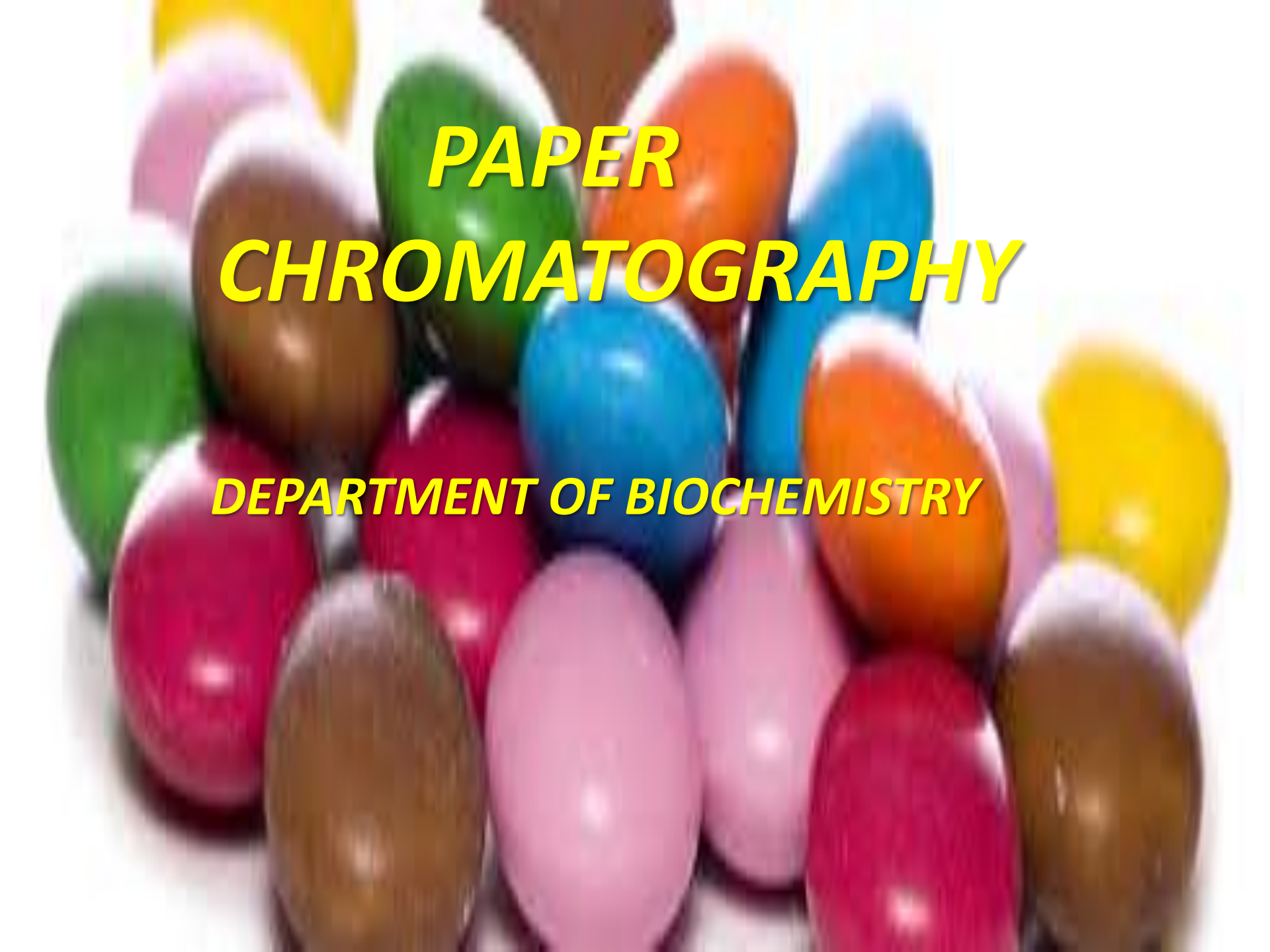

PAPER
CHROMATOGRAPHY
DEPARTMENT OF BIOCHEMISTRY

CONTENTS

- INTRODUCTION
- HISTORY
- PRINCIPLE
- PARTITION COEFFICIENT
- APPLICATIONS
- TYPES OF CHROMATOGRAPHY
- PAPER CHROMATOGRAPHY
- THIN LAYER CHROMATOGRAPHY

