University of Baghdad

Collage of Science

Department of Chemistry

Practical Analytical Chemistry

Gravimetric and Separation Methods in Analytical Chemistry

For

Second year Chemistry

First Semester

اعداد

أ.م.د.أشرف سعد رشيد

أ.م.وسن عبد الامير علوان

م.م. كفاح حسن اسماعيل

2020-2021

Gravimetric methods of analysis

Gravimetric method is one in which the analysis is completed by a weighing operation.

Types of Gravimetric Methods

There are two major types of gravimetric methods:

1- Precipitation methods.

2- Volatilization methods.

In precipitation methods, the analyte is converted to a sparingly soluble precipitate. This precipitate is then filtered, washed free of impurities, and converted to a product of known composition by suitable heat temperature, and the product is weighed.

In volatilization methods, the analyte or its decomposition products are volatilized at a suitable temperature. The volatile product is then collected and weighed or the weight of the product is determined indirectly from the loss in weight of the sample. The two most common gravimetric methods based on volatilization are those for water and carbon dioxide.

Properties of precipitates and precipitating reagents

A gravimetric precipitating agent should react specifically or, if not that, at least selectively with the analyte. ***Specific reagents***, which are rare, react only with a single chemical species. ***Selective reagents***, which are more common, react with a limited number of species. In additional to specificity or selectivity, the ideal precipitating reagent would react with the analyte to give a product that is:

1. Readily filtered and washed free of contaminants.

2.Low solubility so that no significant loss of the solid occurs during filtration and washing.

3. Un reactive with constituents of the atmosphere.

4. Of known composition after its dried or, if necessary, ignited.

Desiccator

Desicator is a glass container, the most common desiccators are circular and made of heavy glass. There is usually a removable platform on which the sample to be stored are placed. The desiccant such as silica gel, fills the space under the platform,

* The desiccators is a convenient way to store the sample and carry it about the lab.

* Dry a material at room temperature.

* prevent absorption of moisture because it contains a drying agents.

Drying Agent

Phosphorus pentoxide P_2O_5 → HPO_3

Barium oxide BaO → $BaO.10H_2O$

Potassium hydroxide KOH → $KOH.XH_2O$

Calcium chloride $CaCl_2.2H_2O$ → $CaCl_2.6H_2O$

Silica gel SiO_2 → $SiO_2.XH_2O$

white or blue Pink

A drying agents should be available, low cost and absorb the moisture very rapidly.

Desiccator

Exp.No.1

Determination of Water of crystallization in copper sulphate hydrous $CuSO_4.XH_2O$ using Volatilization method

Theory:

Water of crystallization is the water forming part of crystal structure of certain materials, known as crystalline hydrate. The contents of such water in these hydrates correspond to definite chemical formulas such as, $BaCl_2.2H_2O$; $Na_2SO_4.10.H_2O$; $Na_2SO_4.7H_2O$, etc. Accordingly, water of crystallization is sometimes called stoichiometric water.

When heated, crystalline hydrates decomposes with libration of water. This is the basis for the method for determining water of crystallization in most crystalline hydrates by volatilization. Copper sulphate hydrate loses all its water of crystallization above 100 °C (100-150 °C). Much higher temperatures (up to 800-900 °C) can be used in this dehydration, for anhydrous copper sulphate is non-volatile and stable even at fairly high temperatures.

$$\mathbf{CuSO_4.\ xH_2O} \xrightarrow{100\text{-}200\ ^oC} \mathbf{CuSO_4 + xH_2O}$$

In the present experiment a weighed sample of $CuSO_4.XH_2O$ contained in a weighing bottle is heated at 100-150 °C in a drying oven until its weight ceases to change (i.e. it is dried to constant weight). When the weight has become constant evidently all the water of crystallization has been removed. Its weight is equal to the weight loss of the sample

Materials and Equipments:

1- porcelain or silica crucible with lid
2- crucible tongs
3- desiccator
4- Analytical balance
5- Copper sulphate crystal
6- Oven (100-200 ^oC)

Procedure:

1- Heat the crucible gently on Oven (100-200 ^oC) and leave it for 30 minutes. Allow to cool in desiccator for 15 minutes and weight the empty crucible.

2- Introduce into the crucible 0.5 g of sample (hydrated Copper sulphate) using an analytical balance.
3- Heat the crucible with sample in oven at temp.(100-200^{o}c) for 1 hour. Then cool in a desiccator for 30 min.
4- Weighed the crucible with the sample after heating and cooling.

Calculations:

1- Calculate the percentage of water content as follows:

2- $H_2O\% = \frac{\text{Weight of the volatile water}}{\text{Weight of sample}} \times 100$

$$H_2O\% = \frac{\text{Weighing the crucible with sample before heating} - \text{weighing the crucible with sample after heating}}{\text{Weighing the crucible with sample before heating} - \text{weighing the empty crucible}}$$

3- Find the number of water of crystallization in the structure according to the following formula.

$$\frac{\text{Weight of hydrous salt } CuSO4.xH2O}{\text{Weight of non hydrous } CuSO4} = \frac{\text{M.wt of hydrouse salt } (CuSO4 + 18H2O)}{\text{M.wt of non hydrouse } CuSO4}$$

Weight of non hydrous= weighing the crucible with sample after heating - weighing the empty crucible

Exp.No.2

Determination of sulfate as barium sulfate

$Ba(SO_4)$

Theory:

This method is based on slowly addition of a dilute solution of barium chloride to a hot solution of the sulphate slightly acidified with (0.05N) HCl:

$$Ba^{2+} + SO_4^{2-} \longrightarrow BaSO_4(s)$$

The barium sulphate, which forms as a crystalline precipitate, is collected on a suitable filter, washed with water, strongly ignited (at 800-1000 °C), and weighted as $BaSO_4$. The reasons of using dilute acid are:

1- to obtain large particle sizes of the precipitate, and
2- The particles of the precipitate are of highly pure.

The solubility of $BaSO_4$ in water is of about 3 mg L^{-1} at the ordinary temperature and this solubility is increased in the presence of mineral acids, because of the formation of the hydrogensulphate ion:($SO_4^{2-} + H^+ \longrightarrow HSO_4^{-1}$); thus the solubilities at room temperature in the presence of 0.1, 0.5, 1.0 and 2.0 M HCl are 10, 47, 87,and 101 mg L^{-1}repectively, but the solubility is less in the presence of a moderate excess of barium ions as a common ion. Nevertheless, it is usual to carry out the precipitation in weakly acid solution in order to prevent the possible formation of barium salts of such anions as chromate, carbonate, and phosphate, which are insoluble in neutral solutions. Moreover, the precipitate thus obtained consists of large crystals, and is therefore more easily filtered (recall Von Weimarn equation).it is also of great important to carry out the precipitation at boiling temperature, for the relative super saturation is less at higher temperature. The concentration of HCl is, of course limited by the solubility of $BaSO_4$, but it has been found that of 0.05 M is suitable; the solubility of the precipitate in the presence of $BaCl_2$ at this acidity is negligible.

This method appears straightforward, but, in fact, it is subjected to numerous interferences, due chiefly to the tendency of $BaSO_4$ to occlude foreign anions and cations. Therefore, the experimental conditions for the precipitation should be carefully controlled to obtain satisfactory results (**Note:** the student is recommended to recall the theoretical related lectures for details).

There are dangerous contaminations by co-precipitation which may occur during the precipitation of $BaSO_4$. For example, $BaCl_2$ and Ba $(NO_3)_2$ are readily co-precipitated by occlusion causing large errors to the weigh of $BaSO_4$, since the chloride is unchanged upon ignition and the nitrate will yield barium oxide. To reduce this contamination at minimum via:

1- Slow addition of hot dilute barium chloride solution to the hot sulphate solution with continuous stirring.
2- The contamination of nitrate must always be removed by evaporation with a large excess of HCl before precipitation.

Pure barium sulphate is not decomposed when heated in dry air until a temperature of about 1400 °C is reached;

$$\mathbf{BaSO_4 \longrightarrow BaO + SO_3}$$

The precipitate is, however, easily reduced to sulphide at temperature above 600 °C by the carbon of the filter paper:

$$\mathbf{BaSO_4 + 4C \longrightarrow BaS + 4CO}$$

The reduction is avoided by first charring the paper without inflaming, and burring off the carbon slowly at low temperature with free access of air.

Materials and Equipments:

1- Porcelain or silica crucible with lid
2- Porcelain triangle
3- Tripod and Bunsen burner
4- Desiccator
5- Analytical balance
6- Beaker (400 mL) and stirring rod
7- Watch glass
8- Funnel
9- Filter paper (Wattmann No. 40 or 540)
10- potassium sulphate or sodium sulphate
11- hydrochloric acid
12- 10% barium chloride
13- dilute silver nitrate solution
14- oven (800-100^{0}c)
15- oven (100-200 ^{0}c)

Procedure:

1-Weigh out accurately 0.3 g of the solid sample (sodium sulphate or any alkali sulphate)
2-Transfer the weighed sample into a 400 mL beaker covered with watch glass and provided with a stirring rod. Then add 200 mL with distilled water and add 1mL of concentrated HCl. **why?**
3-Heat the solution to boiling, then add dropwise from the burette or pipette 10-12 mL of barium chloride heat solution with continuous stirring during the addition.

