
EXPERIMENT 4

PAPER CHROMATOGRAPHIC SEPARATION AND IDENTIFICATION OF METAL IONS

Structure

- 4.1 Introduction
 - Objectives
- 4.2 Theory of chromatography
 - Definition
 - Classification
 - Principle
 - The concept of R_f value
- 4.3 Paper Chromatographic Separation of Metal Ions
 - Principle
- 4.4 Experiment 4a: Separation and Identification of cations of Analytical Group I
 - Requirements
 - Procedure
 - Observations and Calculations
 - Result and Discussion
- 4.5 Experiment 4b: Separation and Identification of Cations of Analytical Group II
 - Requirements
 - procedure
 - Observations and Calculations
 - Result and Discussion

4.1 INTRODUCTION

Experiments 1, 2 and 3 were concerned with solvent extraction methods. Our subsequent experiments 4 to 9 will be concerned with chromatographic separation methods. Here we shall first discuss the basic theory of chromatography with some fundamental concepts and then the principle of liquid chromatography, after that you will be introduced to the actual experiments in which you will perform paper chromatography of metal ions and sugars (Experiments 4 and 5), thin layer chromatography of amino acids (Experiment 6), column chromatography of natural pigments and of inorganic substances (Experiments 7 and 8).

Objectives

After studying and performing this experiments you should be able to:

- define and classify chromatography,
- understand theoretical principle of chromatography,
- calculate the R_f values, and
- Separate and identify cations of analytical group I and II

4.2 THEORY OF CHROMATOGRAPHY

4.2.1 Definition

Chromatography is referred to any of a diverse group of techniques that effect a separation through a distribution of sample between two immiscible phases. One phase is stationary whereas the second is mobile which percolates through the first phase. The stationary phase may be a solid or a liquid while the mobile phase may be a liquid or a gas.

4.2.2 Classification

There are various ways to classify chromatography.

1. On the basis of physical states of mobile phase the chromatography is classified into two board groups.
 - Liquid chromatography in which mobile phase used is in the form of a liquid.
 - Gas chromatography in which mobile phase used is a gas.
2. On the basis of physical states of **stationary phase** and its working principle, chromatography is classified as:
 - Adsorption chromatography in which stationary phase is a solid and works as an adsorbent.
 - Partition chromatography in which stationary phase is a liquid or a liquid supported on an inert solid, and the movement of solute is based on the partition coefficient of the solute into two phases.
 - Ion exchange chromatography in which stationary phase is an ion exchanger and the distribution of solute is based on the ion exchange principle.
 - Gel chromatography in which stationary phase is gel and separation is based on its sieving action.
3. On the basis of the types of column it may be classified as:
 - Column chromatography in which a closed column containing the stationary phase in a cylindrical tube is employed.
 - Sheet chromatography using an open column system in which separations are achieved on sheets of filter paper or thin layers of certain fine solid particles supported on glass or plastic plates.

4.2.3 Principle

Chromatography is essentially a separation process which affects a separation by distributing the sample into two phases. One phase is stationary and second is mobile and flows through the stationary phase. During the process of movement of mobile phase, small differences in adsorption-desorption or partitioning or ion-exchange behaviour of each component of a mixture are multiplied many fold and these parameters distinguish between the different solutes. The ability of chromatography to separate two solutes, depends on the selectivity of the process and the degree to which the system can distinguish between the two solutes. The magnitude of the distribution is determined by the physico chemical nature of the solute and that of the mobile and stationary phases, beside various physical interaction (such as: hydrogen bonding, dipole moment etc.) of the solute with stationary and mobile phases.

Some common steps used in different chromatographic techniques are: application of the sample onto a stationary phase, percolating a mobile phase over the stationary phase, obtaining the separation of components, collection of the different components for qualitative or quantitative purposes. Therefor, the principle of chromatography can be understood by taking a one kind of chromatography. For this

purpose, let us consider a simple example of separation by column chromatography. This can be applied to other methods as well.

