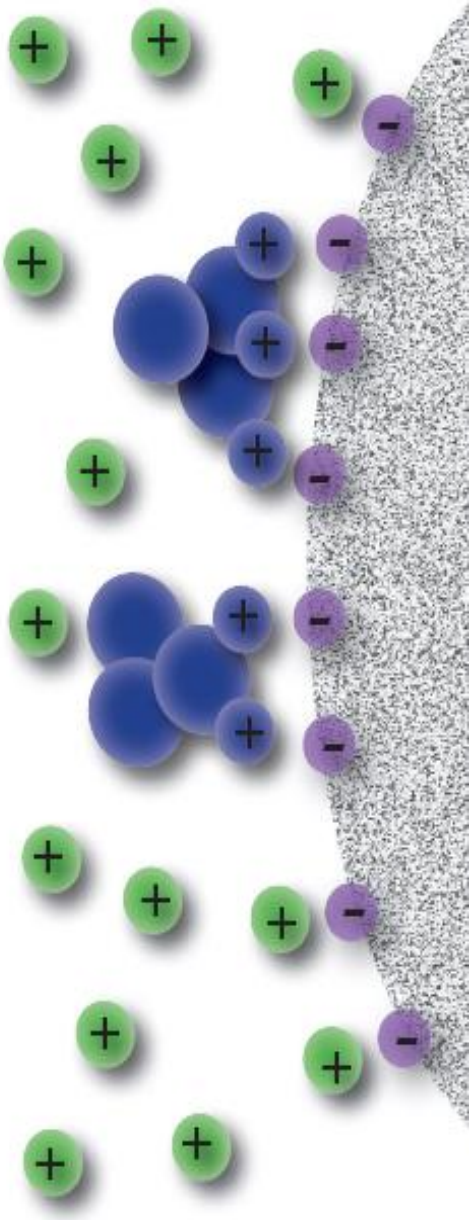
ION EXCHANGE CHROMATOGRAPHY



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- INTRODUCTION OF IEC
- PRINCIPLE OF SEPARATION
- ION EXCHANGE RESINS (CLASSIFICATION OF RESINS)
- PRACTICAL REQUIREMENTS
- FACTORS AFFECTING ION EXCHANGE SEPARATION
- APPLICATIONS

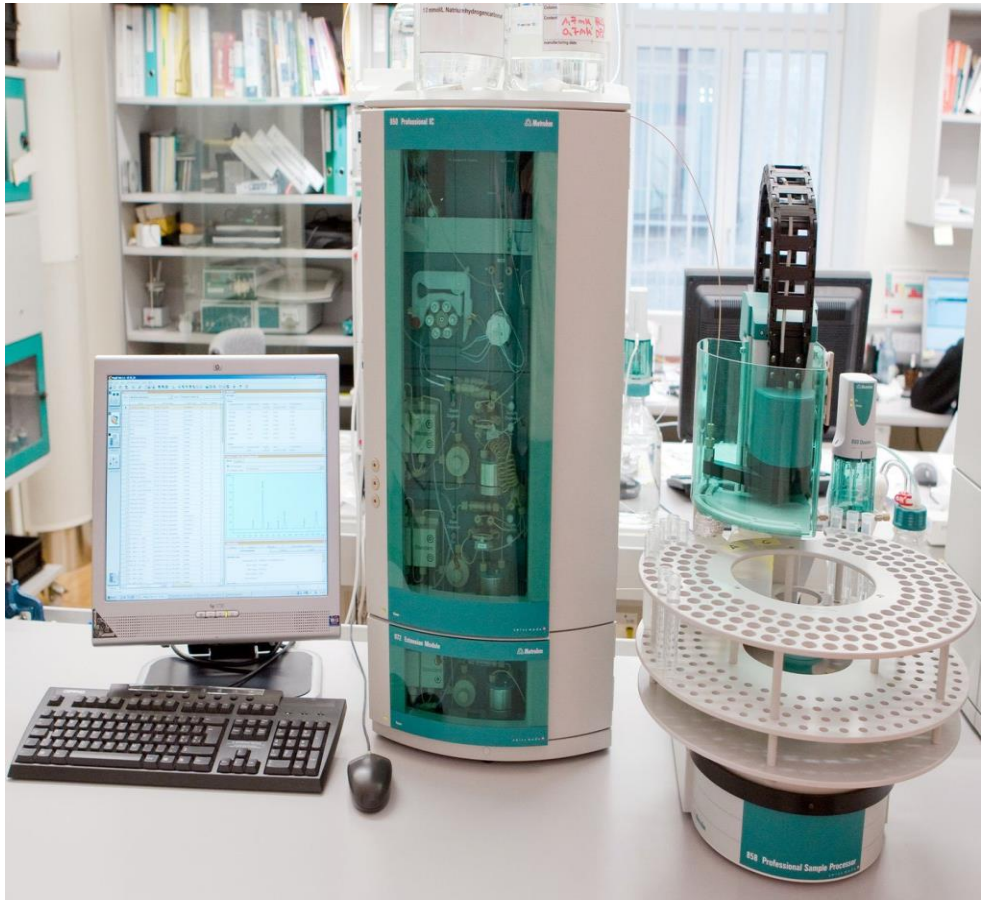
ION EXCHANGE CHROMATOGRAPHY

DEFINITION:

- Ion exchange chromatography is a process by which a mixture of similar charged ions can be separated by using an ion exchange resin which exchanges ions according to their relative affinities.

Definition

- **Ion-exchange chromatography** (or *ion chromatography*) is a process that separates ions and polar molecules based on their affinity to the ion exchanger.



INTRODUCTION

- Ion exchange chromatography is a type of adsorption chromatography.
- There is a REVERSIBLE EXCHANGE OF SIMILAR CHARGED IONS.
- Mostly similar charged ions like cations and anions can be conveniently separated by this technique.
- Many drugs and pharmaceutical agents are weakly or strongly acidic or basic in nature.
- Hence a mixture of similar charged substances can also be separated into pure components.

Types

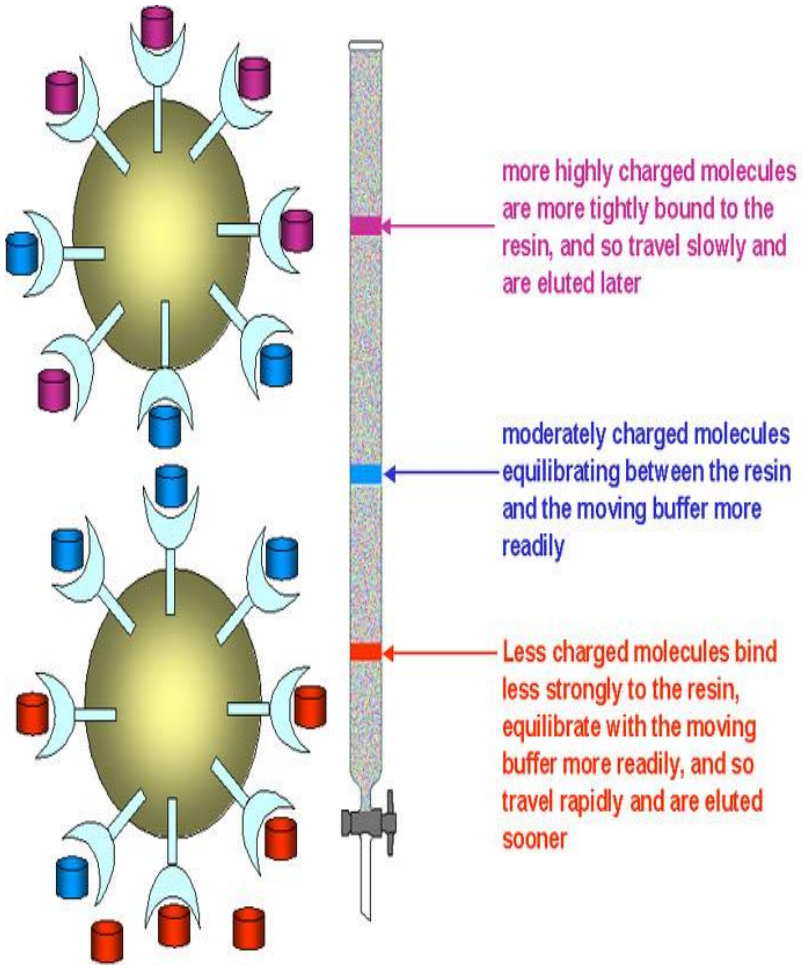
Laboratory

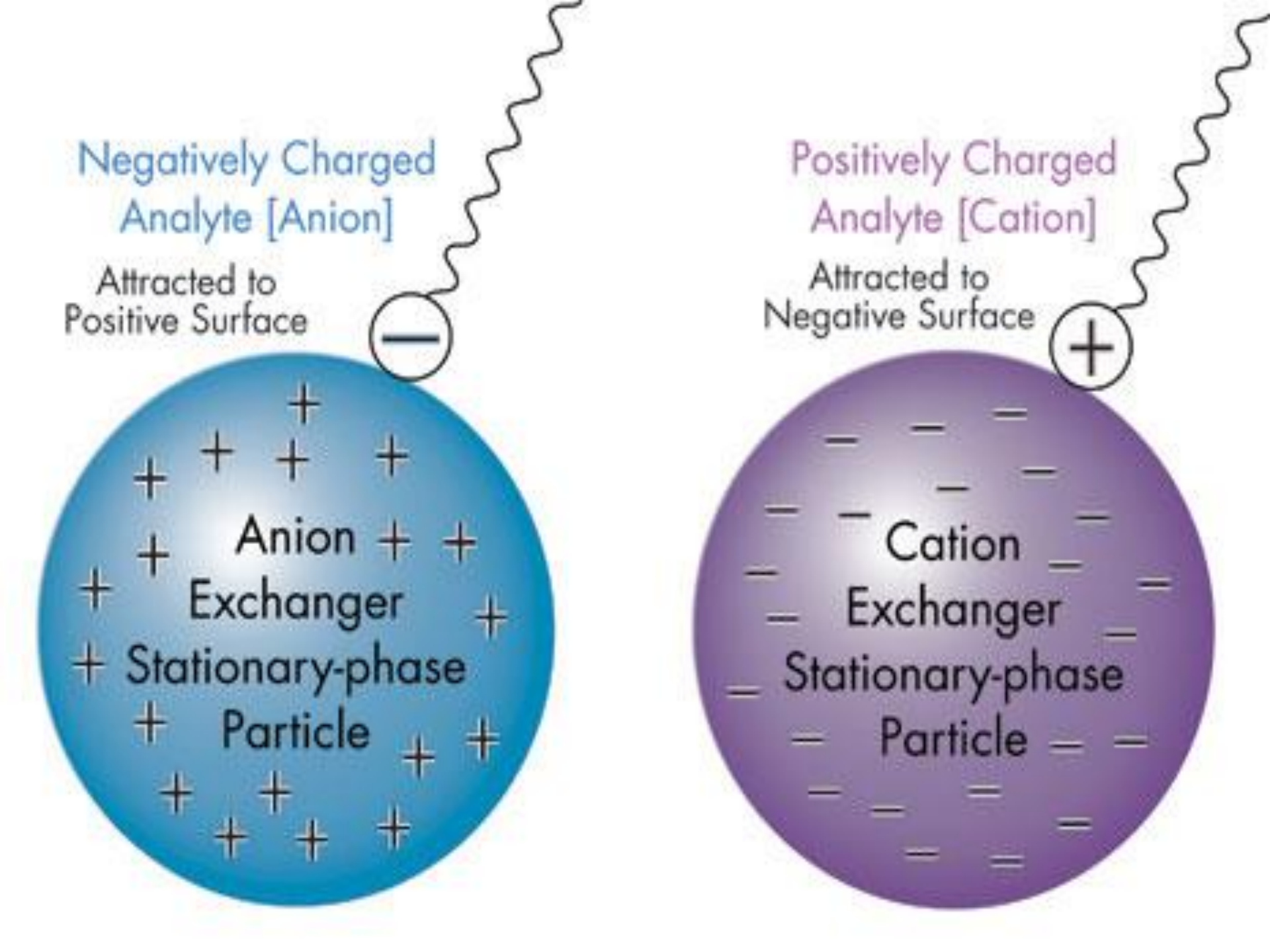
Commercial

Automated

Principle

- Ion exchange chromatography retains analyte molecules based on ionic interactions.
- The stationary phase surface displays ionic functional groups (R-X) that interact with analyte ions of opposite charge.