4-Heat the content gently for half an hour.

5- Allow the precipitate to settle for a minute or two.

6- Test the supernatant liquid for complete precipitation by adding a few drops of barium chloride solution. If a precipitate is formed, add slowly a further 3 mL of the reagent, allow the precipitate to settle as before and test again; repeat this operation until an excess of barium chloride is present.

7- Leave the precipitate overnight to obtain the precipitate characterized by large particle sizes and ease filtration.

8- Filter the precipitate through filter paper NO.40 (Wattmann, ashless). Decant the clear solution through an ashless filter paper and test the filtrate with a few drops of barium chloride to ensure the complete precipitation.

9- Wash the precipitate three times that remained inside the beaker with hot distilled water.

10 -Transfer the precipitate to the filter with the aid of a jet of hot water from wash bottle. Use a rubber-tipped rod (policeman) to remove any precipitate adhering to the walls of the beaker. Wash the precipitate several times with hot distilled water and let each portion of the wash solution run through before adding the next. Continue the washing until about 5 mL of wash solution gives no opalescence with a drop or two of silver nitrate solution (test this by collection of a few drops of the filtrate on watch glass). Why add $AgNO_3$

11- Fold the moist paper around the precipitate and place it in a porcelain crucible previously ignited to redness and weighted.

12- Dry the paper by placing the loosely covered crucible upon a triangle several centimeters above a small flame. Then gradually increase the heat until the paper chars and volatile mater is expelled. Do not allow the paper to burst into a flame, as mechanical loss may thus ensue. When the charring is complete, raise the temperature of the crucible to dull redness and burn off the carbon with free excess of air.

13- Transfer the crucible with its content to muffle furnace at temperature of 800-1000 °C, and leave it inside for 30 minutes. Transfer it to a desiccator, and when cooled, weigh the crucible and contents using a sensitive analytical balance.

Calculations:

Calculate the percentage of sulphate as follows:

$$\% \text{ sulphate} = \frac{Wt.of\ precipitate\ (g)\ x\ GF}{Wt.of\ sample(g)} \times 100$$

GF (gravimetric factor) = $\frac{\text{M.wt. } SO_4^{-2}}{\text{M.wt. } BaSO_4}$

Exp.No.3

Determination of lead as Lead Chromate

Theory:

There are several gravimetric methods of analysis used for determination of Pb in various samples via its precipitation as lead chromate ($PbCrO_4$), lead sulphate ($PbSO_4$) or lead molybdate ($PbMoO_4$) etc…

In the case of the precipitation of lead as lead chromate, the solution should be acidified with dilute acetic acid if it is a neutral or basic. On the other hand, if the solution is acidic with nitric acid, an additional amount of sodium acetate should be added to replace nitric acid with acetic acid followed by addition of an excess of potassium chromate.

$$Pb^{2+} + CrO_4^{2-} \longrightarrow PbCrO_4 \downarrow$$

The most important of this precipitate

1- It dissolves in the strong acids to form a soluble complex.

$$2\ CrO_4^{2-} + 2H^+ \longrightarrow Cr_2O_7^{2-} + H_2O$$

2- Also it dissolves in the strong basess to form a soluble complex.

$$PbCrO_4 + 4OH^- \longrightarrow PbO_2^{-2} + CrO_4^{-2} + 2H_2O$$

Although this method of precipitation is limited in its applicability because of the general insolubility of chromates it is a useful procedure for gaining experience in gravimetric analysis. The best results are obtained by precipitation from homogeneous solution utilizing the homogeneous generation of chromate ion produced by slow oxidation of Cr^{3+} by bromate at 90-95 °C in the presence of an acetate buffer.

$$2Cr^{3+} + BrO_3^- + 5H_2O \longrightarrow 2CrO_4^{2-} + Br^- + 10H^+$$

$$BrO_3^- + 5Br^- + 6H^+ \longrightarrow 2Br_2 + 3H_2O$$

$$Pb^{2+} + CrO_4^{2-} \longrightarrow PbCrO_4$$

Materials and Equipments:

1-Porcelain or silica crucible with lid
2-Porcelain triangle
3-Tripod and Bunsen burner
4-Desiccator

5- Analytical balance
6- Beakers and stirring rod

7- Watch glass

8- Funnel

9- Filter paper (Wattmann No. 40 or 540

10- Lead nitrate Pb $(NO_3)_2$

11- Dilute acetic acid (1:1) CH_3COOH

12- 4% potassium chromate solution (K_2CrO_4)

13- 1% sodium acetate solution (CH_3COONa)

Procedure:

1-Weight accurately 0.15 g on of the lead salts (for example lead nitrate) and add 2 drop NaOH solution (0.1N) dissolve in about 200 mL of distilled water.
2-Add 2.5 mL from buffer solution (acetic acid and sodium acetate)
3-Transfer the weighed salt to a 250 mL beaker and then dissolve it in 250 mL of distilled water.
4-Add 10 mL from chromium nitrite.
5-Add 10 mL of potassium bromate .
6-The mixture heated (90-95^{o}c), during heating chromate ion will be generated, slowly which then react with lead ions to from lead chromate. Precipitation indicated when solution became clear.
7-Cool the solution and filtration by used instrumental filtration then washing of precipitate with nitric acid (1%).
8-Dry the crucible with its contents in the oven at 120 °C (1 hour), cool in a desiccator (30 min.) and weigh as $PbCrO_4$.

Calculations:

The precipitate after drying is $PbCrO_4$and its weight can be found by the weight difference between empty clean sintered glass (W_1) and with precipitate (W_2).

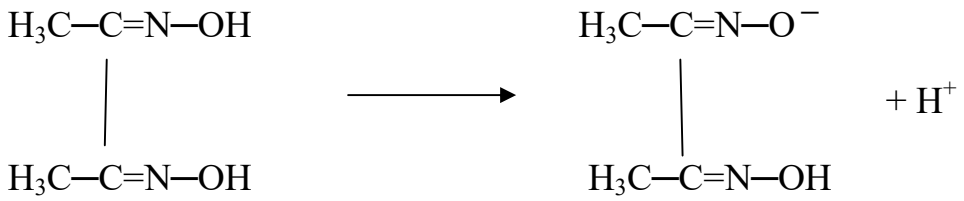
$$\%Pb \text{ in the sample} = \frac{\text{Weight of } PbCrO_4 \times GF}{\text{Weight of sample}}$$

Exp.No.4

Determination of Nickel as dimethylglyoxime complex

Theory:

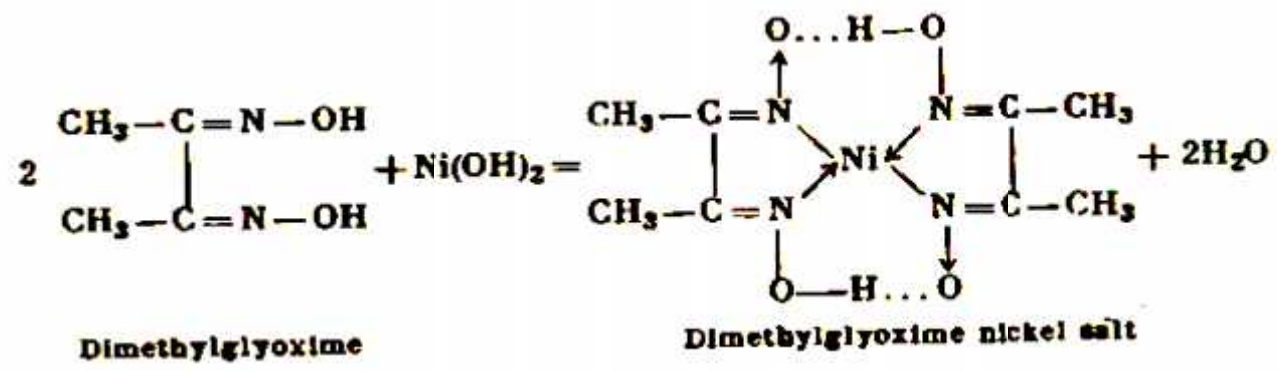
Dimethylglyoxime is considered to be the oldest well-known organic precipitant which discovered by Chugayev (1905)) who first proposed the use of dimethylglyoxime as a precipitant for Ni (II) ion. This reagent is a weak acid, its formula is $CH_3.C$ (: NOH).C (: NOH).CH_3 and symbolized as H_2DMG, low soluble in water giving one H^+ ion when dissociated as follows:



It dissolves in alcohol, so its alcoholic solution is used especially for the precipitating nickel ion quantitatively. The nickel is precipitated by addition of ethanolic solution of dimethylglyoxime to a hot, faintly acid solution of nickel salt, and then adding a slight excess of aqueous ammonia solution (free from carbonate). The precipitate is washed with cold water and then weighed as nickel dimethylglyoximate (red precipitate) after drying at 110-120 °C. With large precipitates, or in work of high accuracy, a temperature of 150°C should be used. Its formation may be represented by the following general equation:

$$Ni^{2+} + 2\ H_2DMG \longrightarrow Ni\ (HDMG)_2 + 2NH_4^+$$

And the overall reaction as follows:



This equation shows that, in addition to replacing two hydrogen atoms in =N─OH groups of two dimethylglyoxime molecules, the Ni^{2+} ion also forms coordination bonds with nitrogen atoms in two more such groups. Therefore, the =N─OH groups are simultaneously acidic and complex-forming.

