

Assignment: Read about thin layer chromatography

#### Ion exchange chromatography

This is a technique employed in analysing compounds that have ionic species. The process is founded on the interchange of ions that have the same signs between the solutions and insoluble solids that are in contact with the solutions.

Conventionally, ion exchange chromatography uses resins that carry charged functional groups which interact with oppositely charged particles of the compound in solution expected to be retained. The resins are used either in batch processes or in a column.

Resins may be classed as cationic or anionic exchangers depending on the nature of ions to be exchanged. Cationic exchanger resins are obtained when acidic functional groups are introduced in the solution. For example strong acids like sulphonic acids are introduced by sulphonation while weak acids like carboxylic acids which introduces the carboxyl functional groups are also used. Anionic exchanger resins on the other hand are obtained by introducing basic functional groups so that anions are exchanged. Resins with strong anions are made by introducing tertiary amines while weak anions are made by introducing secondary amines. Ion exchange chromatography is commonly employed in analytical work involving alkaline earth metals and amino acids.

#### Gel filtration chromatography

This type of chromatography may also be referred to as size exclusion chromatography or gel permeation chromatography. It basically applies by separating particles based on size. In this technique, smaller molecules permeate a porous media taking a longer time to leave the column while larger ones on the other hand pass through the same column within a short time. Often times, a gel, Dextran is used for this purpose. The gel is a three dimensional network of linear polysaccharide molecule. Gel filtration chromatography is employed on molecules with molecular weight lying between 100 to several millions. Such molecules include proteins, peptides, nucleic acids, enzymes and so on.

#### Ascertaining the purity of substances in the laboratory

To be sure that an organic compound being tested is pure some criteria are considered. For solid substances, the melting point is determined. A sharp melting point indicates a pure substance which can then be compared to the standard provided in data books. For example if an unidentified organic compound was determined to have a melting point close to  $133^{\circ}$ , three compounds may be considered namely, 3-phenylpropenoic acid (M.pt  $133^{\circ}$ ), carbamide (M.pt  $132^{\circ}$ ) and Propanedioic acid (M.pt  $134^{\circ}$ ). If a mixture of the organic compound and each of the three known compounds are assessed for their melting point and it was found to be  $114-129^{\circ}$ ,  $131.5-132^{\circ}$  and  $86-99^{\circ}$  respectively. The organic compound which did not change melting point as the known organic compound is accepted as the compound. Hence the unknown organic compound is carbamide.

Similarly, for liquids, the boiling point is determined to ascertain the purity of a compound. Pure substances have fixed boiling points while the presence of impurities elevates or raises the boiling point of the liquid.

### **STEP INVOLVED IN THE DETERMINATION OF THE STRUCTURE OF ORGANIC COMPOUND**

#### **QUALITATIVE AND QUANTITATIVE ANALYSIS OF ORGANIC COMPOUNDS**

Organic compounds are compounds of carbon and hydrogen alongside oxygen, sulphur, nitrogen and the halogens. To determine the exact nature of a pure compound, the knowledge of the elements that are present in the compound and their relative proportions in which they occur is required. This can be done successfully by carrying out qualitative and quantitative analysis on the sample of organic compound provided.

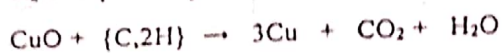
#### **Qualitative analysis of organic compounds**

To adequately identify which elements are present in an organic compound, it is necessary to convert the elements into states that will make them furnish ionic radicals in solution.

##### **a. identifying carbon and hydrogen in an organic compound**



A powdered sample of an organic compound is mixed with an equal volume of dry copper(II) oxide and heating them together in a clean dry test tube. Any carbon or hydrogen present in the compound is converted into  $\text{CO}_2$  and  $\text{H}_2\text{O}$  respectively.



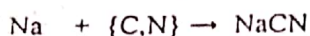
To confirm the presence of carbon as carbon(IV)oxide, use lime water which changes to milky color. For hydrogen to be identified as water, the water which condenses on the cooler parts of the test tube anhydrous copper(II) sulphate or blue cobalt(II)chloride which gives a pink color.

