

A photograph of various pieces of laboratory glassware. In the foreground, there are several Erlenmeyer flasks, some containing blue liquid. In the background, there are graduated cylinders and a burette. The overall scene is set in a laboratory environment. The text 'COLUMN CHROMATOGRAPHY' is overlaid in the center of the image.

COLUMN CHROMATOGRAPHY

CONTENTS

- PRINCIPLE
- PRACTICAL REQUIREMENTS
- FACTORS AFFECTING COLUMN EFFICIENCY
- APPLICATIONS
- ADVANTAGES
- DISADVANTAGES
- PARTITION COLUMN CHROMATOGRAPHY

HISTORY

- This chromatography was first demonstrated by **TSWETT** in 1906.
- In all the early applications, the procedure was limited to the separation of coloured substances, hence the name chromatography .

CHROMATOGRAPHY

It is a technique employed for separation of components of mixture by continuous distribution of components between two phases i.e. one phase moves (**mobile**) over the other (**stationary**) in a continuous manner.

when chromatography is carried out in column it is called **column chromatography**. It is otherwise known as **gravity chromatography**.

TYPES OF COLUMN CHROMATOGRAPHY

- ADSORPTION CHROMATOGRAPHY
- PARTITION CHROMATOGRAPHY
- ION EXCHANGE CHROMATOGRAPHY
- GEL CHROMATOGRAPHY

TERMINOLOGY

- **Stationary phase:** It is also called as **adsorbent**. It is a solid .
- **Mobile phase:** It is also known as **solvent** and **eluent**. It is a liquid.
- **Sample:** It is also known as **adsorbate** which get adsorbs.
- **Elution:** process of removing the components from column.
- **Eluate:** separated component.

ADSORPTION CHROMATOGRAPHY

It is a surface phenomenon. when the stationary phase is solid and mobile phase is liquid or gas it is known as adsorption chromatography.

PRINCIPLE

when a mixture of components dissolved in the mobile phase is introduced into the column, the individual components move with different rates depending upon their relative affinities.

The compound with lesser affinity towards stationary phase moves faster and it is eluted out of the column first. The one with greater affinity towards stationary phase moves slower down the column and hence it is eluted latter. Thus the compounds are separated.

I

II

III

Solvent

Mixture of
compounds
($a+b+c$)

Adsorbent
(stationary
phase)

Glass wool

Plug

a

$b+c$

a

$b+c$

c

- ▣ The rate of movement of component [R] is given by,

$$R = \frac{\text{Rate of movement of a component}}{\text{Rate of movement of mobile phase.}}$$

When a liquid mobile phase is used, equation is written as

$$R = A_m / A_m + \alpha A_s$$

Where α = partition coefficient

A_m = Average cross section of mobile phase

A_s = Average cross section of stationary phase

STATIONARY PHASE

Ideal properties:

- Particles should have uniform size and spherical shape [60-200] microns.
- Should have high mechanical stability, inert, insoluble in mobile phase used.
- Colorless
- Should allow free flow of mobile phase.
- Freely available and in expensive.

TYPES OF ADSORBENTS

WEAK	MEDIUM	STRONG
Sucrose	Calcium carbonate	Activated silica gel
Starch	Calcium phosphate	Activated alumina
Inulin	Magnesium carbonate	Activated charcoal
Talc	Magnesium oxide	Activated magnesia
Sodium carbonate	Calcium hydroxide	Fuller's earth

SELECTION OF ADSORBENT

WEAK ADSORBENT	STRONG ADSORBENT
Few components	More components
Different affinities	Similar affinities
Longer column	Shorter column

For efficient separation –
Adsorbate:Adsorbent=1:20 or 1:30

PREPARATION OF STATIONARY PHASE

- Adsorbents require activation before use.
- This can be achieved by heating, where adsorbent loses water and other adsorbed materials.
- Generally there is an optimum temperature for activation.
- Alumina -400° C
silica gel- 100° C for 1hr.
- Long time heating leads to loss of its activity.

DEVELOPERS

These are the compounds/reagents which are used for the production of colour for colourless substances .