The red precipitate has relatively low solubility in water but soluble in free mineral acids.

Materials and Equipments:

1- Beaker 400 mL
2- Stirring rod
3- watch glass
4- pipette
5- Analytical balance
6- Electric oven
7- Gooch, sintered glass or porcelain filtering Crucible
8- Ammonium nickel sulphate $(NH_4)_2SO_4.NiSO_4.6H_2O$
10-1% Dimethylglyoxime solution.

11-Hydrochloric acid (1:1).

12-Dilute ammonia solution

Procedure:

1-Weigh out accurately 0.2 g of pure ammonium nickel sulphate (as a sample for analysis) into a 400-mL beaker provide with a watch-glass cover and stirring rod.
2- Dissolve the sample in little water, add 5 mL of dilute HCl(1:1) and dilute to 200 mL with distilled water.
3- Heat to 70-80 °C , add a slight excess of the dimethylglyoxime reagent (at least 5 mL for every 10 mg of Ni present), and,
4- Immediately add dilute ammonia solution (1:4) dropwise, directly to the solution and not down the beaker wall, and with constant stirring until the precipitate take place, and then in slight excess.
5- Allow to stand on the steam bath for 20-30 minute.
6- Test the solution for complete precipitation when the red precipitate has settled out and allow the precipitate to stand for 1 hour, cooling at the same time.
7- Filter the cold solution through the sintered glass crucible [(No. 3 or 4), previously heated to 110-120 °C and weighed after cooling in a desiccator] (W_1).
8- Wash the precipitate with cold water until free chloride.
9- Dry it at 110-120 °C for 45-50 minutes.
10- Allow to cool in a desiccator and weigh as $Ni(C_4H_7O_2N_2)_2$

11- Repeat the drying until constant weight is obtained (W_2)

Calculations:

The precipitate after drying is $Ni(C_4H_7O_2N_2)_2$ and its weight can be found by the weight difference between empty clean sintered crucible (W_1) and that with precipitate (W_2).

$$\%Ni \text{ in the sample} = \frac{\text{Weight of } Ni(C_4H_7O_2N_2)_2 \times GF}{\text{Weight of sample}} \times 100$$

GF = 0.2031

Separation Methods In Analytical Chemistry

Chromatography

Chromatography (from Greek *chroma*, color and *graphein* to write) is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a *stationary phase*, which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases. Subtle differences in compounds partition coefficient results in differential retention on the stationary phase and thus **changing** the separation. Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for further use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

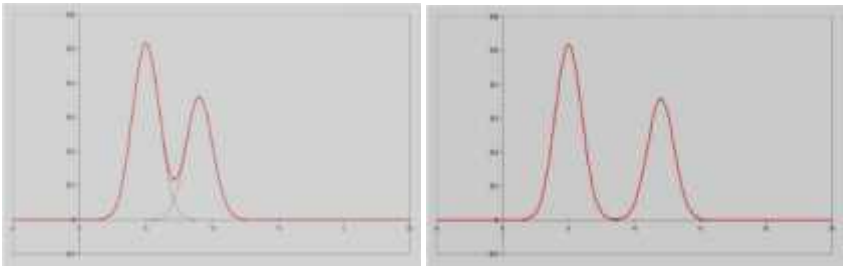
1. History

The **history of chromatography** begins during the mid-19th century. Chromatography, literally "color writing", was used—and named— in the first decade of the 20th century, primarily for the separation of plant pigments such as chlorophyll. New types of chromatography developed during the 1930s and 1940s made the technique useful for many types of separation process. Some related techniques were developed during the 19th century (and even before), but the first true chromatography is usually attributed to Russian botanist Mikhail Semyonovich Tsvet, who used columns of calcium carbonate for separating plant pigments during the first decade of the 20th century during his research of chlorophyll. Chromatography became developed substantially as a result of the work of Archer John Porter Martin and Richard Laurence Millington Synge during the 1940s and 1950s. They established the principles and basic techniques of partition chromatography, and their work encouraged the rapid development of several types of chromatography method: paper chromatography, gas chromatography, and what would become known as high performance liquid chromatography. Since then, the technology has advanced rapidly. Researchers found that the main principles of Tsvet's chromatography could be applied in many different ways, resulting in the different varieties of chromatography described below.

Simultaneously, advances continually improved the technical performance of chromatography, allowing the separation of increasingly similar molecules.

2. Chromatography terms

- The **analyte** is the substance that is to be separated during chromatography.
- **Analytical chromatography** is used to determine the existence and possibly also the concentration of analyte(s) in a sample.
- A **bonded phase** is a stationary phase that is covalently bonded to the support particles or to the inside wall of the column tubing.
- A **chromatogram** is the visual output of the chromatograph. In the case of an optimal separation, different peaks or patterns on the chromatogram correspond to different components of the separated mixture.



Plotted on the x-axis is the retention time and plotted on the y-axis a signal (for example obtained by a spectrophotometer, mass spectrometer or a variety of other detectors) corresponding to the response created by the analytes exiting the system. In the case of an optimal system the signal is proportional to the concentration of the specific analyte separated.

- A **chromatograph** is equipment that enables a sophisticated separation e.g. gas chromatographic or liquid chromatographic separation.
- **Chromatography** is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction.
- The **effluent** is the mobile phase leaving the column.
- An **eluotropic series** is a list of solvents ranked according to their eluting power.
- An **immobilized phase** is a stationary phase which is immobilized on the support particles, or on the inner wall of the column tubing.
- The **mobile phase** is the phase which moves in a definite direction. It may be a liquid (LC and CEC), a gas (GC), or a supercritical fluid (supercritical-fluid chromatography, SFC). The mobile phase consists of the sample being separated/analyzed and the solvent that moves the sample through the column. In the case of HPLC the mobile phase consists of a non-polar solvent(s) such as hexane in normal phase or polar solvents in reverse phase chromotagraphy and the sample being separated. The mobile phase moves through the

chromatography column (the stationary phase) where the sample interacts with the stationary phase and is separated.

- **Preparative chromatography** is used to purify sufficient quantities of a substance for further use, rather than analysis.
- The **retention time** is the characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions. See also: Kovat's retention index
- The **sample** is the matter analyzed in chromatography. It may consist of a single component or it may be a mixture of components. When the sample is treated in the course of an analysis, the phase or the phases containing the analytes of interest is/are referred to as the sample whereas everything out of interest separated from the sample before or in the course of the analysis is referred to as waste.
- The **solute** refers to the sample components in partition chromatography.
- The **solvent** refers to any substance capable of solubilizing other substance, and especially the liquid mobile phase in LC.
- The **stationary phase** is the substance which is fixed in place for the chromatography procedure. Examples include the silica layer in thin layer chromatography

3. Techniques by chromatographic bed shape

3.1- Column chromatography

Column chromatography is a separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube (packed column) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open tubular column). Differences in rates of movement through the medium are calculated to different retention times of the sample. In 1978, W. C. Still introduced a modified version of column chromatography called **flash column chromatography** (flash).The technique is very similar to the traditional column chromatography, except for that the solvent is driven through the column by applying positive pressure. This allowed most separations to be performed in less than 20 minutes, with improved separations compared to the old method. Modern flash chromatography systems are sold as pre-packed plastic cartridges, and the solvent is pumped through the cartridge. Systems may also be linked with detectors and fraction collectors providing automation. The introduction of gradient pumps resulted in quicker separations and less solvent usage. In expanded bed adsorption, a fluidized bed is used, rather than a solid phase made by a packed bed. This allows omission of initial clearing steps such as centrifugation and filtration, for culture broths or slurries of broken cells.

3.2- Planar chromatography

Planar chromatography is a separation technique in which the stationary phase is present as or on a plane. The plane can be a paper, serving as such or impregnated by a substance as the stationary bed (paper chromatography) or a layer of solid particles spread on a support such as a glass plate (thin layer chromatography). Different compounds in the sample mixture travel different distances according to how strongly they interact with the stationary phase as compared to the mobile phase. The specific Retention factor (R_f) of each chemical can be used to aid in the identification of an unknown substance.