#### b. Identifying halogen, nitrogen and sulphur

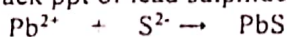
This is made possible by a laboratory test usually referred to as the lassaing's sodium fusion test. In this test, a small piece of bright sodium is taken into a small test tube and heated to redness by a direct flame. The organic compound been tested is dropped directly on the red hot sodium while the heating continues. It is allowed to cool and heated again to red hot. The red hot tube is plunged into a beaker containing about  $25\text{cm}^3$  of distilled water. The broken tube and the products of the lassaing fusion is ground with a pestle and transferred into a boiling tube. The mixture is then boiled, filtered and the filtrate divided into portions for test, the fusion converts halogens and sulphur in the compound into sodium halide and sodium sulphide respectively.



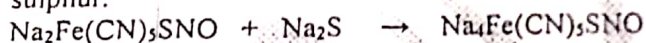
And nitrogen in the presence of carbon gives sodium cyanide



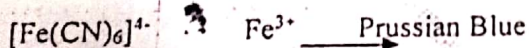
1. To identify halogens-A portion of the filtrate is acidified with dilute  $\text{HNO}_3$  and treated with  $\text{AgNO}_3$  to give a white ppt for  $\text{AgCl}$ , off white for  $\text{AgBr}$  and a yellow ppt for  $\text{AgI}$ . In this case, dilute  $\text{HNO}_3$  is added to destroy any  $\text{NaCN}$  present which could also give white ppt if treated with  $\text{AgNO}_3$ .
2. To identify sulphur, add about  $1\text{cm}^3$  of lead ethanoate solution to a portion of the filtrate which gives a black ppt of lead sulphide.



Also, reacting a filtrate with sodium nitroprusside gives a pink or purple colour indicating the presence of sulphur.



3. To identify nitrogen,  $\text{FeSO}_4$  is added to a portion of the filtrate to give a hexacyanoferrate(II). Adding a few drops of  $\text{FeCl}_3$  and an excess of dilute  $\text{H}_2\text{SO}_4$  gives an intense colored Prussian blue or a green-blue coloration indicating the presence of nitrogen in the organic compound.



#### Quantitative analysis

After successfully identifying the constituent elements in the organic compound, the next step is to know how much of each component is present in the organic compound. Essentially, the quantity of this components are usually expressed in percentages.

#### 1. Carbon and hydrogen

When a known mass of the organic compound, is taken in a stainless combustion tube and allowed to heat up in a stream of dry oxygen to about  $900^\circ\text{C}$ , the hydrogen and carbon in the organic compound burns to form water and Carbon(IV) oxide which are then absorbed into previously weighed absorption tubes which contain magnesium chlorate(VII) which is a neutral dessicant and soda-lime or a strong base like  $\text{NaOH}$ . After which the tubes are then reweighed to give the masses of water and Carbon(IV) oxide absorbed. Therefore by relating the masses to their relative molar masses, the percentage of the carbon and hydrogen in the original compound can be determined. This is generally expressed in the following equation;

$$\text{Mass of C} = \frac{\text{Relative atomic mass of carbon} \times \text{mass of carbon(IV)oxide obtained}}{\text{Relative molar mass of Carbon (IV)Oxide}}$$

And



$$\text{Mass of H} = \frac{\text{Relative atomic mass of hydrogen} \times \text{mass of water absorbed}}{\text{Relative molar mass of water}}$$

Consequently, with the known mass of the compound taken, the % Composition of carbon and hydrogen could be determined as indicated in the relationship below;

$$\% \text{ C} = \frac{\text{Relative atomic mass of Carbon} \times \text{mass of carbon(IV)oxide obtained} \times 100}{\text{Relative molar mass of Carbon (IV)Oxide} \times \text{Known mass of organic compound}}$$

OR

$$\% \text{ C} = \frac{\text{Mass of carbon} \times 100}{\text{Known mass of Organic compound}}$$

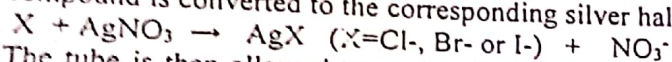
Similarly for water,

$$\% \text{ H} = \frac{\text{Relative atomic mass of hydrogen} \times \text{mass of water obtained} \times 100}{\text{Relative molar mass of water} \times \text{Known mass of organic compound}}$$

$$= \frac{\text{mass of hydrogen} \times 100}{\text{Known mass of organic compound}}$$

## 2. Halogens (Carius method)

To determine halogens quantitatively in an organic compound, take a known quantity of material that will sufficiently yield 0.2-0.5g of the silver halide. Heat a mixture of fuming  $\text{HNO}_3$  and an excess of silver nitrate ( $\text{AgNO}_3$ ) in a sealed tube for several hours at  $270^\circ\text{C}$ . Any halogen present in the compound is converted to the corresponding silver halide.