CHROMATOGRAPHY

- ***INTRODUCTION:***
- ***Chromatography (from Greek chroma-color and graphein-to write) is a collective term for a set of laboratory technique for separation of mixtures.***
- ***Chromatography may be defined as the technique of separation of substances according to their partition coefficients between two immiscible phases.***

HISTORY

- Chromatography literally means "color writing" was first employed by **Russian Biochemist Michael Tswett** who separated chlorophyll from a mixture of plant pigments in 1906.
- The concept of **Gas-liquid chromatography** was first introduced by **Martin and Synge in 1941**.
- In **1959** a technique known as **Gel Filteration chromatography** was observed which is used to separate low molecular weight substances from a high molecular weight.
- In **1960** further improvement in liquid chromatography led to development of **High Performance Liquid Chromatography.**

PRINCIPLE

- *Chromatography is based on the principle of separations of compounds into different bands and then identification of those bands*
- *The preferential separation is done due to differential affinities of compounds towards stationary and mobile phases.*
- *The differences in affinities arise due to relative adsorption or partition coefficient in between components towards the both phases.*

PARTITION COEFFICIENT

- *Partition coefficient is a definitive term used to describe the way in which given compound distributes itself between two immiscible phases the stationary phase and mobile phase.*
- *The concentration of the compound in each of the phases is described by the "partition coefficient "K.*
- *The ratio of concentration of the solute in two phases at equilibrium at a given temperature is called "partition coefficient".*
- *K=Cs/Cm Cs and Cm –conc. of compound in stationary and mobile phase respectively*

APPLICATIONS

- ***The chromatographic technique is used for the separation of Amino acids, proteins, and carbohydrates.***
- ***It is also used for analysis of drugs, hormones, vitamins.***
- ***Helpful for qualitative and quantitative analysis of complex mixtures.***
- ***The technique is also used for the determination of molecular weight of proteins.***
- ***Used in quality control of drugs in pharmaceutical industries.***

TYPES OF CHROMATOGRAPHY

- PAPER CHROMATOGRAPHY
- THIN-LAYER CHROMATOGRAPHY
- GEL FILTERATION CHROMATOGRAPHY
- COLUMN CHROMATOGRAPHY
- ION EXCHANGE CHROMATOGRAPHY
- GAS LIQUID CHROMATOGRAPHY
- AFFINITY CHROMATOGRAPHY
- HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

ON THE BASIS OF TECHNIQUE

Chromatography are of two types:

- ***PLANE CHROMATOGRAPHY***
- ***COLUMN CHROMATOGRAPHY***

There are two variations of paper chromatography:

- ***PAPER CHROMATOGRAPHY***
- ***THIN LAYER CHROMATOGRAPHY***

PAPER CHROMATOGRAPHY

- *This technique was introduced by Martin and Synge in 1941*
- *It is a type of partition chromatography in which substances are distributed between two liquids one is stationary liquid which is held in the pores of paper called stationary phase the other is moving liquid and is called mobile phase*
- *The component of the mixture to be separated migrate at different rates and appear as spot at different points on paper*

PAPER CHROMATOGRAPHY

PRINCIPLE OF SEPERATION

The principle of separation is mainly partition rather than adsorption.