Negatively Charged
Analyte [Anion]
Attracted to
Positive Surface
Anion
Exchanger
Stationary-phase
Particle
Positively Charged
Analyte [Cation]
Attracted to
Negative Surface
Cation
Exchanger
Stationary-phase
Particle

PRINCIPLE OF SEPARATION

The principle of separation is by reversible exchange of ions between the ions present in the solution and those present in the ion exchange resin.

CATION EXCHANGE:

The separation of cations using cation exchange resin. The cations to be separated are present in solution and exchanges for similar ions present in cation exchange resin, a solid matrix. the exchange can be represented by the following equation:

$$X^{+} + R^{-}K^{+} \longrightarrow X{+}R^{-} + K^{+}\ \text{(solution)}$$

(solution)

- The cations retained by the solid matrix of ion exchange resin can be eluted by using buffers of different strength and hence separation of cations can be effected.

ANION EXCHANGE:

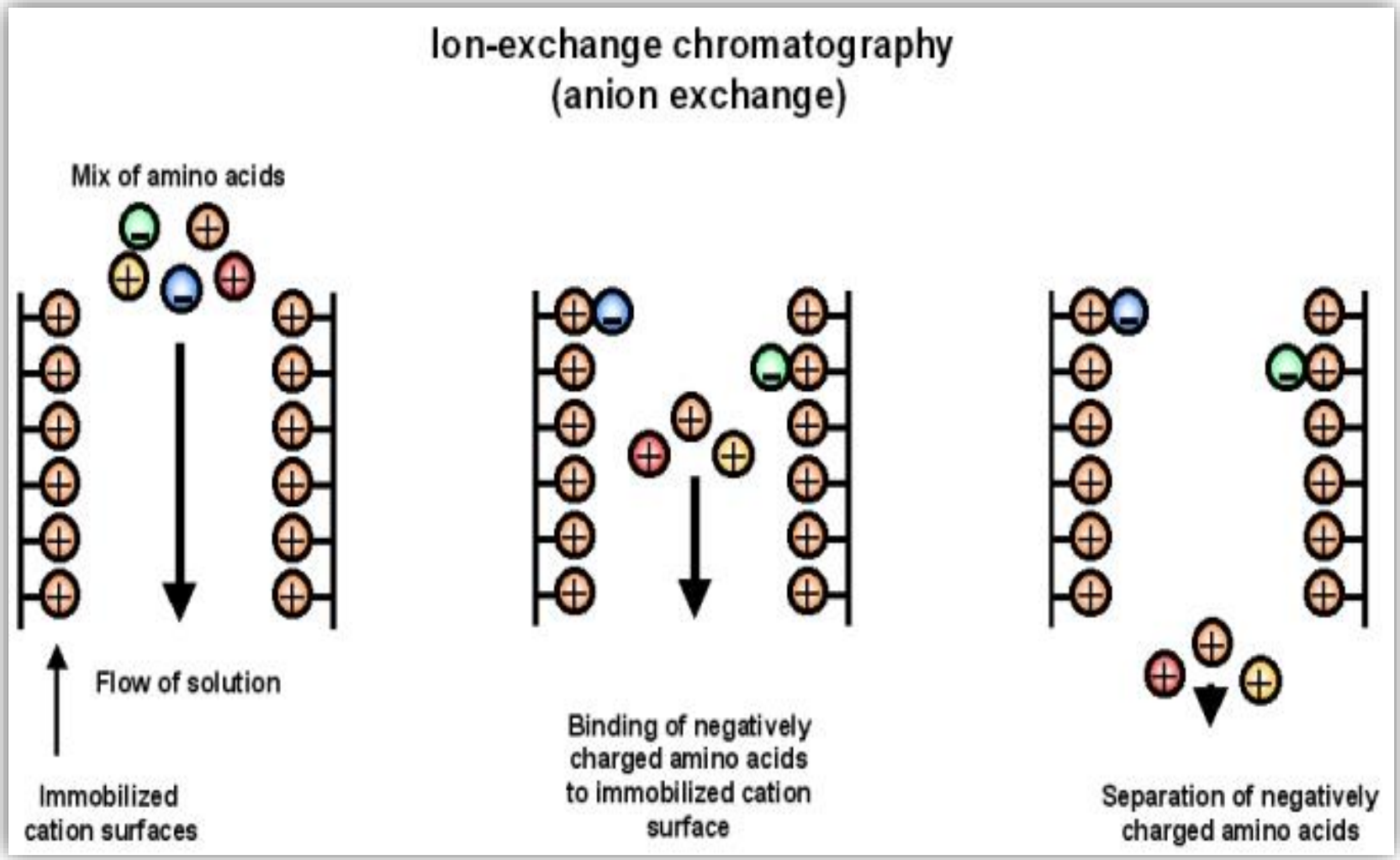
Separation of anion using anion exchange resin can be carried out. The anions to be separated are present in solution and exchanges for similar ions present in anion exchange resin, a solid matrix the exchange can be represented by the following equation:

(solid)

$$X^- + R^+Cl^- \longrightarrow X\text{-}R^+ + Cl^- \text{ (anion exchange)}$$

(solution)

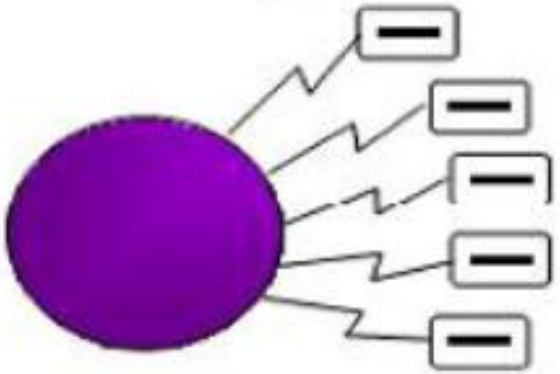
- The anions retained by the solid matrix of ion exchange resin can be eluted by using buffers of different strength and hence separation of anions can be effected.



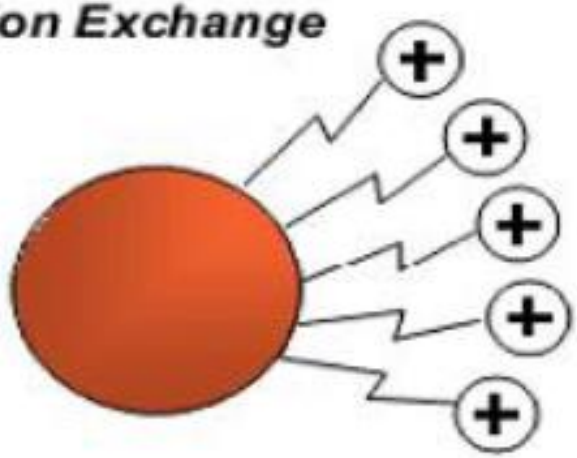
Ion Exchange Theory

Cation Exchange vs Anion Exchange

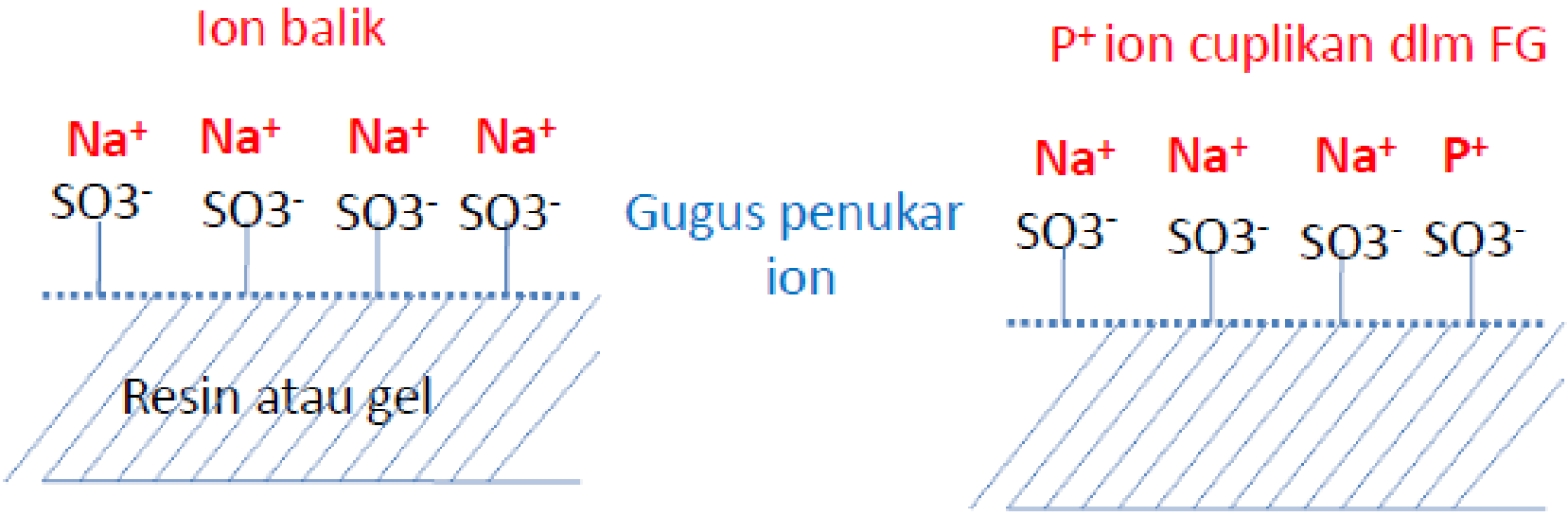
Cation Exchange



Anion Exchange



Cation exchange columns have a negative charge to attract cations.
Anion exchange columns have a positive charge to attract anions


Ion balik
Na+ Na+ Na+ Na+
SO3- SO3- SO3- SO3-
Resin atau gel
Gugus penukar ion
P+ ion cuplikan dlm FG
Na+ Na+ Na+ P+
SO3- SO3- SO3- SO3-
Satu ion cuplikan pengganti ion Na+

Recommended Buffers for Polypeptide Ion-Exchange Chromatography

A wide range of buffers are available for use with ion-exchange chromatography. Recommended buffers for various ranges of pH are listed below.

Anion-Exchange Chromatography Buffers

Buffers for anion exchange are generally basic amines.

Buffer	Concentration	Anion	pKa	Buffering Region
L-histidine	20 mM	Cl-	6.15	5.5 - 6.8
bis-Tris	20 mM	Cl-	6.50	5.8 - 7.0
bis-Tris propane	20 mM	Cl-	6.80	6.4 - 7.3
Triethanolamine	20 mM	Cl-	7.77	7.3 - 8.2
Tris	20 mM	Cl-	8.16	7.5 - 8.8
diethanolamine	20 mM	Cl-	8.88	8.4 - 9.4

Cation Exchange Chromatography Buffers

Buffers for cation-exchange chromatography are acids.

