

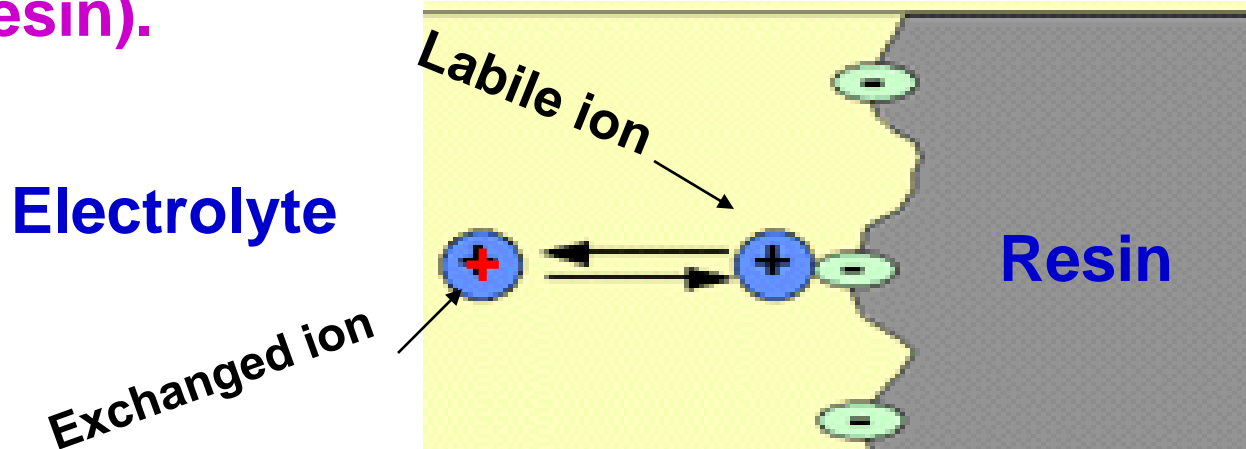
# **Chromatographic Methods of Analysis**

## **Section - 4 : Ion Exchange Chrom.**

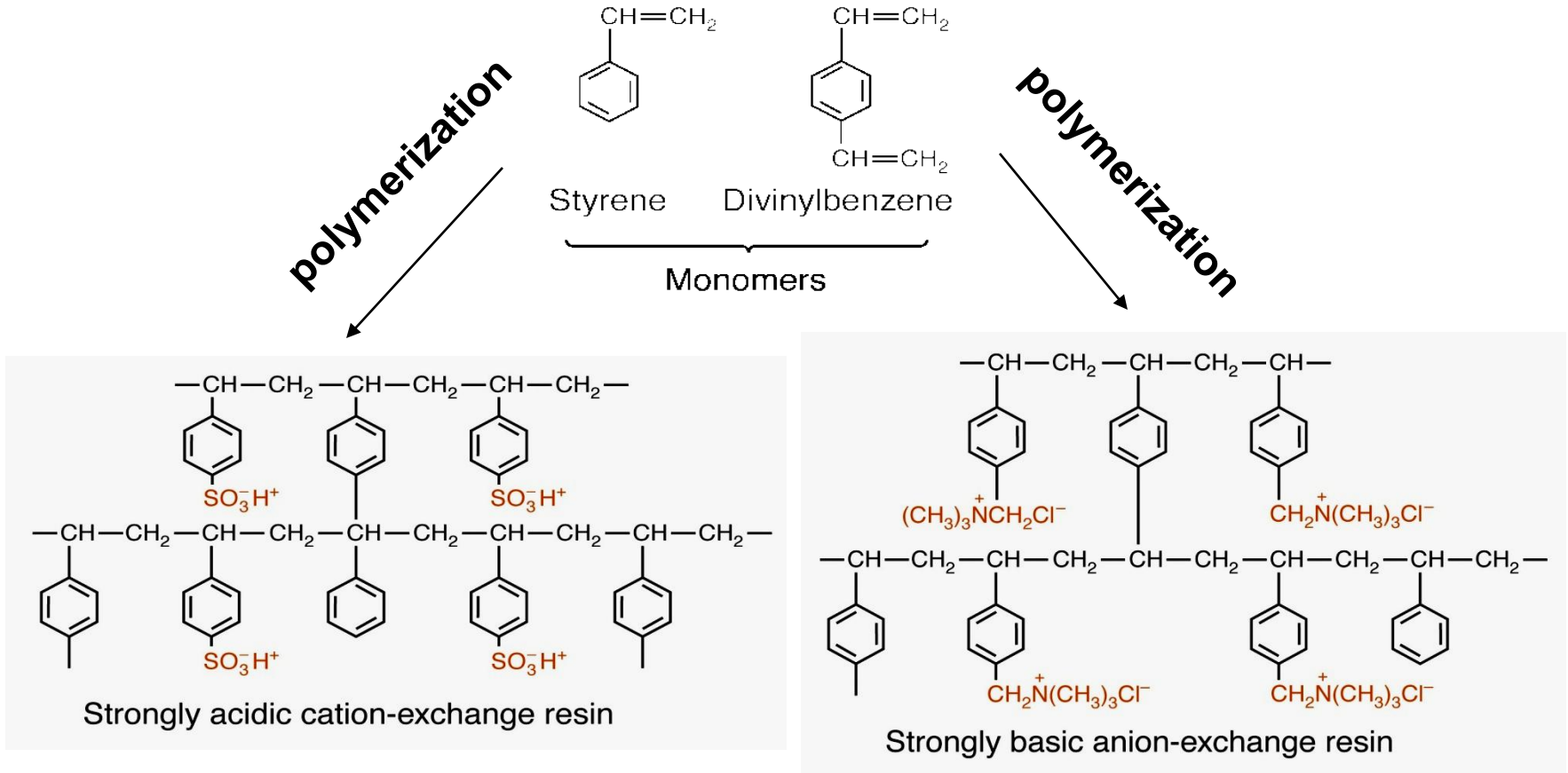
Prof. Tarek A. Fayed

# Ion Exchange Chromatography (IEC)

- In this type of chromatography, the solid stationary phase (**organic resin**) is containing covalently bonded anions or cations onto it. Solute ions of the opposite charge in the mobile liquid phase (**electrolyte**) are attracted to the resin by electrostatic forces, and then exchanged
- Thus solutes are separated due to the differences in the type and magnitude of their ionic charges. **The separation is due to exchange of ions in the sample with the labile ions onto the stationary phase (exchange resin).**