Cellulose layers in filter paper contains moisture which acts as stationary phase & organic solvents/buffers are used as mobile phase

PAPER CHROMATOGRAPHY

- PRACTICAL REQUIREMENTS
- 1)Stationary phase & papers used
- 2)Application of sample
- 3)Mobile phase
- 4)Development technique
- 5)Detecting or Visualizing agents

PAPER CHROMATOGRAPHY

- STATIONARY PHASE AND PAPERS USED

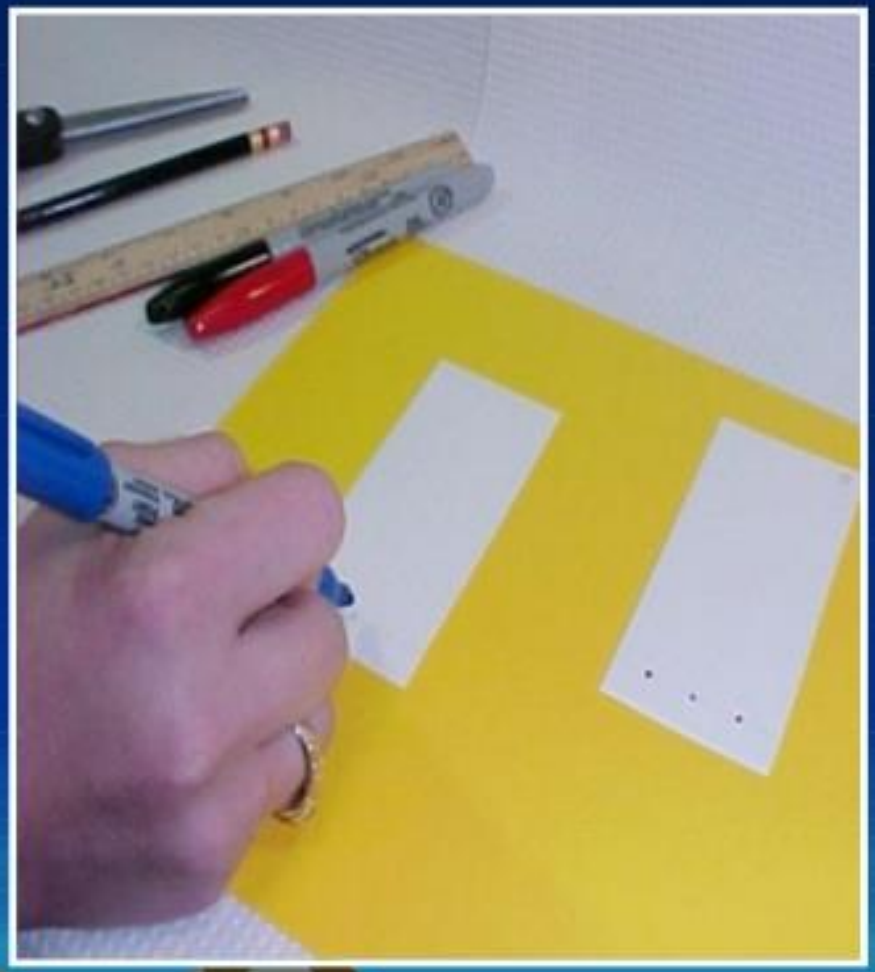
  Whatman filter papers of different grades like No.1, No.2, No.3, No.4, No.20, No.40, No.42 etc are used. In general this paper contains 98-99% of α-cellulose, 0.3 – 1% β -cellulose

  Factors that governs the choice of paper:

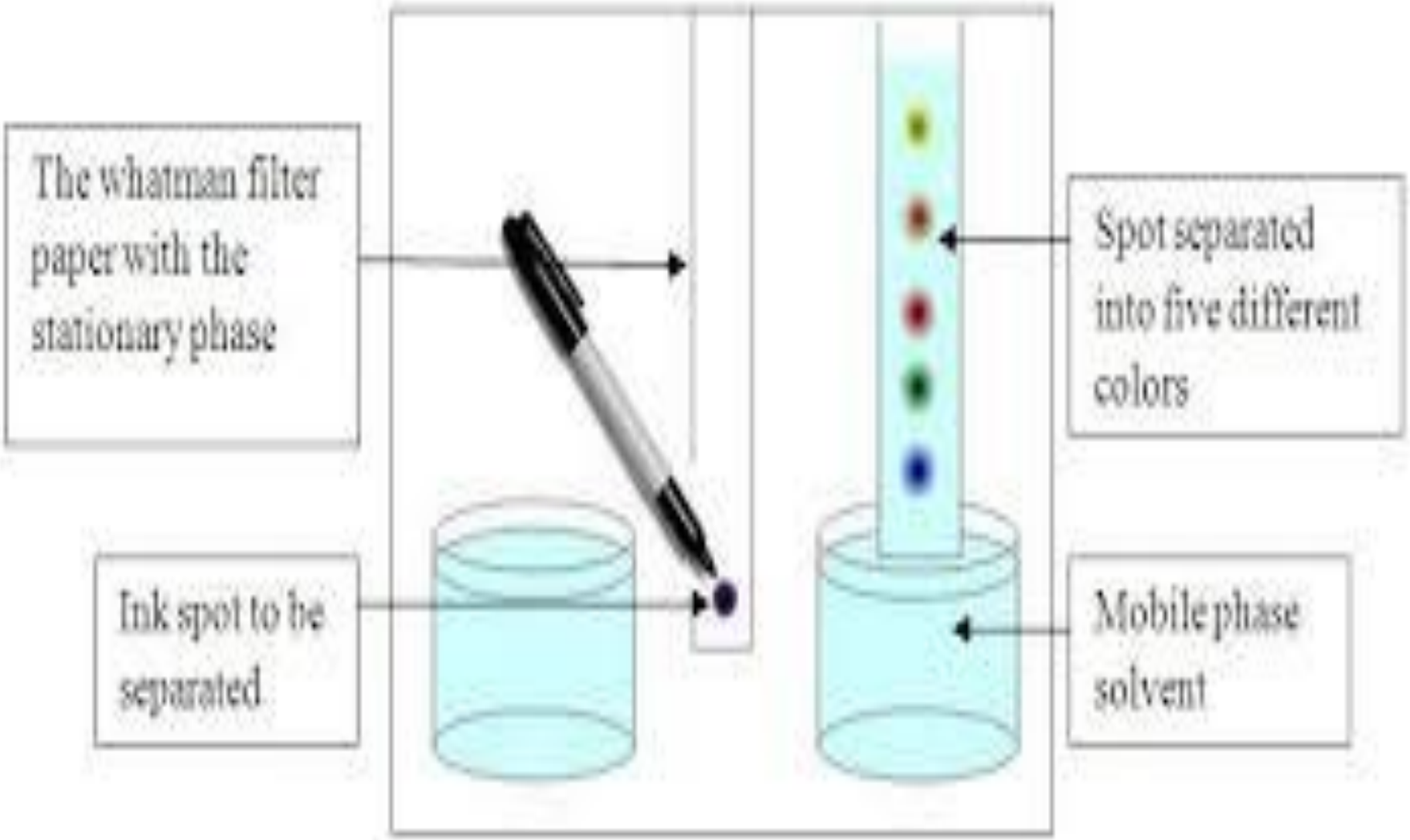
  - Nature of Sample and solvents used.
  - Based on Quantitative or Qualitative analysis.
  - Based on thickness of the paper.

PREPARATION OF PAPER

- Cut the paper into desired shape and size depending upon work to be carried out.
- The starting line is marked on the paper with an ordinary pencil 5cm from the bottom edge.
- On the staring line marks are made 2cm apart from each other.