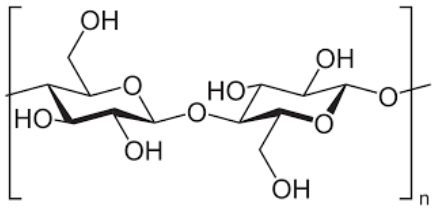
Buffer	Concentration	Cation	pKa	Buffering Region
formate	20 mM	Na+	3.75	3.3 - 4.3
acetate	20 mM	Na+	4.76	4.2 - 5.2
MES	20 mM	Na+	6.15	5.5 - 6.7
phosphate	20 mM	Na+	2.1/7.2	2.0 - 7.6
HEPES	20 mM	Na+	7.55	7.6 - 8.2

Column Chemistry

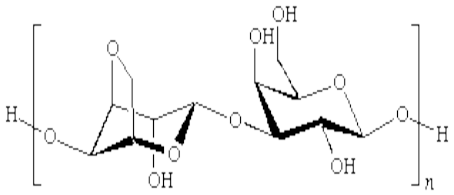
Stationary phase material

- Hydrophilic
- Physically strong

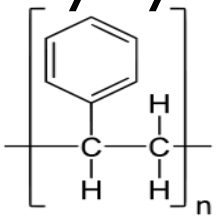
Cellulose



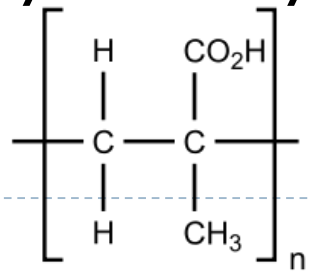
Agarose



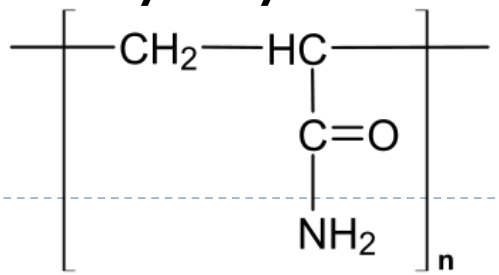
Polystyrene

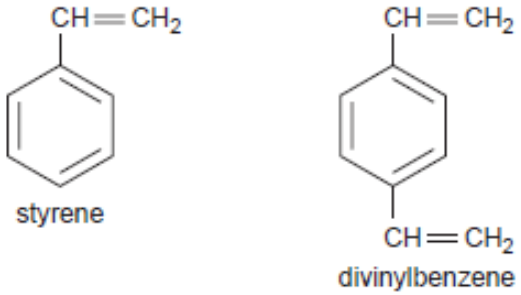


Polymethacrylate

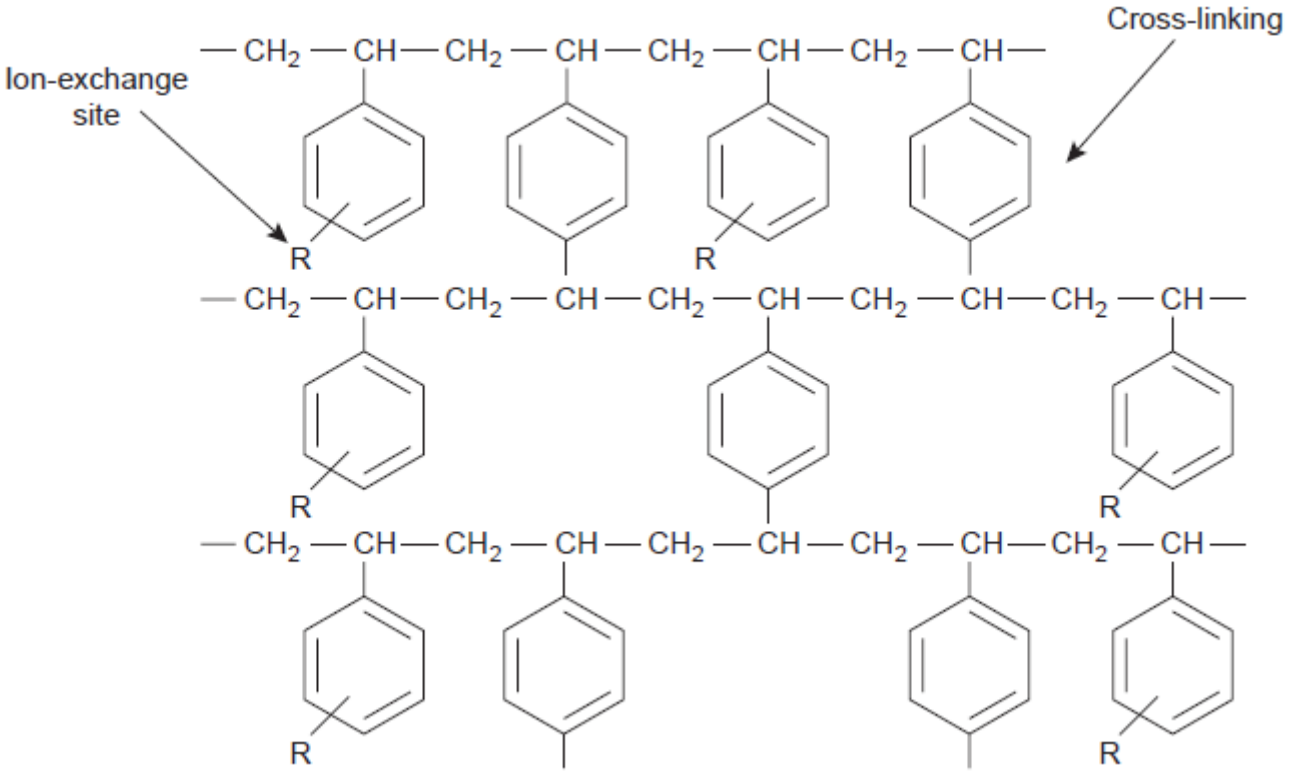


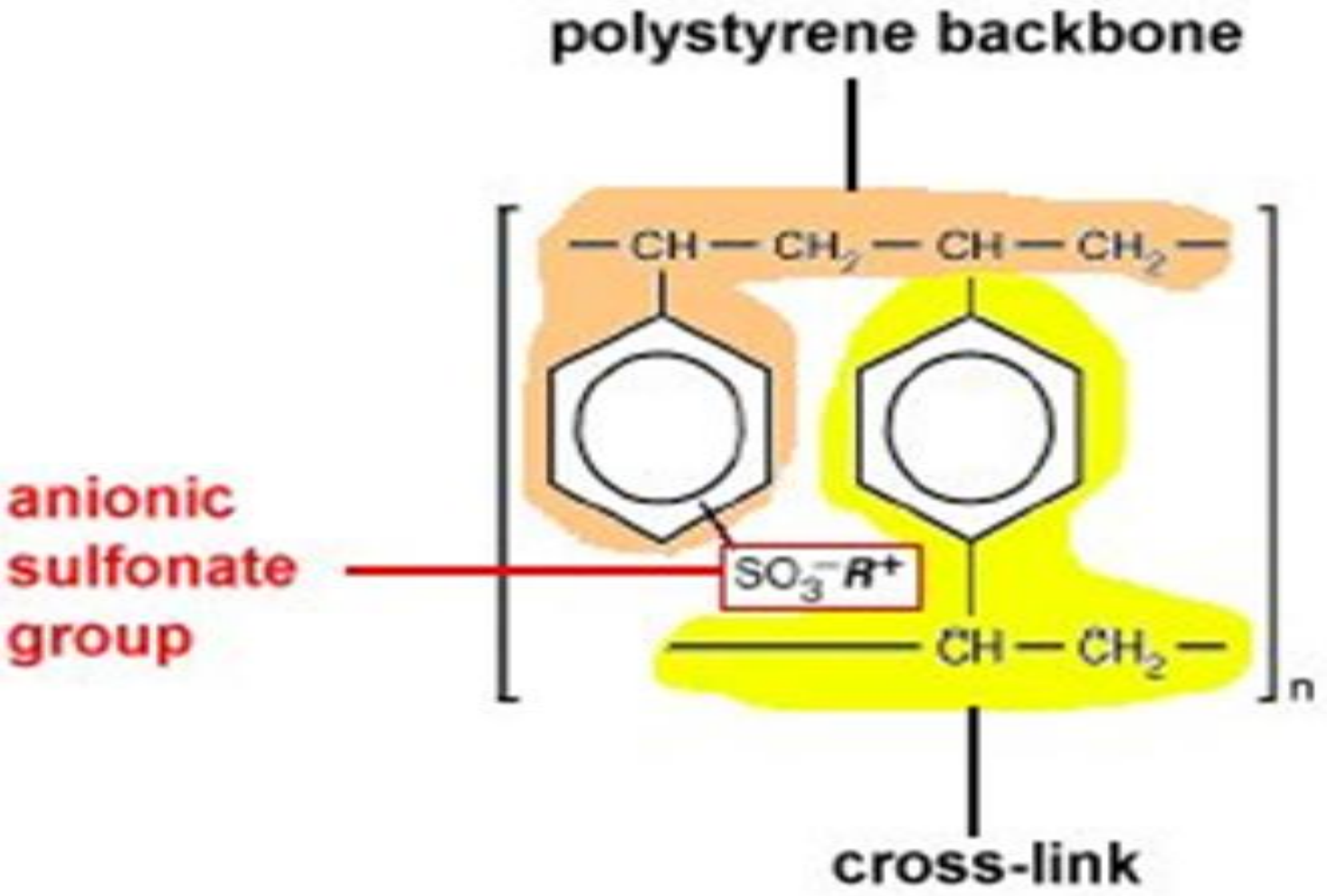
Polyacrylamide

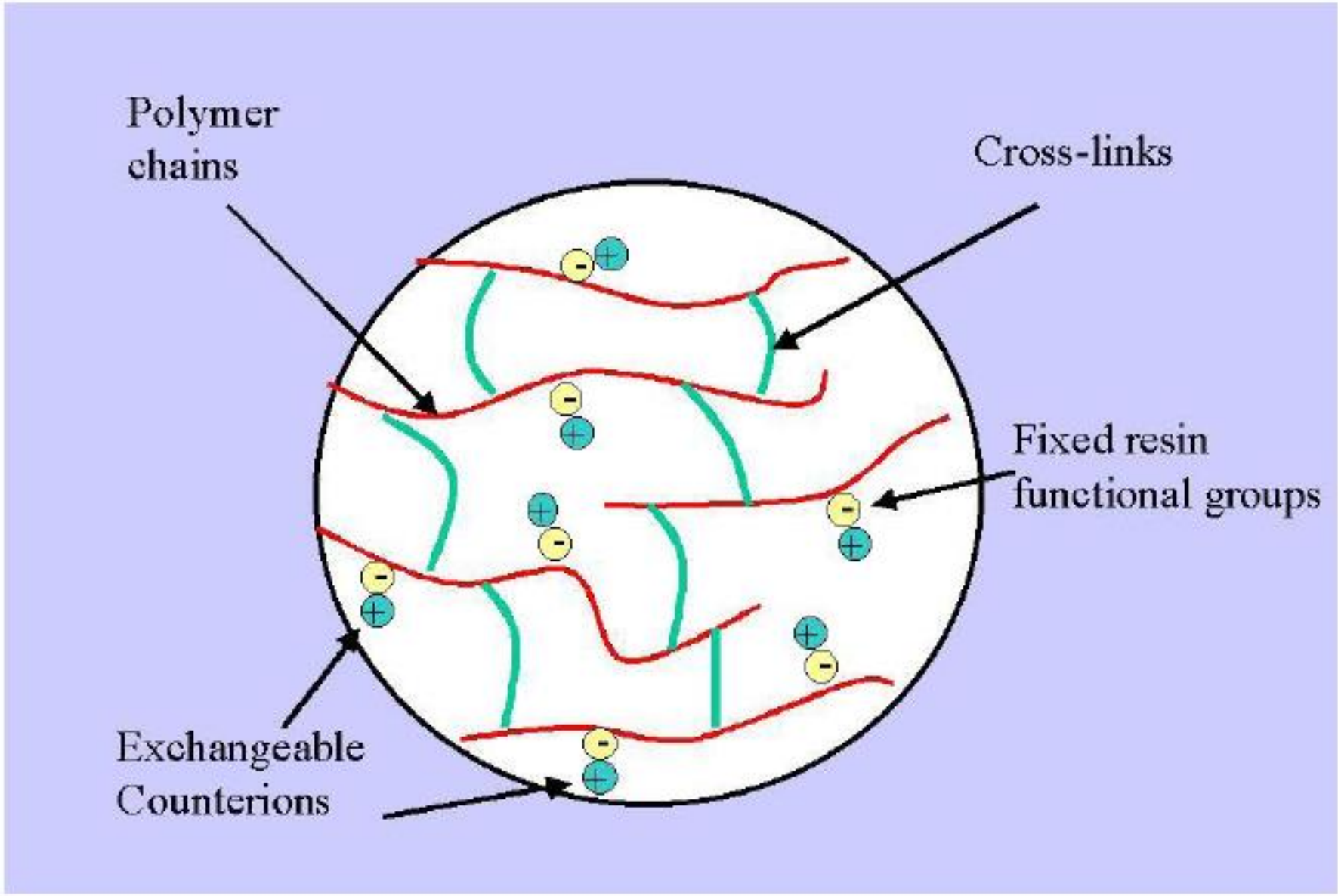




Structures of styrene, divinylbenzene, and a styrene-divinylbenzene co-polymer modified for use as an ion-exchange resin. The ion-exchange sites, indicated by R, are mostly in the *para* position and are not necessarily bound to all styrene units.



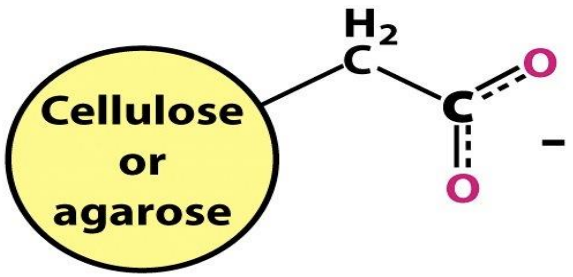

polystyrene backbone
anionic sulfonate group
SO3− R+
cross-link
n


Polymer chains
Cross-links
Fixed resin functional groups
Exchangeable Counterions

Ion exchangers – Functional groups

Cation exchanger

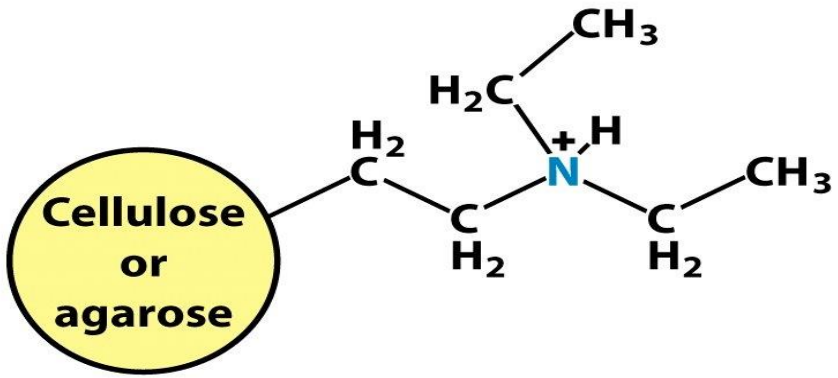
- Methylsulfonate(S-)
- Sulphopropyl (SP-)
- Carboxymethyl (CM-)



Carboxymethyl (CM) group (ionized form)

Anion exchanger

- Quaternary aminoethyl (QAE-)
- Diethylaminopropyl (DEPE-)
- Diethylaminoethyl (DEAE-)



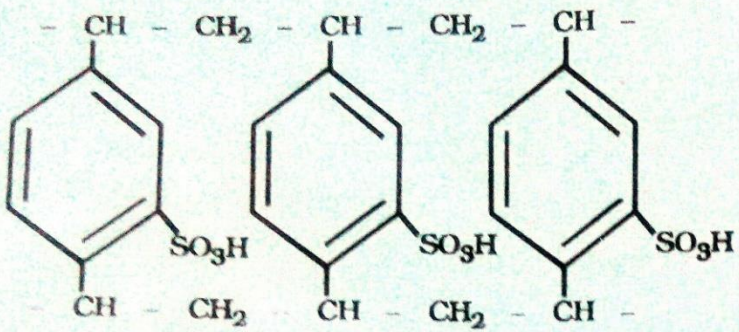
Diethylaminoethyl (DEAE) group (protonated form)

Ion Exchange - Bonded Functionalities