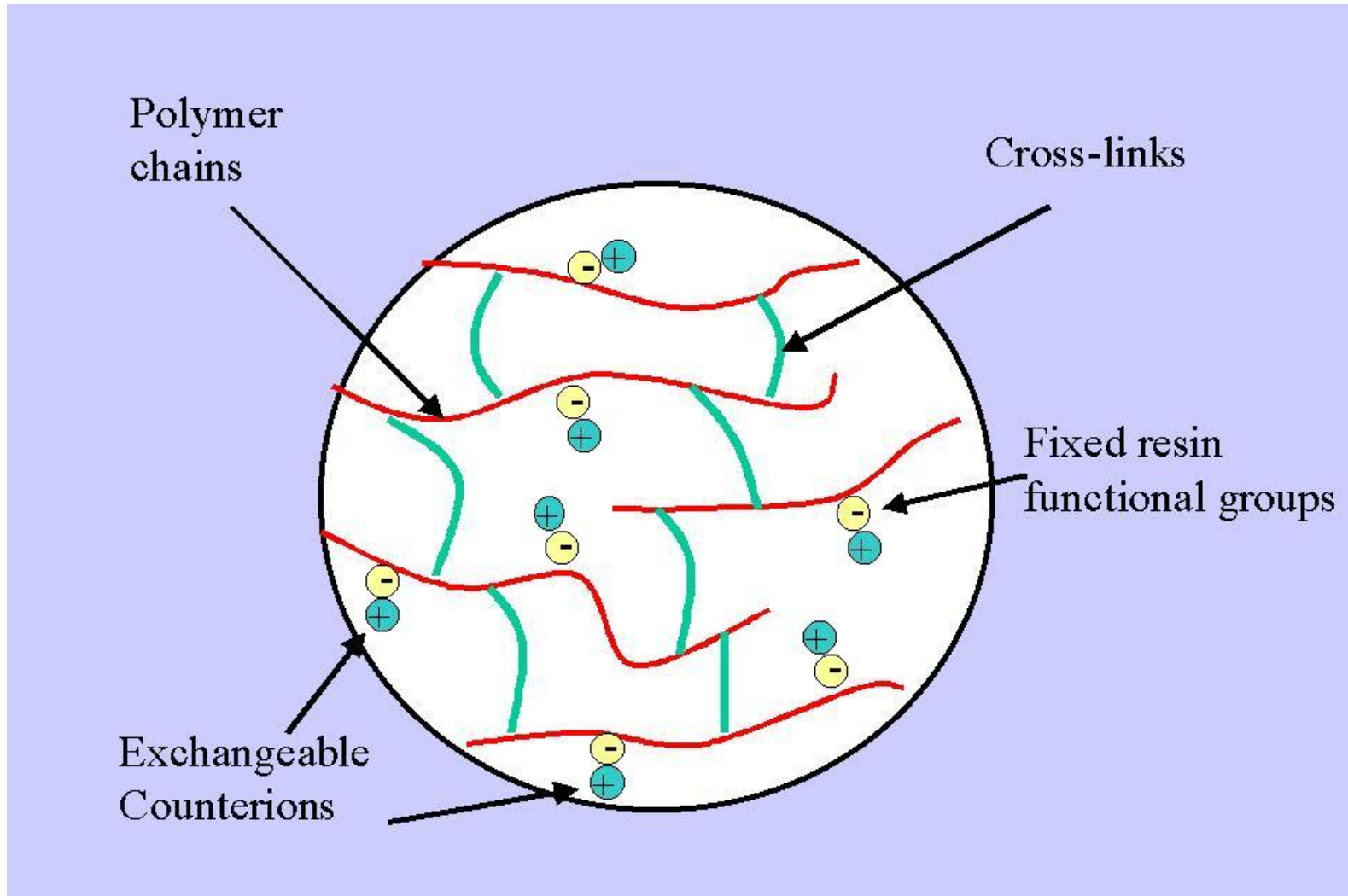
# Structure of Ion-Exchangers



Cation exchangers:  $-\text{SO}_3^-$ ,  $-\text{CO}_2^-$  the labile ion is  $\text{H}^+$  or  $\text{Na}^+$

Anion exchangers:  $-\text{NR}_3^+$  the labile ion is  $\text{OH}^-$  or  $\text{Cl}^-$

# Shape of Ion Exchangers

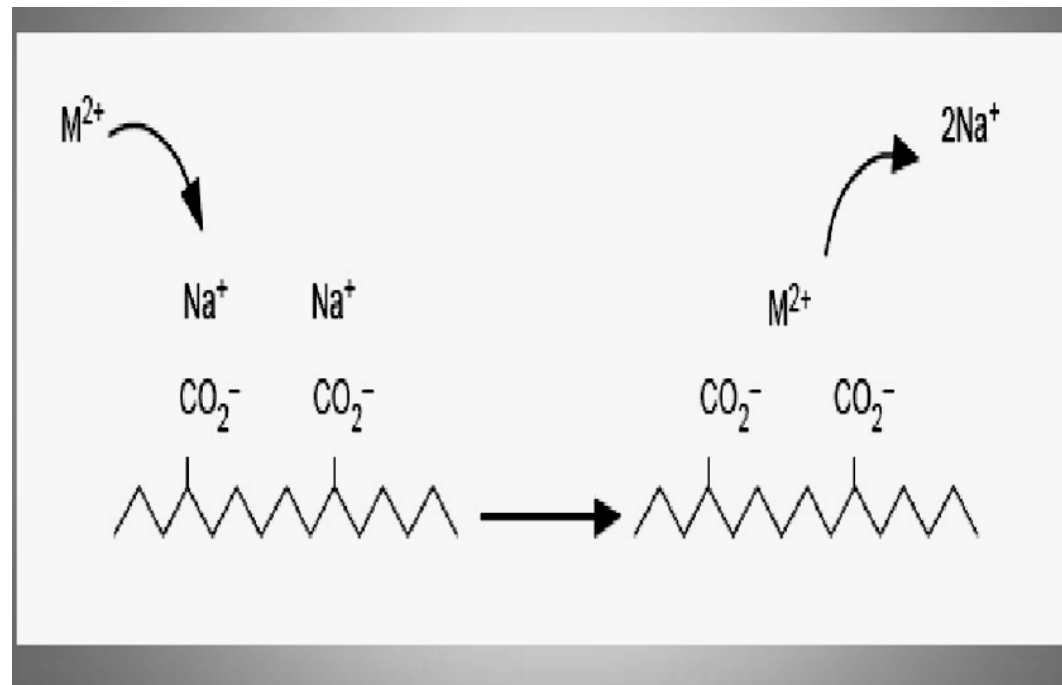


Separation in ion exchange chromatography depends upon the reversible adsorption of charged solute molecules to the immobilized ion exchange groups of opposite charge onto the resin beads.