3.2.1- Paper chromatography

Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of *chromatography paper*. The paper is placed in a jar containing a shallow layer of solvent and sealed. As the solvent rises through the paper, it meets the sample mixture which starts to travel up the paper with the solvent. This paper is made of cellulose, a polar substance, and the compounds within the mixture travel farther if they are non-polar. More polar substances bond with the cellulose paper more quickly, and therefore do not travel as far.

3.2.2- Thin layer chromatography

Thin layer chromatography (TLC) is a widely employed laboratory technique and is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate. Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents. For even better resolution and to allow for quantification, high-performance TLC can be used.

4. Techniques by physical state of mobile phase

4.1-Gas chromatography

Gas chromatography (GC), also sometimes known as Gas-Liquid chromatography, (GLC), is a separation technique in which the mobile phase is a gas. Gas chromatography is always carried out in a column, which is typically "packed" or "capillary" (see below) .Gas chromatography (GC) is based on a partition equilibrium of analyte between a solid stationary phase (often a liquid silicone-based material) and a mobile gas (most often Helium). The stationary phase is adhered to the inside of a small-diameter glass tube (a capillary column) or a solid matrix inside a larger metal tube (a packed column). It is widely used in analytical chemistry; though the high temperatures used in GC make it unsuitable for high molecular weight biopolymers or

proteins (heat will denature them), frequently encountered in biochemistry, it is well suited for use in the petrochemical, environmental monitoring, and industrial chemical fields. It is also used extensively in chemistry research.

4.2- Liquid chromatography

Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. Liquid chromatography can be carried out either in a column or a plane. Present day liquid chromatography that generally utilizes very small packing particles and a relatively high pressure is referred to as high performance liquid chromatography (HPLC). In the HPLC technique, the sample is forced through a column that is packed with irregularly or spherically shaped particles or a porous monolithic layer (stationary phase) by a liquid (mobile phase) at high pressure. HPLC is historically divided into two different sub-classes based on the polarity of the mobile and stationary phases. Technique in which the stationary phase is more polar than the mobile phase (e.g. toluene as the mobile phase, silica as the stationary phase) is called normal phase liquid chromatography (NPLC) and the opposite (e.g. water-methanol mixture as the mobile phase and C18 = octadecylsilyl as the stationary phase) is called reversed phase liquid chromatography (RPLC). Ironically the "normal phase" has fewer applications and RPLC is therefore used considerably more. Specific techniques which come under this broad heading are listed below. It should also be noted that the following techniques can also be considered fast protein liquid chromatography if no pressure is used to drive the mobile phase through the stationary phase. See also Aqueous Normal Phase Chromatography.

5. Techniques by separation mechanism

5.1- Ion exchange chromatography

Ion exchange chromatography uses ion exchange mechanism to separate analytes. It is usually performed in columns but can also be useful in planar mode. Ion exchange chromatography uses a charged stationary phase to separate charged compounds including amino acids, peptides, and proteins. In conventional methods the stationary phase is an ion exchange resin that carries charged functional groups which interact with oppositely charged groups of the compound to be retained. Ion exchange chromatography is commonly used to purify proteins using FPLC.

5.2- Size exclusion chromatography

Size exclusion chromatography (SEC) is also known as **gel permeation chromatography** (GPC) or **gel filtration chromatography** and separates molecules according to their size (or more accurately according to their hydrodynamic diameter or

hydrodynamic volume). Smaller molecules are able to enter the pores of the media and, therefore, take longer to elute, whereas larger molecules are excluded from the pores and elute faster. It is generally a low-resolution chromatography technique and thus it is often reserved for the final, "polishing" step of a purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins, especially since it can be carried out under native solution conditions.

6. Special techniques

6.1- Reversed-phase chromatography

Reversed-phase chromatography is an elution procedure used in liquid chromatography in which the mobile phase is significantly more polar than the stationary phase.

6.2- Two-dimensional chromatography

In some cases, the chemistry within a given column can be insufficient to separate some analytes. It is possible to direct a series of unresolved peaks onto a second column with different physico-chemical (Chemical classification) properties. Since the mechanism of retention on this new solid support is different from the first dimensional separation, it can be possible to separate compounds that are indistinguishable by one-dimensional chromatography

Ion exchange

Ion exchange: is a process by which ions held on a porous, essentially insoluble solid, are exchanged for ions in a solution that is brought in contact with the solid. The solid material is called ion exchange resin.

Ion exchange resins

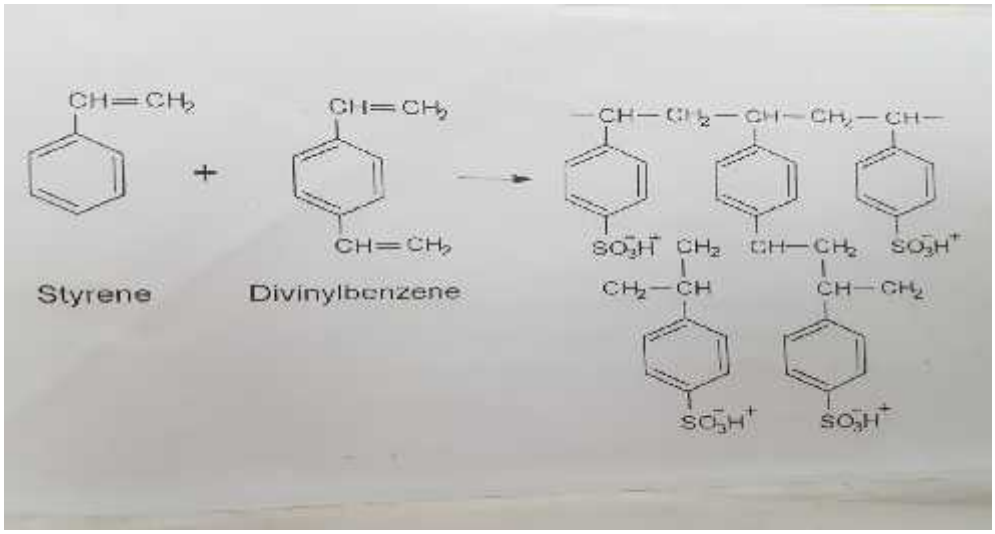
Synthetic ion-exchange resins are high-molecular weight polymers that contain large numbers of an ionic functional groups per molecule.

There are three types of ion exchange resin:

Cation exchange resins: These resins contain acidic functional groups. The strong –acid cation exchangers have sulfonic acid groups, ($-SO_3^-H^+$), which are strong acids. The weak –acid cation exchanger have carboxylic acid groups, (-COOH), which are only partially ionized. The protons on these groups can exchange with other cations. Cation-exchange resin is prepared by a polymerization of styrene and divinylbenzene, followed by sulfonation.

Anion exchange resins : anion exchangers contain quaternary ammonium [$-N^+ (CH_3)_3OH^-$] groups (strong-base). Whereas weak-base types contain secondary or tertiary amines.

Mixed exchange resins: these resins contain acidic and basic functional groups (HO----R-----H).



The important properties which determine the behavior of a resin can be summarized:

1. Size of particles- rate of exchange and permeability of packed column.

2. Degree of cross-linking- rigidity, porosity, swelling.

3. Nature of functional group- kind of ion exchanged.

4. Strength of functional group- distribution coefficient.

5. Number of functional groups-capacity of resin.

Experiment No.1

Cation Exchange Column Preparation and Determination of Total Capacity by Used Sodium Chloride

Theory

The total capacity of an ion exchange resin is defined as the total number of chemical equivalents or charged sites available for exchange per some unit weight or unit volume of resin. High capacity resins require rather concentrated eluents to elute sample ions from the column, but they do have the ability to handle large sample loads

Procedure

1- Wash column containing resin cation exchange using 25 mL from HCl (0.1 N) at flow rate (20 drops in minute).
2- Wash column by distilled water to remove all acid by using pH-paper
3- Add sample (NaCl) and wash by distilled water (using pH-paper).
4- Titrate the flowing solution with NaOH (0.1N) using indictor ph.ph.

Calculations

1- Calculate the weight of resin
$W = r^2 \times \pi \times L$

2- Calculate total capacity
$T.C. = N \times V / W$

$N = 0.1$ N NaOH

V = volume of titration

Experiment No.2

Determination of Percentage From Sulfate Ions by Used Cation Exchange Chromatography

$$RSO_3^- H^+ + Na_2SO_4 \longrightarrow H_2SO_4 + RSO_3^- Na^+ \quad \text{.....(1)}$$

$$H_2SO_4 + NaOH \xrightarrow{\text{ph.ph}} Na_2SO_4 + H_2O \quad \text{.....(2)}$$

Procedure

1. Wash column containing resin cation exchange using 25 mL HCl (1:10) at flow rate (20 drops in minute).
2. Wash column by distilled water to remove all acid using pH-paper.
3. Weigh 0.15 g of sample (dissolve by 10 mL distilled water).
4. Transfer the sample to column and Collect the flowing solution (acid) from the column by conical flask.
5. Wash column by distilled water and Collect the flowing solution from the column in conical flask to ensure the down flow of all acid using pH-paper.
6. Titrate with NaOH (0.1N) by using ph.ph.
7. Wash column by 25 mL HCl (1:10).