EX- hydrogen sulphide, potassium ferrocyanide

MOBILE PHASE

- To introduce the mixture into column as **solvent**.
- To develop the zones for separation as **developing agent**.
- To remove pure component out of the column as **eluant**.

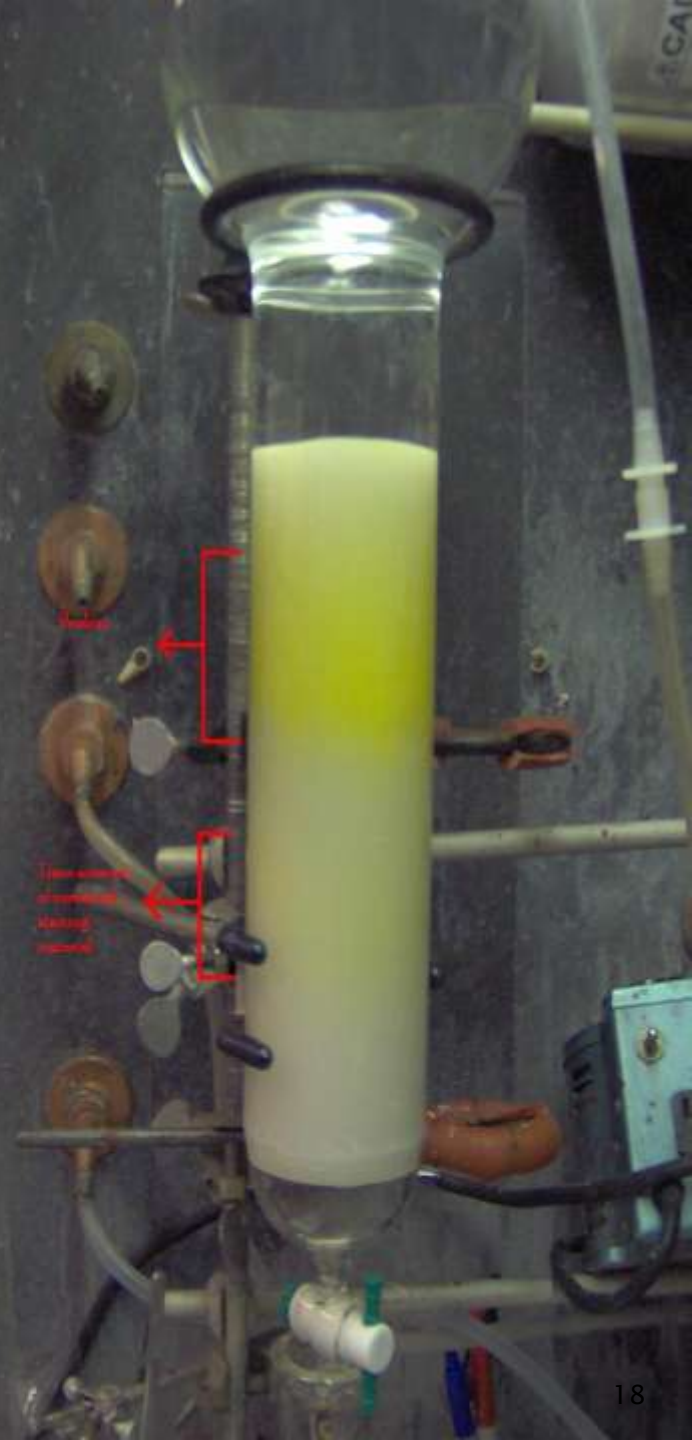
Different mobile phases used:

Ex; In increasing order of polarity or elution strength.

cyclohexane < carbondisulphide < ether <
Benzene < toluene < esters < alcohols <
chloroform < acetone < water < pyridine <
organic acids.