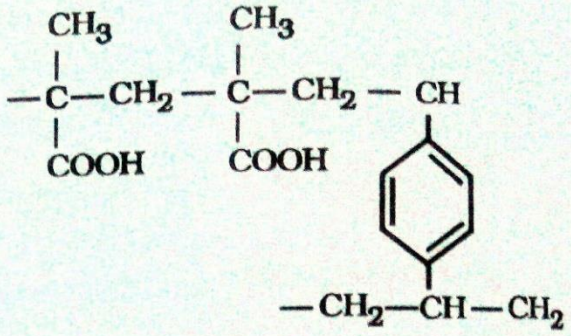
	Cation	Anion
WEAK	~~COO⁻ Na⁺ Carboxylic Acid	~~N⁺(R)(H)-R Cl⁻ Primary, Secondary or Tertiary Amine
STRONG	~~SO_3^- Na^+ Sulfonic Acid	~~N⁺(R)(R)-R Cl⁻ Quaternary Amine

Typical chemical functionalities used for commercial exchangers.

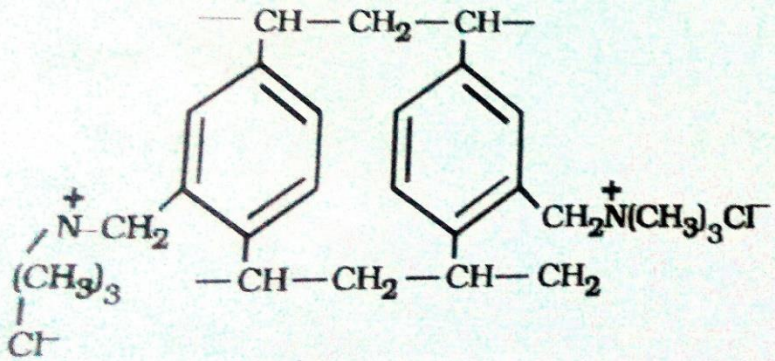
Chemical structure of resins



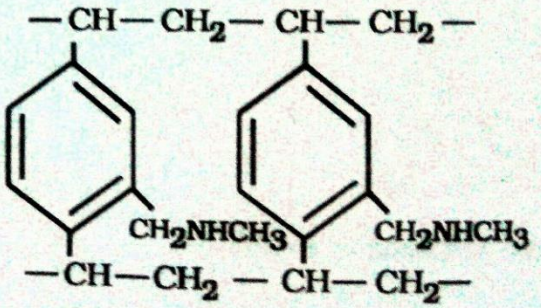
Strong acidic cation exchange
(Sulfonic acid group)



Weakly acidic cation exchange
(Methacrylate polymer)



Strong basic anion exchange
(Quaternary ammonium)

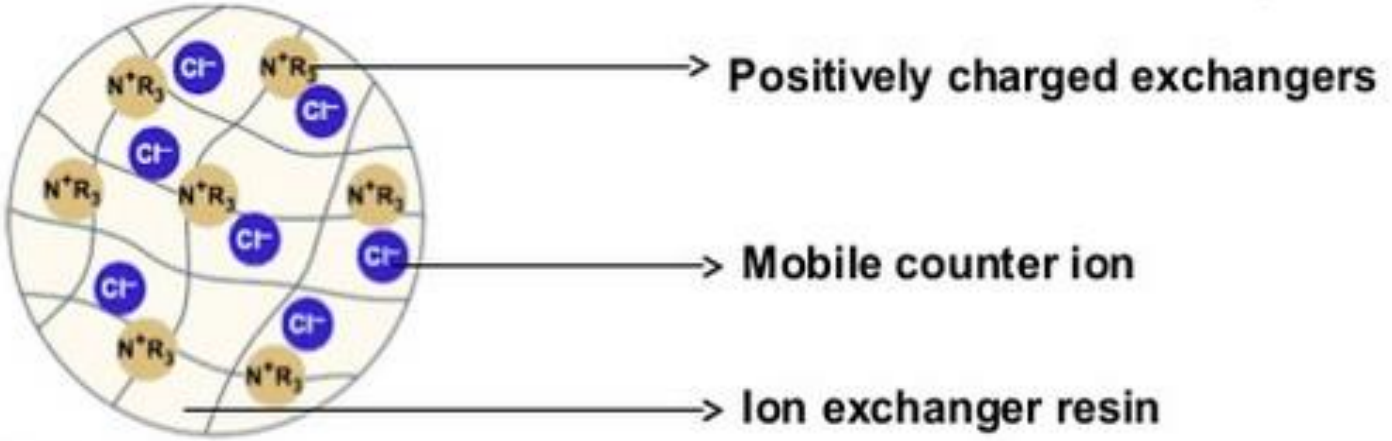


Weakly basic anion exchange
(Methyl Amino)

1. Anion Exchangers

- The anion exchangers have positively charged exchanger with negatively charged mobile counter ion available for exchange.
- If the basic functional groups are introduced, the resin becomes anion exchanger.
- Tertiary amines ⟶ Strong anion exchangers

  Secondary amines ⟶ Weak anion exchangers



Examples: Anion Exchangers

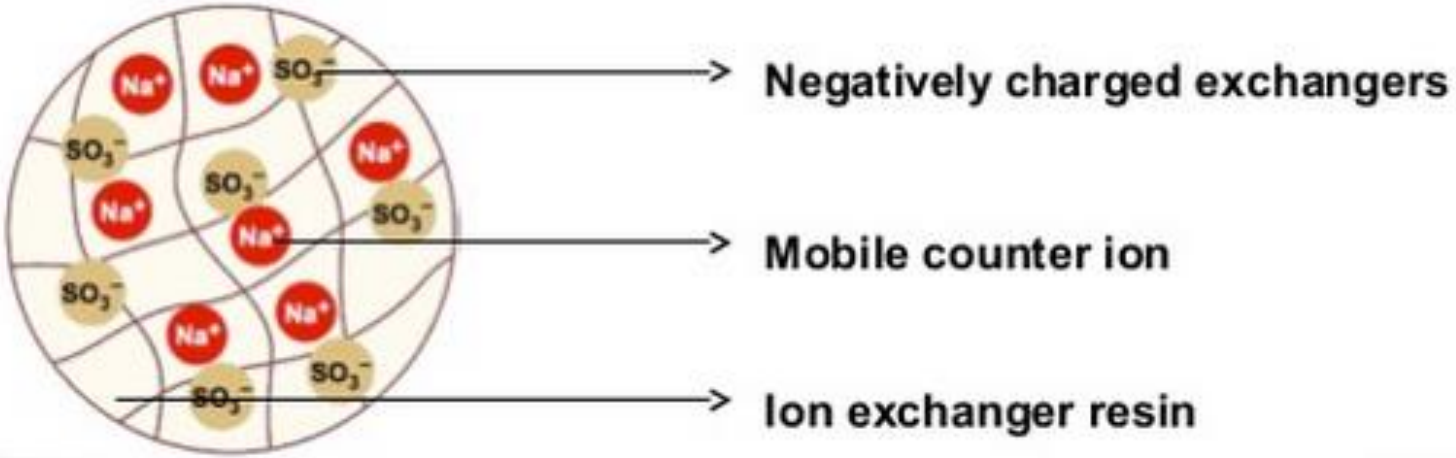
Type	Functional groups	Functional group name	Matrices
Weakly basic (anion exchanger)	$-CH_2CH_2N^+H_3$	Aminoethyl	Agarose
	$-CH_2CH_2N^+H$ $\vert$ $(CH_2CH_3)_2$	Diethylaminoethyl	Cellulose
			Dextran
			Polystyrene
			Polyacrylate
Strongly basic (anion exchanger)	$-CH_2N^+(CH_3)_3$	Trimethylaminomethyl	Cellulose
	$-CH_2CH_2N^+(CH_2CH_3)_3$	Triethylaminoethyl	Dextran
	$-CH_2N^+(CH_3)_2$ $\vert$ CH_2CH_2OH	Dimethyl-2-hydroxyethyl-aminomethyl	Polystyrene

Source: Wilson, K., & Walker, J. (Eds.). (2010). *Principles and techniques of biochemistry and molecular biology*. Cambridge University Press.