Calculations

Calculate the percentage of sulfate ion:

$$\% SO_4^{2-} = \frac{\text{wt. of } SO_4^{2-}}{\text{Wt. of sample}} \times 100$$

$$\text{Wt. of } SO_4^{2-} = \frac{N_{NaOH} \times V_{NaOH} \times \text{eq.wt } SO_4^{2-}}{1000}$$

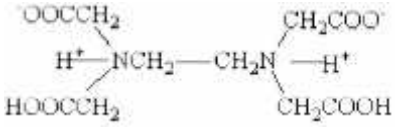
Experiment No.3

Ion Exchange Chromatography and Complex Titration

Theory

1. Chelometric Titrations

Titrations with Ethylenediaminetetraacetic Acid (EDTA)



The introduction of ethylenediaminetetraacetic acid, as a titrant in analytical chemistry has done a great deal to simplify the analysis of metal ions. This reagent and the other chelons are unique in that they form soluble but very slightly dissociated complex ions with many polyvalent cations and consequently can be used effectively as titrating agents. Procedures, for example, have been developed for the volumetric determination of alkaline earths, rare earths, Mn, Fe, Cu, Hg, Cr, Co, Ni, Zn, Ce, Pb, Al, Ga, In, Ti, Pd, Bi, Zr, Sc, and others; but the first and probably still the most practiced is the determination of hardness (I.e. , Ca^{++} and Mg^{++}) in water.

2. Titration of Zinc and Magnesium

Ethylenediaminetetraacetic acid, abbreviated as EDTA or H_4Y, forms a series of salts in neutralization with sodium hydroxide. One of these salts, $Na_2H_2Y\ 2H_2O$, is commercially available in a high degree of purity and is used in the preparation of the titration solution.* By proper purification and drying, this compound can be prepared sufficiently pure to serve as a primary standard. When this solution reacts with zinc and magnesium ions, the reactions can be represented by the following equations:

$$H_2Y^{=} \rightleftharpoons HY^{3-} + H^{+} \qquad pK_3 = 6.16$$

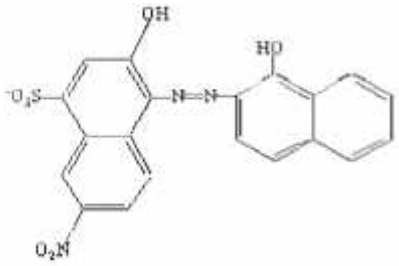
$$HY^{3-} \rightleftharpoons Y^{4-} + H^{+} \qquad pK_4 = 10.26$$

$Y^{4-} + Zn^{++} \rightleftharpoons ZnY^{=}$ log K = 10.64

$Y^{4-} + Mg^{++} \rightleftharpoons MgY^{=}$ log K = 8.7

3. Use of Metal Ion Indicator

A very satisfactory indicator for the titration of the sum of Zn^{++} and Mg^{++} at pH 10 is Eriochrome Black T (also called CI203, its color index number).



It is a tribasic acid and forms colored soluble complexes with both zinc and magnesium ions. The magnesium complex with the indicator is more stable than the complex of zinc with the indicator but less stable than the magnesium EDTA complex. Thus during a titration, the EDTA reacts first with the free zinc ions, then with the free magnesium ions, and finally with the magnesium in the indicator complex.

$Mg^{++} + HIn^{=} \rightleftharpoons MgIn^{-} + H^{+}$

(blue) (pink)

$+$

HY^{3-}

$\downarrow\uparrow$

$MgY^{=}$

$+$

H^{+}

When the end point is approached, the solution turns from a pink to a purple to a pure blue. The final pure blue is the end point. The position of the equilibrium in the above equation shifts to the left as the magnesium ions react with EDTA, thereby causing the solution to become pure blue when a quantity of EDTA equivalent to the magnesium content has been added. In other words, titrate until all of the pink tinge (Mg^{++}- indicator complex) is completely discharged and the addition of an extra drop of EDTA does not produce a color change. Since the magnesium indicator complex is wine red in color and th free indicator is blue between pH's of 6.5 and 11.5.

$$H_2In^- \rightleftharpoons HIn^= \rightleftharpoons In^{3-}$$

pH 6.5 pH 11.5

(red) (blue) (red)

the color of the solution changes from wine red to blue at the end point. A pH of 10 is best for the titration of the sum of zinc and magnesium. In more alkaline solutions, zinc and magnesium hydroxides may be precipitated, whereas in more acid media, the magnesium is not bound strongly enough to the indicator to give the wine red compound. The optimum pH can be readily obtained and maintained by the addition of a sufficient amount of an ammonia-ammonium chloride buffer.

4. Titration of Mgnesium Only

For this titration the solution is buffered at a pH of 10 and an excess of KCN is added to complex the zinc so tightly that EDTA cannot titrate it. In this way the magnesium is titrated alone. The CN^- thus acts as a "masking agent" for Zn^{++} ion. By the use of such masking agents, EDTA can be used as a more selective titrant. In a similar manner CN^- will mask Ni^{++}, Co^{++}, and Cd^{++}, and triethanolamine will mask Fe^{+++}, Al^{+++}, and Mn^{++}, Zn^{++}, and Cd^{++} can be selectively "demasked" by the action of formaldehyde.

Another method by which the EDTA titration can be made more selective is pH selection. Since EDTA is an acid, the H^+ concentration enters into the equilibrium expressions for the stability of the metal chelates. Thus the alkaline earths which form relatively weak complexes with EDTA can only be titrated in alkaline solution where the H^+ concentration is low

$$HY^{3-} + Mg^{++} \rightleftharpoons MgY^{=} + H^+$$

and the above equilibrium is shifted to the right. However, metals like Bi^{3+} which form very strong complexes with EDTA can be titrated in acid solution. The common indicators are similarly affected, and thus it is possible to titrate many metal ions (rare earths, transition and heavy metal ions) in acid solution without interference from the alkaline earths.

Procedure

1. Wash column containing resin cation exchange by 25 mL HCl (6 N).
2. Wash column with distilled water to remove all acid using pH-paper.
3. add (10 mL) sample solution contains (H^+,Na^+,Ca^{++},Mg^{++}) to column, and Collect the flowing solution (acid) from the column by conical flask .
4. Wash column by distilled water and Collect the flowing solution from the column in conical flask to ensure the down flow of all acid using pH-paper.
5. Titrate flowing solution with NaOH (0.1N) using ph.ph(*).
6. Transfer 5 mL from solution unknown to conical flask and titrate with NaOH (0.1N) using ph.ph (**).

7. Transfer 5 mL the unknown solution to conical flask and add 2 mL buffer solution (pH= 10) and titrate with E.D.T.A (0.02M) by using E.B.T.(***).
8. Transfer 5 mL the unknown solution to conical flask and add 2 mL NaOH (4 M , pH= 12) and titrate with E.D.T.A (0.02M) using Murexide.(****).

Calculation

1- concentration of H^+
2- concentration of Na^+
3- concentration of Ca^{++}
4- concentration of Mg^{++}

Notes

1- (*) = volume of NaOH ***Equivalent*** (H^+,Na^+)
2- (**) = volume of NaOH ***Equivalent*** H^+
3- (***) = volume of E.D.T.A ***Equivalent*** (Mg^{++},Ca^{++})
4- (****)= volume of E.D.T.A ***Equivalent*** Ca^{++}

Experiment No.4

Determination of chloride by anion exchange chromatography

Theory

$RN^+(CH_3)_3OH^- + NaNO_3 \longrightarrow RN^+(CH_3)_3 NO_3^- + NaOH$……..…(1)

$RN^+(CH_3)_3 NO_3^- + NaCl \longrightarrow RN^+(CH_3)_3 Cl^- + NaNO_3$ ……..…(2)

$RN^+(CH_3)_3 Cl^- + NaNO_3 \longrightarrow RN^+(CH_3)_3 NO_3^- + NaCl$……..……(3)

$NaCl + AgNO_3 \longrightarrow AgCl\downarrow + NaNO_3$ ……..….(4)

$AgNO_3 + K_2CrO_4 \longrightarrow Ag_2CrO_4\downarrow + KNO_3$ ……...……..…..(5)

Procedure

1. Wash column containing resin anion exchange by 25 mL sodium nitrate (0.5M).
2. Wash column by 30mL of distilled water to remove all sodium hydroxide to ensure using ph.ph.
3. Add sample solution (0.01gm of unknown dissolves in 10mL distilled water) to column , and Collect the flowing solution from the column by conical flask.
4. Wash column by $NaNO_3$ (0.5M) to remove chloride ions and Collect the flowing solution from the column in conical flask to ensure the down flow of all chloride ions using $AgNO_3$* .
5. Titrate flowing solution with $AgNO_3$ (0.1N) using K_2CrO_4.