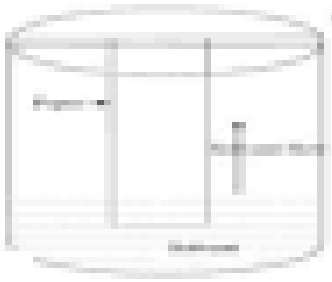
SAMPLE APPLICATION

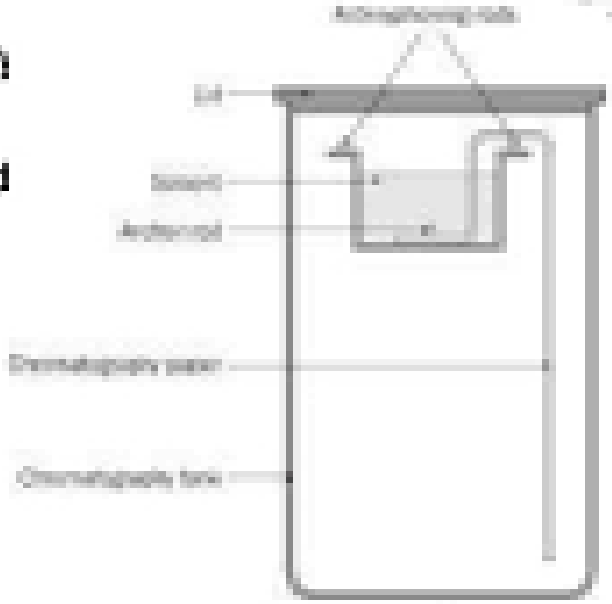


DEVELOPMENT TECHNIQUE

Development technique in paper chromatography

- **Ascending chromatography:**
- **Descending chromatography**
- **Ascending- descending mod**





Descending chromatography

ASCENDING TECHNIQUE

- ***In this technique the paper is allowed to hang in or is suspended in manner that the base of paper is in contact with the solvent at base of chamber***

- ***The sample spot should be in position just above the surface of solvent so that as solvent moves vertically up by capillary action separation of sample is achieved.***

DESCENDING TECHNIQUE

- ***In this technique the end of the paper near which samples are located is held in trough at the top of tank.***
- ***Rest of paper allowed to hang vertically but not in contact with solvent in base of tank.***
- ***Separation of sample is achieved as solvent moves downward under gravity***.