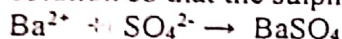


The tube is then allowed to cool and then opened. The Ag halide precipitate is filtered off, washed, dried and weighed. The percentage of halide present in the compound can be determined from the relationship;

$$\% \text{ X} = \frac{\text{Relative atomic mass of X} \times \text{mass of AgX obtained} \times 100}{\text{Relative molar mass of silver halide} \times \text{known mass of organic compound}}$$

## 3. Sulphur

Sulphur can also be determined quantitatively by the Carius method. For this, a sample of the organic compound is heated in a tube along with fuming  $\text{HNO}_3$  for several hours at  $270^\circ\text{C}$ . This converts carbon, hydrogen and sulphur in the organic compound to  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and sulphate ions respectively. After cooling to room temperature, the tube is opened and the contents treated with an excess of  $\text{BaCl}_2$  solution so that the sulphate is precipitated as  $\text{BaSO}_4$ .



The precipitate formed is now filtered off, washed, dried and weighed.

$$\% \text{ S} = \frac{\text{relative atomic mass of Sulphur} \times \text{mass of BaSO}_4 \text{ obtained} \times 100}{\text{relative molar mass of BaSO}_4 \times \text{known mass of Organic compound}}$$

## 4. Nitrogen

Nitrogen is determined quantitatively by two methods namely Dumas method and Kjeldahl method. The Dumas method requires that excess copper (II)Oxide is passed through a washed sample of organic compound converting the nitrogen to gaseous nitrogen. Oxides of nitrogen present are reduced by passing the gases over heated copper and collecting the nitrogen in a graduated tube known as a nitrometer for measurement. All  $\text{CO}_2$  and  $\text{SO}_2$  present are absorbed in 50%  $\text{KOH}$ . Nitrogen in the organic compound can be calculated since 1 mole of nitrogen i.e 28g of nitrogen occupies  $22400\text{cm}^3$  at STP.

The Kjeldahl method involves converting nitrogen in a weighed sample of an organic compound into ammonium sulphate by boiling Concentrated  $\text{H}_2\text{SO}_4$  and  $\text{K}_2\text{SO}_4$ . The mixture is then diluted and boiled with an excess of  $\text{NaOH}$  so that the ammonia evolved is absorbed in a known volume of standard acid and estimated by back titration.

## 5. Oxygen

The quantity of oxygen in an organic compound is often found by difference. This implies that the % of carbon and hydrogen and other elements in the organic compound are subtracted from 100 to give the % value of oxygen.

**Determination of empirical and molecular formulae of an organic compound**

The empirical formulae of a compound expresses the smallest whole number ratio of atoms of each element present in a molecule while the molecular formulae of a compound expresses the actual number of atoms of each element present in the molecule. The molecular formulae and empirical formulae are related in the equation;

$$\text{Molecular formula} = (\text{empirical formula})_n$$

Where n is a whole number which can be calculated if the R.M.M of the compound is known;



$$n = \frac{\text{Relative Molar mass}}{\text{Empirical formula weight}}$$

It should be noted that for a whole number ratio of an empirical formula to be attained, mathematical manipulations may be performed by multiplying each number by the smallest integer that can convert the ratios to the nearest whole numbers. This is necessary because some ratios may be fractions which on approximation may not give a reliable whole number.

### Example

If an organic compound has RMM 60 and containing C, 40.1% and H, 6.67%. What is (i) empirical formula (ii) Molecular formula of the compound if only carbon, hydrogen and oxygen are present?

### Solution

$$\% \text{O} = 100 - (40.1 + 6.67) = 53.23\%$$

	Carbon	Hydrogen	Oxygen
Ratio of masses (%):	40.1	6.67	53.23
Divide masses by R.A.M:	$\frac{40.1}{12}$	$\frac{6.67}{1}$	$\frac{53.23}{3.33}$
	3.34	6.67	3.33
Divide through by the Smallest	$\frac{3.34}{3.34}$	$\frac{6.67}{3.33}$	$\frac{3.33}{3.33}$
	1.003	2.003	1.000

(i) The empirical formulae therefore is  $\text{CH}_2\text{O}$

(ii) to determine the molecular formulae, first find the empirical formulae weight

$$12 + (2 \times 1) + 16 = 30$$

$$\text{Molecular formula} = (\text{CH}_2\text{O})_n$$

$$n = \frac{59}{30} = 2$$

The molecular formula therefore is  $\text{C}_2\text{H}_4\text{O}_2$ .