Calculations

Calculate the percentage of chloride ion:

$$\%Cl^- = \frac{N_{AgNO3} \times V_{AgNO3} \times (A.wt\ Cl^- / 1000)}{wt.\ of\ sample} \times 100$$

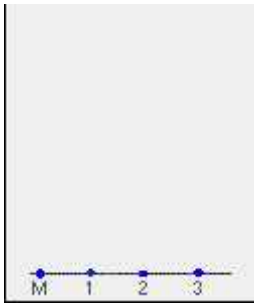
Experiment No.5

Paper chromatography

Chromatography is used to separate mixtures of substances into their components. All forms of chromatography work on the same principle. They all have a ***stationary phase*** (a solid, or a liquid supported on a solid) and a ***mobile phase*** (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates. We'll look at the reasons for this further down the page. In paper chromatography, the stationary phase is a very uniform absorbent paper. The mobile phase is a suitable liquid solvent or mixture of solvents.

Producing a paper chromatogram

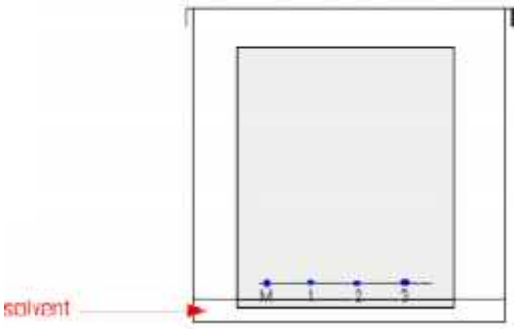
You probably used paper chromatography as one of the first things you ever did in chemistry to separate out mixtures of coloured dyes - for example, the dyes which make up a particular ink. That's an easy example to take, so let's start from there.Suppose you have three blue pens and you want to find out which one was used to write a message. Samples of each ink are spotted on to a pencil line drawn on a sheet of chromatography paper. Some of the ink from the message is dissolved in the minimum possible amount of a suitable solvent, and that is also spotted onto the same line. In the diagram, the pens are labelled 1, 2 and 3, and the message ink as M.



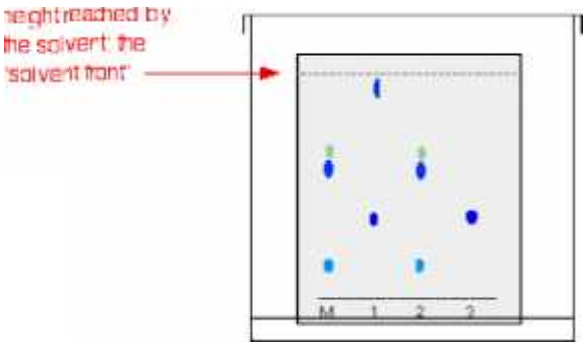
Note: The chromatography paper will in fact be pure white - not pale grey. I'm forced to show it as off-white because of the way I construct the diagrams. Anything I draw as pure white allows the background colour of the page to show through.

The paper is suspended in a container with a shallow layer of a suitable solvent or mixture of solvents in it. It is important that the solvent level is below the line with the spots on it. The next diagram doesn't show details of how the paper is suspended because there are too many possible ways of doing it and it clutters the diagram. Sometimes the paper is just coiled into a loose cylinder and fastened with paper clips top and bottom. The cylinder then just stands in the bottom of the container. The reason

for covering the container is to make sure that the atmosphere in the beaker is saturated with solvent vapour. Saturating the atmosphere in the beaker with vapour stops the solvent from evaporating as it rises up the paper.



As the solvent slowly travels up the paper, the different components of the ink mixtures travel at different rates and the mixtures are separated into different coloured spots.The diagram shows what the plate might look like after the solvent has moved almost to the top.



It is fairly easy to see from the final chromatogram that the pen that wrote the message contained the same dyes as pen 2. You can also see that pen 1 contains a mixture of two different blue dyes - one of which *might* be the same as the single dye in pen 3.

R_f values

Some compounds in a mixture travel almost as far as the solvent does; some stay much closer to the base line. The distance travelled relative to the solvent is a constant for a particular compound as long as you keep everything else constant - the type of paper and the exact composition of the solvent, for example. The distance travelled relative to the solvent is called the R_f value. For each compound it can be worked out using the formula:

$$R_f = \frac{\text{distance travelled by compound}}{\text{distance travelled by solvent}}$$

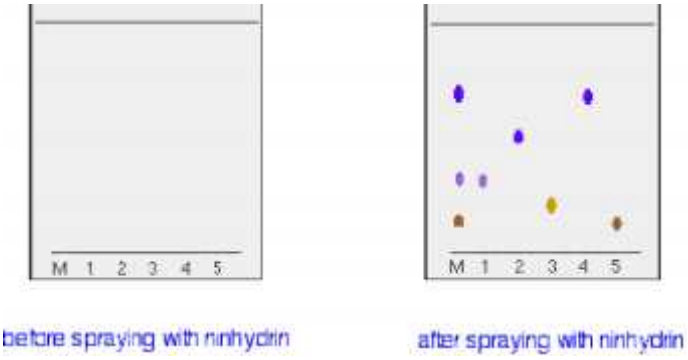
For example, if one component of a mixture travelled 9.6 cm from the base line while the solvent had travelled 12.0 cm, then the R_f value for that component is:

$$R_f = \frac{9.6}{12.0} = 0.80$$

In the example we looked at with the various pens, it wasn't necessary to measure R_f values because you are making a direct comparison just by looking at the chromatogram. You are making the assumption that if you have two spots in the final chromatogram which are the same colour and have travelled the same distance up the paper, they are most likely the same compound. It isn't necessarily true of course - you could have two similarly coloured compounds with very similar R_f values. We'll look at how you can get around that problem further down the page.

What if the substances you are interested in are colourless?

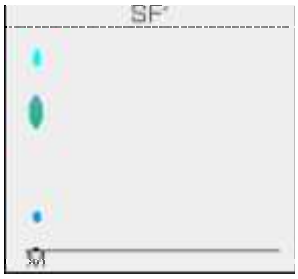
In some cases, it may be possible to make the spots visible by reacting them with something which produces a coloured product. A good example of this is in chromatograms produced from amino acid mixtures.Suppose you had a mixture of amino acids and wanted to find out which particular amino acids the mixture contained. For simplicity we'll assume that you know the mixture can only possibly contain five of the common amino acids.A small drop of a solution of the mixture is placed on the base line of the paper, and similar small spots of the known amino acids are placed alongside it. The paper is then stood in a suitable solvent and left to develop as before. In the diagram, the mixture is M, and the known amino acids are labelled 1 to 5. The position of the solvent front is marked in pencil and the chromatogram is allowed to dry and is then sprayed with a solution of ***ninhydrin***. Ninhydrin reacts with amino acids to give coloured compounds, mainly brown or purple. The left-hand diagram shows the paper after the solvent front has almost reached the top. The spots are still invisible. The second diagram shows what it might look like after spraying with ninhydrin.



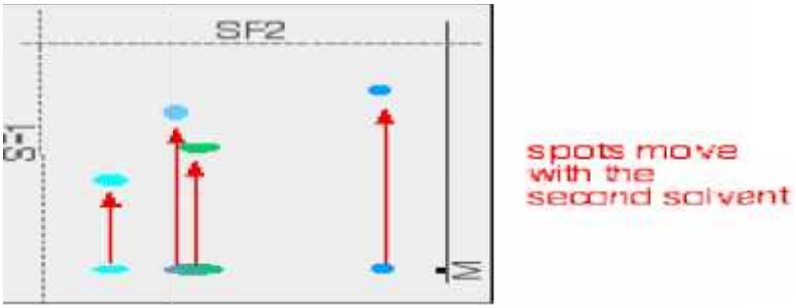
There is no need to measure the R_f values because you can easily compare the spots in the mixture with those of the known amino acids - both from their positions and their colours. In this example, the mixture contains the amino acids labelled as 1, 4 and 5. And what if the mixture contained amino acids other than the ones we have used for comparison? There would be spots in the mixture which didn't match those from the known amino acids. You would have to re-run the experiment using other amino acids for comparison.

Two way paper chromatography

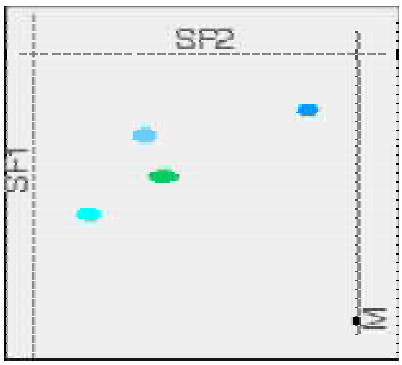
Two way paper chromatography gets around the problem of separating out substances which have very similar R_f values. I'm going to go back to talking about coloured compounds because it is much easier to see what is happening. You can perfectly well do this with colourless compounds - but you have to use quite a lot of imagination in the explanation of what is going on! This time a chromatogram is made starting from a single spot of mixture placed towards one end of the base line. It is stood in a solvent as before and left until the solvent front gets close to the top of the paper. In the diagram, the position of the solvent front is marked in pencil before the paper dries out. This is labelled as SF1 - the solvent front for the first solvent. We shall be using two different solvents.



