M.Sc. Semester II

Forensic Science

Paper: FS-201: Forensic Analytical Chemistry

Chromatography

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1.

Ion exchange chromatography

→Mixture of similar charged ions separated by using ion exchange resin

→Reversible change of similar charged ions

→Cations or anions can be separated

Principle

Reversible change of ions between ions present in solution and ion exchange resin.

Classification of resins

According to their nature they are classified as follows-

→Strong anion exchange resin: contain sulphonic groups

→Weak anion exchange resin: contain carboxylic or phenolic groups

→Strong cation exchange resin: contain quaternary ammonium groups

→Weak ion exchange resin: contain amino, substituted groups.

According to the source they can be classified as-

Natural →

Cation- zeolytes and clay

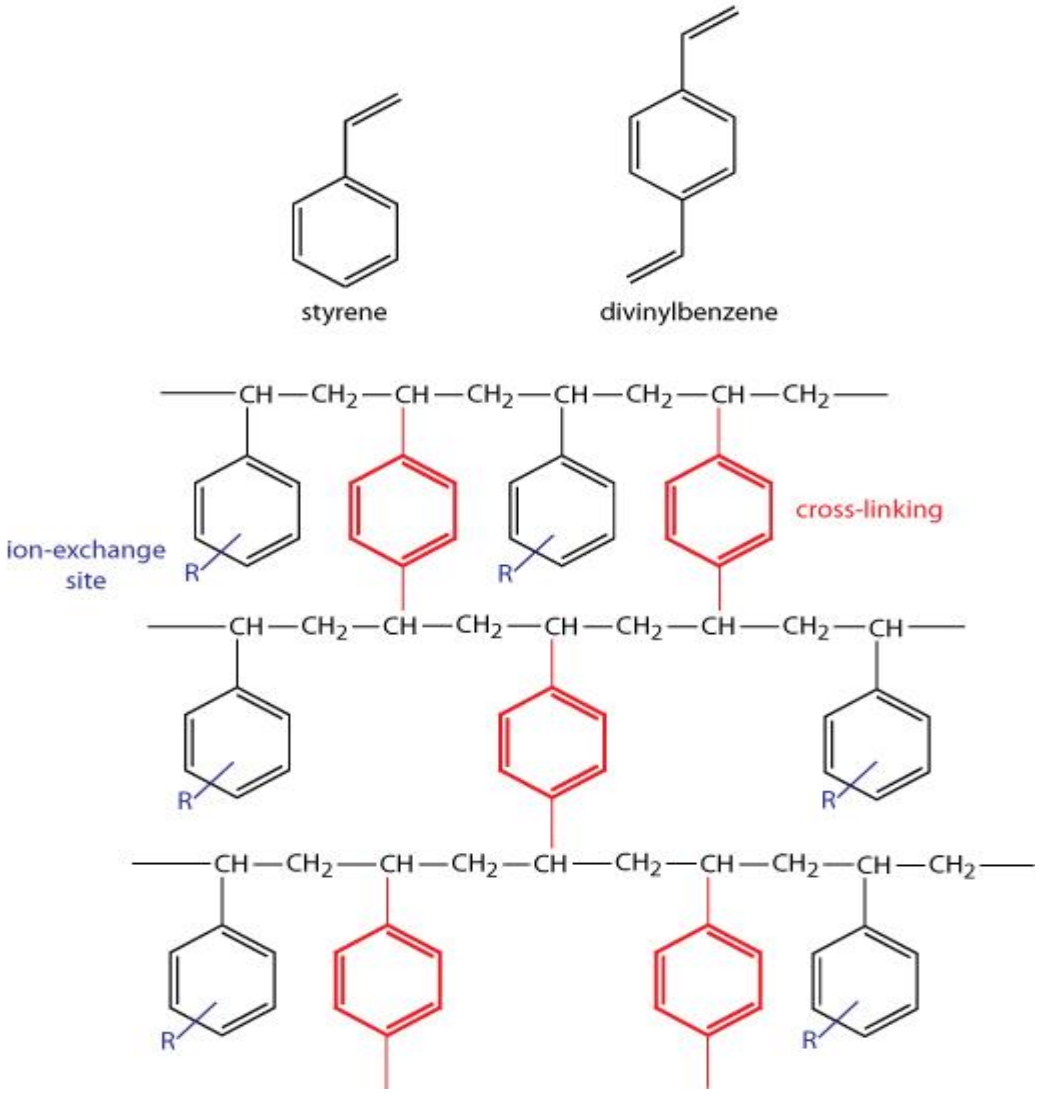
Anion- dolomite

Synthetic→

organic and inorganic resins

Organic resins are polymeric resin matrix- These are complex in nature. The polymer carries an electric charge that is exactly neutralized by the charge on counter ions. These active ions are cations in cationic exchanges and anions in anionic exchanges.

The resins are composed of Polystyrene (sites for exchangeable function groups), Divinyl Benzene (cross linking agent) which offers stability.



Ion exchange resin should have following requirements-

→It must be chemically stable

→It should be insoluble in common solvents

→It should have sufficient degree of cross linking

→The swollen resin must be denser than water

→It must contain sufficient no of ion exchange groups

Structural types of ion exchange resins-

a) Pellicular type with ion exchange resins:

30-40 μ with 1-2 μ thickness

Very low exchange capacity

b) Porous resin coated with exchange beads:

Size 5-10 μ

Porous and highly efficient

c) Macroreticular resin bead:

Not highly efficient and low exchange capacity

d) Surface sulfonated and bonded electrostatically with anion exchange:

Less efficient and low exchange capacity

Physical properties of ion exchange resins-

Cross linking:

It affects strength solubility and swelling

Swelling:

When resin swells, polymer chain spreads apart

Polar solvents → swelling

Non polar solvents → contraction

Swelling also affects electrolyte concentration

Particle size and porosity:

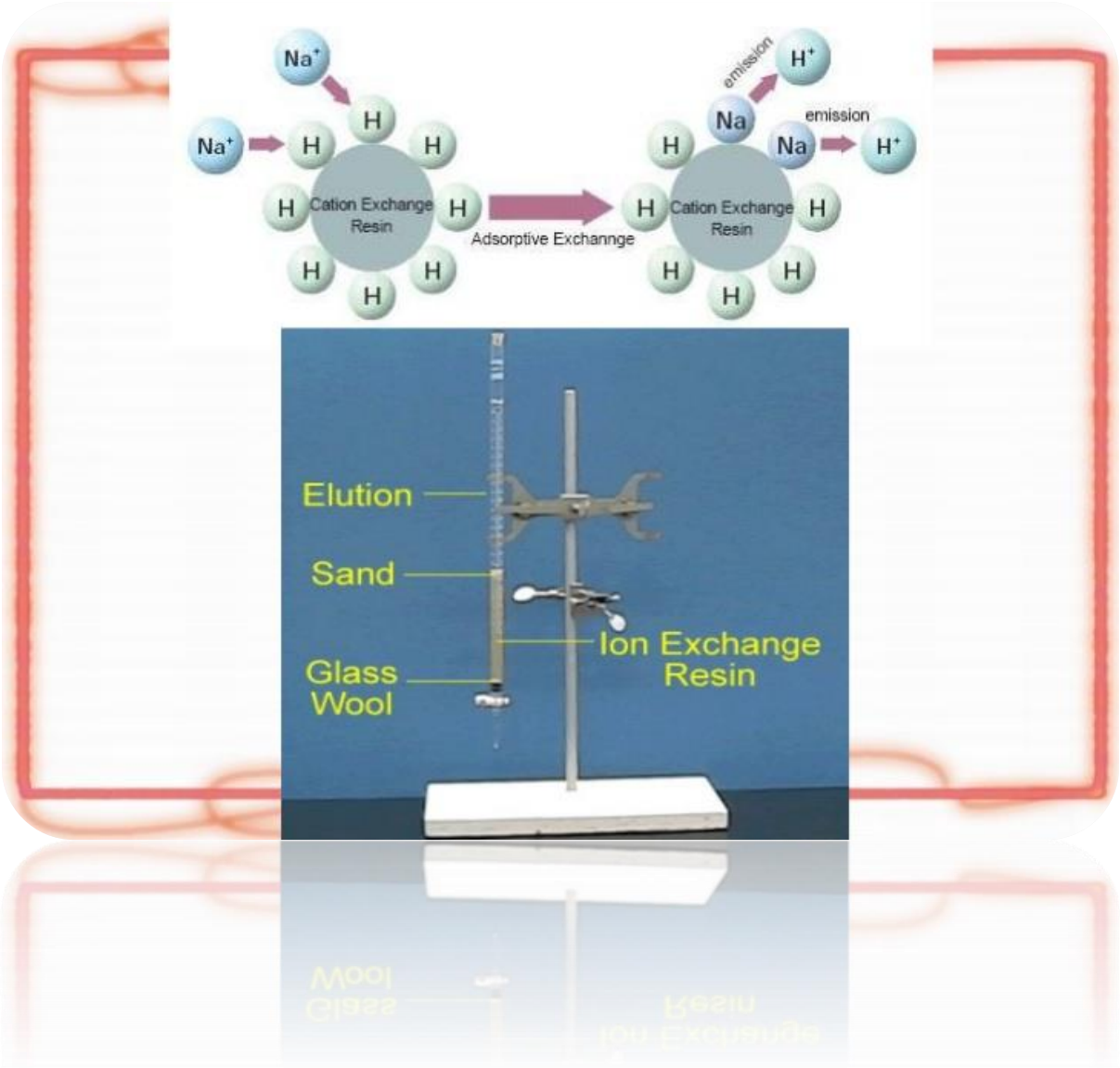
Increase in surface area and decrease in particle size will increase the rate of ion exchange

Particle size-: 50-100 mesh / 100-200 mesh

Regeneration:

Cation exchange resins are regenerated by treatment with acids then washing with water.

Anion exchange resins are regenerated by treatment with NaOH, then washing with water until neutral.



Practical requirements:

1. **Column-**

Glass, stainless steel or polymers

Length: diameter ratio 20:100 to 100:1

2. **Packing ratio-**

Wet packing method

3. **Application of the sample-**

After packing sample is added to the top of the column using syringe or pipette

4. **Mobile phase-**

Acids, alkalis, buffers

5. **Elution-**

Components of mixture separate and move down the column at different rates depending upon the affinity of ion for the exchanger of ion

6. **Analysis of the eluate-**

Spectrophotometric, flame photometry, polarographic, conductometric etc.

Factors affecting ion exchange separations-

a) Nature and properties of ion exchange resins-

→Cross linking and swelling is important.
→If more cross linking, they are more rigid, but their swelling is less.

Swells less → separation of ions of different sizes becomes difficult

b) Nature of exchanging ions-

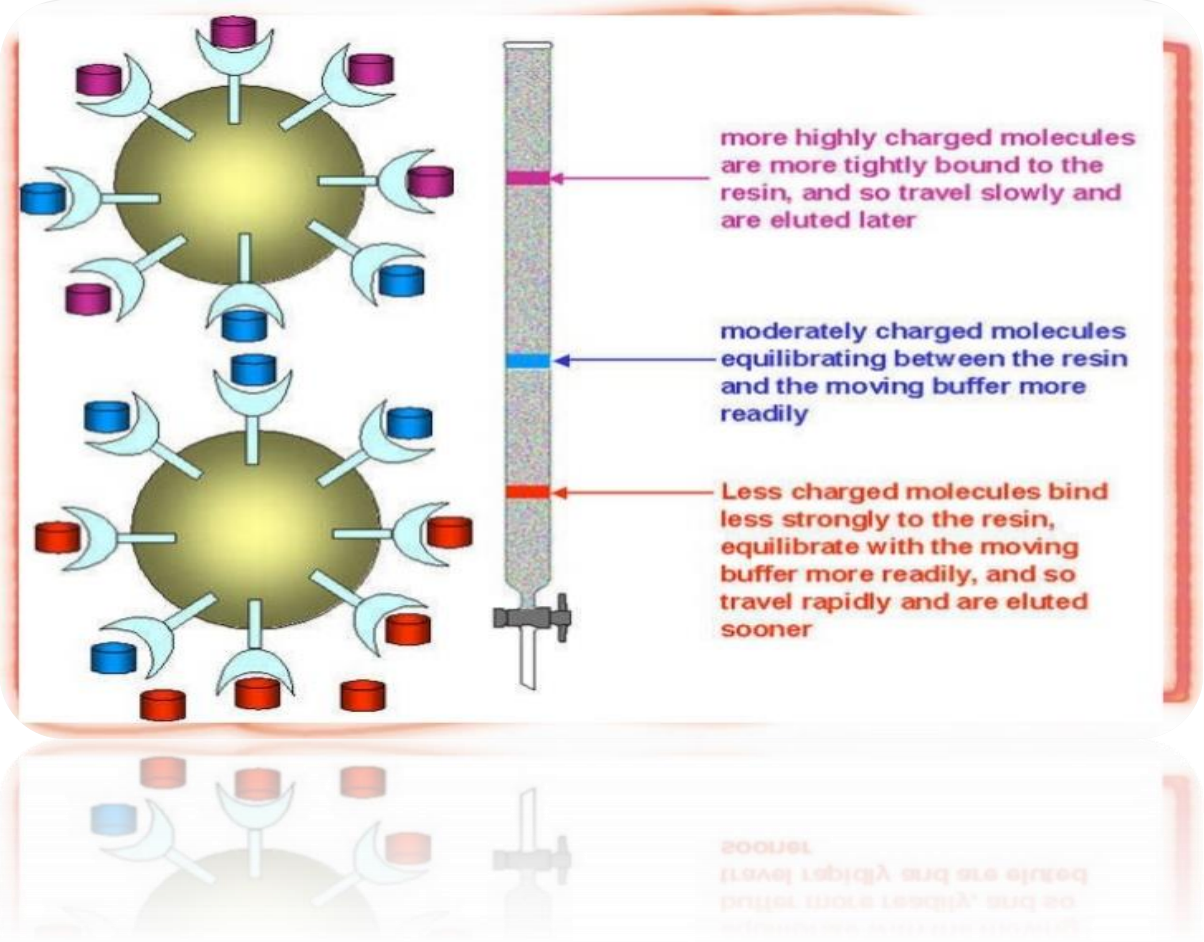
1. Valency of ions:
   At low concentration and normal temperature the exchange increases with the valency.
   $Na^{+} < Ca^{2+} < Al^{3+} < Th^{4+}$
2. Size of ions

Under same conditions and with same valency the exchange increases with decrease in size of hydrated cation. $Li^+ < H^+ < Na^+ < NH4^+ < K^+ < Rb^+ < Cs^+$

But in divalent ions the ionic size is an important factor but the incomplete dissociation of their salts also plays an important part.
$Cd^{2+} < Be^{2+} < Mn^{2+} < Mg^{2+} = Zn^{2+} < Cu^{2+} = Ni^{2+} < Co^{2+} < Ca^{2+} < Sr^{2+} < Pb^{2+} < Ba^{2+}$

Selectivity coefficient or equilibrium concentration of reaction determines the preference of ion. The ion with higher selectivity coefficient replaces ion with lower selectivity coefficient in the resin molecule.

c) **Polarizability**
d) **Concentration of solution**
e) **Concentration and charge of ions:** To exchange a higher valent ion on the exchanger for one of the lower valent ion in solution the exchange will be favoured by increasing the concentration.



Applications

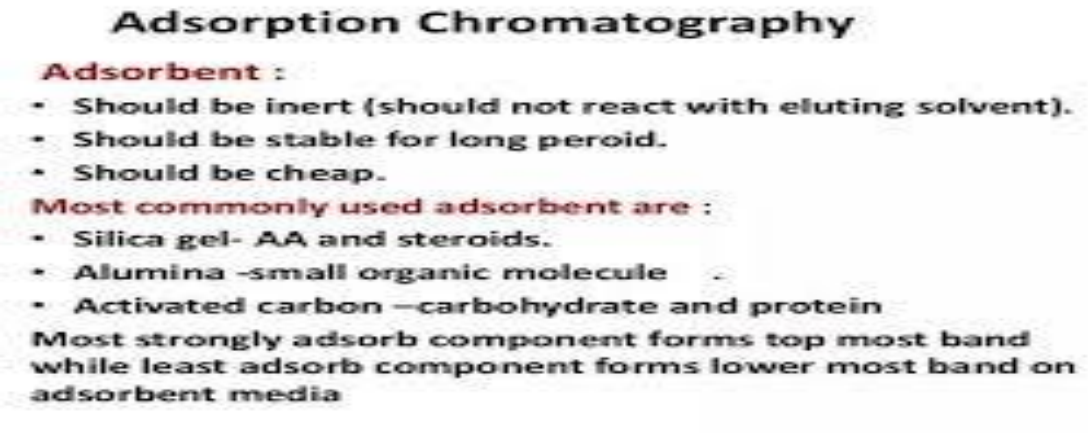
→Softening of water
→Deminelarisation of water

→Purification of solutions free from ionic impurities
→Separation of ionic ions
→Separation of sugars and amino acids
→Ion exchange column in HPLC

Adsorption chromatography

Principle: It involves the analytical separation of a chemical mixture based on the interaction of the adsorbate with the adsorbent. The mixture of gas or liquid gets separated when it passes over the adsorbent bed that adsorbs different compounds at different rates.

1. **Adsorption versus Absorption**: In absorption one substance penetrate in to the bulk of another substance. Adsorption is a surface phenomenon where interaction takes place only on the surface of one substance.
2. The stationary phase in adsorption chromatography is called Adsorbent and it is the oldest type of chromatography. Actually Tswett's work was a kind of adsorption chromatography.
3. **Adsorbents** (Stationary phase):



4. Types of adsorbents: The Ideal adsorbent must fulfill the following requirements: Insoluble in mobile phase; Inert to solutes (adsorptive); Colourless especially when work with coloured mixtures; Suitable particle size enough to give good separation and reasonable flow rate.

   Some examples of adsorbents are:

   a) **Silica gel:** It is the most widely used adsorbent. Silica gel is prepared by acidification of sodium silicate with sulphuric acid followed by washing with water and drying. The active sites of silica gel are the hydroxyl groups attached to silicon atoms "Silanol groups" . These groups are 5 0 Å apart and form hydrogen bonding groups with solutes. Silica gel reaches its maximum power when heated between 150 -250 ^{0} C to get rid of water. If silica gel contains water it then act by partition not by adsorption. Decrease in particle size increases the surface area and consequently increases separation power.

   b) **Derivatives of silica gel:** All are based on reaction with the Si – OH groups (Silanol groups) to block them.

   Reversed phase silica gel (RP): In this type a straight chain aliphatic groups are attached to the OH of silica gel by silylation. RP silica gel are named according to the length of the carbon chains. C4(RP4) , C8 (RP8), C18 (RP18)

Si-O-Si–$(CH_2)_3$ –CH_3 , Si-O-Si-$(CH_2)_7$-CH_3 , Si-O-Si-$(CH_2)_{17}$-CH_3

c) **Cyano silica gel:** O Si-O–Si –$(CH_2)_3$-CN O Si

d) **Alumina:** It is aluminum oxide (Al_2O_3). Alumina activated by heating at 400 ^{0}C overnight. It is prepared by washing aluminum oxide with 2N HCl then with distilled water.

Advantages of alumina: 1- large capacity; 2- Insoluble; 3- Relatively inert; 4- Available;

Adsorption is different from silica gel due to the strong positive field of Al and the influence of basic sites which easily affect polarized compounds. It is good in separation of aromatics from olefins.

Disadvantages : Not suitable for base labile compounds. Cause rearrangement and ring expansion of unsaturated compounds. React chemically with acidic compounds.

e) **Charcoal:** There are two types of charcoal based on temperature of activation: 1- Non–polar Charcoal prepared by activation at 1000 ^{0}C and act by adsorption through hydrogen bonds and electrostatic forces. 2- Polar charcoal prepared at lower temp and contains water so act by partition.

5. **Forces:**

In adsorption chromatography there are two types of forces: Forces attracting solutes to adsorbent (Stationary Phase). Forces tending to remove solutes from adsorbent to move with the mobile phase.

Forces of attraction: They may be classified according to their strength: Dipole–dipole attraction: It is a force that is present between polar adsorbent and polar solutes.

Hydrogen bonding: It is a type of bond weaker than covalent bonds. Hydrogen bonds are formed between the OH group hydrogen (as in silica) and electronegative atoms such as Oxygen, nitrogen in solutes.

Polarizability forces: A force that occurs between polar adsorbents and solutes that can polarize such as aromatic compounds. Weak covalent bonds: As those take place during complex formation.

Van der Waals forces: Non polar attraction forces occur between the atoms of nuclei and electrons of another atoms.

Forces causing solutes movements: Elution: It is the tendency of solutes to dissolve and move with the mobile phase. The solvent used as mobile phase must be just good enough to dissolve the solutes to allow competition with the adsorption power of the stationary phase. If very strong solvents are used they will wash out all solutes together without separation.

6. **Solvents:** For better separation, the solution of the mixture should be prepared in a relatively non-polar solvent and polar solvent should be used for elution of the adsorbed material.

   Elutropic Series of solvents: Solvent are arranged in this series according to their strength in ascending (increasing) order.

   Arrangement of polar groups according to their binding to adsorbent

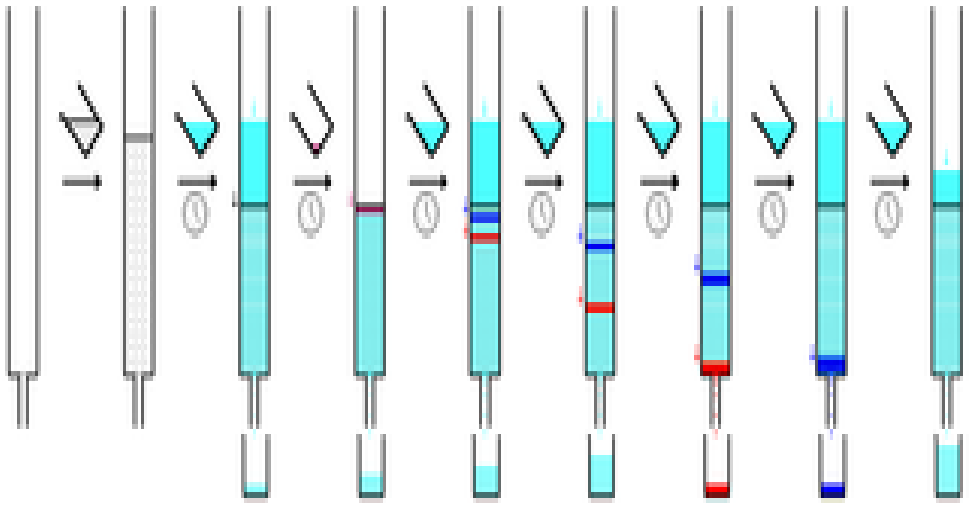
   carboxylic < Petroleum ether < Cyclohexane < amines < CCl_4 < carbon disulphide < ether < acetone < benzene < esters of organic acid < $CHCl_3$ < alcohols< water < pyridine < organic acid
   Ether/ hydrocarbons / carbonyl solvents are of common use.

   Displacement: In this case solvent molecules compete with the solutes for the adsorption sites of the stationary phase. This competition makes solutes move in different speeds.

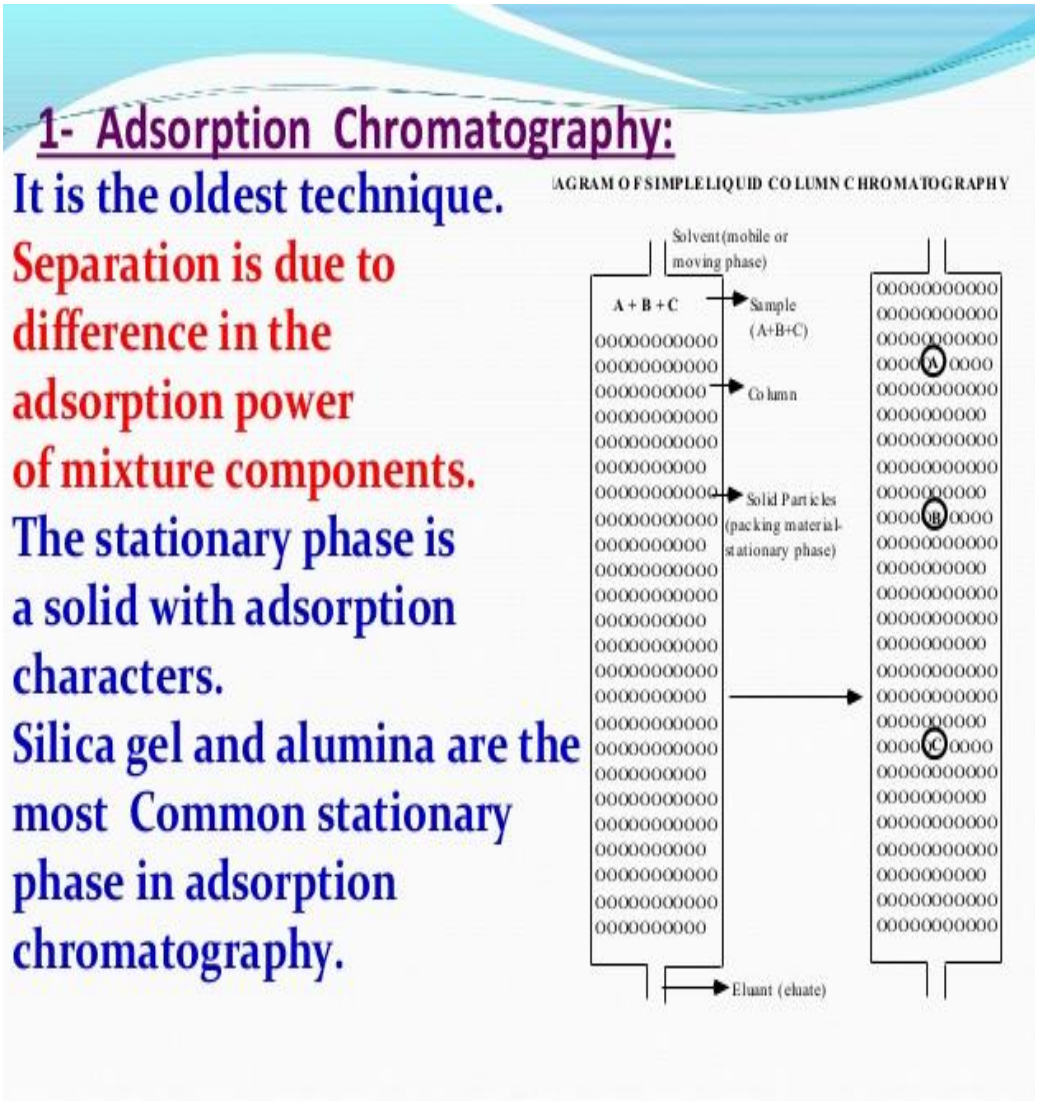
7. **Technique:**

   Column adsorption chromatography uses a column packed with the solid stationary phase, a liquid (the mobile phase) runs through this column and specific molecules will adsorb to the solid. Some components will adsorb more readily (or stronger) than other components. The compounds that absorb stronger will form a line first, at the top of the column. Compounds that absorb weaker will form a line towards the bottom of the column. Compounds that aren't adsorbed at all will simply run through the entire column and collect in a beaker at the bottom.

   Additional solvents are then added to the column, one at a time. The first solvent can break the weakest interaction between the mobile and stationary phase, bringing that compound with it into the collection beaker. Stronger and strong solvents are added until all of the compounds have been eluted (run through) the column.



Column chromatography will separately collect each compound based on how strongly it adsorbs to the stationary phase



8. Applications

Adsorption chromatography has many applications. Generally, it is used for determining the concentration of a compound (or its purity), separating out a mixture into individual components, and identifying what is in a mixture.

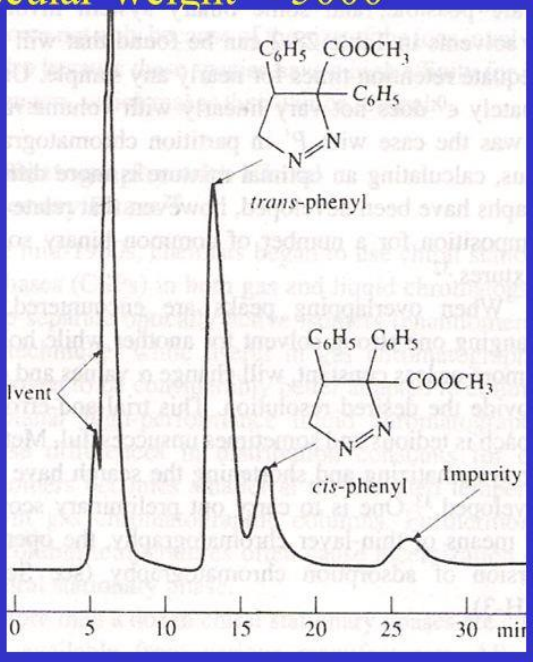
Drug testing can be performed using column adsorption chromatography. Urine, or other body samples, are run through the column, where the high pH of the urine makes it elute out first, followed by other substances. The other substances that elute out can be tested to see if any drugs are present. The nice thing about this type of chromatography is that the separated out substances can be used for further testing.

Adsorption chromatography is used for separation of amino acids. It is used in the isolation of antibiotics. It is used in the identification of carbohydrates. It is used to separate and identify fats and fatty acids.

Application of Adsorption Chromatography:

- best suited for nonpolar and water insoluble compounds having molecular weight < 5000

• particular strength of adsorption chromatography, not shared by other methods, is its ability to differentiate among the components of **isomeric mixture**



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