## Properties of Resin

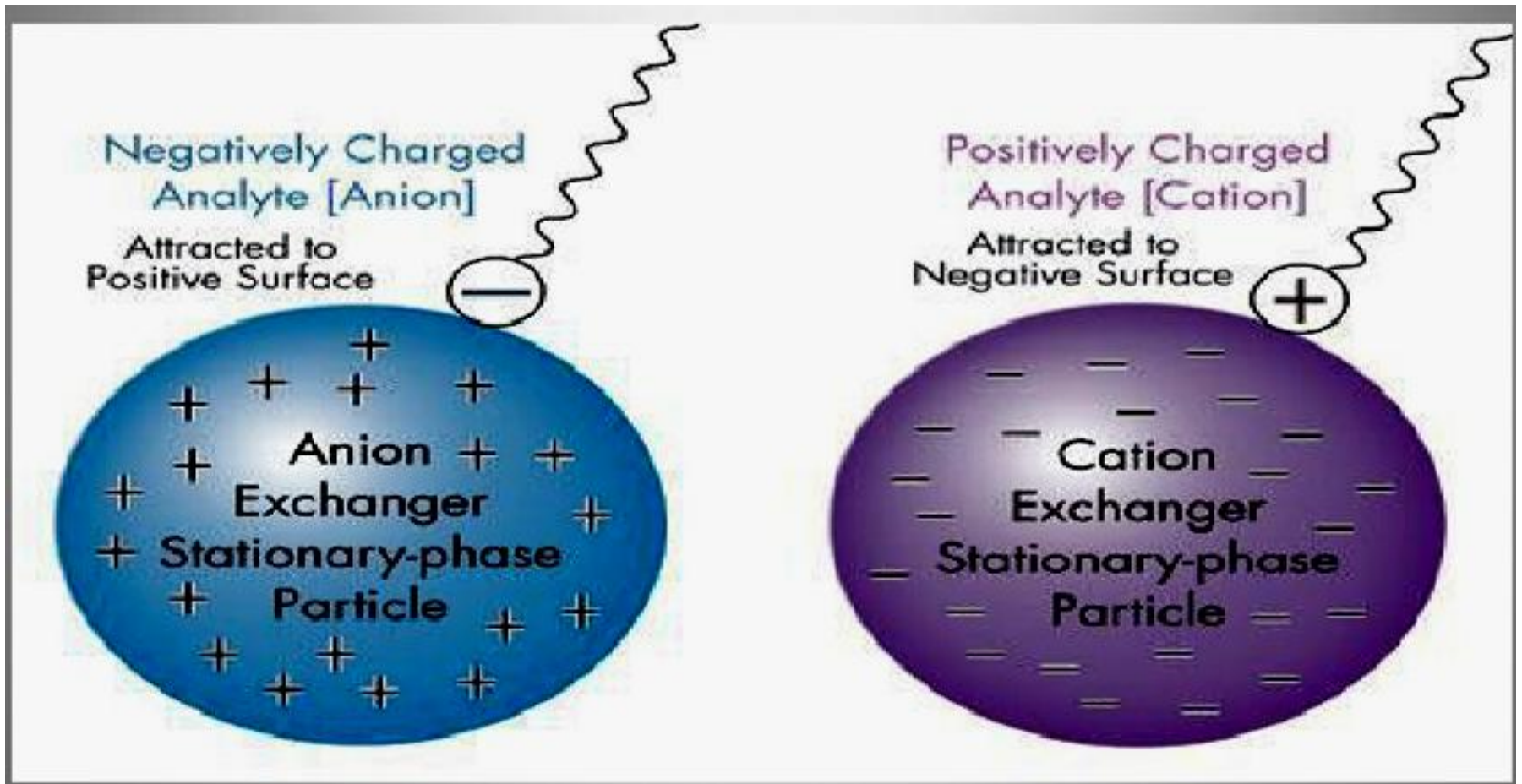
Any ion exchanger should be;

- Chemically and mechanically stable.
- Chemically inert.
- Have the same degree of cross-linking and mesh size.
- Homogenous (one type; either cationic or anionic)



**Anion exchangers** are positively charged exchangers and have negatively charged counter labile-ions (anions) available for exchange.

**Cation exchangers** are negatively charged exchangers and have positively charged counter labile-ions (cations) available for exchange.