2. Cation Exchangers

- The cation exchangers have negatively charged exchanger with positively charged mobile counter ion available for exchange.
- If acidic functional group are introduced, then the resin becomes cation exchangers.
- Sulphonic acid ⟶ Strong cation exchangers

  Carboxylic acid ⟶ Weak cation exchangers



Examples: Cation Exchangers

Type	Functional groups	Functional group name	Matrices
Weakly acidic (cation exchanger)	$-COO^-$	Carboxy	Agarose
	$-CH_2COO^-$	Carboxymethyl	Cellulose
			Dextran
			Polyacrylate
Strongly acidic (cation exchanger)	$-SO_3^-$	Sulpho	Cellulose
	$-CH_2SO_3^-$	Sulphomethyl	Dextran
	$-CH_2CH_2CH_2SO_3^-$	Sulphopropyl	Polystyrene
			Polyacrylate

Source: Wilson, K., & Walker, J. (Eds.). (2010). *Principles and techniques of biochemistry and molecular biology.* Cambridge University Press.

CLASSIFICATION OF ION EXCHANGE RESINS:

There are 6 types,

1. Source of the resin
2. Chemical nature
3. Functional group
4. Structural type
5. Physical properties
6. Chemical structure

Ion exchange resin should have following requirements

- It must be chemically stable.
- It should be insoluble in common solvents.
- It should have a sufficient degree of cross linking.
- It must contain sufficient no. of ion exchange groups.

I

1. Source of the Resin

Divided in to two,

1. Natural
   - ✓ cation – Zeolytes, Clay, etc
   - ✓ Anion – Dolomite
2. Synthetic
   - ✓ In organic and Organic resins

2. Synthetic

- ✓ In organic and Organic resins

Organic resins are **polymeric resin matrix.**

The resin composed of –

- **Polystyrene** (sites for exchangeable functional groups)
- **Divinyl benzene**(Cross linking agent)-offers stability.

2 According to chemical nature:

4 types:

1. Strong cation exchange resin
2. weak cation exchange resin
3. Strong anion exchange resin
4. weak anion exchange resin

Table 12.5 Examples of Common Ion-Exchange Resins

Type	Functional Group	Examples
strong acid cation exchanger	sulfonic acid	$-SO_3^-$ $-CH_2CH_2SO_3^-$
weak acid cation exchanger	carboxylic acid	$-COO^-$ $-CH_2COO^-$
strong base anion exchanger	quaternary amine	$-CH_2N(CH_3)_3^+$ $-CH_2CH_2N(CH_2CH_3)_3^+$
weak base anion exchanger	amine	$-NH_3^+$ $-CH_2CH_2NH(CH_2CH_3)_2^+$

- **Strongly acidic cation exchanger** ---sulphonic acid groups attached to styrene and di vinyl benzene copolymer.
- **Weakly acidic cation exchanger**---carboxylic acid groups attached to acrylic and divinyl benzene co-polymer
- **Strongly basic anion exchanger**-----quaternary ammonium groups attached to styrene and divinyl benzene co-polymer N+
- **Weakly basic anion exchanger**-----poly alkyl amine groups attached to styrene and divinyl benzene co-polymer

FUNCTIONAL GROUPS PRESENT IN DIFFERENT ION EXCHANGE RESINS:

Strong cation exchange resin- SO_3H

Weak cation exchange resin- COOH,OH,SH,PO_3H_2

Strong anion exchange resin- $N+R_3$,NR_2

Weak anion exchange resin- NHR,NH_2

Class of Resin	Nature	pH Range	Applications
Cation- strong	Sulfonated polystyrene	1-14	• Fractionation of Cations • in organic separations • peptides, amino acids, B Vitamins.
Cation – weak	Carboxylic methacrylate	5 – 14	• Fractionation of Cations • Bio chemical separations • Organic bases, Anti biotics
Anion – Strong	Quaternary ammonium Polystyrene	0 – 12	• Fractionation of anions • Alkaloids, Vitamins • Fatty acids
Anion – weak	Polyamine Polystyrene (or) Phenol Formaldehyde	0 – 9	• Fractionation of anionic complexes • Anions of different valency • Vitamins, Amino acids.

- Sulphonate groups of strongly acidic resins remain ionized even in strongly acidic solutions , where as carboxyl groups are protonated near pH 4 and loose their cation exchange capacity

- Strongly basic quaternary ammonium groups remain cationic at all values of pH, where as weakly basic tertiary ammonium anion exchangers are deprotonated in moderately basic solutions and loose their ability to bind anions

CATIONS

RETENTION & ELUTION STRENGTH

MONO-VALENT

$Li^+ > H^+ > Na^+ > NH_4^+ > K^+ > Rb^+ > Cs^+ > Ag^+$

DI-VALENT

$Be^{++} > Mn^{++} > Mg^{++} > Zn^{++} > Co^{++} > M^{++} > Ca^{++} > Sr^{++}$

TRI-VALENT

$Al^{+++} > M^{+++} > Ce^{+++}$

transition metals

Transition metals

ANIONS

RETENTION & ELUTION STRENGTH

$$F^- , OH^- > OAc^- > H_2PO_4^- > HCO_3^- > Cl^- > NO_2^- >$$

$$HSO_3^- > CN^- > Br^- > NO_3^- > I^-$$

$$A^{---} > A^{--} > A^-$$

Effect of pH on ion exchange

- Varying pH is usually a preferred way to change selectivity in ion exchange separations
- An increase in the pH leads to greater sample ionization and retention in anion exchange HPLC
- Eg: antibiotics containing COOH groups
- Decrease in pH favours retention of bases by cation exchange HPLC
- Eg: local anesthetics containing NH_2 groups.
- Only the ionized form of acid or base will be retained significantly

Physical properties of resins

1. Cross linking and swelling
2. Particle size & Porosity
3. Regeneration

Physical properties of ion exchange resins

Cross linking & Swelling:

It affects swelling & strength & solubility

When resin swells, polymer chain spreads apart

Polar solvents → swelling

Non-polar solvents → contraction

Swelling also affected electrolyte conc.

- When more cross linking agent is present, they are more rigid, but swells less.
- When swelling is less, separation of ions of different sizes is difficult as they cannot pass through the pores present.
- When less cross linking agent is present, they are less rigid but swell more.
- when swelling is more, separation will not be efficient as exchange of functional groups does not take place due to wide pore.
- Hence an optimum quantity of cross linking agent should be added to the polymeric ion exchange resin for the separation to be effective.

Particle size & Porosity

↑surface area & ↓particle size will ↑rate of ion exchange

- available fine powder of uniform Particle size from 50-200 mesh

Regeneration

- Cation exchange resin are regenerated by treatment with acid, then washing with water
- Anion exchange resin are regenerated by treatment with NaOH, then washing with water until neutral

PRACTICAL REQUIREMENTS:

1. Column material and dimension
2. Type of ion exchange resin & the selection depend on following properties
    a.) type of ions
    b.) nature of ions
    c.) efficiency of resin
    d.) Particle size
    e.) Structural type
3. Packing of column
4. Mobile phase – acids, alkali, and buffers.
5. Development of chromatogram
6. Analysis of the elute
7. Regeneration of ion exchange resin

1. Column material and dimensions:

Columns used in the laboratories are made up of glass. In industries are made up of either high quality stainless steel or polymers which are resistant to strong acids and alkalis.

The column dimensions are also important and a length: diameter ratio of 20: 1to 100: 1 for higher efficiency can be used.

2. Type of ion exchange resin:

- Type of ions ⟹ cations (or) anions
- nature of ions ⟹ Strong (or) weak
- Efficiency of the resin ⟹ It is measured by ion exchange capacity

3. PACKING OF THE COLUMN:

- Wet packing method is used.
- Resin + Mobile phase – packing in the column uniformly.

4. MOBILE PHASE:

- ✓ Organic solvents are less useful and they are not used at all.
- ✓ Only strengths of acids, alkalies and buffers are used as eluting solvents.

  E.g. 0.1N HCL, 1N NaOH, Phosphate buffer, Acetate buffer, Borate buffer, phthalate buffer, etc

5. DEVELOPMENT OF THE CHROMATOGRAM AND ELUTION

After introduction of the sample, development of the chromatogram is done by using different mobile phases. As, mentioned earlier, organic solvents are less useful and only acids, alkalis and buffers of different pH are used.

There are two elution technique:

1. Isocratic elution
2. Gradient elution

Isocratic elution:

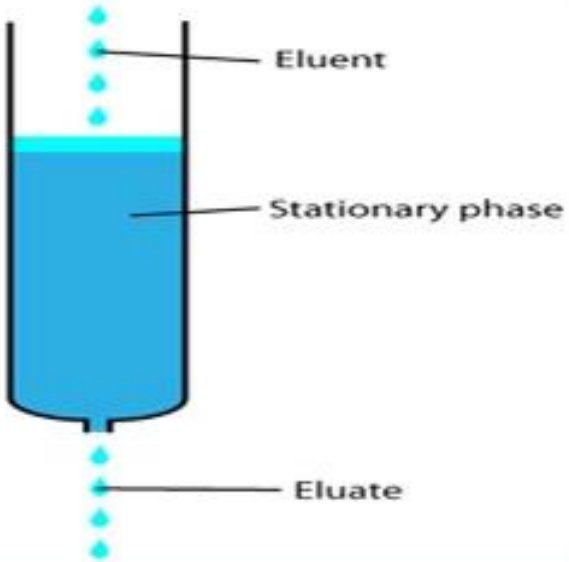
Same solvent composition is used. i.e., same solvent of acid or alkali or buffer.

Gradient elution:

In gradient elution technique, initially less acidic or basic character is used followed by increasing the acidity or basicity of the mobile phase.

this elution technique is usually used for complex mixtures. The different fractions of the eluent is collected volume wise or time wise and analysed.