Let us suppose that we wish to separate a mixture of two (say coloured) components A and B. A small amount of the sample solution is introduced at the top of the column which is packed with suitable adsorbent (stationary phase). First a narrow band is formed at the top of the column. The developer (mobile phase) is now poured into the column and is allowed to flow through the column. The two important steps are: (i) formation of the initial zone (ii) development of the initial zone and allowing the components to appear as separate zones.

As soon as the solution comes in contact with the stationary phase (column material or adsorbent) and the mobile phase the following reversible reaction occurs:



For which the equilibrium concentrations are related as

$$K' = \frac{C_m}{C_s} \text{ or } K = \frac{C_s}{C_m} \quad \dots(4.1)$$

where, $K' = 1/K$ and K' is equilibrium constant

K is called as the distribution coefficient, C_s is the concentration of the solute in the stationary phase and C_m that in mobile phase.

For two solutes A and B there is competition of A and B (i) for the stationary phase and (ii) for the mobile phase.

If the stationary phase takes B more likely than A, the value of K_A will be less than K_B . Thus when the developer leaves the narrow band it is richer in A than B. The developer flowing downwards now comes in contact with fresh stationary phase and the solutes A and B face the new competition and when the mobile phase leaves this part of the stationary phase, each time it becomes richer and richer in A. Small differences in the interactions of A and B with stationary and mobile phases become exaggerated as the developer proceeds down the column, soon after the well separated bands of A and B are obtained.

If the development further proceeds (elution analysis) the components A and B of the mixture are elute out of the column. Solute A which has smaller K emerges first followed by B which has a larger K . The two fractions may be quantitatively analysed for A and B respectively.

4.2.4 The concept of R_f Value

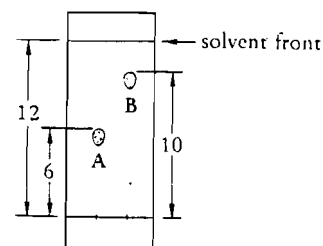
R_f value of a solute is the ratio of the rate of movement of the solute peak to the rate of movement of the eluting solvent.

However, one can not readily detect the position of the solvent front or of the solute on the column chromatography. These are better measured now in terms of retention volume or retention time.

In paper chromatography R_f value is constantly quoted as a characteristic of the solute. It describes the migration of solute relative to that of developer and is given by

$$R_f = \frac{\text{distance moved by the centre of the solute zone}}{\text{distance moved by the solvent front}}$$

With this background, now we will take up experiment based on different types of chromatographic separations in detail.



$$R_f = \frac{\text{Distance to leading edge of spot}}{\text{Distance to solvent front}}$$

$$A R_f = \frac{6}{12} = 0.50$$

$$B R_f = \frac{10}{12} = 0.83$$

**Measuring R_f values in
paper chromatography.**

4.3 PAPER CHROMATOGRAPHIC SEPARATION OF METAL IONS

Paper chromatography (PC) is a simple technique to separate complex mixtures of metal ions, amino acids, sugars, dyes and drugs. A very small amount of sample is required for the analysis. PC has become a popular technique for the separation of metal cations. In this experiment, the use of PC will be illustrated to separate a mixture of cations of group I and group II. Next experiment concerned with PC separation of the sugars.

4.3.1 Principle

In paper chromatography of cations, the principles of partition, adsorption and ion exchange may be exploited, out of these the most important is partition the involves the distribution of a solute between a mobile liquid phase and a gel (a kind of water cellulose complex) as the stationary phase. The different components of the sample are distributed across the paper depending on their partition coefficients.

Attempts have been to employ paper chromatography for the systematic qualitative analysis of metal cations. However, it is not possible to separate all the cations simultaneously and therefore, the separation of a group of cations can be handled. Most frequent of these is the preliminary separation into the current analytical groups, each of which is then subjected to a separate chromatographic analysis. In the two parts of this Experiment (4a and 4b) you will learn the use of paper chromatography for the separation of cations of analytical group I and group II, respectively.