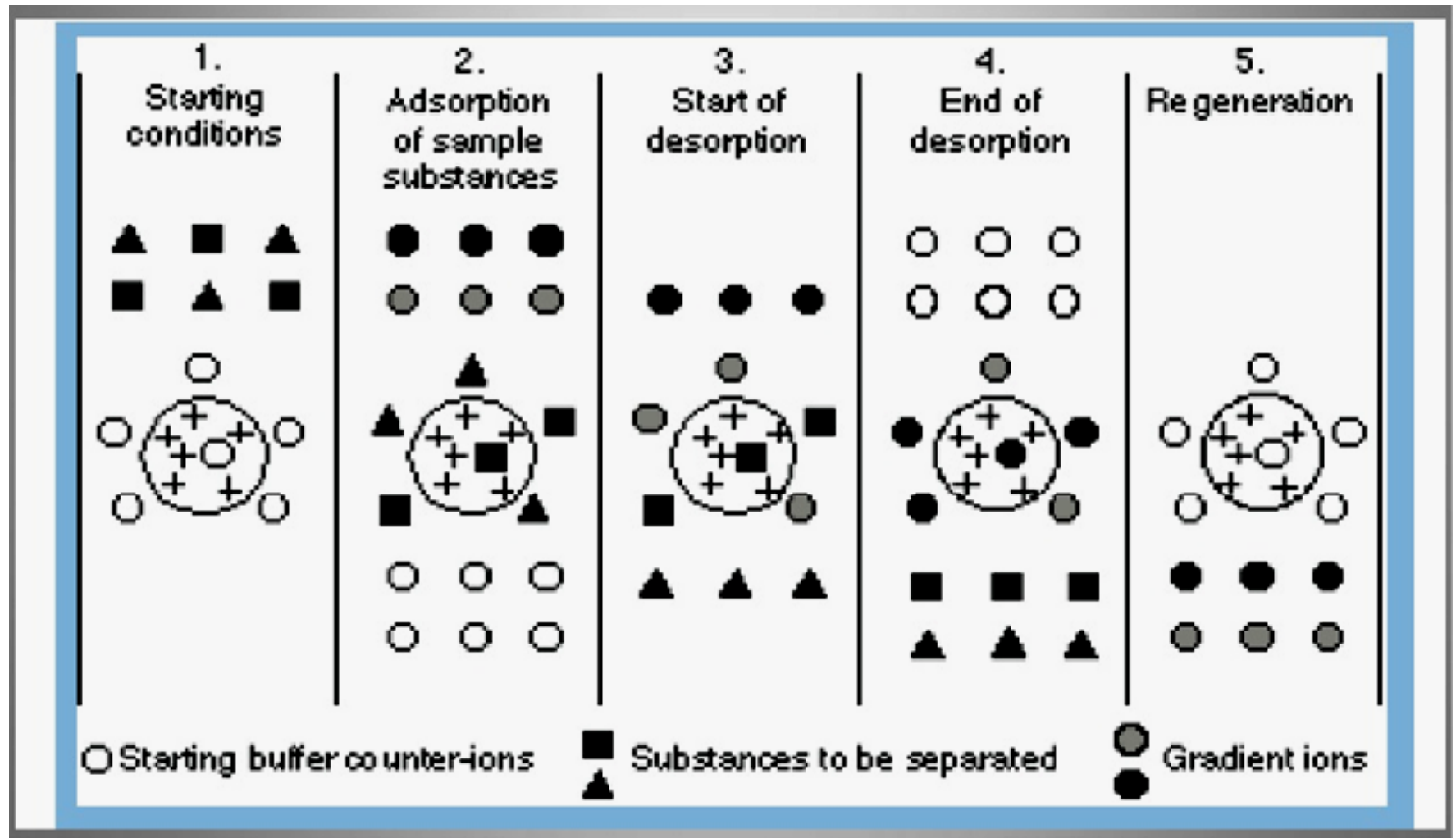


**Table 26-1 Ion-exchange resins**

Resin type	Chemical constitution	Usual form as purchased	Common trade names		Selectivity	Thermal stability
			Rohm & Haas	Dow Chemical		
Strongly acidic cation exchanger	Sulfonic acid groups attached to styrene and divinylbenzene copolymer	Aryl-SO <sub>3</sub> <sup>-</sup> H <sup>+</sup>	Amberlite IR-120	Dowex 50W	Ag <sup>+</sup> > Rb <sup>+</sup> > Cs <sup>+</sup> > K <sup>+</sup> > NH <sub>4</sub> <sup>+</sup> > Na <sup>+</sup> > H <sup>+</sup> > Li <sup>+</sup> Zn <sup>2+</sup> > Cu <sup>2+</sup> > Ni <sup>2+</sup> > Co <sup>2+</sup>	Good up to 150°C
Weakly acidic cation exchanger	Carboxylic acid groups attached to acrylic and divinylbenzene copolymer	R-COO <sup>-</sup> Na <sup>+</sup>	Amberlite IRC-50	—	H <sup>+</sup> >> Ag <sup>+</sup> > K <sup>+</sup> > Na <sup>+</sup> > Li <sup>+</sup> H <sup>+</sup> >> Fe <sup>2+</sup> > Ba <sup>2+</sup> > Sr <sup>2+</sup> > Ca <sup>2+</sup> > Mg <sup>2+</sup>	Good up to 100°C
Strongly basic anion exchanger	Quaternary ammonium groups attached to styrene and divinylbenzene copolymer	Aryl-CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>3</sub> <sup>+</sup> Cl <sup>-</sup>	Amberlite IRA-400	Dowex 1	I <sup>-</sup> > phenolate <sup>-</sup> > HSO <sub>4</sub> <sup>-</sup> > ClO <sub>3</sub> <sup>-</sup> > NO <sub>3</sub> <sup>-</sup> > Br <sup>-</sup> > CN <sup>-</sup> > HSO <sub>3</sub> <sup>-</sup> > NO <sub>2</sub> <sup>-</sup> > Cl <sup>-</sup> > HCO <sub>3</sub> <sup>-</sup> > IO <sub>3</sub> <sup>-</sup> > HCOO <sup>-</sup> > acetate <sup>-</sup> > OH <sup>-</sup> > F <sup>-</sup>	OH <sup>-</sup> form fair up to 50°C Cl <sup>-</sup> and other forms good up to 150°C
Weakly basic anion exchanger	Polyalkylamine groups attached to styrene and divinylbenzene copolymer	Aryl-NH(R) <sub>2</sub> <sup>+</sup> Cl <sup>-</sup>	Amberlite IR-45	Dowex 3	Aryl-SO <sub>3</sub> H > citric > CrO <sub>3</sub> > H <sub>2</sub> SO <sub>4</sub> > tartaric > oxalic > H <sub>3</sub> PO <sub>4</sub> > H <sub>3</sub> AsO <sub>4</sub> > HNO <sub>3</sub> > HI > HBr > HCl > HF > HCO <sub>2</sub> H > CH <sub>3</sub> CO <sub>2</sub> H > H <sub>2</sub> CO <sub>3</sub>	Extensive information not available; tentatively limited to 65°C

SOURCE: Adapted from J. X. Khym, *Analytical Ion-Exchange Procedures in Chemistry and Biology* (Englewood Cliffs, NJ: Prentice Hall, 1974).

# Ion exchange process is performed in five main steps:







- **1. The first step (assurance of resin homogeneity)**

is an equilibration in which the ion exchanger is brought to a starting state, which allows the binding of the desired solute molecules. The exchanger groups are associated at this time with exchangeable counter labile-ions (usually simple anions or cations, such as chloride or sodium).