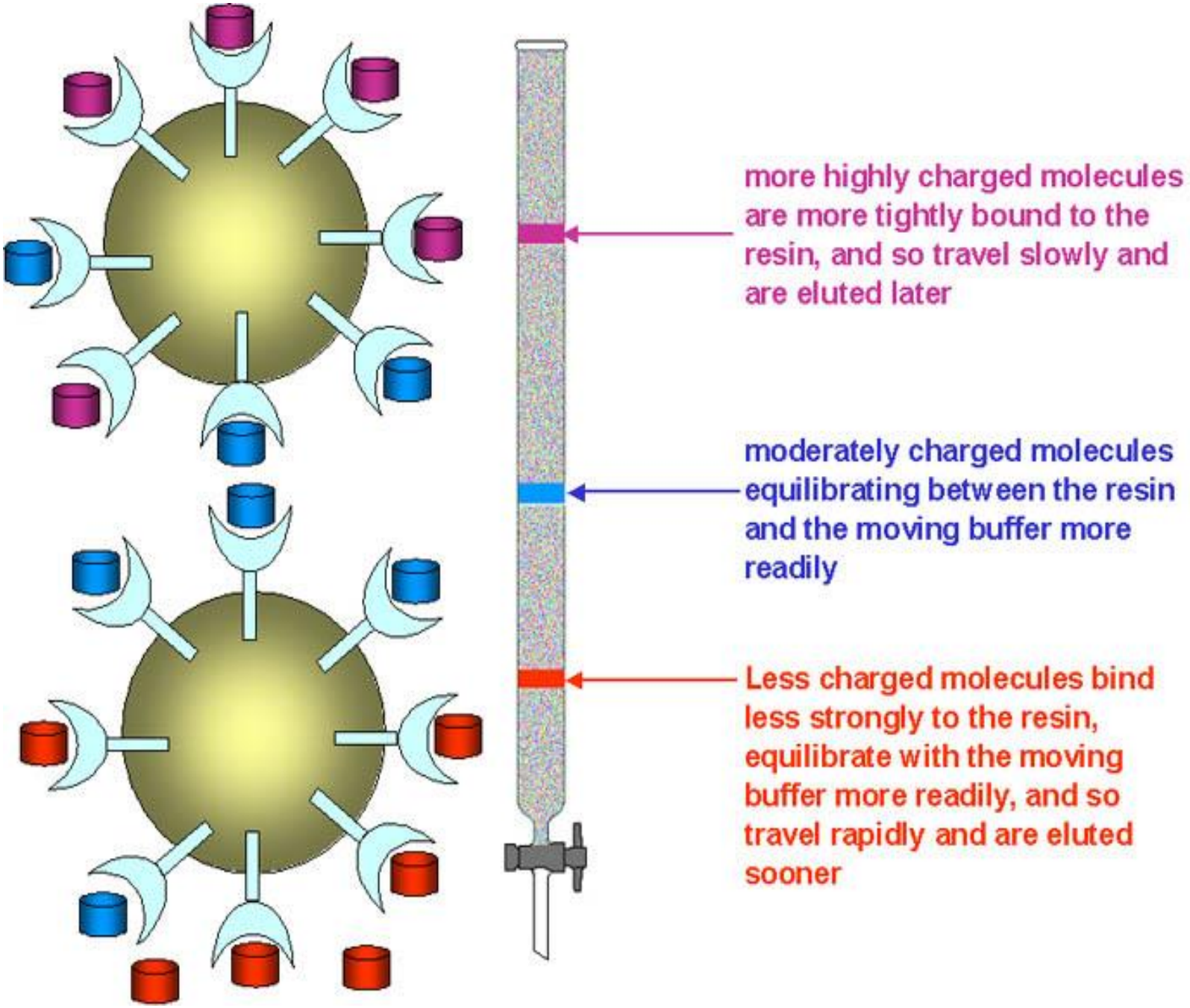
ELUTION



- The process of extracting a substance that is adsorbed to another by washing it with a solvent.

Eluent :The substance used as a solvent in elution

Eluate :The solution of the solvent and the substance that was adsorbed to another is


more highly charged molecules are more tightly bound to the resin, and so travel slowly and are eluted later
moderately charged molecules equilibrating between the resin and the moving buffer more readily
Less charged molecules bind less strongly to the resin, equilibrate with the moving buffer more readily, and so travel rapidly and are eluted sooner

ANALYTES OF THE ELUTE:

several methods of analysis can be used which depends up on the nature & the quantity of the sample.

1. Spectrophotometric method
2. Polarographic method
3. Conductometric method
4. Amperometric method
5. Flame photometric method
6. Radio chemical methods

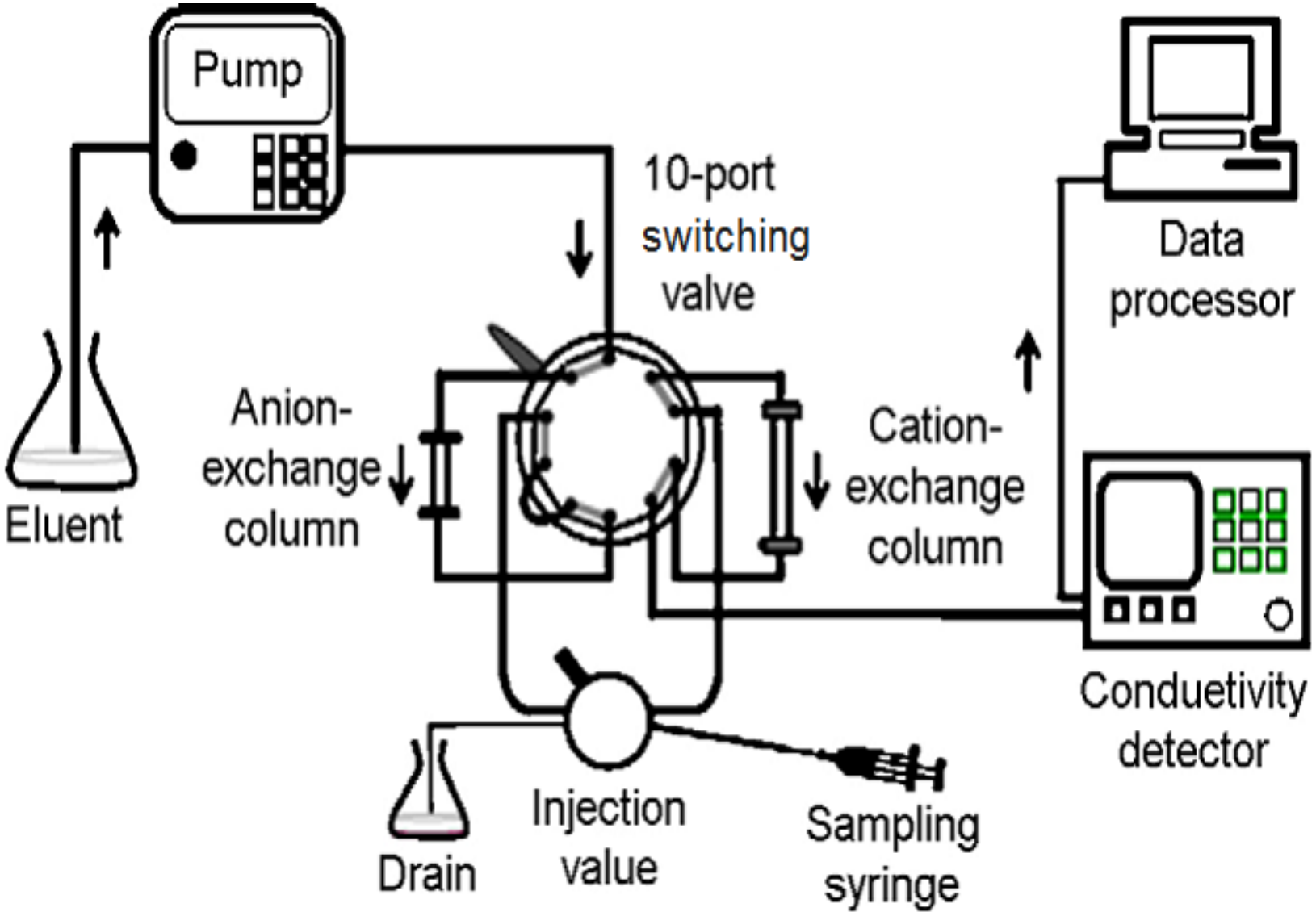
- Geiger muller counter
- ionization chamber method.

after analyzing similar fraction are mixed in order to get pure ions or compound of each type.

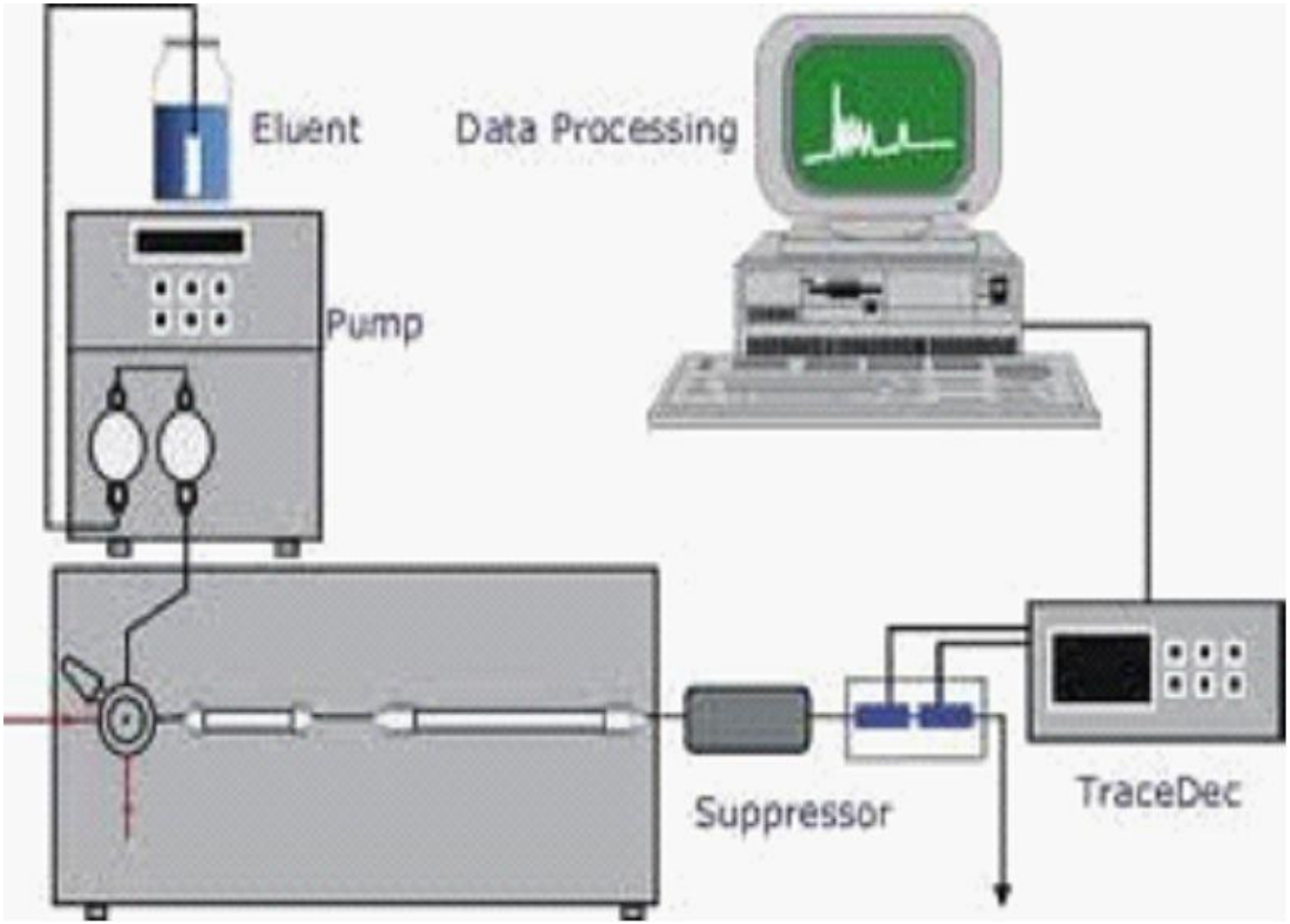
REGENERATION OF THE ION EXCHANGE RESIN:

- Regeneration makes the used ion exchange resin to be as efficient as a virgin resin.
- Regeneration refers to the replacement of the exchangable cations or anions present in the original resin.
- Hence regeneration of the cation exchange resin is done by the charging the column with strong acid like HCl acid.
- Regeneration of anion exchange resin is done by using strong alkali like sodium hydroxide or potassium hydroxide.