### Example 2

0.02592g of pure Aspirin-a compound which contains carbon, hydrogen and oxygen only-yielded 0.05702 carbon(IV)oxide and 0.01037g water on combustion. What is (a) the percentage composition and (b) the empirical formula.

### Solution

$$(a)(i) \% \text{C} = \frac{\text{R.A.M of carbon} \times \text{mass of CO}_2 \text{ obtained} \times 100}{\text{R.M.M of CO}_2 \times \text{Known mass of organic compound}}$$

$$= \frac{12 \times 0.05702 \times 100}{44 \times 0.02592}$$

$$= \frac{68.424}{1.14048} = 60.00\%$$

$$(ii) \% \text{H} = \frac{\text{R.A.M of H} \times \text{mass of H}_2\text{O obtained} \times 100}{\text{R.M.M of H}_2\text{O} \times \text{known mass of organic compound}}$$

$$= \frac{2 \times 0.01037 \times 100}{18 \times 0.02592}$$

$$\% \text{O} = 100 - (60.00 + 4.45)$$

$$= 35.55\%$$

	Carbon	Hydrogen	Oxygen
% mass	60.00	4.45	35.55
Amount of the atom	$\frac{60}{12}$	$\frac{4.45}{1}$	$\frac{35.55}{16}$
	5.00	4.45	2.22

Divide by the  
Smallest number

$$\frac{5}{2.22}$$

$$\frac{4.45}{2.22}$$

$$\frac{2.22}{2.22}$$

2.25

2.00

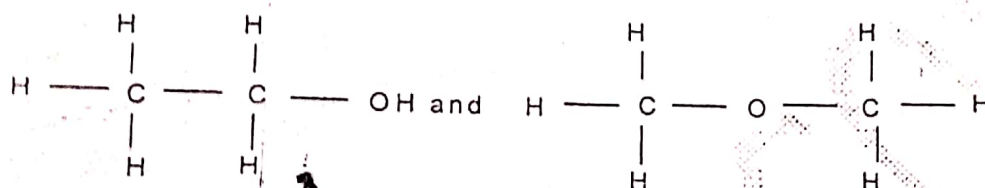
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2.25 is a fraction since to make it a whole number will require subtracting 0.25 or adding 0.75. Hence a factor must be made to multiply through to get a reliable whole number which is 4. Hence the ratio of C:H:O will then be 9:8:4. thus the empirical formula for aspirin is  $C_9H_8O_4$ .

N.B: fractions in the 0.2-0.8 range of decimals should be multiplied by a conversion factor. 0.1 and 0.9 could be approximated to the nearest whole number.

### ASSIGNMENT OF STRUCTURAL FORMULA

The structural formula of a compound expresses the arrangement of atoms or how atoms are linked together. For simple compounds having limited number of isomers, possible structural formulae could be deduced from the molecular formula on the valencies of the elements that are known. Consider a compound with molecular formula  $C_2H_6O$ , two possible structures are expected.



i.e either ethanol or dimethyl ether. Generally, for an assignment of structure to be appropriate, the knowledge of the following is required;

- The **chemical reactions** that the compound undergoes such as hydrolysis, oxidation, reduction among others based on the presence of some functional groups like the hydroxyl, carboxyl or amino groups. This information will enable the analyst eliminate possible isomers so that the choice of a probable structural formulae becomes possible for the compound. When complex molecules are involved, heating, hydrolysis, oxidation, reduction or any degradation mechanism could help to easily identify the compound.
- The **physical properties** shown by the compound such as boiling point and melting point can help to assign the structural formulae. Consider ethanol and ether which are isomers. Ethanol has a boiling point of  $78^\circ\text{C}$  while ether has a boiling point of  $-25^\circ\text{C}$ . This can help to assign their structural formulae. In addition, colour and smell can provide a guide as to which family an organic compound belongs. For example, esters have pleasant fruity smell making them different from their isomers, the carboxylic acids which do not have a characteristic smell.
- Results obtained from **spectroscopic studies** such as UV-Visible spectroscopy, Nuclear magnetic resonance spectroscopy, mass spectroscopy and infra-red spectroscopy.