- **2. The second step (adsorption of sample)**

Is the sample application and adsorption, in which solute molecules carrying the appropriate charge displace counter-ions and bind reversibly to the resin surface. Unbound substances can be washed out from the exchanger bead using eluent (buffer or electrolyte)



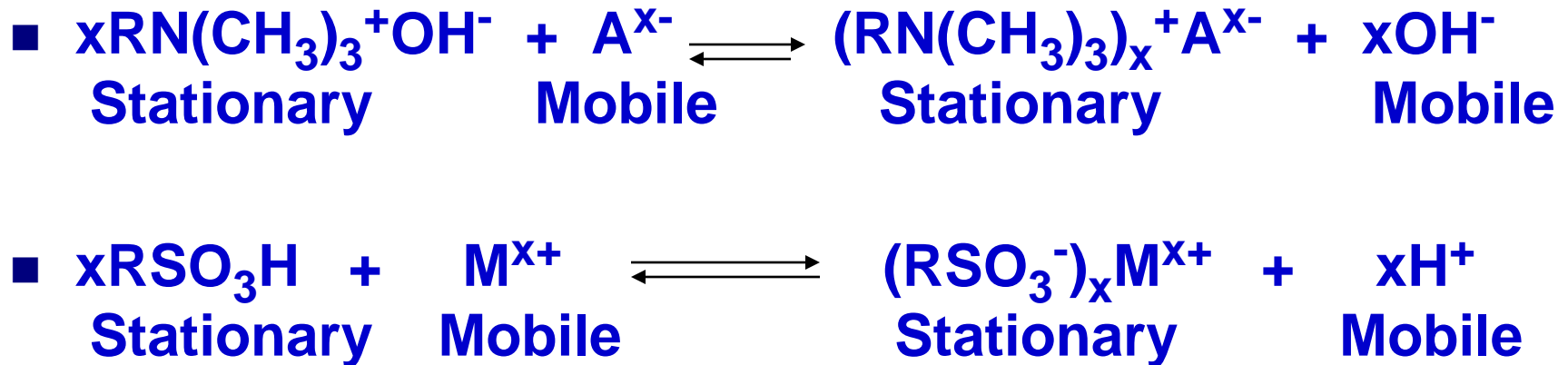
- **3. The third step (desorption and elution of components)**

The substances are removed from the column by changing to elution conditions for ionic bonding of the solute molecules. This normally involves increasing the ionic strength of the eluting buffer or changing its pH.

- **4. The fourth and fifth steps (regeneration)**

Are the removal of substances from the column that are not eluted under the previous experimental conditions and re-equilibration at the starting conditions for the next purification.

# Exchange constant or selectivity coefficient (K)



For the above equilibria;

$$K = \frac{[\text{R-M}^+][\text{H}^+]}{[\text{R-H}^+][\text{M}^+]}$$

# Factors affecting ion exchange selectivity (K)

- Three major factors are affecting ion exchange selectivity;

## 1. Nature and properties of ion exchange resins

- Cross linking and swelling are important factors, when more cross linking agent is present, the resin becomes more rigid and swells less (has small pore size). This makes separations of ions of different sizes more difficult as they can not pass through the pores present and it becomes selective to ions of different (smaller) sizes.
- The nature of resin whether cationic or anionic exchanger, which determines strongly its selectivity. Cationic resin is selective for cations and vice versa.
- Also, the resin capacity (number of me-equivalents of replaceable ions per gram of dry resin) is important. How it could be determined?

## 2. Nature of exchanging ions in the sample

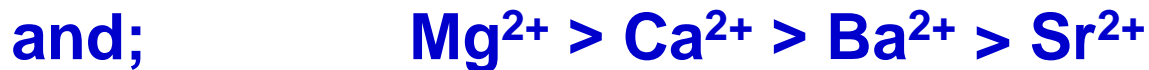
### a. Valence of ions:

- At low concentrations, the extent of exchanges increases with increase in valence; ions with higher charge is more selective;



### b. Size of ions:

- For similarly charged ions; the exchange selectivity increases with decrease in the size of hydrated ions;



### **c. Polarizability:**

Highly polarizable ions are more selective. Exchange is preferred for greater polarizable ions, as;  $I^- > Br^- > Cl^- > F^-$

### **d. Concentration of solutions:**

In dilute solutions poly-valent ions are generally absorbed preferentially.

## **3. Nature of mobile phase and pH**

- The presence of other ions that compete with the sample for binding to the ion exchanger (using of electrolyte).
- The pH of the solution which influences the net charge of the sample (as in case of amino acids).

**Table 26-3** Relative selectivity coefficients of ion-exchange resins

Sulfonic acid cation-exchange resin				Quarternary ammonium anion-exchange resin	
Cation	Relative selectivity for divinylbenzene content			Anion	Relative selectivity
	4%	8%	10%		
Li <sup>+</sup>	1.00	1.00	1.00	F <sup>-</sup>	0.09
H <sup>+</sup>	1.30	1.26	1.45	OH <sup>-</sup>	0.09
Na <sup>+</sup>	1.49	1.88	2.23	Cl <sup>-</sup>	1.0
NH <sub>4</sub> <sup>+</sup>	1.75	2.22	3.07	Br <sup>-</sup>	2.8
K <sup>+</sup>	2.09	2.63	4.15	NO <sub>3</sub> <sup>-</sup>	3.8
Rb <sup>+</sup>	2.22	2.89	4.19	I <sup>-</sup>	8.7
Cs <sup>+</sup>	2.37	2.91	4.15	ClO <sub>4</sub> <sup>-</sup>	10.0
Ag <sup>+</sup>	4.00	7.36	19.4		
Tl <sup>+</sup>	5.20	9.66	22.2		