If you look closely, you may be able to see that the large central spot in the chromatogram is partly blue and partly green. Two dyes in the mixture have almost the same R_f values. They could equally well, of course, both have been the same colour - in which case you couldn't tell whether there was one or more dye present in that spot. What you do now is to wait for the paper to dry out completely, and then rotate it through 90°, and develop the chromatogram again in a different solvent. It is very unlikely that the two confusing spots will have the same R_f values in the second solvent as well as the first, and so the spots will move by a different amount. The next diagram shows what might happen to the various spots on the original chromatogram. The position of the second solvent front is also marked.



You wouldn't, of course, see these spots in both their original and final positions - they have moved! The final chromatogram would look like this:



Two way chromatography has completely separated out the mixture into four distinct spots.If you want to identify the spots in the mixture, you obviously can't do it with comparison substances on the same chromatogram as we looked at earlier with the pens or amino acids examples. You would end up with a meaningless mess of spots.You can, though, work out the R_f values for each of the spots in both solvents, and then compare these with values that you have measured for known compounds under exactly the same conditions.

Experiment No.5

Separation of a Mixture of Halides by Paper Chromatography

Procedure

1. Take 5 clean test tubes and 5 capillary tubes.
2. Obtain 5 a rectangular pieces of Whatman filter paper and by using a pencil (not a pen) and a ruler, draw a line 1 cm from one end of the paper represents the location of solute
3. Capillary tube is used to put a drop of 3 halides on the three paper

(NaI, NaBr, NaCl)

and put a drop of a fourth mix pigments on paper fourth, the fifth paper by putting your drop of the unknown.

4. Put in each test tubes 2 cm of solvent, which is a

(Acetone 5, Water 1)

And using long-leg funnel to prevent the fall of the solvent on the internal walls of the tubes .

5. Put all 5 papers in test tubes and take care not to touch the solvent to the of sites halide and leave for 30 minutes.
6. Get out the papers and leave it to dry and put them silver nitrate and leave it to dry again.

Calculations

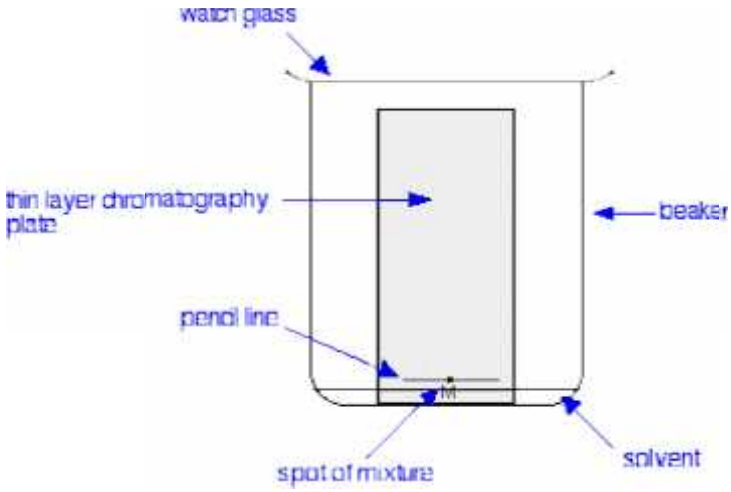
$$R_f = \frac{\text{distance travelled by compound}}{\text{distance travelled by solvent}}$$

Thin layer chromatography

They all have a ***stationary phase*** (a solid, or a liquid supported on a solid) and a ***mobile phase*** (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates. We'll look at the reasons for this further down the page. Thin layer chromatography is done exactly as it says - using a thin, uniform layer of silica gel or alumina coated onto a piece of glass, metal or rigid plastic. The silica gel (or the alumina) is the stationary phase. The stationary phase for thin layer chromatography also often contains a substance which fluoresces in UV light - for reasons you will see later. The mobile phase is a suitable liquid solvent or mixture of solvents.

Producing the chromatogram

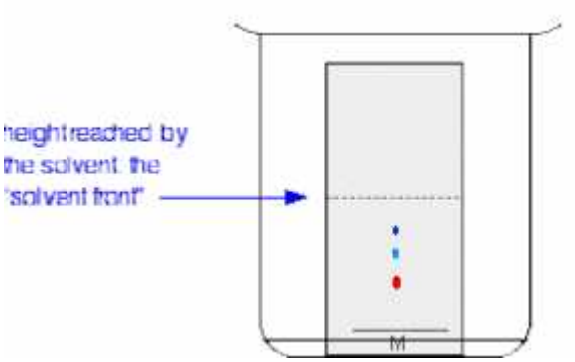
We'll start with a very simple case - just trying to show that a particular dye is in fact a mixture of simpler dyes.



Note: The chromatography plate will in fact be pure white - not pale grey. I'm forced to show it as off-white because of the way I construct the diagrams. Anything I draw as pure white allows the background colour of the page to show through

A pencil line is drawn near the bottom of the plate and a small drop of a solution of the dye mixture is placed on it. Any labelling on the plate to show the original position of the drop must also be in pencil. If any of this was done in ink, dyes from the

ink would also move as the chromatogram developed. When the spot of mixture is dry, the plate is stood in a shallow layer of solvent in a covered beaker. It is important that the solvent level is below the line with the spot on it. The reason for covering the beaker is to make sure that the atmosphere in the beaker is saturated with solvent vapour. To help this, the beaker is often lined with some filter paper soaked in solvent. Saturating the atmosphere in the beaker with vapour stops the solvent from evaporating as it rises up the plate. As the solvent slowly travels up the plate, the different components of the dye mixture travel at different rates and the mixture is separated into different coloured spots.

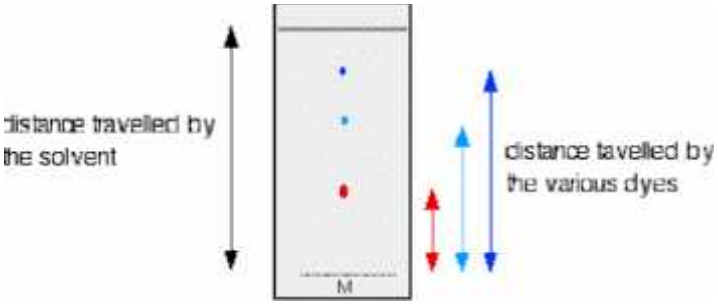


The diagram shows the plate after the solvent has moved about half way up it.

The solvent is allowed to rise until it almost reaches the top of the plate. That will give the maximum separation of the dye components for this particular combination of solvent and stationary phase.

Measuring R_f values

If all you wanted to know is how many different dyes made up the mixture, you could just stop there. However, measurements are often taken from the plate in order to help identify the compounds present. These measurements are the distance travelled by the solvent, and the distance travelled by individual spots. When the solvent front gets close to the top of the plate, the plate is removed from the beaker and the position of the solvent is marked with another line before it has a chance to evaporate.These measurements are then taken:



The R_f value for each dye is then worked out using the formula:

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

For example, if the red component travelled 1.7 cm from the base line while the solvent had travelled 5.0 cm, then the R_f value for the red dye is:

$$R_f = \frac{1.7}{5.0}$$
$$= 0.34$$

If you could repeat this experiment under *exactly* the same conditions, then the R_f values for each dye would always be the same. For example, the R_f value for the red dye would always be 0.34. However, if anything changes (the temperature, the exact composition of the solvent, and so on), that is no longer true. You have to bear this in mind if you want to use this technique to identify a particular dye. We'll look at how you can use thin layer chromatography for analysis further down the page.

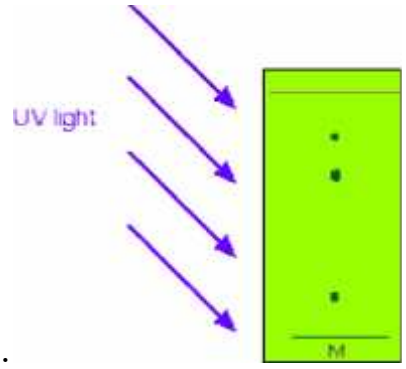
What if the substances you are interested in are colourless?

There are two simple ways of getting around this problem.

Using fluorescence

You may remember that I mentioned that the stationary phase on a thin layer plate often has a substance added to it which will fluoresce when exposed to UV light. That means that if you shine UV light on it, it will glow. That glow is masked at the position where the spots are on the final chromatogram - even if those spots are

invisible to the eye. That means that if you shine UV light on the plate, it will all glow apart from where the spots are. The spots show up as darker patches.