There are different forms of electromagnetic radiations that constitute the electromagnetic spectrum. The range of electromagnetic radiations include visible light, infra red (IR), Ultra-violet (UV) rays and radio waves. To achieve transitions are possible therefore x-ray radiations and the UV-Visible radiations produce electronic transitions, Infra red radiations bring about molecular vibrations while microwave and radio waves bring about molecular inversions or rotations.

### ASSIGNMENT

- An organic liquid provided to Student in the laboratory during practical contained 62.1 % C and 10.3 % H. Suggest possible structural formulae for the liquid and briefly describe the chemical tests appropriate for distinguishing the isomers of this liquid.

### SOME SPECTROSCOPIC TECHNIQUES INVOLVED IN DETERMINATION OF STRUCTURE OF COMPOUND

#### 1. Ultraviolet and Visible region spectroscopy

This is a portion of the electromagnetic spectrum which absorbs energy that causes electronic transitions or promotions from ground state to higher energy levels or excited states. The effectiveness of this technique depends on the absorbing group or chromophore, intensity of absorption of electromagnetic radiation (which is determined



by how tightly electrons are coupled within a molecule). Ultraviolet spectroscopy is often used for the detection of Nitro groups and other multiple bonds while visible spectroscopy is employed only for coloured substances. Spectra in the visible and ultraviolet regions are applied for quantitative analysis of solutions because intensity of absorption is related to the concentrations of the solution(Beer-Lambert law).

## **2. Infra red (IR) spectroscopy**

This spectroscopic technique shows more absorption peaks hence yielding more information on the structure of compounds than the UV- visible spectra. Infra- red spectroscopy involves absorbing radiations that cause changes in the vibrational energy levels of the bonds in a molecule of a compound. The absorption peaks associated with this vibrational changes in specific bonds and functional groups occur within the wave number ranges. This is usually referred to as the finger print region. Infrared spectroscopy is applied for the identification of functional groups, geometrical isomers, position of substitution in aromatic compounds, demonstration of hydrogen bonding and detection of impurities among others.

## **3. Nuclear Magnetic Resonance(NMR) Spectroscopy**

This spectroscopic technique helps to observe the characteristics shown in hydrocarbon skeletons. The magnetic properties of the hydrogen nucleus enable the observation of the environment of the hydrocarbon nuclei as it is bonded in a molecular system. The technique determines physical and chemical properties of atoms or molecules. It essentially relies on nuclear magnetic resonance to provide information on structure, dynamics, reaction states and chemical environment of molecules. When an active nuclei  $^1\text{H}$  or  $^{13}\text{C}$  is placed in a magnetic field, it absorbs electromagnetic radiation at a frequency which is characteristic of the isotope. As a result NMR can therefore be used for functional group analysis, bonding connectivity and orientation among others.

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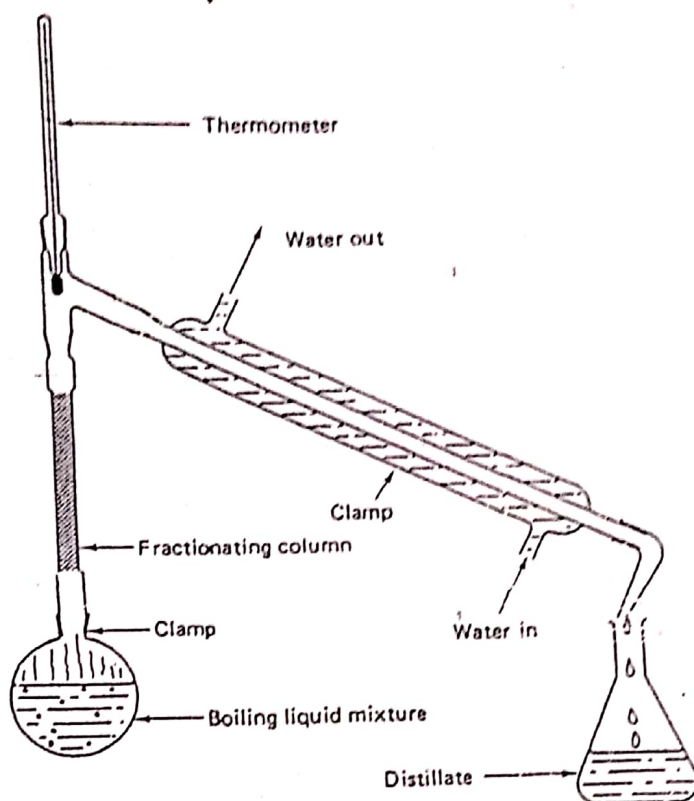