COLUMN CHARACTERISTICS

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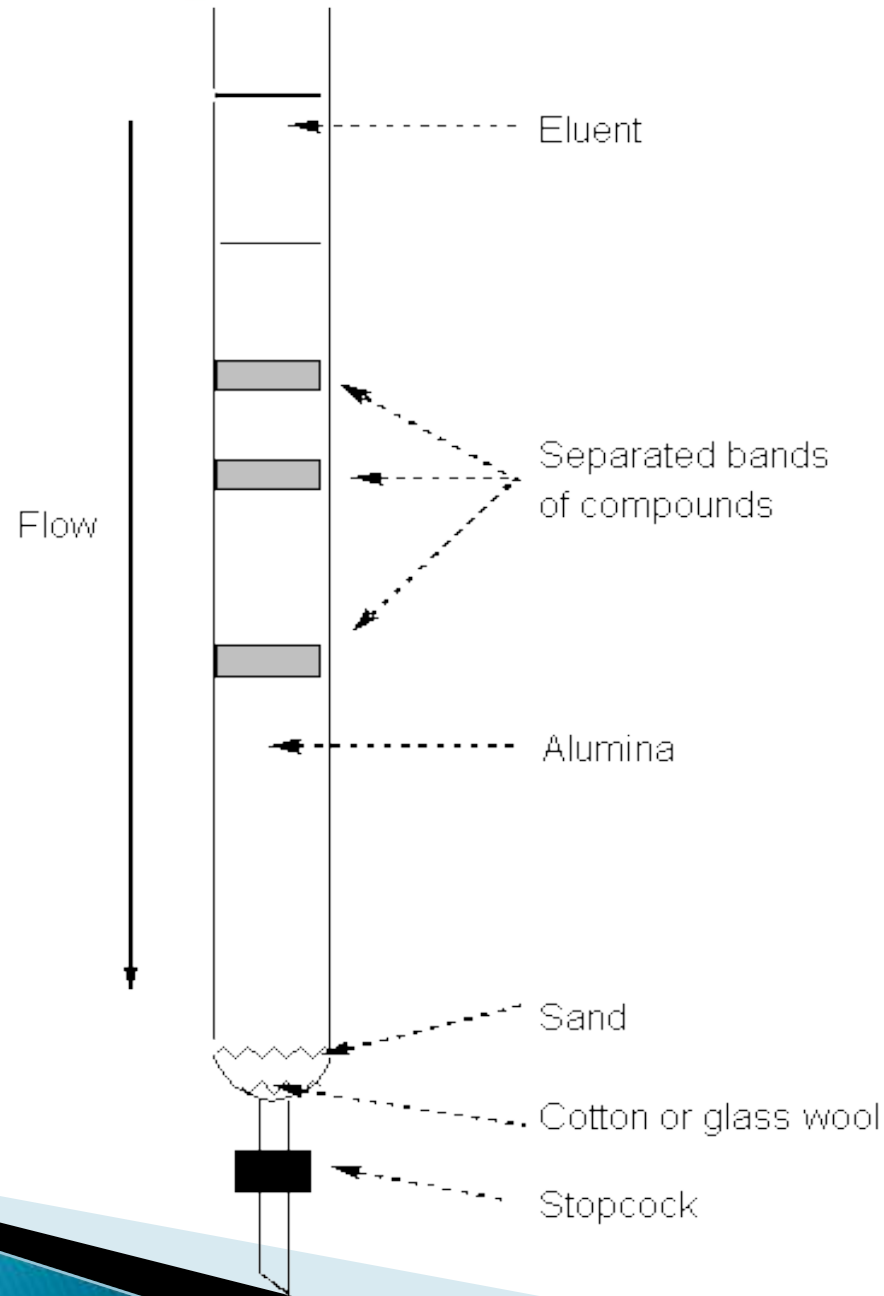


- Column material is made of good quality neutral glass since it should not be affected by solvents, acids or alkalies.
- Column dimensions are important for effective separations.
- Length: diameter – 10:1 to 30:1
- For more efficient separation
Length :diameter -100:1

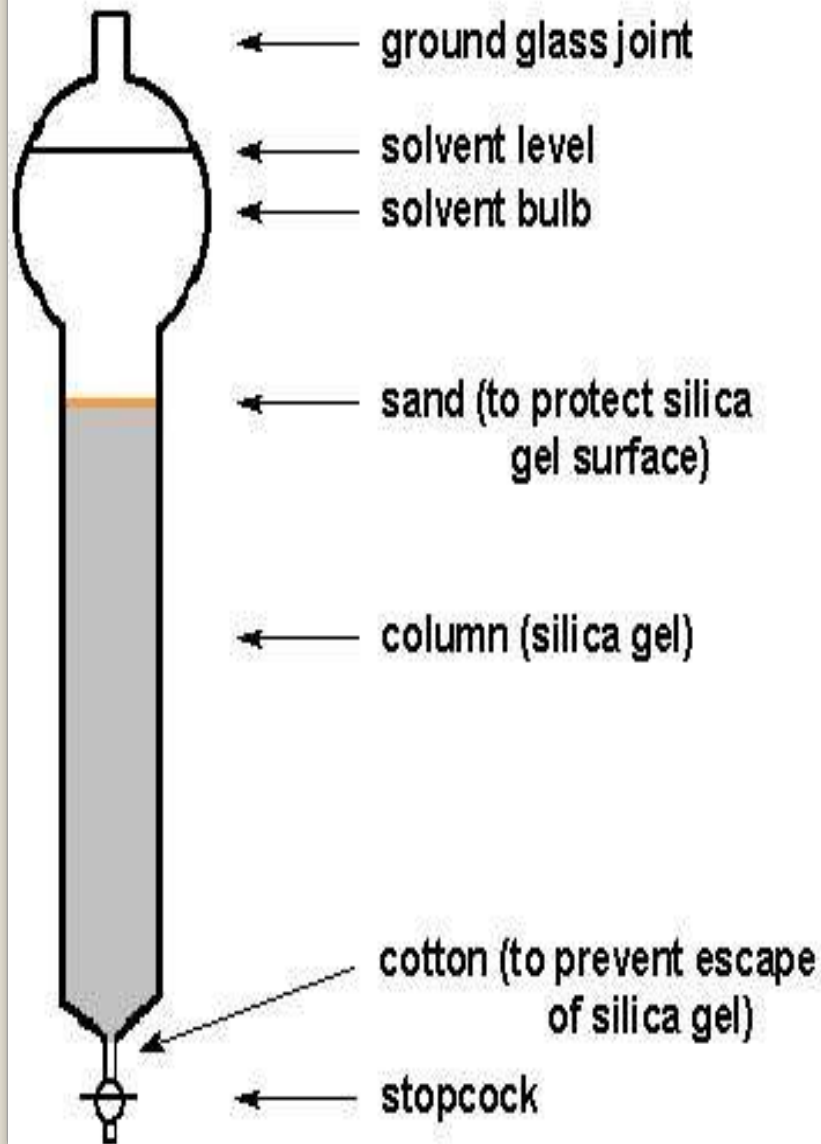




PACKING OF THE COLUMN



The Chromatography Column



Chromatography is a separation method which depends on polarity of substances.

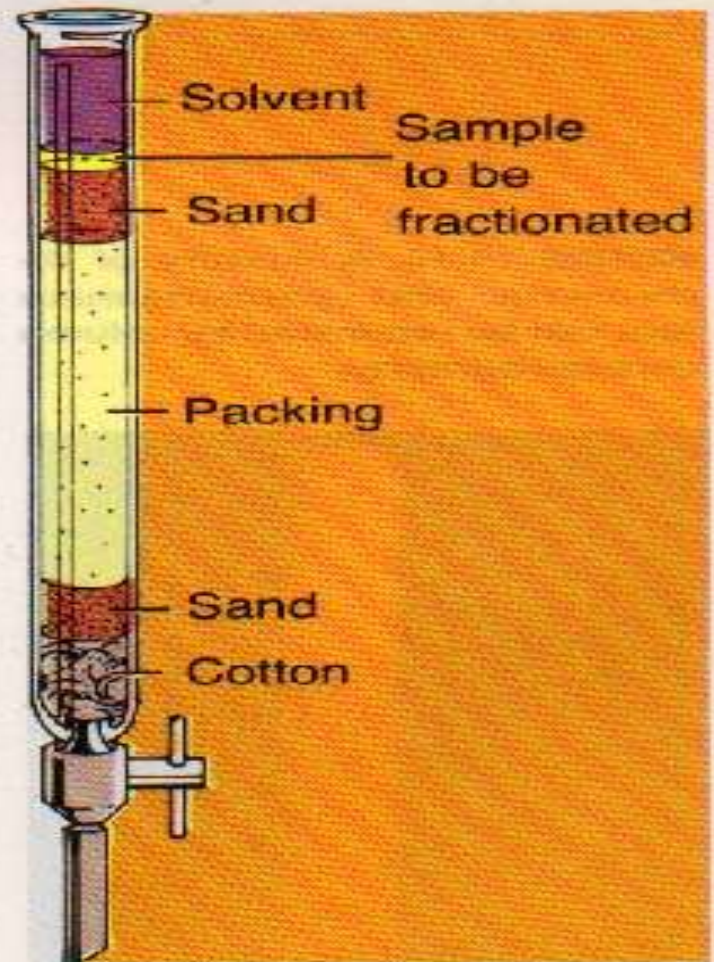


FIGURE 14-14. The packing components for a typical chromatography column are shown.

IMPORTANCE OF PACKING

- The column must be packed as uniformly as possible to minimize the distortion of the chromatographic boundaries
- Channeling is usually caused by the inclusion of air bubbles during packing.

TYPES OF PACKING

DRY PACKING :In this the required quantity of adsorbent is poured as fine dry powder in the column and the solvent is allowed to flow through the column till equilibrium is reached.

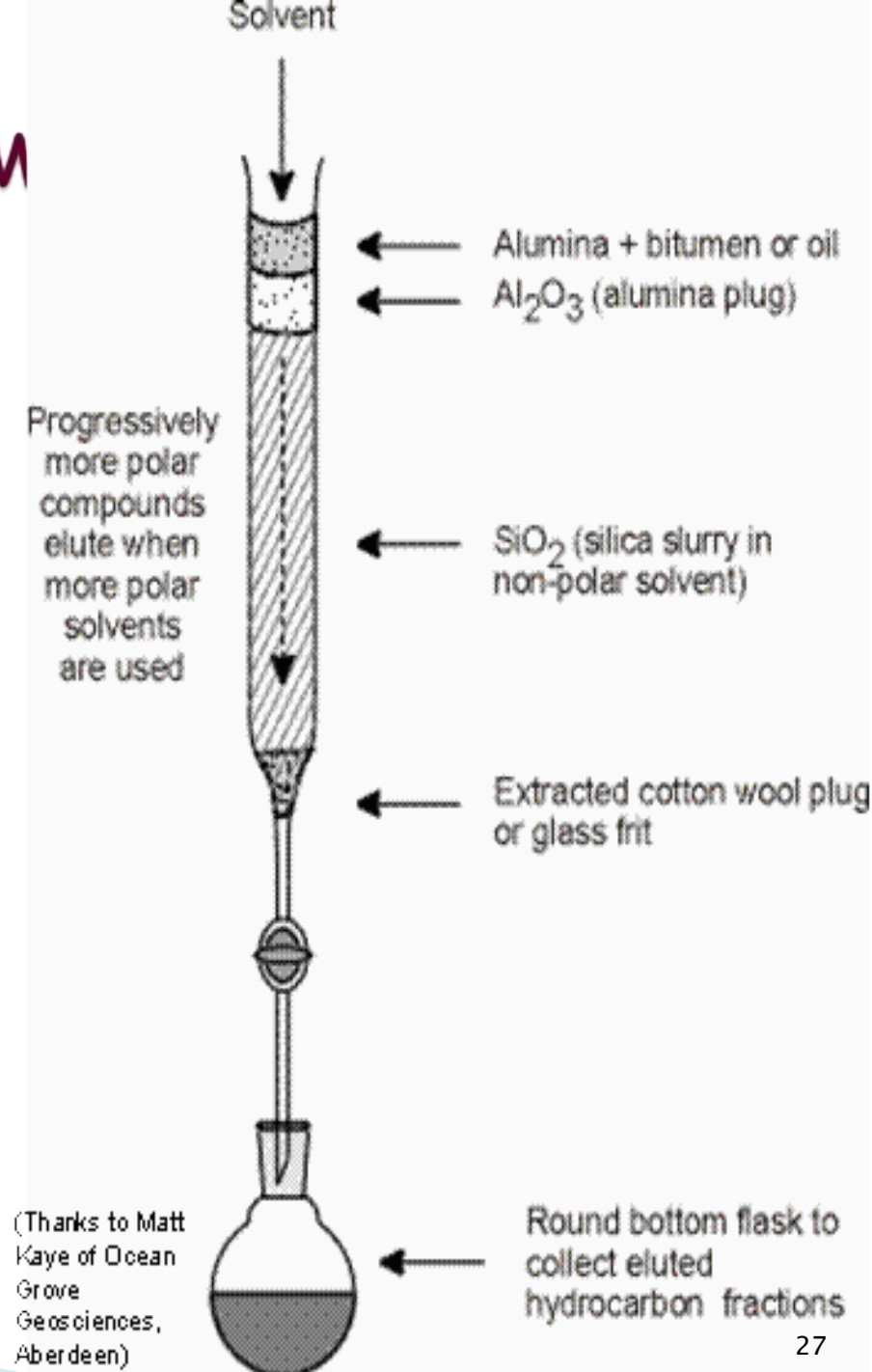
DEMERITS :

- Air bubbles are entrapped between mobile phase and stationary phase and the column may not be uniformly packed.
- Cracks appear in the adsorbent present in the column. Hence flow characteristics and clear band of the separated component may not be obtained.

WET PACKING :

- This is the ideal technique.
- The slurry of adsorbent with the mobile phase is prepared and is poured into the column.
- The stationary phase settles uniformly in the column and there is no entrapment of air bubbles.
- There will not be any crack in the column of adsorbent.
- The bands eluted from the column will be uniform and ideal for separation.

INTRODUCTION OF SAM



ELUTION

- ▣ After the introduction of the sample, by the elution techniques the individual components are separated out from the column.

Two types of elution techniques.

Isocratic elution technique : Iso means same/ similar.

In

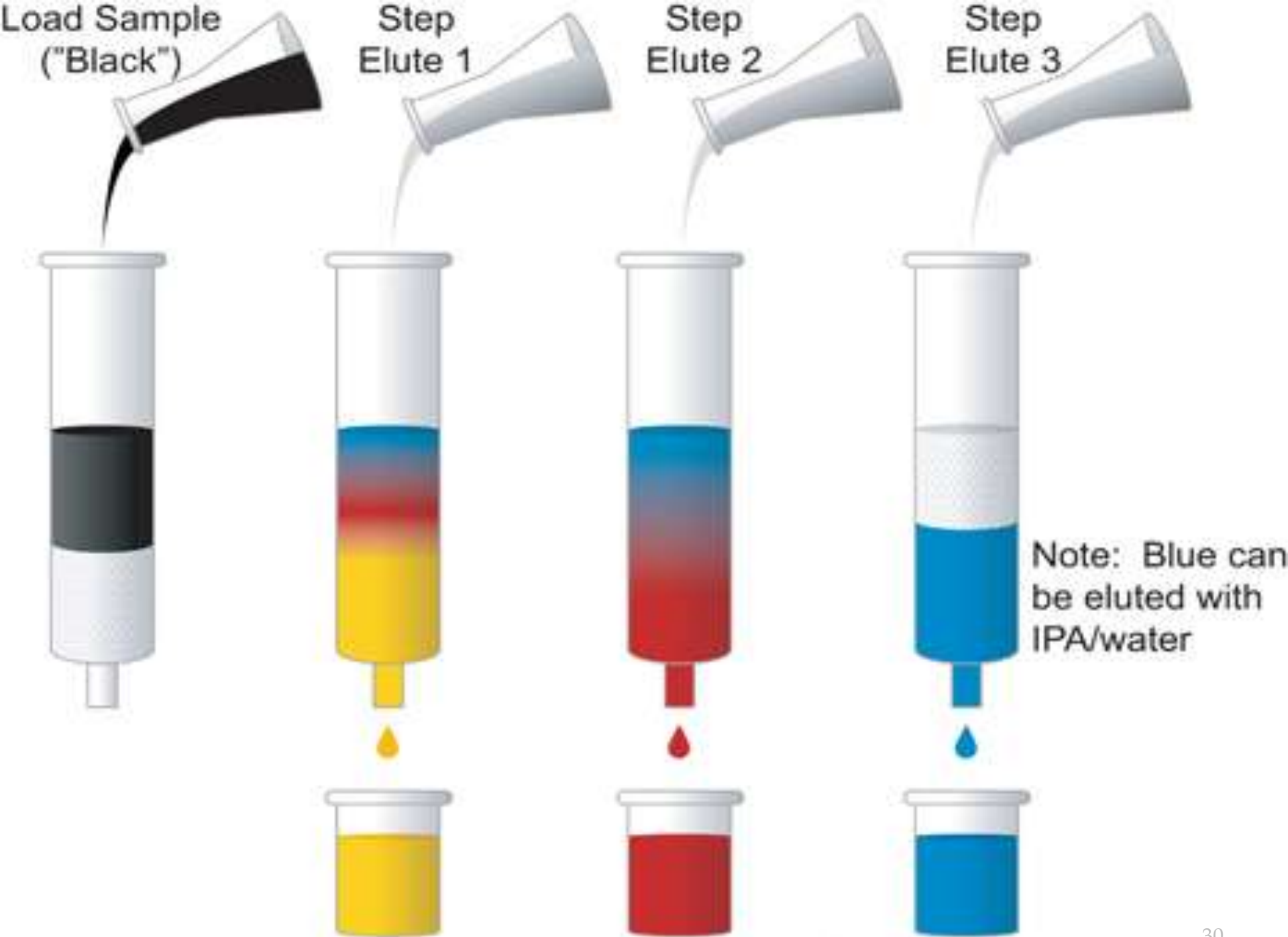
this elution technique, the same solvent or solvent system of same polarity is used throughout the process of separation.

Ex- chloroform only, petroleum: ether=1:1

Gradient/Stepwise elution technique

- ▣ The solvents of gradually increasing polarity or increasing elution strength are used during the process of separation. Initially low polar solvent is used followed by gradually increasing the polarity.

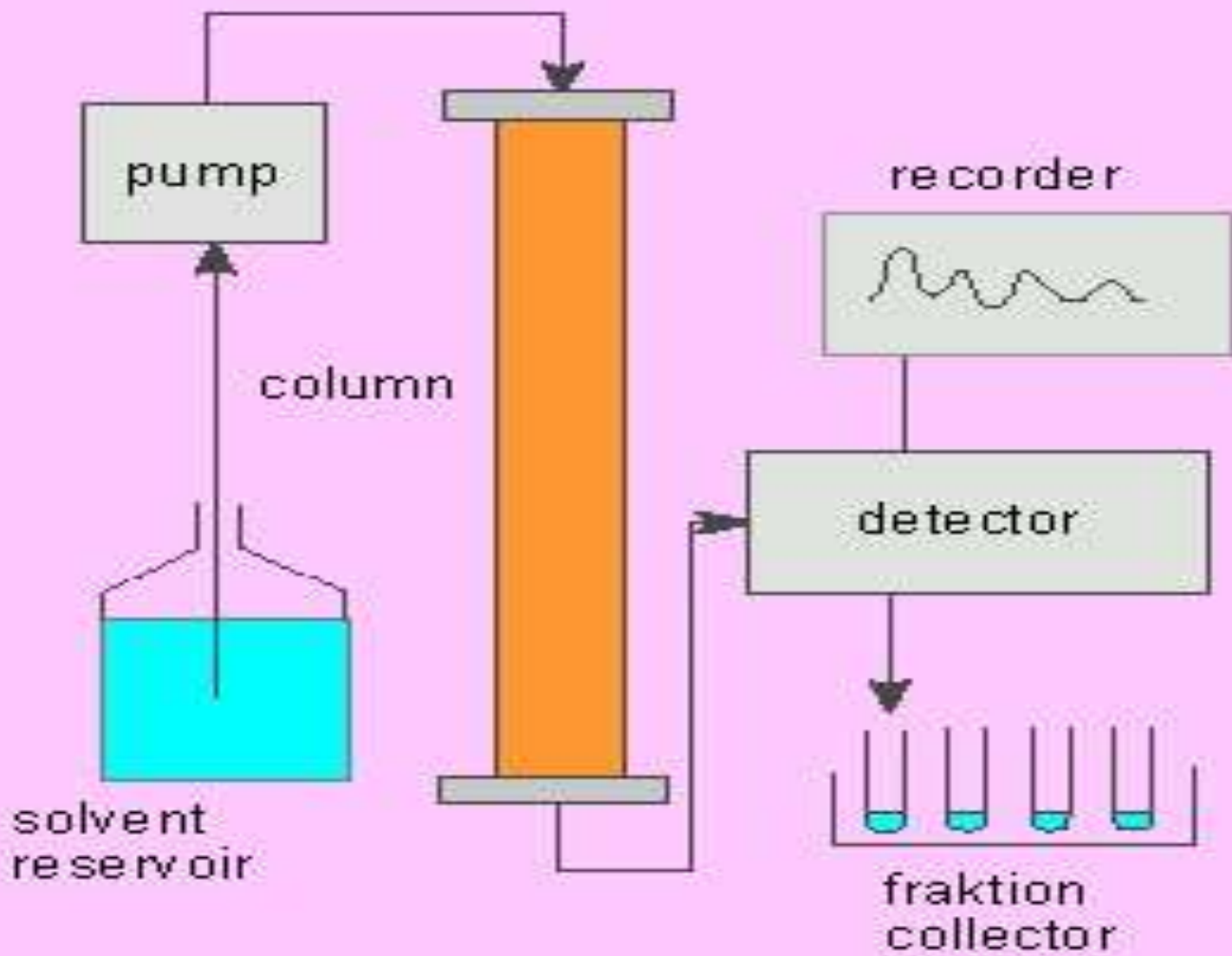
Ex-Initially Benzene then Chloroform, Ethyl acetate, Methanol etc.



One cartridge can separate all three dyes

DETECTORS

The detection of colored components can be done visually. Different colored bands are seen moving down the column which can be collected separately. But for colorless compounds the technique depends upon the properties of the components. And monitoring the fractions by TLC.



RECOVERY OF COMPONENTS

- ▣ The best technique is to recover the components by a process called as elution. The components are called as eluates, the solvent called as eluent and the process of removing the components from the column is called as elution.
- ▣ Recovery is done by collecting different fractions of mobile phase of equal volume like 10ml, 20ml etc or unequal volume. They can also be collected time wise i.e. a fraction for every 10min or 20min etc. The recovered fractions are detected by using different techniques.

FACTORS AFFECTING COLUMN EFFICIENCY

- Dimensions of the column
- Particle size of the adsorbent
- Nature of the solvent
- Temperature of the column
- Pressure

APPLICATIONS

- Separation of mixture of compounds.
- Removal of impurities or purification process.
- Isolation of active constituents.
- Isolation of metabolites from biological fluids.
- Estimation of drugs in formulation or crude extracts.

ADVANTAGES

- Any type of mixture can be separated by column chromatography.
- Any quantity of the mixture can also be separated.
- Wider choice of mobile phase.
- In preparative type, the sample can be separated and reused.
- Automation is possible.

DISADVANTAGES

- Time consuming method.
- More amount of solvents are required which are expensive.
- Automation makes the technique more complicated and expensive.

PARTITION COLUMN CHROMATOGRAPHY

- ❖ It is similar to adsorption column chromatography except that, the stationary phase is liquid. A solid support like silica gel or cellulose is used to hold the liquid stationary phase like water, buffer solutions are used as thin film on the surface. Mobile phase is similar to that of column chromatography. But the gradient elution technique is not used since the equilibrium will be disturbed. But this technique is not widely used.

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