INSTRUMENTATION

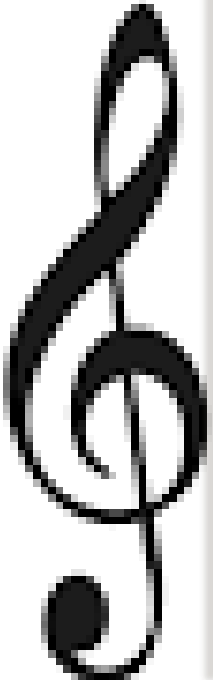


INSTRUMENTATION



INSTRUMENTATION

- Pump
- Injector
- Column
- Detector
- Recorder



1. PUMP

It provide a continuous constant flow of the eluent through the IC injector, column and detector.differnt types of pumps is used.
They are

constant flow pumps, reciprocating piston pumps, dual piston pumps.

2. INJECTOR

it should provide the possibility of injecting the liquid sample with in the range of 0.1 to 100ml of volume with high reproducibility and high pressure up to 4000 psi.

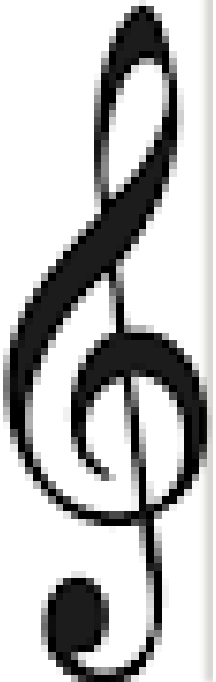
the widely used sampling device for modern LC is the microsampling injector valve.

3. COLUMNS

ion exchange columns vary widely in size, packing material and material of construction.

Depending on its ultimate use and area of application, the column material may be stainless steel, titanium, glass.

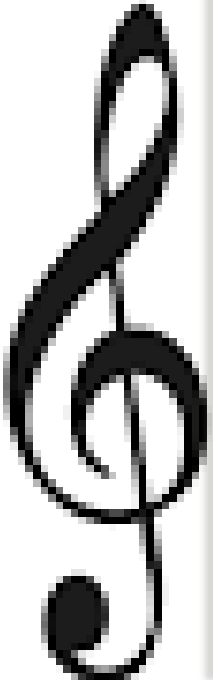
the column can vary in diameter from about 2mm to 5 cm and in length from 3 to 50 cm depending on whether it is to be used for normal analytical purposer.



4. DETECTOR:

- Electrical conductivity detector
- Amperometric detector

5. RECORDER



FACTORS AFFECTING ION EXCHANGE SEPARATIONS:

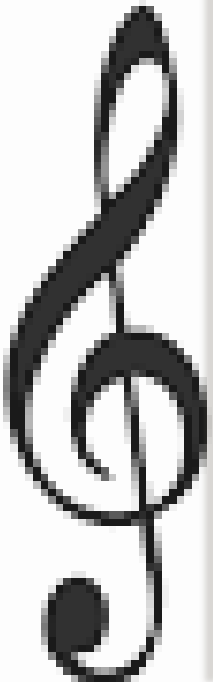
1. Nature and properties of ion exchange resins.
2. Nature of exchanging ions.

1. Nature and properties of ion exchange resins:

Cross linking and swelling is important factor which depends on the proportion of cross linking agent is and polystrene.

when more cross linking agent is present, they are more rigid, but swells less.

When swelling is less, separation of different sizes is difficult as they cannot pass through the pores present and it becomes selective to ions of different sizes.



When less cross linking agent is present, they are less rigid, but swell more.

When swelling is more, separation will not be efficient as exchange of functional groups does not take place due to wide pore.

Hence an optimum quantity of cross linking agent should be added to the polymeric ion exchange resins for the separation to be effective.

2. Nature of exchanging ions:

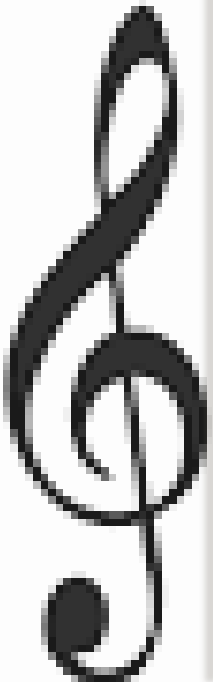
a. Valency of ions

b. Size of ions

c. Polarizability

d. Concentration of solution

e. Con. & charge of ions



VALENCY OF IONS:

At low concentrations and at ordinary temperatures, extent of exchange increases with increase in valency.

$Na^+ < Ca^{2+} < Al^{3+} < Th^{4+}$

SIZE OF IONS:

For similar charged ions, exchange increases with decrease in the size of hydrated ion.

$Li^+ < H^+ < Na^+ < NH_4^+ < K^+ < Rb^+ < Cs$

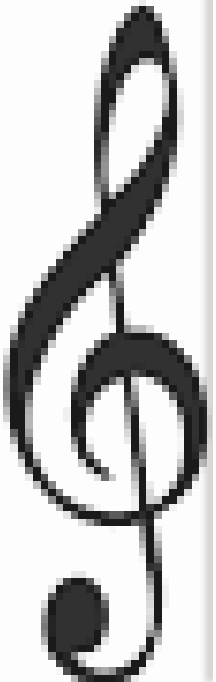
POLARIZABILITY:

Exchange is preferred for greater polarizable ion

e.g. $I^- < Br^- < Cl^- < F^-$

CONCENTRATION OF SOLUTION:

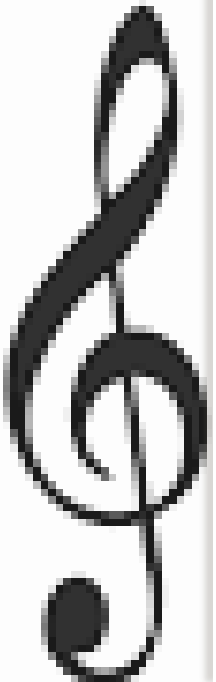
In dilution solution, polyvalent anions are generally adsorbed preferentlly.



CONCENTRATION AND CHARGE OF IONS:

- If resin has higher +ve charge and solution has lower +ve charge, exchange is favoured at higher concentration.

- If the resin has lower +ve charge and solution has high +ve charge, then exchange is favoured at low concentration.



ADVANTAGES:

- ✓ It is a non-denaturing technique. It can be used at all stages and scales of purification
- ✓ An IEX separation can be controlled by changing pH, salt concentration and/or the ion exchange media
- ✓ It offers high selectivity; it can resolve molecules with small differences in charge.

DISADVANTAGES

- costly equipment and more expensive chemicals
- Nature and properties of ion exchange resins

Separating proteins

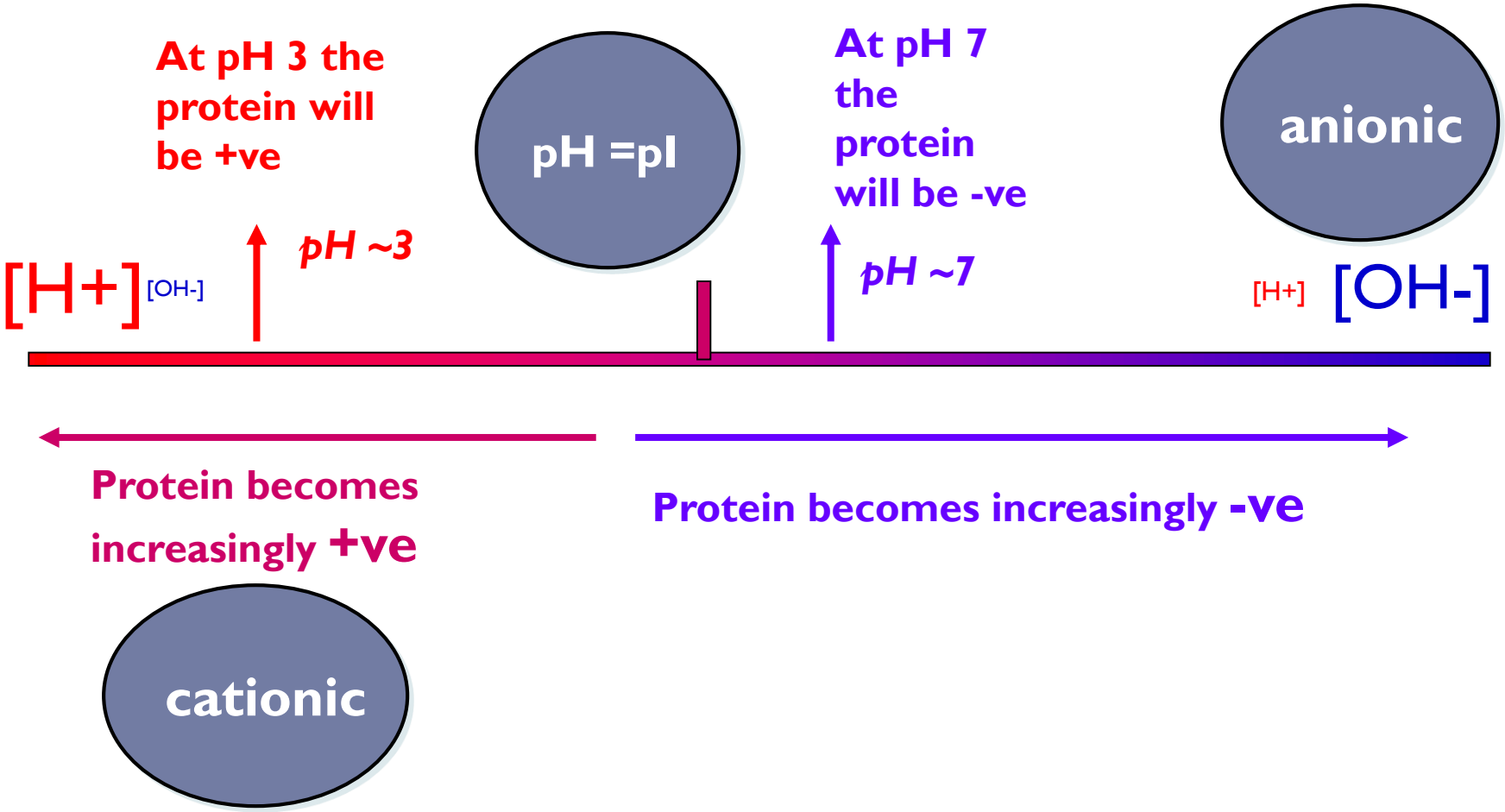
- Proteins have numerous functional groups that can have both positive and negative charges.
- Ion exchange chromatography separates proteins according to their net charge, which is dependent on the composition of the mobile phase.
- By adjusting the pH or the ionic concentration of the mobile phase, various protein molecules can be separated.

Mechanism of protein separation

- pH based separation:

- Salt based separation:

Proteins are charged molecules. At specific pH, it can exist in **anionic** (-), **cationic** (+) or **zwitterion** (no net charge) stage.



