



CHROMATOGRAPHY

Student Learning Outcomes (SLOs)

After completing this lesson, the student will be able to:

- Describe how paper chromatography is used to separate mixture of soluble substances, using a suitable solvent.
- Describe the use of locating agents when separating mixtures containing colourless substances. (F or context, knowledge of specific locating agents is not required)
- Interpret simple chromatograms (for context, students should identify:
 - a) unknown substance with known substances
 - b) pure and impure substances.
- State and use the equation for R_f .

INTRODUCTION

The word chromatography has been originated from two words one is Chroma means colour the other graphein means writing.

Chromatography has been originated as a very strong analytical tool in the analytical chemistry. This is not only used to analyse a compounds, it is used for separation of the compounds as well. Nowadays this technique can be coupled with other techniques like mass spectrometry.

19.1 PRINCIPLE OF CHROMATOGRAPHY

Chromatography works on the principle of partitioning where the components of a mixture distribute themselves between two phases one is called stationary phase and the other one is said to be mobile phase.

Stationary phase:

Stationary phase in chromatography is a solid phase or a liquid phase coated on the surface of a solid phase. Like in paper chromatography water molecules are adsorbed on paper material cellulose.

Mobile phase:

It is a liquid or gas that moves through or over the stationary phase.

As the mixture is carried by the mobile phase through the stationary phase, each component interacts differently with the two phases, leading to different migration rates. This results in the separation of the components based on their affinity for the stationary phase relative to the mobile phase.

19.2 Paper chromatography

In paper chromatography the stationery phase is the water absorbed on filter paper. The mobile phase can be a solvent or a mixture of solvents. A mixture of inks or some metal ions can be separated with the help of paper chromatography.

Procedure

1. Take 1 dm³ measuring cylinder fitted with the rubber bung carrying a glass hook at the lower end
2. Pour 25 cm³ of water and ethanol solvent mixture (6:4) in it and close it. Allow it to stand for 15 minutes.
3. Take a strip of paper (what Mann filter paper number one) 2.5 cm wide and lengthier than the length of the cylinder.
4. Mark a line 5 centimetre from one end its mid point with pencil.
5. Prepare a mixture of various inks like blue, black, green, red etc by taking two drops of each in a clean China dish. Mix them thoroughly.
6. Apply a very tiny drop of this mixture at the midpoint by means of capillary tube.
7. Dry the strip in the air for about 15 minutes and suspend it by the glass hook with the impregnated end dipping into the solvent to a depth of 5-6 mm.

8. When the solvent front has risen for about an hour or the solvent front is 2-3cm below the upper end of the filter paper.
9. Remove the paper strip and dry it in air. It is seen that every ink gives coloured bands at different regular intervals.
10. Measure the distance of these coloured bands from the baseline and the distance travelled by the solvent front from the baseline.
11. Calculate the R_f values for various inks by the formula given. In this way we can separate a mixture of various inks by chromatography.

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

R_f factor (retardation factor) is a measure of affinity of a component with mobile phase or stationary phase. If R_f value is greater it means that the component is more affine towards the mobile phase than to stationary phase.

Locating agent

A locating agent is a substance or a solution which is used to locate the components which have been separated by chromatography but are not visible due to being colourless. For example, a mixture of amino acids can be separated by paper chromatography or by thin layer chromatography (TLC) but they are not visible, to locate them ninhydrin is sprayed on the chromatogram. Ninhydrin reacts with amino acids and develops into a colourful chromatogram.

Reading a chromatogram

Measure the distance each compound spot travelled from the starting point (origin) to its current position. This is known as the R_f (Retention Factor) value and is calculated as the distance travelled by the compound divided by the total distance travelled by the solvent. Compare the R_f values of the separated compounds to known standards data to identify the components within your chromatogram.