Fig. 2.1. Fractional distillation apparatus

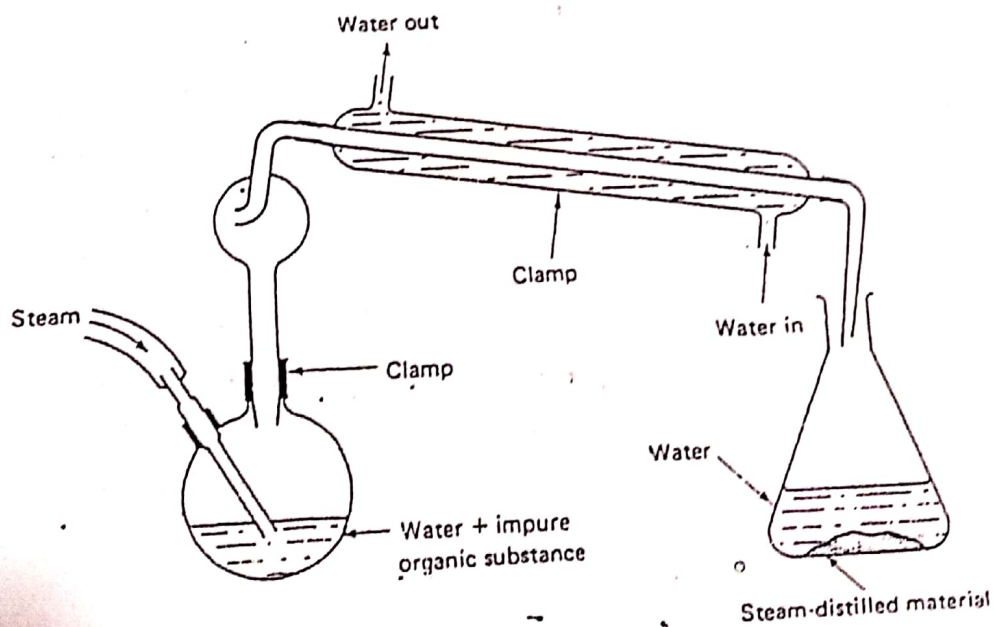


Fig. 2.2 Steam-distillation apparatus

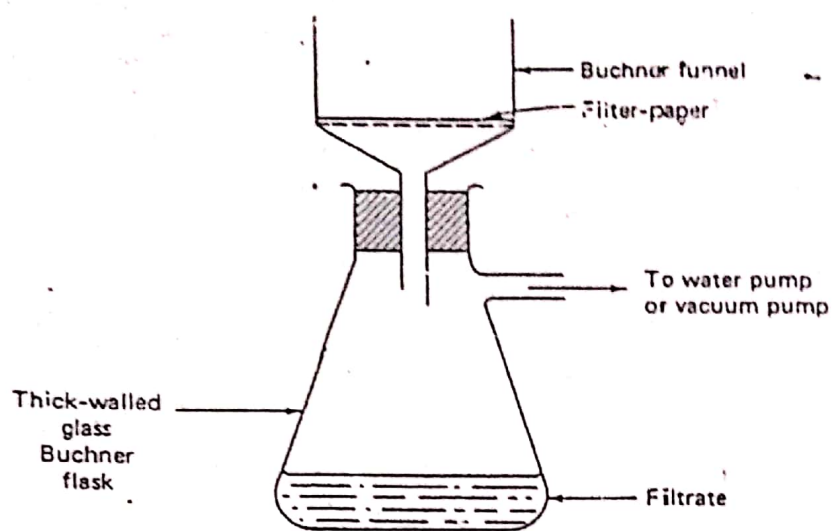