Factors to be considered during protein separation:

- **The pH of buffers**
  should be
  one unit below pI for cation exchangers and
  one unit above pI anion exchangers

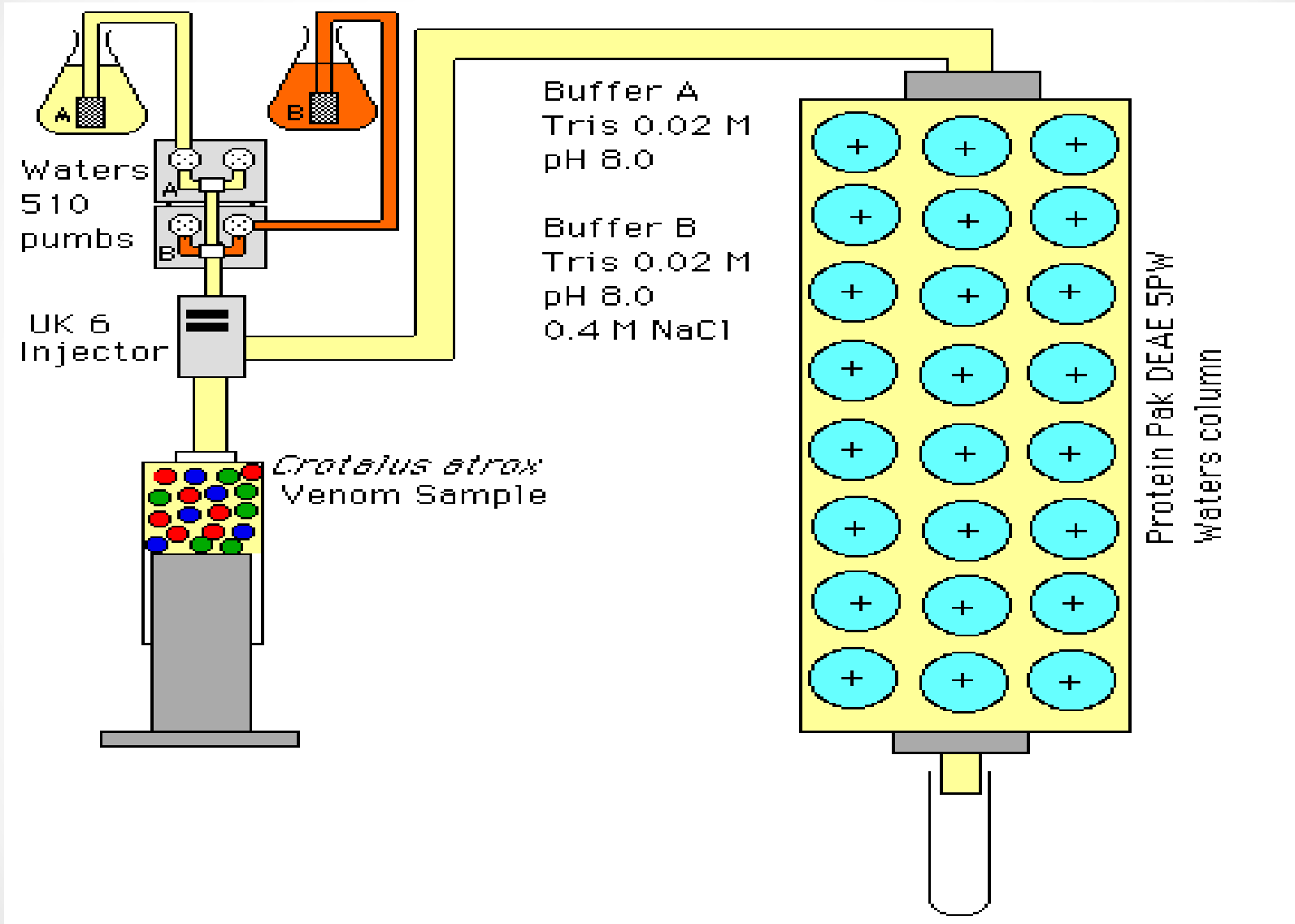
- **Stability of proteins**
  stable below pI value, use **cation-exchanger**
  stable above pI value, use **anion-exchanger**

- **Molecular size of proteins**
  <10,000 mw, use matrix of small pore size
  10,000-100,000 mw, use Sepharose equivalent grade

Buffers Used in Ion Exchange Chromatography

- Use Cationic Buffers in Anion Exchanger :
  Eg: Alkyl Amines, Tris, Amino Ethyl Alcohol

- Use Anionic Buffers in Cation Exchanger:
  Eg: Phosphate, Acetate, Citrate, Barbiturate


A
B
Waters
510
pumbs
A
B
UK 6
Injector
Buffer A
Tris 0.02 M
pH 8.0
Buffer B
Tris 0.02 M
pH 8.0
0.4 M NaCl
Crotalus atrox
Venom Sample
Protein Pak DEAE 5PW
Waters column

Journal of Chromatography A

Volume 16, 1964, Pages 111–125

Ion-exchange chromatography of nucleotides on poly-(ethyleneimine)-cellulose thin layers ☆

K. Randerath, E. Randerath

Abstract

A great number of naturally occurring mononucleotides can be separated and identified by poly(ethyleneimine)-cellulose thin-layer chromatography. R_F data for 33 compounds are given, and the factors are discussed which influence the mobility under different elution conditions. The method is compared with other present techniques for separating nucleotides.

Separation of Amino Acids:

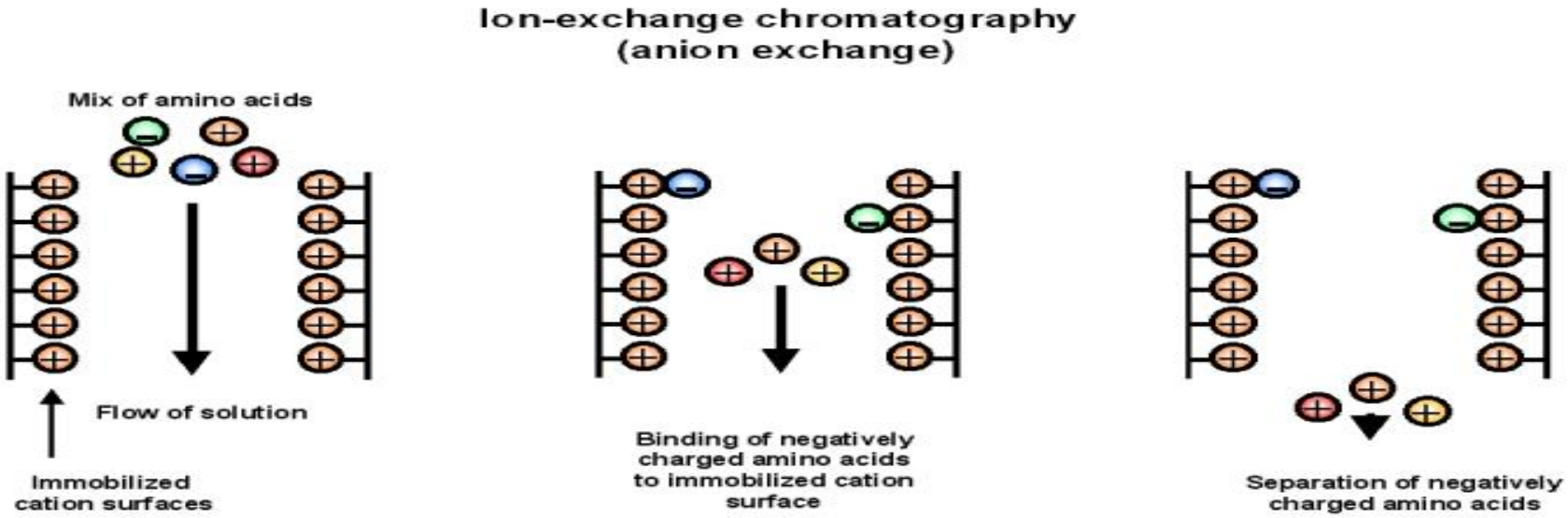
Journal of Neurochemistry JI

THE FREE AMINO ACIDS OF HUMAN SPINAL FLUID DETERMINED BY ION EXCHANGE CHROMATOGRAPHY

Johanne C. Dickinson and Paul B. Hamilton

Issue
JNC Journal of Neurochemistry

The free amino acids in human CSF from eighteen subjects have been determined. Amino acids always found in readily detectable amounts were: taurine, threonine, serine, glutamine, glutamic acid, citrulline, glycine, alanine, α-NH_2-*n*- butyric acid, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, ethanolamine, ornithine, lysine, histidine and arginine.



Analysis of Water-Soluble Vitamins by High-Speed Ion-Exchange Chromatography

R.C. Williams
D.R. Baker
J.A. Schmit

Abstract

High-speed liquid chromatography with superficially porous ion-exchange column packings has been used to separate and quantitatively analyze the water soluble vitamins nicotinic acid, thiamine, riboflavin, ascorbic acid, folic acid, pyridoxine, pyridoxal and pyridoxamine. Chromatograms and chromatographic conditions for these as well as the phosphoric acid esters of riboflavin and pyridoxamine are shown. The reproducibility of retention time and peak area has been demonstrated to be better than 1.5% while the lower limits of sensitivity for the test vitamin compounds were better than 50 nanograms. Advantages of the technique include fast analysis times and minimum of sample clean up. Applications of the methods to vitamin analysis of food and pharmaceutical products are discussed.

0ther applications

- Ion exchange chromatography is used to convert one salt to other.

  Eg; we can prepare tetra propyl ammonium hydroxide from a tetra propyl salt of some other anion.

- For the measurement of drugs and their metabolites in serum and urine, for residue analysis in food raw materials.

- For the measurement of additives such as vitamins and preservatives in foods and beverages.

APPLICATIONS OF IE CHROMATOGRAPHY

- Separation of similar ions
  - A mixture of sodium, hydrogen and potassium can be separated using cation exchanger resin.
  - A mixture of Chloride, bromide, and iodide can be separated using basic anion exchange resin.
- METHOD: Mixture of chloride, bromide & iodide is passed through basic anion exchanger using 0.5M sodium nitrate as eluant. Chloride will first elute. Raise the conc of Sodium Nitrate, Bromide will elute, raise the conc of Sodium Nitrate further, iodide ion will elute.

- Removal of interfering radicals: Phosphate ion is the interfering with the calcium & barium ions. Phosphate is removed using sulphonic acid cation exchanger.

  Calcium & barium ions exchanged with H+ ions while phosphate ion pass through the column.
- Softening of hard water:

  Hardness of water due to cal, mg and other divalent ions. This water is passed through cation exchanger charged with the sodium ions. Ca & Mg ions retained in the column while sodium is exchanged.

- Complete demineralization of water:

  Removal of both cations & anions.

  Step A) Hard water is first passed through an acidic cation exchanger- Ca, Mg & Na are exchanged by H^+ ions.

  Step B) This water is then passed through a basic anion exchanger – Cl, NO_2, SO_4^- are exchanged by OH^- ions of the exchanger.

- Separation of Lanthanides- La, Y, Ce, Rb etc
- Separation of sugars:

  sugars-borate complexes. This complex is separated on Dewax. In this disaccharides separated from mono.

- Separation of Amino Acids: protein after hydrolysis is introduced to a short column on special polystyrene sulphonic acid resin at pH 2 and eluted with 0.35N sodium citrate buffer of pH 5.25. acidic & neutral AAs first leave the column as unseparated then others.
- Other applications
  - For the measurement of various active ingredients in medicinal formulations,
  - For the measurement of drugs and their metabolites in serum and urine, for residue analysis in food raw materials,
  - For the measurement of additives such as vitamins and preservatives in foods and beverages.

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- Principles of instrumental analysis , skoog , latest edition, pno. 641-647


Thank you!