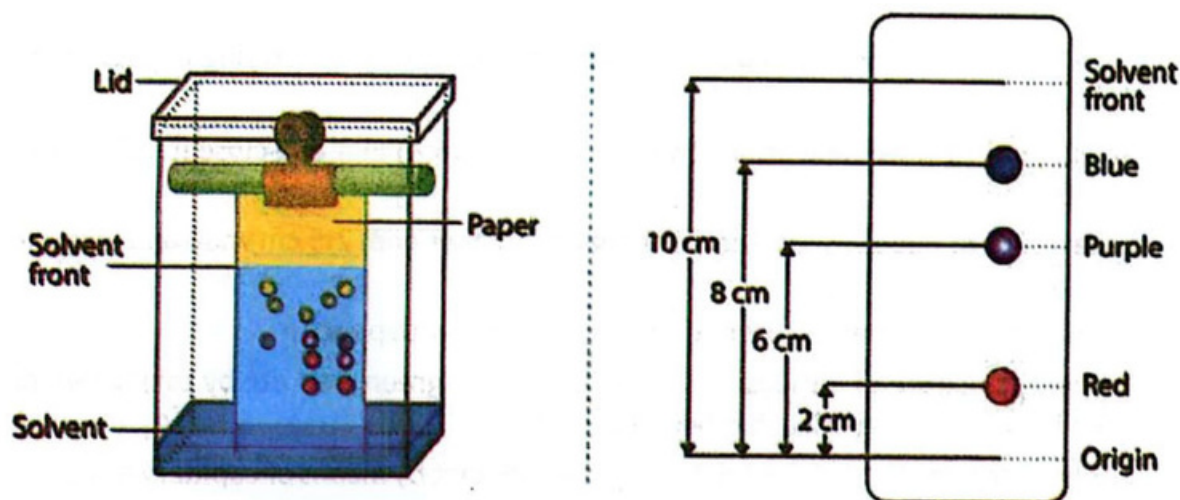


Figure 19.1: Paper chromatography

For example, in the above given chromatogram solvent front travelled 10cm and the blue coloured component travelled 8 cm, so the R_f is:

$$R_f (\text{blue}) = 8/10 = 0.8$$

$$R_f (\text{purple}) = 6/10 = 0.6$$

$$R_f (\text{red}) = 2/10 = 0.2$$

Blue component has the greatest affinity with the mobile phase and the red has the greatest affinity with stationary phase.

Identification of an unknown substance

A paper chromatogram is used to identify an unknown substance by comparing it with known substances. Two substances are likely to be same, if their R_f value is same. Therefore, R_f value of an unknown substance is determined. This R_f value is then cross-matched with already known R_f values to identify the potential substance.

A pure substance produces only one spot on the paper chromatogram. An impure substance can produce more than one spots depending on the compounds present in it.

KEY POINTS

- Chromatography works on the principle of partitioning where the components of a mixture distribute themselves between two phases one is called stationary phase and the other one is said to be mobile phase.
- In paper chromatography the stationary phase is the water absorb on filter paper
- A locating agent is a substance or a solution which is used to locate the components which have been separated by chromatography but are not visible due to being colourless
- R_f (Retention Factor) value is calculated as the distance travelled by the compound divided by the total distance travelled by the solvent

REVIEW QUESTIONS

1. Give short answer.
 - (i) Define chromatography
 - (ii) Define locating agent
 - (iii) What is R_f value?
 - (iv) Define paper chromatography
 - (v) What do you mean by paper chromatogram?
2. Explain in detail paper chromatography.
3. How can you identify an unknown substance by chromatography?
4. Differentiate between stationary and mobile phase.



