatograms are prepared by applying the mixture of metal ions [Ni(II) + Cu(II) + Cd(II)], [Co(II) + Zn(II) + Bi(III)] and [Mn(II) + Hg(II) + Fe(III)] on Whatman No.1 filter paper impregnated with zirconium trioxalato aluminate as above.

2.3.6 Metal dithizonates on Whatman No.1 filter paper impregnated with zirconium trioxalato aluminate

The chromatograms are prepared by applying the mixture of metal dithizonates $[Ni(HDz)_2 + Cu(HDz)_2 + Cd(HDz)_2]$, $[Co(HDz)_2 + Zn(HDz)_2 + Bi(HDz)_3]$ and $[Mn(HDz)_2 + Hg(HDz)_2 + Fe(HDz)_3]$ on Whatman No.1 filter paper impregnated with zirconium trioxalato aluminate as above.

Nature of the present work

The candidate proposes the study of chromatographic separation of metal dithizonates on Whatman No.1 filter paper by using appropriate solvent compositions so as to give the optimum separation.

The results will be compared with those of metal ions separated on Whatman No.1 filter paper as is reported in the literature.

For the first time, we are reporting the use of potassium trioxalato aluminate as a material for impregnation of paper. This material forms a gel and hence may serve as a useful material for chromatographic study. Separation of metal ions and metal dithizonates will be studied and optimum conditions for separation will be worked out.

Zirconium trioxalato aluminate is a still better medium for chromatographic studies. Separation of metal ions and metal dithizonates will be studied on Whatman No.1 filter paper impregnated with zirconium trioxalato aluminate and optimum conditions for separation will be worked out.