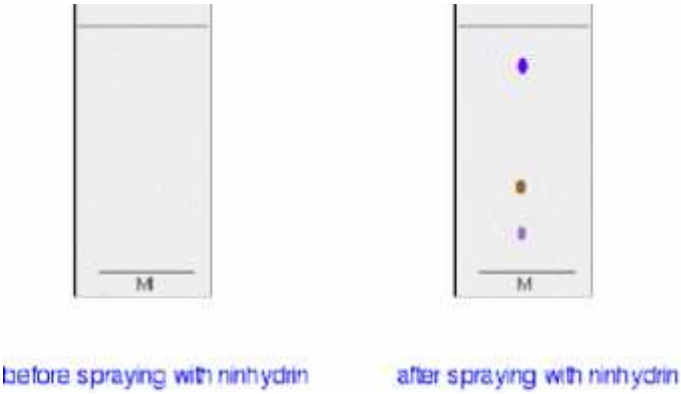


While the UV is still shining on the plate, you obviously have to mark the positions of the spots by drawing a pencil circle around them. As soon as you switch off the UV source, the spots will disappear again.

Showing the spots up chemically

In some cases, it may be possible to make the spots visible by reacting them with something which produces a coloured product. A good example of this is in chromatograms produced from amino acid mixtures.

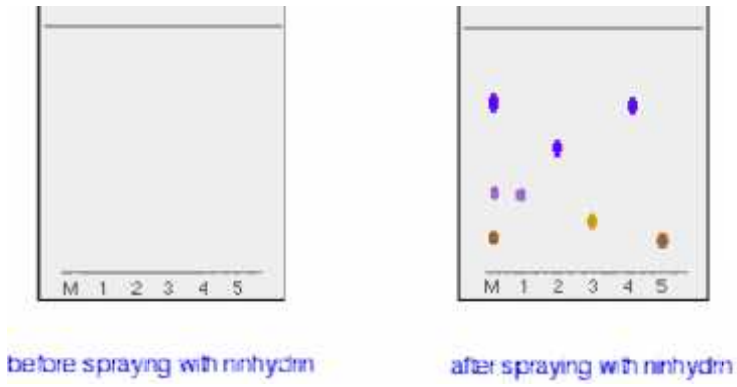
The chromatogram is allowed to dry and is then sprayed with a solution of ***ninhydrin***. Ninhydrin reacts with amino acids to give coloured compounds, mainly brown or purple.



In another method, the chromatogram is again allowed to dry and then placed in an enclosed container (such as another beaker covered with a watch glass) along with a few ***iodine crystals***. The iodine vapour in the container may either react with the spots on the chromatogram, or simply stick more to the spots than to the rest of the plate. Either way, the substances you are interested in may show up as brownish spots.

Using thin layer chromatography to identify compounds

Suppose you had a mixture of amino acids and wanted to find out which particular amino acids the mixture contained. For simplicity we'll assume that you know the mixture can only possibly contain five of the common amino acids. A small drop of the mixture is placed on the base line of the thin layer plate, and similar small spots of the known amino acids are placed alongside it. The plate is then stood in a suitable solvent and left to develop as before. In the diagram, the mixture is M, and the known amino acids are labelled 1 to 5. The left-hand diagram shows the plate after the solvent front has almost reached the top. The spots are still invisible. The second diagram shows what it might look like after spraying with ninhydrin.

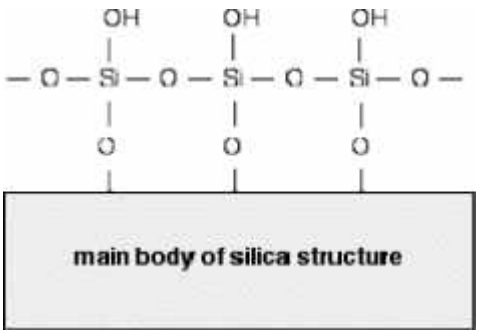


There is no need to measure the R_f values because you can easily compare the spots in the mixture with those of the known amino acids - both from their positions and their colours. In this example, the mixture contains the amino acids labelled as 1, 4 and 5. And what if the mixture contained amino acids other than the ones we have used for comparison? There would be spots in the mixture which didn't match those from the known amino acids. You would have to re-run the experiment using other amino acids for comparison.

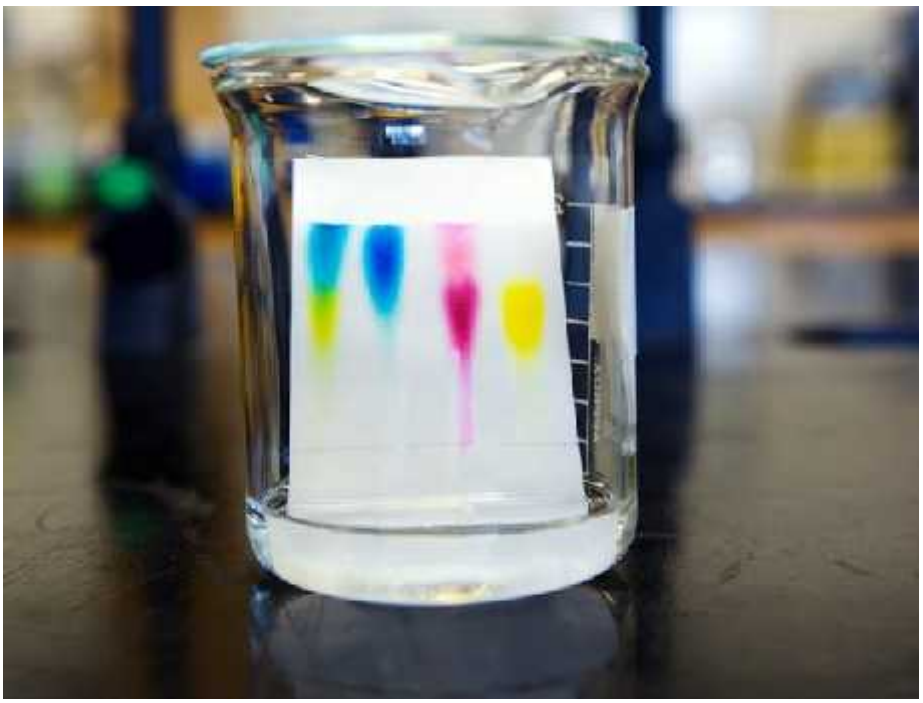
How does thin layer chromatography work?

The stationary phase - silica gel

Silica gel is a form of silicon dioxide (silica). The silicon atoms are joined via oxygen atoms in a giant covalent structure. However, at the surface of the silica gel, the silicon atoms are attached to -OH groups. So, at the surface of the silica gel you have Si-O-H bonds instead of Si-O-Si bonds. The diagram shows a small part of the silica surface



The surface of the silica gel is very polar and, because of the -OH groups, can form hydrogen bonds with suitable compounds around it as well as van der Waals dispersion forces and dipole-dipole attractions.The other commonly used stationary phase is alumina - aluminium oxide. The aluminium atoms on the surface of this also have -OH groups attached. Anything we say about silica gel therefore applies equally to alumina.



Paper Chromatography

Experiment No.6

Separation of a Mixture of Dyes by TLC

Procedure

1. Take 2 glass slides and clean with distilled water and acetone and wipe with a piece of cotton to dry.
2. Prepare the silica gel by dissolves silica gel 30 grams in 100 mL chloroform in a homogeneous.
3. stick the slides to each other and put them in silica gel and get out (in medium speed) and the process is repeated for six times , and then separate the slides from each other and then leave a go dry .
4. appointed positions the solute on the slides at 0.5 cm from edge of slides by a pin , capillary tube using to put a drop of three dyes on the first slide :

(Methylene blue - Alizarine – Fluoresciene)

and put a drop of fourth mix pigments and the unknown on the second slide .

5. put in beaker a small amount of solvent which is a

(Butanol 60 – Ammonia 20 – Ethanol 20)

then put the first and second slides in beaker and prevent contact with the solvent for dyes .

6. the put the lid on beaker to prevent evaporation of solvent and left for 20 min. and get out the slides from beaker and then left to dry

Calculations

$$R_f = \frac{\text{distance travelled by compound}}{\text{distance travelled by solvent}}$$

Experiment No.7

Separation of Black Ink Components by Paper Chromatography

Procedure

1. Take four clean test tubes and 4 capillary tubes.
2. Obtain four a rectangular pieces of Whatman filter paper and by using a pencil (not a pen) and a ruler, draw a line 1 cm from one end of the paper represents the location of solute
3. Capillary tube is used to put a drop ink on four papers:
4. Put in each test tubes 2 cm of different solvents, (some suggested solvents are:

   (Methanol, Ethanol, 50:50 water-methanol and 0.1M HCl).

5. Put all four papers in test tubes and leave for 30 min.
6. Get out the papers and leave it to dry.

Calculations

$$R_f = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent}}$$

How to write a report

The student should write a report after the completion of each experiment as follows:

Name of the Laboratory:

Name of Experiment:

Date:

Name of the Student:

Number of the Sample:

The purpose of an experiment

Theory:

Materials and Equipments:

Procedure:

Environmental conditions:

Calculations:

Discussion:

Chemical Hazards: