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Analytical Chemistry

5th Semester

Chromatography:

Introduction:

=> In 1903 a Russian botanist Mikhail Tswett introduced this term.

=> Chroma → color

graphy → writing

=> "It is an analytical technique which is used for separation, isolation and identification of chemical components of complex mixture"

=> 1st time it was used for analysis of pigments of plants.

=> In 1952 A.J.P Martin and R.L.M Synge were awarded Nobel prize for work on chromatography

Principle of Chromatography:

1) When the mobile phase containing the sample components passes through the stationary phase, the rate of travel of an individual component directly related to the partition i.e. distribution of that component b/w the mobile phase and stationary phase.

2) Partition coefficient can be denoted by "K"

$$K = \frac{C_s}{C_m}$$

C_s = Conc. of component in S. phase

C_m = Conc. of component in m. phase

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Paper Chromatography:

=> In 1944 Cosdon, Gordon and Martin introduced this paper partition chromatography.

=> It is also known as liquid-liquid chromatography.

Phases:

=> Stationery phase → solid/liquid

=> mobile phase → liquid

=> Mainly partition co-efficient depends upon two phases.

=> Adsorption effect occurs when stationery phase is not completely inert.

Development of Chromatography:

=> A strip of cellulose filter paper (30 x 5 cm) is taken.

=> By using a pencil a line is drawn 5 cm above the end point.

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- ⇒ A drop of sample is applied on base line.
- ⇒ Strip dipped upto 1-2 cm on solvent containing vessel.
- ⇒ Vessel is closed with a cardboard.

Mechanism:

- ⇒ In chromatographic vessel solvent rises over strip by capillary action at different rates according to their partition coefficient.
- ⇒ When solvent reached at the top of the edge, strip is removed and solvent front is marked.
- ⇒ Dries the strips.
- ⇒ Mark is visualized by sustaining techniques.

R_f value :-

By using formula R_f value can be calculated.

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$$R_f = \frac{x}{d}$$

" R_f is the ratio

b/w distance travelled by the component to the distance travelled by solvent.

$R_f = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by component}}$
Factors Effecting on R_f value:-

- i) Solute concentration
- ii) Temperature
- iii) Direction of flow of solvent
- iv) pH

TYPES of Chromatography:

⇒ There are two types of paper chromatography depending upon the separation of components into two or one direction:-

One-direction paper chromatography:

⇒ 3- different process are used for one dimensional p.c depending upon the direction of flow of solvent

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i) Ascending Method:

- ⇒ In this method mobile phase moves upward.
- ⇒ It is so convenient and suitable method for P.C.
- ⇒ It is used for slow moving components, having low R_f.
- ⇒ In this method solvent travels upto limited level.

ii) Descending Method:

- ⇒ Mobile phase moves downward.
- ⇒ Since bottom of paper is dips into the solvent, the separation is wider.
- ⇒ This method is used for similar and low R_f values.
- ⇒ R_f values are determined by standard references i.e. glucose.

iii) Radial or Horizontal Method:

- ⇒ It is convenient and used for rapid separation.

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- ⇒ Circular filter paper is taken.
- ⇒ A thin strip is cut along its radius which act as a wick for solvent movement.
- ⇒ A drop of sample is applied near the center.
- ⇒ It is dipped into a petri dish containing solvent.
- ⇒ Solvent moves upward through capillary action and component of mixture are separated in the form of semi-circular bands or radial development.

2-D Paper Chromatography:

- ⇒ It is use for sample having similar R_f values.
- ⇒ Two mixtures are separated at right angle to each other by using different solvent.
- ⇒ 1st separation is done in one direction
- ⇒ Volatile solvent is evaporated.

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- > Paper is dried.
- > Paper is turned at right angle and separation is done with a second solution.
- > A spot is located and a map is obtained.
- > Map is compared with by Standard map.
- > This comparison shows the separation of mixture in 2-D Paper Chromatography.

Applications:

Paper Chromatography is used to:-

- i) Separate organic acid
- ii) Separate Amino Acids
- iii) Separation of Sugars.
- iv) Separation of Ions.

Choice of Solvent:

- > Choice of solvent depends upon the sample investigated.
- > Having suitable solvent.
- > CH_3COOH and NH_3 are used

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to create strong acids or basic mediums.

Choice of Papers:

⇒ The R_f value depends upon the choice of paper.

Types:

- ① Whatman No. 1 → analytical uses
- ② Whatman No. 0.3 → large quantities
- ③ Whatman No. 4, 5 → Rapid separation
- ④ Ion-exchanging → Commercial purpose

② Pure cellulose paper:

It is a systematic arrangement of fibers having surface and amorphous regions that hold hold the H_2O , which are responsible for retention of R_f .

③ Hydrophobic substance can be separated by using modified cellulose paper which consist on cellulose paper and

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Silica gel and alumina.

Diagrams:

Column Chromatography:

⇒ In 1897 an American chemist D.T. Dewey introduced this technique by doing experiment on petroleum.

⇒ "Uniform percolation of a liquid through a column of finely divided substance."

Phases:

⇒ Stationary phase → Solid

⇒ Mobile phase → Liquid

⇒ It is also known as column adsorption chromatography.

Formation of Chromatogram:

⇒ Sample containing absorbent poured into the column.

⇒ Component having maximum adsorption absorb in upper portion and vice versa.

⇒ Components of mixture are separated in the form of band or zones.

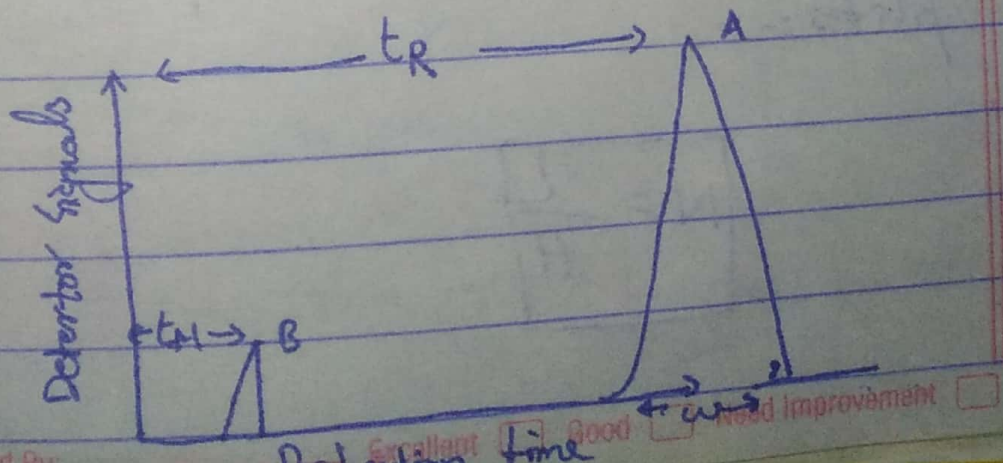
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- ⇒ By using pure solvent bands becomes well defined.
- ⇒ Banned column is called chromatogram and process is called development of chromatogram.
- ⇒ (Elution) The removing of remaining component can be done by flowing solvent through column.
- ⇒ (Elute) Solute containing eluted component called elute.
- ⇒ Pure component is obtained by removing solvent from Elute.
- ⇒ By using detectors quantity of component is analysed.
- ⇒ A graph b/w detector signal and retention time is obtained.



Theoretical plates:

⇒ Chromatographic column consist of discrete section at which partition of solute b/w 'stationary phase' and mobile phase occurs. That is called theoretical plates.

⇒ No. of theoretical plates can be calculated by using this formula.

$$N = 16 \left(\frac{t_R}{W} \right)^2$$

t_R = Retention time

W = Base line width

Efficiency:

Efficiency of column can be determined in term of No. of and height of theoretical plates:-

$$N = \frac{L}{H}$$

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Factors:-

Separation depends upon

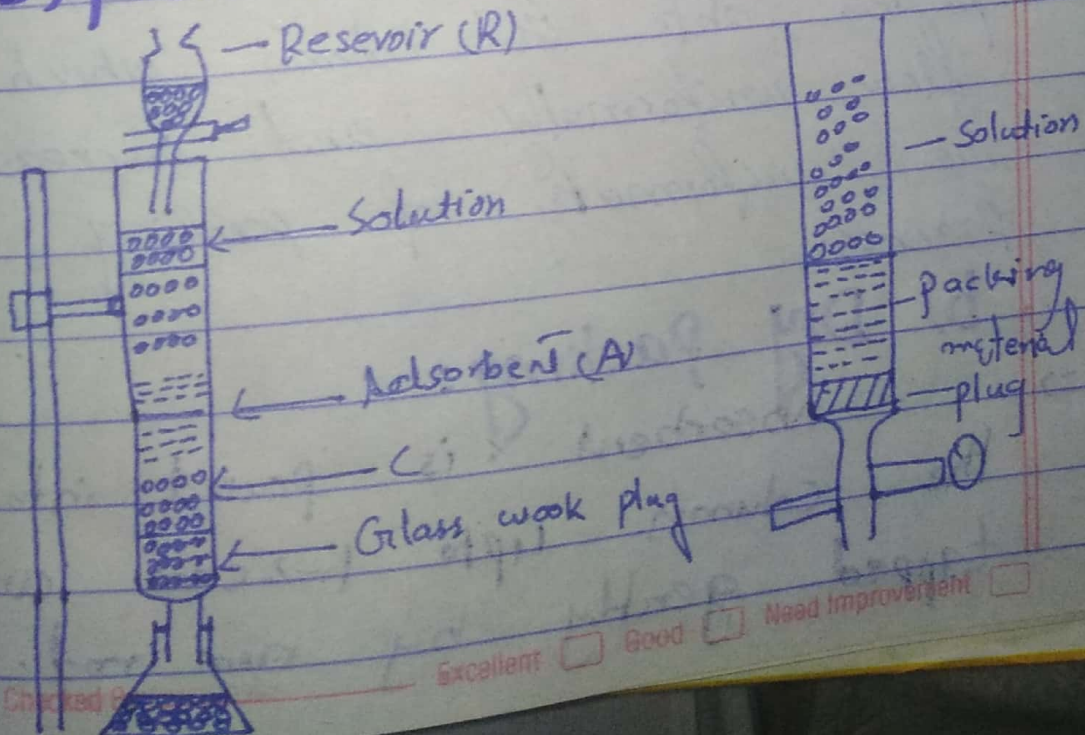
following factors:-

- i) nature of adsorbent
- ii) nature of solvent used to elute
- iii) Sample
Rate of flow of solvent through
Column.
- iv) Geometry of column.

Priority of separation:

Acid, base > alcohol > aldehyde >
unsaturated C-H > Saturated C-H.

Experimental Setup:



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Column consist of:

- i) glass tube (C)
- ii) plug of glass wool (G)
- iii) filter flask connected to glass tube by rubber stopper
- iv) solvent containing reservoir

Procedure:-

i) packing of column:-

A column or long tube is taken and absorbent is uniformly placed on it until air bubbles and channels removes.

a) wet packing:-

Slurry (Solvent + absorbent) poured into the column which flow uniformly and increase the uniformity of packing process.

b) Dry packing:-

⇒ Absorbent is poured into the column upto 1-2 cm and tapped gently by hand.

⇒ Add another portion of absorbent.

ii) Development of Chromatogram:

⇒ Solvent containing separating mixture poured into the column.

⇒ The column is filled so that liquid level coincide with the top of the bed.

⇒ A small portion (1-2 cm) piped above the bed.

⇒ The position of reservoir is settled.

⇒ By applying suction flow is obtained by gravity.

⇒ Solvent runs steady into the column.

⇒ As developer solvent percolates through column, components of mixture get separated.

⇒ Colored component forms colored zones.

⇒ Colorless components can be

visualize via UV Lamp.

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⇒ A well developed column is called chromatogram.

iii) Elution and Recovery of Different components:-

⇒ After obtaining the well developed chromatogram, solvent is passed through the column and remove all the components from adsorbent surface. (Elution)

⇒ Solvent is evaporated and components are recovered in pure form.

Absorbents:-

- i) BaSO_4
- ii) ZnO
- iii) ThO_2
- iv) BeO

Choice of Solvent:-

Choice of solvent depends upon following thing:-

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It should be:-

- i) Non-reactive
- ii) Not decomposed the substance
- iii) Insoluble in solvent
- iv) colorless in colored zones
- v) Having same properties through out the reaction.
- vi) Having particle size uniform.

Classification of absorbent:-

a) Weak absorbent

Sucrose, insulin, starch, etc.

b) Intermediate absorbent:-

CaCO_3 , magnesia, slacked lime

c) Strong absorbent:-

alumina, Charcoal.

Developers:

⇒ These are used to give color to the colorless compounds.

⇒ They have less affinity with solvents.

i.e Hydrogen Sulphide, ammonium

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Sulphide, potassium ferrioxalate,
potassium Thiocyanide.

Solvents:-

Alcohols, petroleum ether
Acetone etc.

Applications of Column

Chromatography:-

This method is used to :-
purification of compounds
Determination of homogeneity
of compounds.

Comparison of compounds thought
to be identical.

Concentration of substance.

—x—x—x—x—

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Thin Layer Chromatography:

⇒ In 1956, Stahl and Demolin introduced this technique.

Phases:-

- i) Stationary phase → Solid
⇒ glass plates coated with thin film of absorbent i.e. Silica gel, alumina, cellulose paper.
- ii) Mobile phase → liquid.

Choice of absorbent:-

⇒ For weak polar substances:-
Alumina is used.

⇒ For strong polar substances:-
Silica gel is used.

Choice of Solvents:-

Choice of solvent depends upon following factors:-

- i) nature of substance to be separated.

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- ii) Nature of absorbent.
- (ii) Combination of Solvents gives better results than single Solvents.

Experimental Set up:-

Requirements:-

- i) Suitable absorbent
- ii) Suitable Solvent
- iii) glass plate
- iv) Suitable device to apply thin layer of absorbent.
- v) Mean of Holding plates in jar or tank.

Preparation of Chromaplates:

- => Glass plates (20 x 5 cm) of uniform thickness is taken.
- => plates are dipped into the slurry or slurry of absorbent with binders (gypsum, resins) is poured onto the plates by using spreader up to the 0.1 to 0.3 mm thickness.
- => Plates are put into the oven upto 100°C, cooled and kept in dissociator.
- => Solid stationary phase is obtained.
- => It is also known as activation of plates.

Development of Chromatogram:

- => This process is done by using developing tank.
- => 1st atmosphere of tank is made saturated so that Rf value is not effected.

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Chromoplates are dipped into solvent upto 0.5 cm keeping the baseline above the surface.

Lid is put on the tank.

Solvent moves upward via capillary action and dissolve the spot on the baseline.

Movement of Solute depends upon its solubility and interaction with solid phase.

Component of mixture are separated.

By using air currents plates surface is dried and position of components is marked.

Chromatogram is developed on Chromoplates.

Calculation of R_f value :-

$R_f = \frac{\text{Distance travelled by Component to baseline}}{\text{Distance travelled by Solvent to baseline}}$

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R_f value depends upon:-

- i) Partical size of absorbent
- ii) Quality and composition of solvent
- iii) Degree of saturation of atmosphere of tanks.
- iv) preparation, activation and storage of plates.

Location of Compounds:-

- => Location of colored components can be located easily
- => Colorless components can be located by using chemicals or physical means.
 - i) By using Spraying Agents
 - ii) Ninhydrin test for Amino Acid.
 - iii) By using I₂ vapores gives dark brown color
 - iv) Organic components treating with H₂SO₄ gives black spot
 - v) Ready made TLC plates having florecents dyes.

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Application of TLC:

- This technique is used for:-
- Isolation, Separation and identification of mixture
 - Checking the purity of compound by comparing R_f value with authentic values.
 - To check the rate of reaction
 - Identification of plants extract
 - Separation of metal ions
i.e. Ni, Co, Zn etc.

Superiority of TLC over P.C

- It is rapid than P.C
- It takes less case as compared to P.C
- Clean spots are obtained
- Capacity of TLC is greater than Paper Chromatography (P.C)

④ Ion Exchange Chromatography:

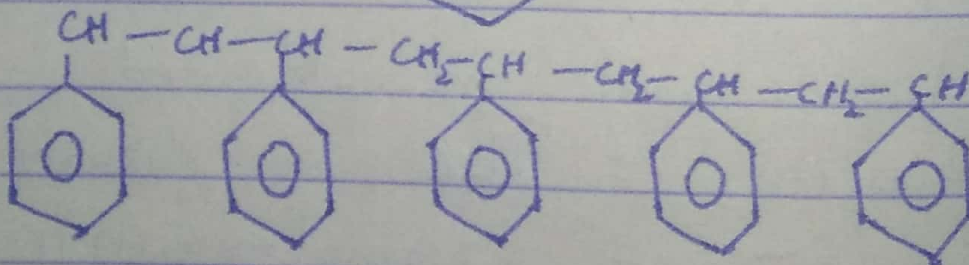
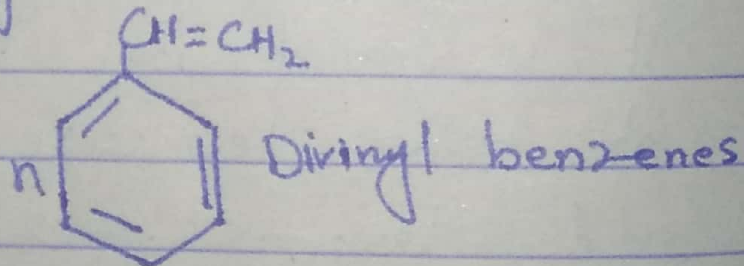
In 1956, Peterson and Sober introduced this technique for the separation of ions.

Phases:

- ⇒ Stationary phase is → Solid
 Mobile phase → Liquid
 It is known as Solid-Liquid Chromatography.

Stationary phase:

- ⇒ It is a resin which consists of insoluble polystyrene.
 ⇒ Polystyrene is a polymer of divinylbenzene.



Polystyrene

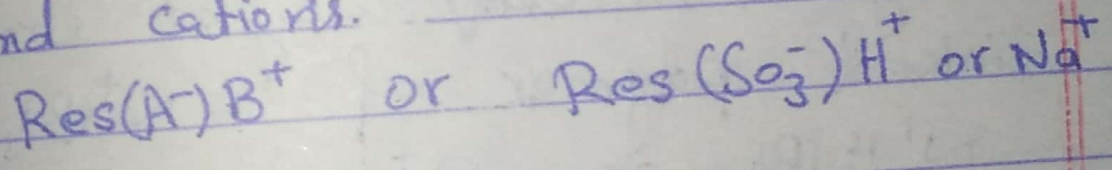
- ⇒ Hydrophilic ions are active groups attached with resins.

Types of Ion Exchange resins:-

- Two types:-
- i) Anion Exchange resins
 - ii) Cation Exchange resins

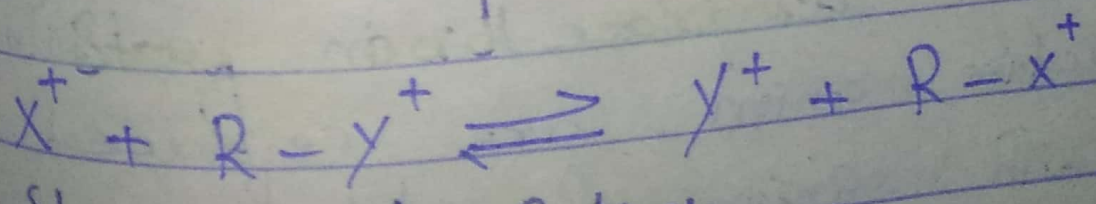
Cation Exchange Resins:-

It is a high molecular weight cross linked polymer. It consist of Sulphonic carboxylic, phenolic group with resins and cations.



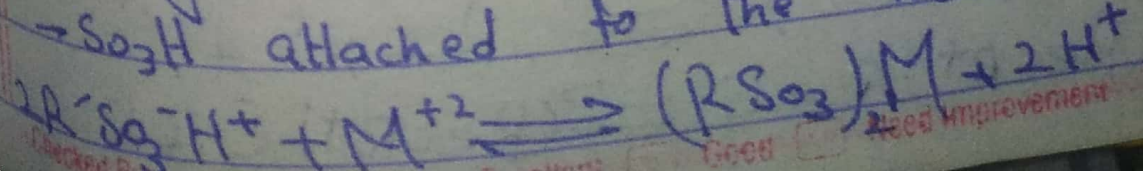
Mechanism:-

When a cation exchange resin is placed in a solution active cations are replaced with cations of solutions



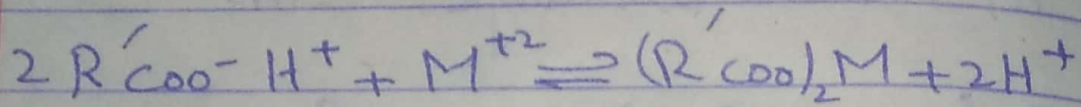
Strong acid Cations:

-SO₃H attached to the matrix.



⇒ Weak acid cations:-

- coolt group attached to the matrix.



Equilibrium can be shifted left or right side by increasing $[H^+]$ and $[M^{+2}]$ respectively.

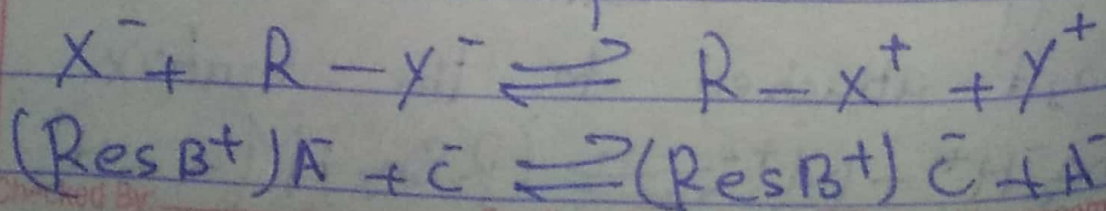
Anion Exchanger Resin:-

⇒ High molecular weight cross-linked polymer of $(-N^+(R)_3, -N^+H(R)_2$ or $-R^+H_3)$ with (Cl^-, SO_4^{2-}, OH^-) with resin.

Resin + polymer $(ResB^+)A^-$ or $ResN^+R_3$

Mechanism:-

When a anion exchange resin is placed in a soln active anion are replaced with anions of solutions:-



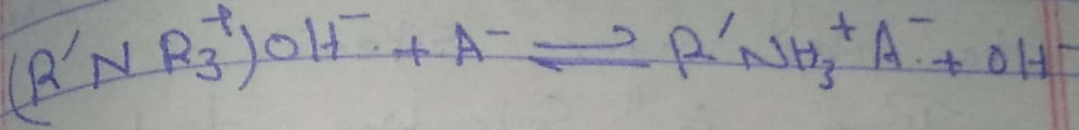
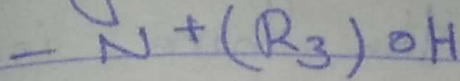
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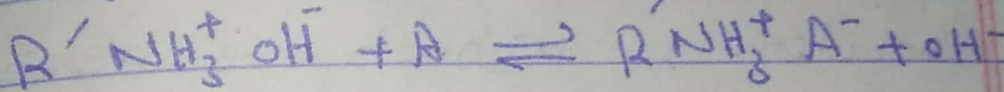
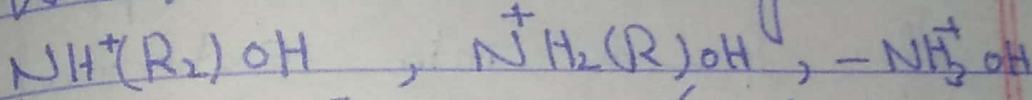
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Strong anion resin:



Weak anion exchanger.



Commercially used Resins:

Strong

Weak:

	Zerolite ZCS	Zerolite 226
Cations:	Amberlite 120	Amberlite 50
	Zerolite EE	Zerolite H
Anions	Amberlite 400	Amberlite 45

General Mechanism:

In Homogeneous solution diffusion of ions into exchanger surface

takes place.

Diffusion through matrix takes place and it depends upon the degree of cross linkage and conc. of solution.

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⇒ The more charged ion exchanges the more ions binds to exchanger site.

⇒ There is selective desorption by the eluent and diffusion of species into external solution, which is achieved by changing pH and ionic concentration.

Ion Exchange Capacity:

Capacity of Ion exchange resin depends upon the no. of active groups.

$$\text{ion Exchange Capacity} = \frac{V \times N}{W}$$

V = volume of solution

N = ionic strength

W = weight of solution

Nature of Ion Exchange Resin

⇒ It quality depends upon ion

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exchange capacity which further depends upon no. of active groups.

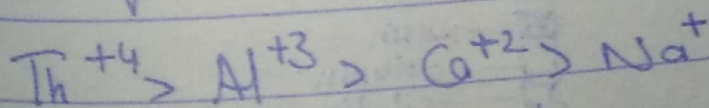
1) Greater the active groups greater will be capacity.

2) Its Efficiency depends upon degree of cross linkage.

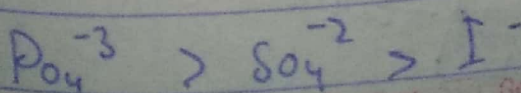
Factor Effecting on ion exchange Equilibria:-

These factors effects the distribution of ions b/w ion exchange resin and solution.

① Resin prefers the ions of the highest charge ion with H^+ .



② Capacity of anions having same size decrease in following order.



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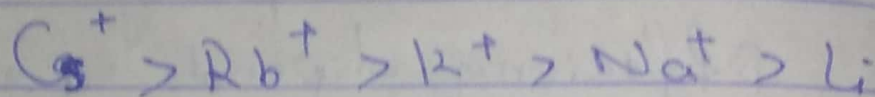
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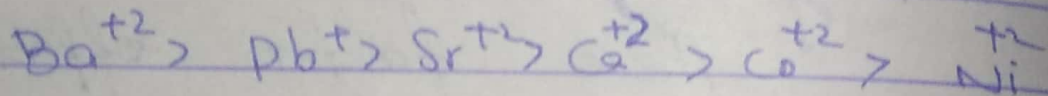
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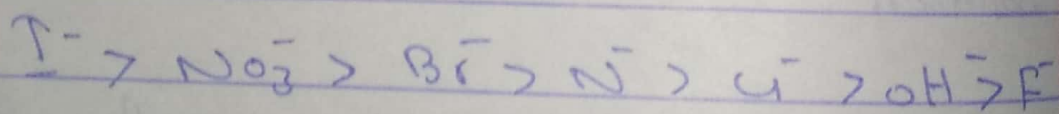
③ Ions having same charges but smallest hydrated radius are strongly held by resins and vice versa



④ Among doubly charged cations capacity has been found to decrease



⑤ Among univalent anions:-



Experiment Set up:-



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- ⇒ Column is used
- ⇒ Resin in the form of slurry is applied
- ⇒ packed the column carefully.

Packing of column:-

- ⇒ Water is drained through tube to remove air bubbles.
- ⇒ Filled the tube about 3-4 quarters with H₂O.
- ⇒ Slurry is poured into column and resin bed about 15ml is formed.
- ⇒ Set the resin by gentle tapping.

Precautions:-

- (i) Avoid air bubbles in tube
- (ii) Avoid to form channels
- (iii) used ionized H₂O
- (iv) Don't use dry form b/c it cause bursting of column.

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Applications:-

- ⇒ This method is used to
- i) Separation of similar ions
i.e. Li^+ , Na^+ , K^+ etc
 - ii) Removal of Interfering Radicals
 - iii) Softening of H_2O
 - iv) Demineralization of H_2O
 - v) Separation of Lanthanides and actanides elements:-