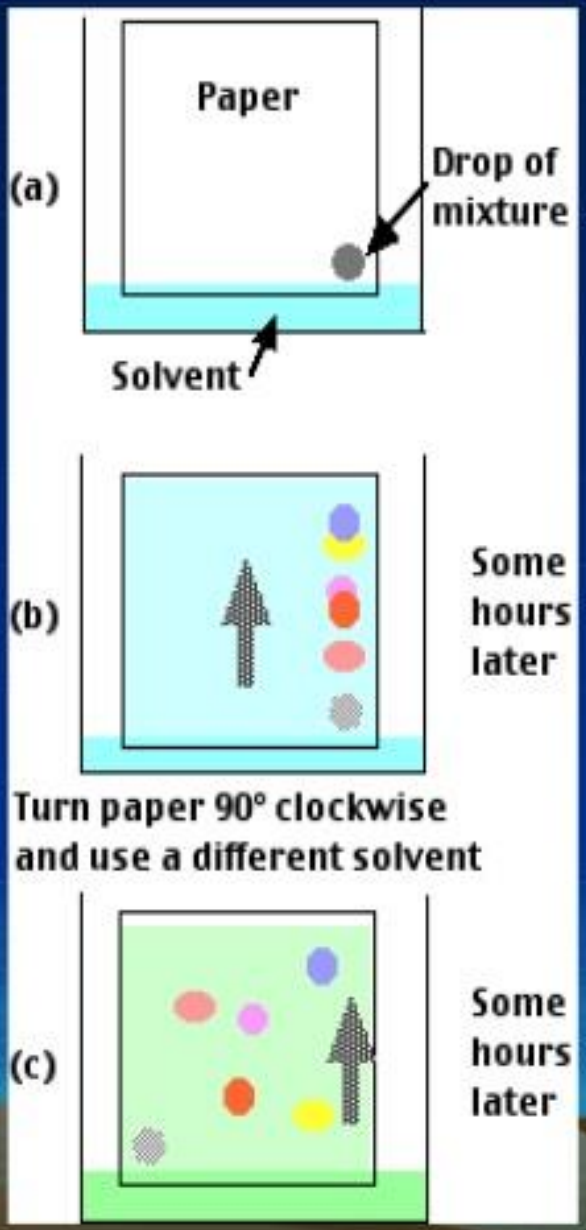
PAPER CHROMATOGRAPHY

5)TWO DIMENSIONAL DEVELOPMENT

In this method the paper is developed in one direction and after development, the paper is developed in the second direction allowing more compounds to be separated into individual spots.

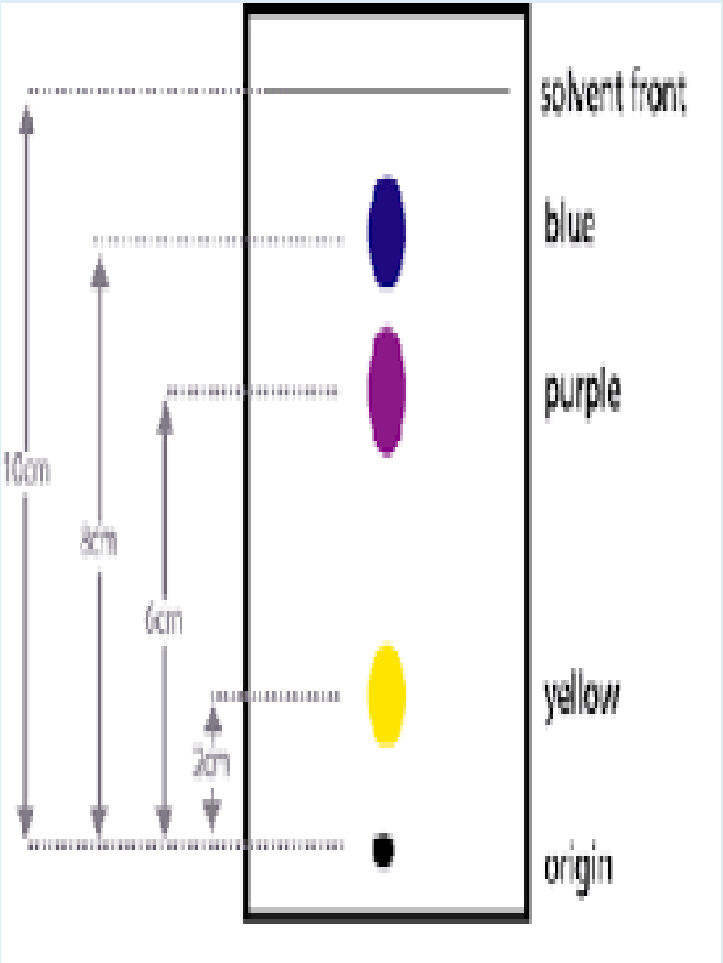
in the second direction, either same solvent/different solvent system can be used for development.

TWO DIMENSIONAL DEVELOPMENT



IDENTIFICATION

Rf= Distance travelled by solute
Distance travelled by solvent front



APPLICATION

- *Used to check the control of purity of pharmaceuticals.*
- *To the detection of adulterants.*
- *To detect the contaminants in food and drinks.*
- *To study the ripening and fermentation.*
- *To the detection of drugs and dopes in animals.*
- *To the analysis of cosmetics*.

REFERENCE

- *Biophysical Chemistry Principles and techniques Upadhyay , upadhyay, Nath , Himalaya publishing house, 2014*
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- *http://www.chem.ucla.edu/~bacher/General/30BL/tips/TLC1.html*

THANK YOU FOR WATCHING MY PRESENTATION