Fig. 2.3 A Buchner funnel and flask

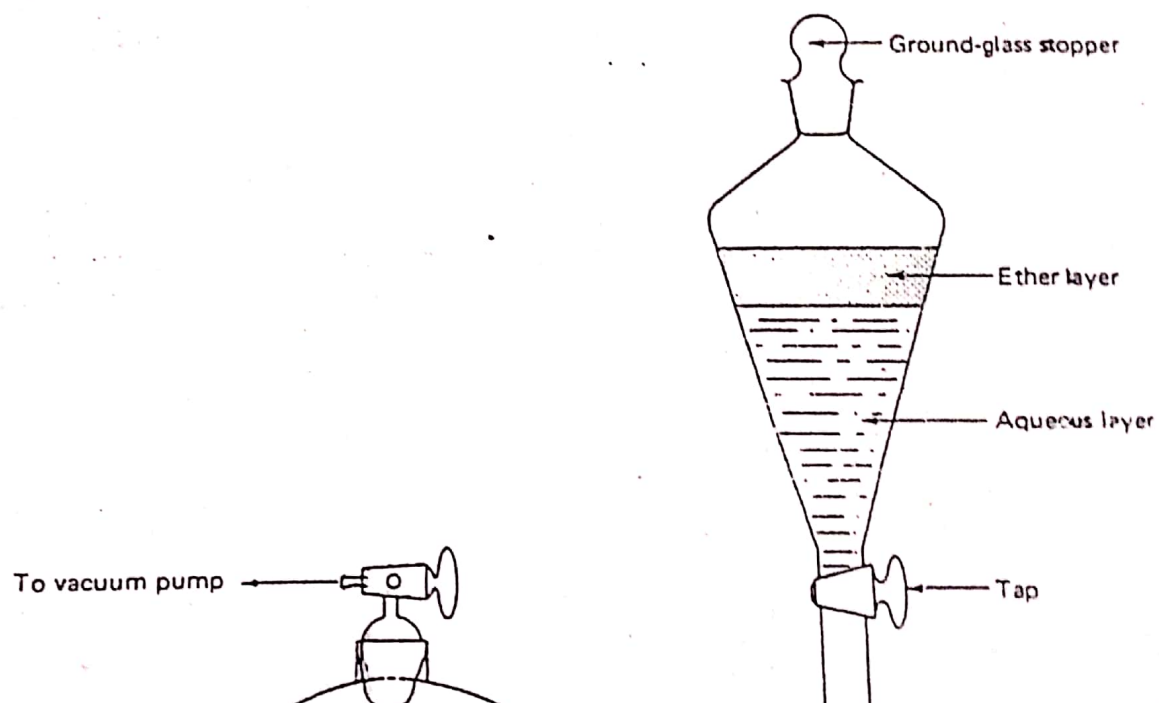


Fig. 2.5 A separating-funnel

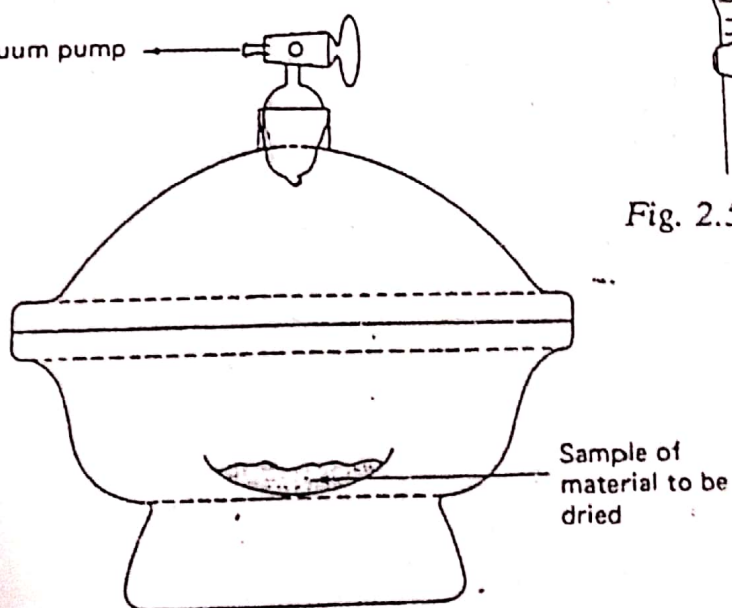


Fig. 2.4 A vacuum desiccator