4.4 EXPERIMENT 4A: SEPARATION AND IDENTIFICATION OF CATIONS OF ANALYTICAL GROUP I

This experiment of an easy ascending paper chromatographic technique is rapid and requires no special apparatus or reagents. A very simple procedure for this separation has been employed with the use of only distilled water as the developer. However, other developers can also be utilised depending on the availability of the reagents and precautions in their use [e.g. butanol - 1 + pyridine + water (15:1:9)]. In this experiment you will perform the separation of cations of group I.

4.4.1 Requirements

Apparatus		Chemical
Boiling tubes or chromatographic jar	5	Potassium chromate
Measuring cylinder	1	Lead nitrate
Pipette	1	Silver nitrate
Spotting capillaries	5	Mercurous nitrate
Small test tubes	5	Nitric acid
Whatman No. 1 Filter paper		

Solution provided

- 1) **Unknown solution:** It can be prepared by dissolving any one or two nitrate of analytical group I in water.
- 2) **Detector:** 0.25 M aqueous solution of potassium chromate $K_2Cr_2O_4$ is prepared by dissolving 24.25 g K_2CrO_4 in distilled water in 250 cm^3 volumetric flask.

4.4.2 Procedure

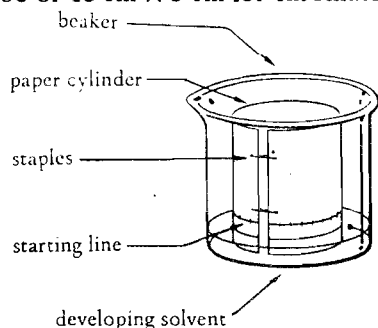
Proceed according to the following steps:

1) **Preparation of solutions**

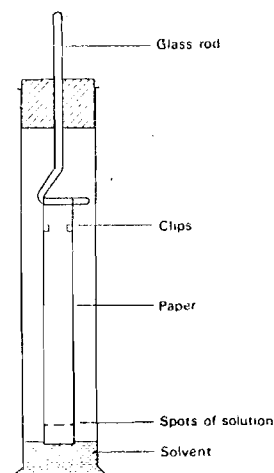
i) Prepare 1 cm³ aqueous solutions of (i) lead nitrate (ii) silver nitrate (iii) mercurous nitrate by dissolving about .1g crystals in a small test tube. Add few drops of HNO₃ to prevent hydrolysis. For the preparation of mixture of cations, add few drops of each cation solutions in a test tube.

ii) Developer: Distilled water.

2. Cut whatman No. 1 filter paper strips of the required size: 15 cm × 2 cm to fit usual boiling tube or 15 cm × 3 cm for chromatographic jar.



An Alternate method for developing paper chromatogram by making paper cylinder. Beaker can be covered by aluminum foil.



Apparatus for paper chromatography

Application of the sample on the paper should be on a small area. Larger spots lead to poorer separations.

Please indicate the name of test sample on the top of the paper strips.

The paper in the boiling tube or in the jar should be vertically changed and it should not touch the sides of the tube.

The spot of the solute (at the point of application) should always be above the level of the developer otherwise the solute will mix with the developer and error will be resulted.

Ensure that the spot gets dried before placing the paper in the boiling tube.

Do not allow the paper to come in contact with impurities.

3. On each strip draw a line at about 1 cm of the one end and put a dot in the centre of line. This will be the bottom of the strip and development will take place from this end.
4. Apply the solutions of Pb²⁺, Ag⁺ and Hg₂²⁺ separately on 3 strips with the help of a fine capillary. Use a fresh capillary for each solution. The teacher is supposed to demonstrate the technique of application of the solution.
5. On the 4th paper strip apply the mixture of the three cations.
6. Apply the unknown solution (e.g. containing any one or two) on the 5th paper strip.
7. Place 5 dry boiling tubes vertically in a stand.
8. Add distilled water, with the help of a pipette, to each of the boiling tubes so that the height of the developer (distilled water) in each of the boiling tubes is less than 1 cm. The sides of the boiling tube must be dry as far as possible.
9. Suspend the spotted and dried paper strips in the respective boiling tubes containing distilled water with the upper end pinned to the cork and the lower end touching the developer. Care is taken to see that this is done gently and the strip is vertical. The spot should always be above the developer level.
10. Allow the developer to rise along the paper and wait till the developer (solvent front) reaches near the upper end of the paper.
11. Remove the paper from the boiling tube and mark the solvent front with the help of a pencil.
12. Get the dried tubes to evaporate the developer.
13. Take potassium chromate solution in a petri dish (or a watch glass) and dip the dried paper in the detector.
14. Encircle the coloured zones with pencil and mark the centre of the zone.
15. Calculate the R_f values.

16. Compare the R_f values of individual cations with that of their R_f values in known mixture and in unknown.
17. Identify the cations present in the unknown on the basis of R_f values.

4.4.3 Observations and Calculations

Observe the coloured spots of different cations. Pb^{2+} will appear as yellow; Ag^+ as orange-red and Hg_2^{2+} as orange zones. Measure the distance of the centre of each solute zone from the point of application call this distance ds . Measure the distance between the solvent front and the starting line and call this distance as dm .

Calculate the R_f value of each solute by the relation:

$$R_f = \frac{\text{Distance travelled by the centre of solute zone}}{\text{Distance travelled by the solvent front}} = \frac{ds}{dm}$$

Record your data in the following way:

Observation Table
PC of Metal ions of group I

Sample	ds	dm	$R_f = ds/dm$ Remark
Ag^+			
Hg_2^{2+}			
Pb^{2+}			
Mixture			R_f resembles with
Unknown			R_f resembles with
1.			
2.			

4.4.4 Result and Discussion

Metal ions present in the unknown sample are:

1.
2.

The metal ions of analytical group I move along the paper in distilled water at different rates. On dipping in detector. K_2CrO_4 solution the coloured precipitates of the chromates of lead, mercurous and silver appear as yellow, orange and orange-red zones on the paper. Sometimes, when the coloured spot of Hg_2^{2+} is not intense, the paper is exposed to ammonia vapours: the mercurous compound gives black spot.

Lead migrates with the fastest rate and appears at the upper most area on the paper. Mercurous follows the lead. Silver migrates with the slowest rate and appears below the mercury zone.

4.5 EXPERIMENT 4B: SEPARATION AND IDENTIFICATION OF CATION OF ANALYTICAL GROUP II

In this experiment you will perform the separation of Bismuth (III), copper (II), cadmium (II), lead (II) and mercury (II) using paper chromatography. This separation is achieved by 1- butanol saturated with 3M HCl as developer. However, another simple developer containing: ethanol + water + 1 M HCl (18:1:1) can also be used to separate Pb^{2+} , Cu^{2+} , Cd^{2+} (excluding Hg_2^{2+} from Group II).

4.5.1 Requirements

Apparatus		Chemicals
Boiling tubes	5	1-Butanol
Measuring cylinder	1	3M HCl
Pipette	1	bismuth chloride
Cupric chloride		Lead chloride
Spotting capillaries	7	Mercuric chloride
Small test tubes	7	

Pettridish

Whatman No. 1. filter paper sheets.

Solution provided

1. **Unknown solution:** It can be prepared by dissolving any one or two chlorides of analytical group II in water.
2. **Preparation of Detector 1:** H₂S water. It is prepared by passing H₂S gas in water and add a few drops of ammonia to it. Keep it in a covered container.
3. **Preparation of Detector 2:** Dithizone. Prepare a conc. solution of dithizone in chloroform or carbon tetrachloride.

4.5.2 Procedure

Proceed according to the following steps.

1. i) **Preparation of Solution:**

Sample Preparation : prepare 1cm³ concentrated aqueous solutions of (i) bismuth chloride (ii) cadmium chloride (iii) cupric chloride (iv) lead chloride and (v) mercuric chloride. Add 1-2 drops of hydrochloric acid to prevent hydrolysis. For the preparation of the mixture of these cations, add few drops of each cation solutions a test tube.

ii) **Preparation of Developer:** Developer (1-butanol saturated with 3 M HCl): Prepare this developer by taking equal volumes of 1-butanol and 3 M hydrochloric acid in a separatory funnel. Shake well and allow to stand to separate the layers clearly. Reject the lower aqueous layer and use the upper (organic) layer as the developer.

2. Cut whatman No. 1 filter strips of about 15×2 cm to be placed in usual boiling tubes (or chromatographic jars).
3. On each strip draw a line with pencil at a bout 1 cm of one end and mark a point in the centre of line. This point is the point of application of the solute/sample solution.
4. Apply the test solution to the point of application with the help of of a fine capillary. Apply bismuth. Cadmium, copper, lead and mercury solutions separately on 5 strips. Use a fresh capillary for each solution.
5. Apply the mixture solution and unknown solution separately on other strips. Application of solution can be repeated twice or thrice if the solutions are dilute.
6. Take the clean and dry boiling tube and place (10-15 cm³ of) the developer in each of these boiling tube.

7. Suspend the spotted and dried paper strips in the respective boiling tubes containing distilled water with the upper end pinned to the cork and the lower end touching the developer. Care is taken to see that this is done gently and the strip is vertical. The spot should always be above the developer level.
8. Allow the developer to rise along the paper and wait till the developer (solvent) reaches near the upper end of the paper.
9. Remove the paper strip and mark the solvent front with the help of a pencil.
10. Leave the paper for some time to get it dried.
11. Hold the paper in an atmosphere of H_2S gas until the zones of metallic sulphides are seen. An alternative way is to use H_2S water in which the paper is dipped to locate the zones. Another alternative method is use dithizone for detecting the zones of metal ions. Spray the paper (or dip in) with a concentrated solution of dithizone in chloroform.
12. Encircle the coloured zones and mark the centre of each zone. Calculate the R_f values of individual cations with that of their R_f values in mixture to identify the cations in the mixture and unknown sample solution.

4.5.3 Observations and Calculations

Observe the colour of the spots of various cations of group II. With H_2S bismuth (III) will appear as dark brown: cadmium (II) as yellow: copper (II) as chocolate brown: lead (II) as black and mercury (II) as black zones.

Measure the distance travelled by the centre of the solute zone (ds) and the distance travelled by the solvent front (dm) on the paper chromatogram.

Calculate the R_f values of each solute by the relation: $R_f = ds/dm$.

Record your data in the following way:

Observation Table
PC Separation of Pb^{2+} , Cu^{2+} , Cd^{2+} , Bi^{3+} , and Hg^{2+}

Sample cation	ds	dm	$R_f = ds/dm$	Remark
Hg^{2+}				
Cd^{2+}				
Bi^{3+}				
Pb^{2+}				
Cu^{2+}				
Mixture				
Unknown				R_f resembles with
1.				R_f resemble with
2.				

Result and Discussion

Metal ions present in the unknown sample are:

1.

2.

The rate of migration of group II metal ions in butanol saturated with 3M HCl appears in the order $Hg^{2+} < Cd^{2+} < Bi^{3+} < Pb^{2+} < Cu^{2+}$

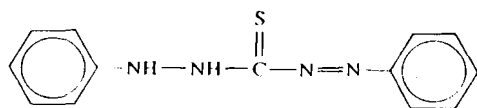
When H_2S is used as detector the coloured zones of metal sulphides are seen: HgS black, CdS yellow, Bi_2S_3 dark brown, PbS black and CuS Chocolate brown.

When dithizone is used as detector, coloured complexes of metal ions are formed with dithizone. Dithizone is the commercial name of diphenyl-1-thiocarbazono (see formula in below).

**Paper Chromatographic
Separation and Identification of
Metal Ions**

Metal ion + Dithizone \longrightarrow coloured complex

Colours of the complexes of metal ions are: mercury-pink, cadmium- purple, bismuth-purple and copper brown. Lead is not clearly detected for which rhodizonic acid may be used.



Diphenylthiocarbazono
(dithizone)