Acknowledgment

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CHAPTER 19

CHROMATOGRAPHY

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GLOSSARY

Allotropes	are different physical forms of a pure element.
Alkanes	the simplest hydrocarbons in which all carbon-carbon bonds are single.
Alkenes	The family of hydrocarbons which contain one carbon-carbon double bond.
Alkynes	unsaturated hydrocarbons with a carbon-carbon triple bond.
Arrhenius Acid	a substance that produces H^+ ions in water.
Arrhenius base	a substance that produces OH^- ions in water.
Base	a substance that can accept a proton from an acid.
Bronsted lowry acid	a substance that can donate a proton.
Bronsted lowry base	a substance that can accept a proton.
Alpha particle	is doubly positively charged helium nuclei.
amu	is the abbreviation for atomic mass unit. It is exactly $1/12^{th}$ mass of one atom of carbon-12.
Anion	is an atom or group of atoms having a negative charge
Atmosphere	is the unit of pressure of air at the earth's surface.
Atom	is the smallest particle of an element to retain the properties of the element.
Atomic mass	is the exact mass of one atom of an element.
Atomic number	is the number of protons in the nucleus of an atom.
Bonding	is the term used to describe ways in which atoms are held together in elements and compound.
Chemical equilibrium	-a state of a chemical reaction at which rate of forward reaction equals the rate of reverse reaction.
Cation	is an atom or group of atoms having a positive charge.
Chemical bond	is the force that holds two or more atoms together in a substance.
Chemistry	is the study of matter, its composition, properties and the interaction of atoms as they react to form new substances.
Colloid	is a heterogeneous mixture of a substance dispersed through a medium.
Compound	is a substance made up of elements in fixed ratio.
Concentration	is the measure of the amount of solute dissolved in a solution.
Condensation	is the change of a gas into a liquid.
Conductor	is a substance that can conduct electric current.
Covalent bond	is a bond formed by the mutual sharing of electrons between two atoms.
Distillation	is the process for purifying a liquid by evaporating it, condensing the vapours and collecting the liquid in a clean container.
Double bond	is a covalent bond formed by two pairs of shared electrons.
Electrolyte	is a substance which in fused form or in solution form allows current to flow through it.
Electron	is the sub-atomic negatively charged particle in an atom.
Electron configuration	is the arrangement of electrons in orbitals.
Electron shells	are sets of energy levels corresponding to a single value of n .
Electronegativity	is the power of an atom to attract electrons to itself.
Element	is the pure substance in which all the atoms have same atomic number.

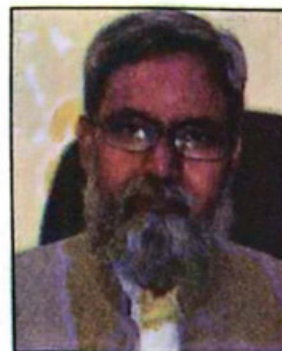
Energy levels	are the discrete energies of electron in atom
Enhanced greenhouse effect	an upset in the natural balance of the concentration of greenhouse gases in the atmosphere.
Evaporation	is the escape of molecules of a liquid through the surface.
Formula mass	is the sum of atomic masses of all the atoms present in the compound. It can also be defined as the mass of one formula unit or molecule in amu or mass of one mole of the compound in grams.
Formula unit	is the simplest formula of an ionic compound.
Freezing point	is the temperature at which a liquid changes to a solid.
Functional groups	are groups of atoms in an organic molecule which give particular properties.
Gas	is one of the physical states of matter that has neither any definite shape nor fixed volume.
Gram formula mass	is the mass of 1mole of a compound or element in grams.
Group	refers to the vertical column on the periodic table.
Halogens	are the elements present in VIIA group on the periodic table.
Hydrochloric acid	is an aqueous solution of hydrogen chloride.
Hydrogen bond	is an intermolecular force due to the interaction of lone pair present on N, O or F atom and the partially positively charged hydrogen of another molecule attached with N, O or F.
Inorganic	refers to a compound made up primarily of atoms other than carbon.
Intermolecular forces	are the attractive forces between the molecules.
Ion	is an atom or a molecule possessing an electric charge.
Ionic bond	is the bond formed by the attraction of oppositely charged ions.
Isotopes	are atoms of an element that differ in the number of neutrons.
Liquid	is one of the states of matter that has definite volume but no definite shape.
Lone pair	is a pair of non-bonding valence electron or a pair of electron that are not shared in covalent bonding.
Main group elements	are elements belonging to sub-group A on the periodic table.
Mass number	is the sum of the number of protons and neutrons in an atom.
Matter	is anything that occupies space and has mass
Melting point	is the temperature at which a substance melts.
Metals	are elements that are good conductor of electricity and heat and tends to form positive ions.
Molar mass	is the mass of one mole of a compound or element in grams.
Molarity	is the number of moles of solute per dm ³ of solution.
Mole	is Avogadro's number of atoms, molecules or formula units. It can also be defined as the atomic mass, formula mass or molecular mass of a substance expressed in grams.
Molecular mass	is the sum of atomic masses of all the atoms present in a molecule.
Molecule	is a group of atoms held by a chemical bond and behaves as an independent unit.
Neutron	is a sub-atomic neutral particle in the nucleus of an atom.
Non-metal	is an element that is a bad conductor of electricity and tends to form negative ion.
Nucleus	is the positively charged center of an atom.
Octet	is eight electron configurations in the valence shell.
Organic chemistry	is the study of carbon compound or the study of hydrocarbons and