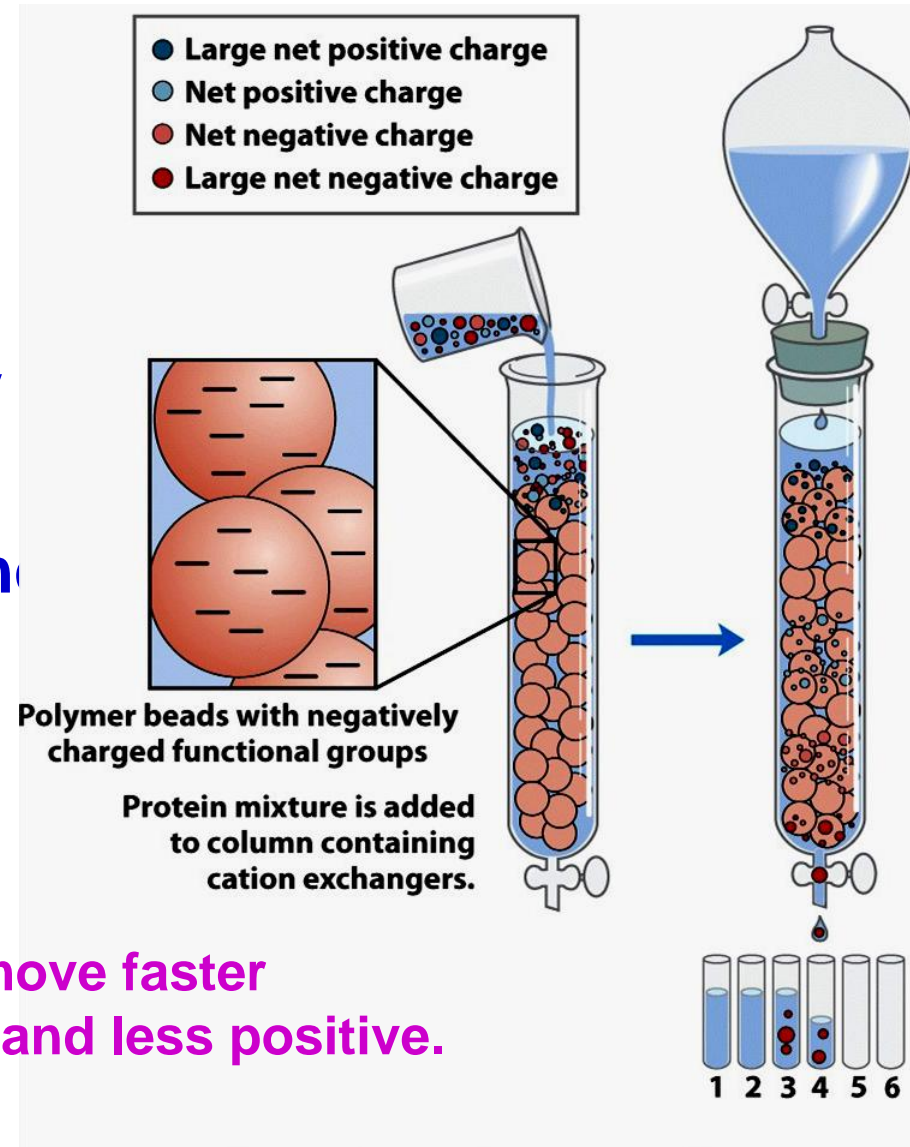
SOURCE: *Amberlite Ion Exchange Resins—Laboratory Guide* (Rohm & Haas Co., 1979).

# Applications of Ion Exchange Chromatography

**1- Clinical applications:  
As in separation of amino  
acids and proteins.**

**Ion exchange chromatography  
is a principle technique for  
analyses of the amino acids and  
proteins.**

**Proteins move with rates determined  
by their net charge at the used pH of  
the eluent. With cation exchangers,  
proteins with more negative charge move faster  
and eluted earlier, then less negative and less positive.  
Finally, the more positive ones.**



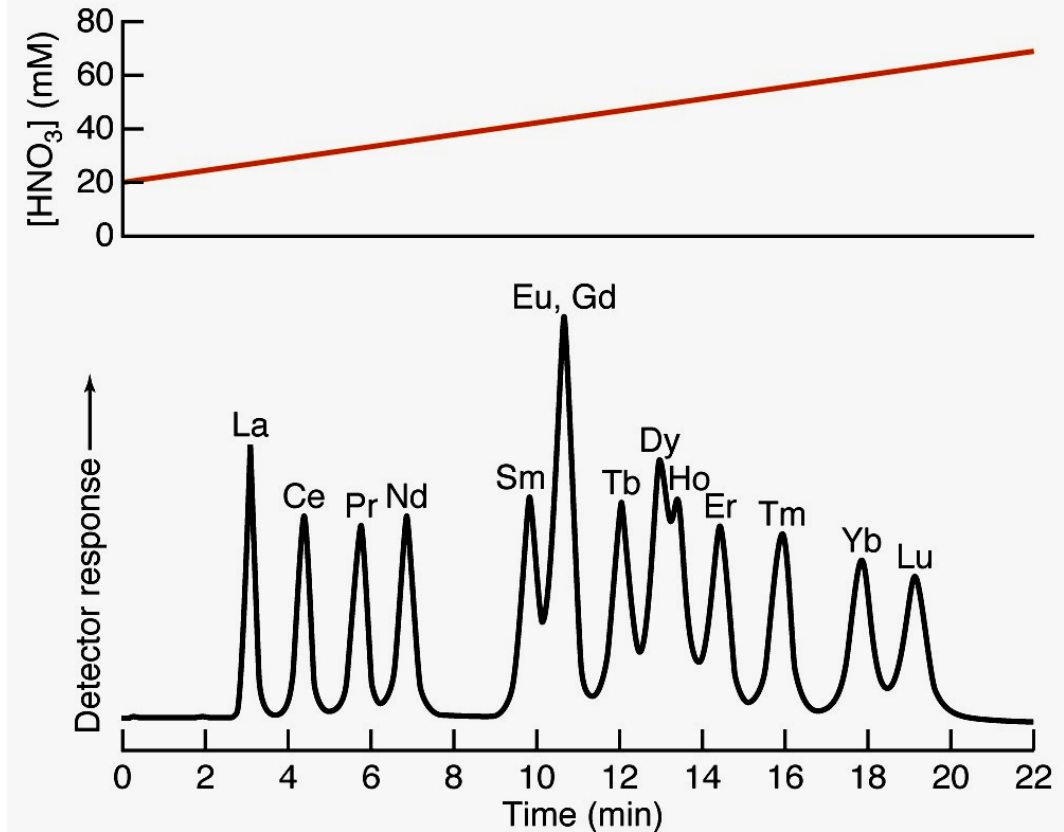


## **For separation of amino acids mixture in protein hydrolysate using cation exchanger;**

- **Amino acids have functional groups that can carry both positive or negative charges (amphoteric) depending on the pH or the ionic concentration of the mobile phase.**
- **Ion exchange chromatography separates amino acids according to their net charge and iso-electric points (PI) which is dependent on the pH of the mobile phase.**
- **For example; if a mixture of aspartic, glycine and arginine has been introduced into cationic resin in a strong acidic buffer, then on gradient elution using increasing pH, results in sequential elution of more acidic aspartic, followed by neutral glycine and finally the more basic arginine .**

## 2- Separation of similar ions like lanthanides

Lanthanides have very similar properties and can not be separated by usual techniques, but can be separated by ion exchange chromatography. This depends on the Acidity (ionic strength) of the medium.



### 3- Removal of interfering ions

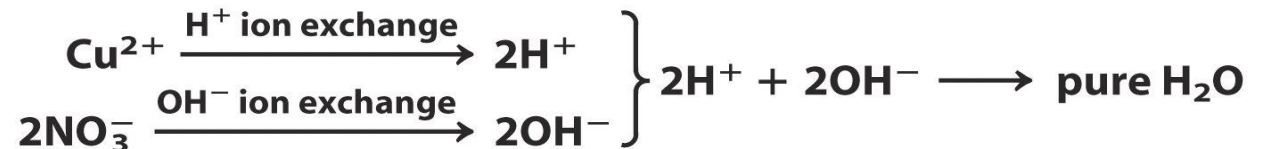
For estimation of  $\text{Ca}^{2+}$ ,  $\text{Ba}^{2+}$  ions by the oxalate or sulphate method in quantitative analysis, phosphate ions are found to interfere and can be removed by passing the solution through  $\text{H}_2\text{SO}_4$ .