Organic compounds	their derivatives. are compounds mainly composed of carbon atoms except bicarbonates, carbonates, oxides and carbides.
Oxidation	is the loss of electron by an element.
Oxidation state	is the actual charge on an ion or apparent charge on an atom in a molecule.
Oxidizing agent	is the reactant containing the element that is reduced.
Period	refers to the horizontal row in the periodic table.
Periodic table	is a systematic arrangement of elements based on electron configuration and periodic behaviour.
Polar covalent bond	is a bond in which electrons are shared unequally between two atoms. Thus one atom bears partial positive charge and the other a partial negative charge.
Proton	is the positively charged sub-atomic particle in the nucleus of an atom.
Reactant	is a substance consumed in a chemical reaction.
Redox reaction	is an oxidation and reduction reaction.
Reduction	is the gain of electron by an atom.
Reducing agent	is the reactant containing the element that is oxidized.
Refining	a process of separation of crude oil into useful fractions.
Solid	is one of the three states of matter, it has a definite shape and definite volume.
Solubility	is the amount of solute that dissolved in 100g of water at a particular temperature.
Solute	is the component of solution that is present in relatively small amount.
Solvent	is the component of solution that is present in relatively larger amount.
S-orbital	is a spherical shape orbital.
Structural formula	shows the arrangements of atoms in a molecule
Sublimation	is the conversion of a solid directly into a gas.
Technology	is the application of scientific knowledge to serve some practical purpose.
Triple bond	is a covalent bond involving the sharing of three pairs of electrons.
Unsaturated hydrocarbons	molecules containing only carbon and hydrogen in which at least two carbon atoms form double or a triple bond.
Universal indicator	an indicator that changes colour at each pH value.
Valence electrons	are the electron in the outer most energy level of the atom.
Valence shell	is the outermost energy level of the atom.
Vapour pressure	is the pressure exerted by the vapours of a liquid in equilibrium with its liquid at a particular temperature.
Zero group	refers to VIII A group on the periodic table

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He is an accomplished alumnus of Quaid-i-Azam University Islamabad, obtaining a master's degree in Chemistry in 1979 and an M.Phil degree in 1988. Commencing his teaching career in 1980, he served in various capacities, including as Professor/Principal at Islamabad College for Boys, G-6/3, Islamabad. Recognized for his teaching excellence, he received the best teacher in Chemistry award and a gold medal during the Silver Jubilee celebrations of I.C.B, G-6/3. His extensive experience also led him to contribute as a member of the National Review Committee from 2002-2006, finalizing Chemistry textbook manuscripts for secondary and higher secondary classes, and managing/co-authoring numerous textbooks published by NBF.



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