### 4- Pre-concentration of solutions

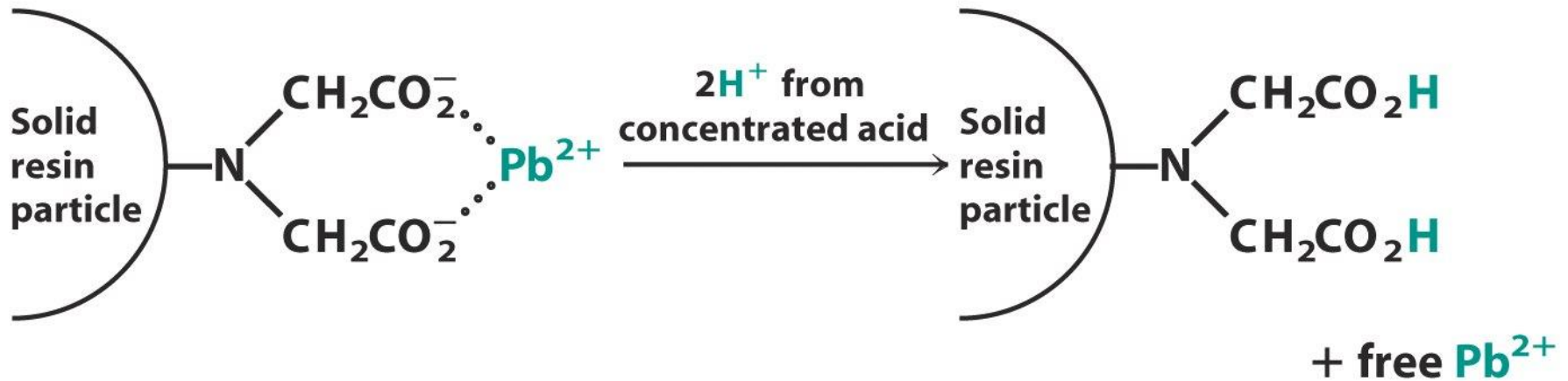
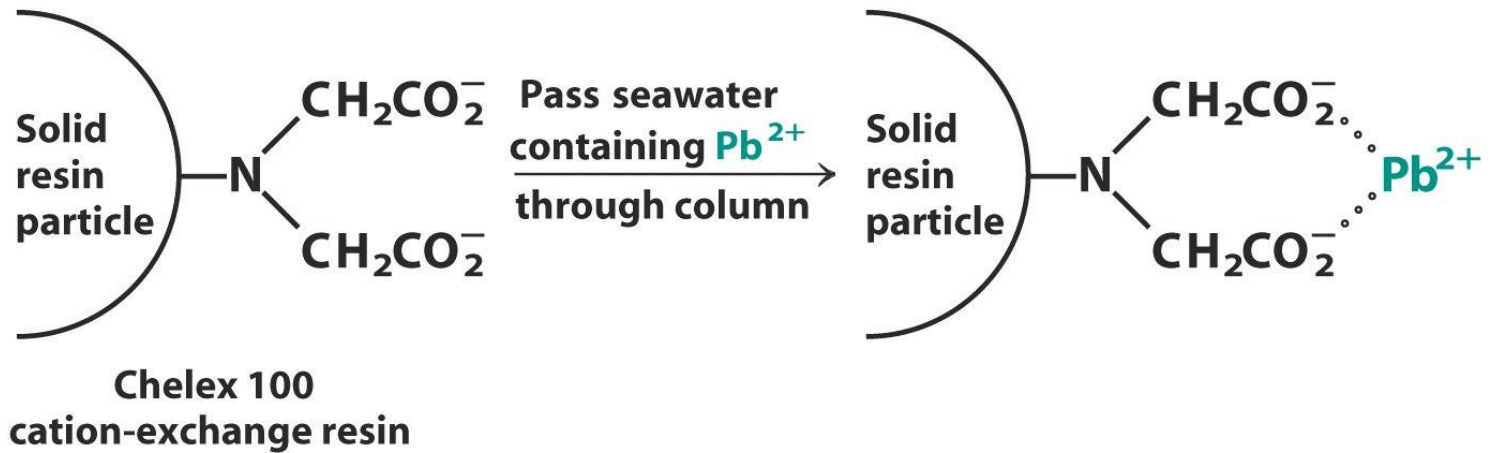
By passing much water over a resin and then elute with a high concentration of acid. Cation exchangers trap cations. It is important for trace analysis, where solubility [s] is extremely low. It is important for environmental problem.

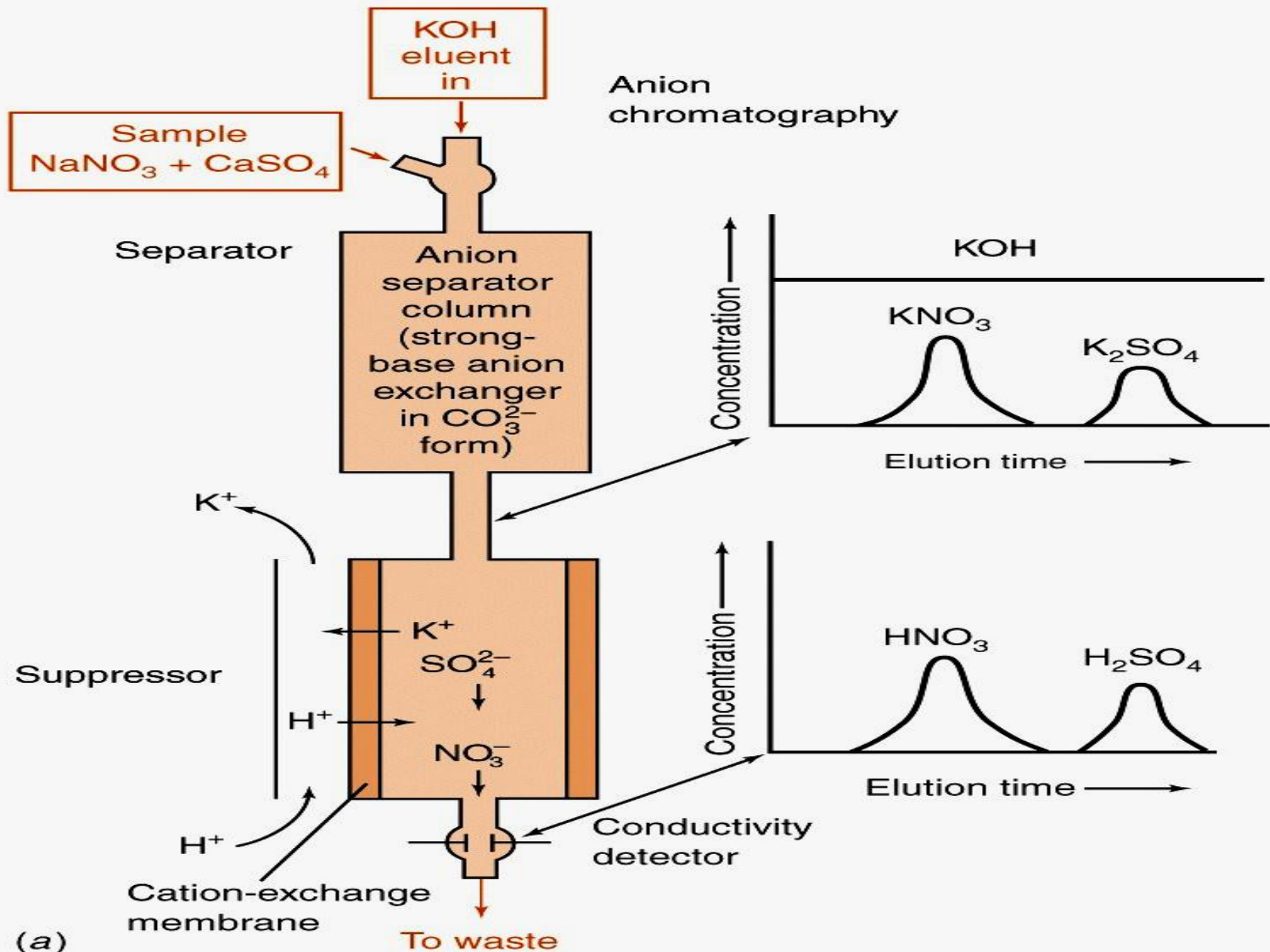
### 5- Water deionization and softening

Removal of cations by cation exchangers and anion exchangers for anion removal.

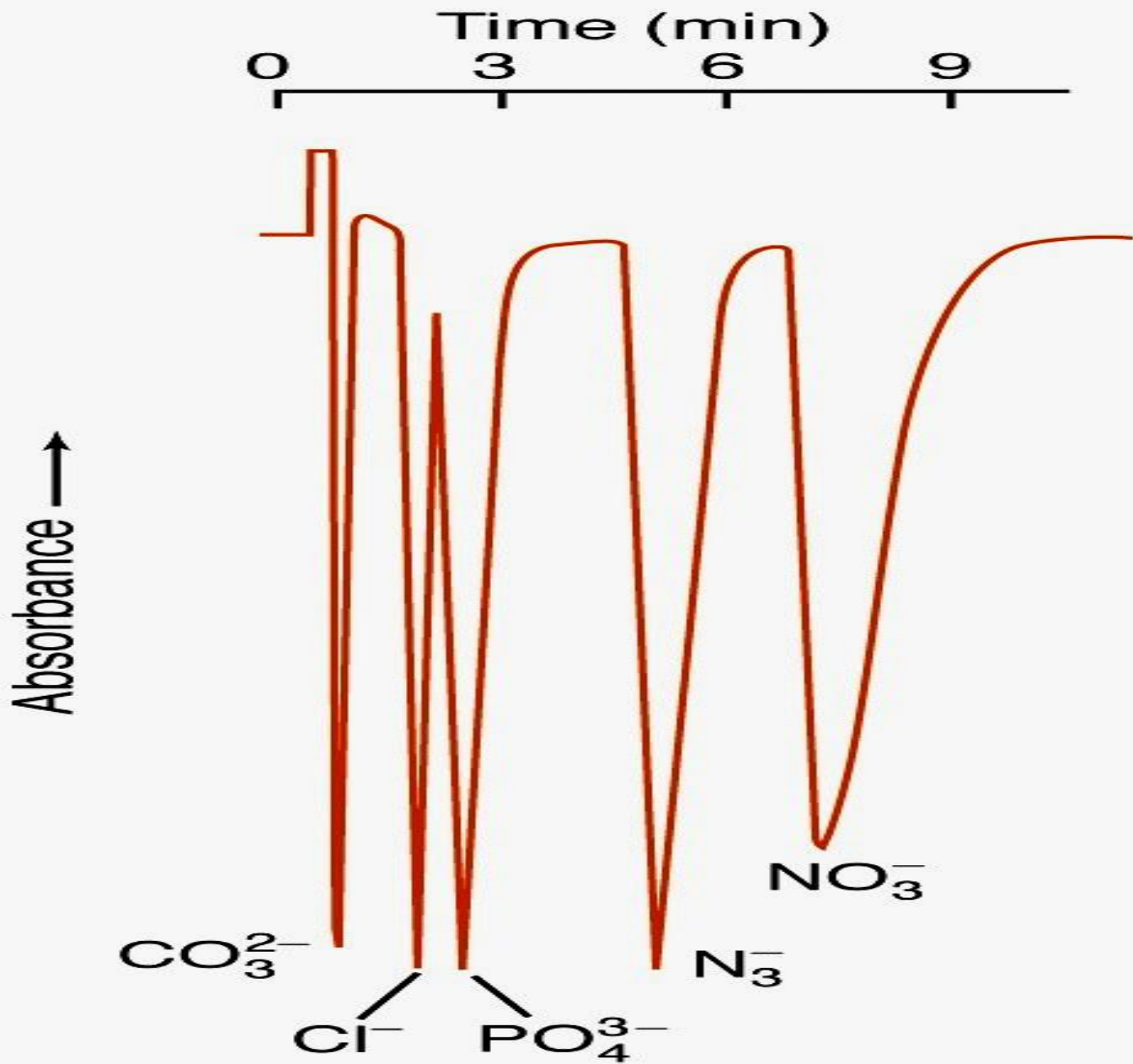


# ex: Removing of toxic lead ions from seawater





**Schematic representation of ion exchange Chromatograph, as HPLC**



The chromatogram of some anions separated by using anion exchanger.