Types of chromatography

CHEM 313-4

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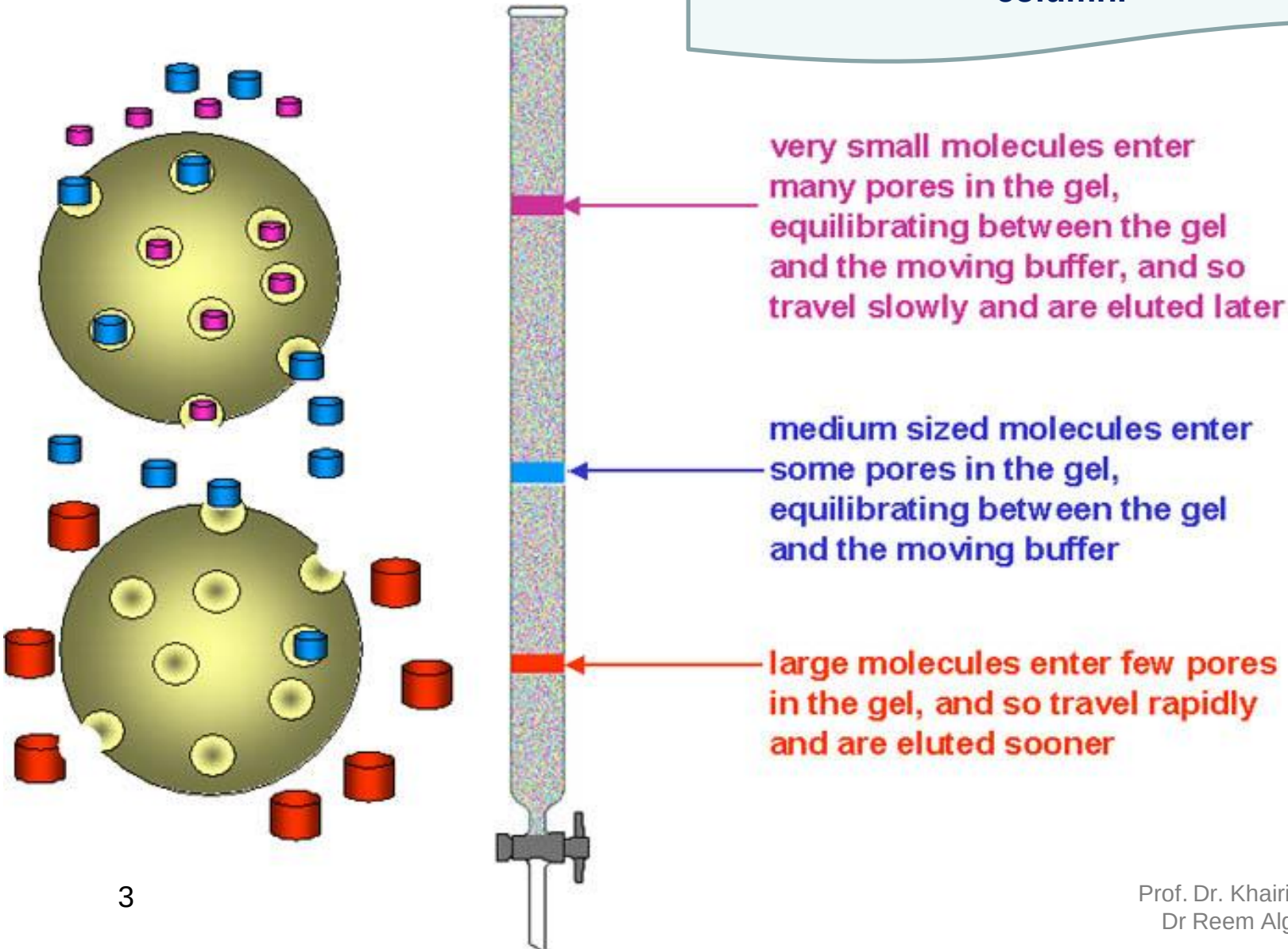
17.5 SIZE EXCLUSION CHROMATOGRAPHY

- Size exclusion chromatography (also called **molecular or gel chromatography**) is a type of chromatography in which the stationary phase is a molecular sieve.
- These are **polymeric carbohydrates** and **acrylamides** that have an open **network formed** by the **cross-linking** of the polymeric chains.
- They are **hydrophilic** and therefore, capable of absorbing water, whereupon **swelling** causes an opening of this structure.
- The degree of cross-linking will determine the size of the holes.
- Solvated molecules larger than the largest pores of the swollen gel cannot penetrate the gel particles and, therefore, will pass straight through the column through the spaces between the individual particles.
- Smaller molecules, however, will penetrate the open network in the particles to a **varying degree**, depending on their **size and shape**.
- They are retarded to varying degrees and will be **eluted in order of decreasing molecular size.**
- Gel with a high degree of swelling are used to fractionate large molecules (generally high-molecular-weight substances), whereas the denser (lower swelling) gels are used for separation of low-molecular weight compounds.

Molecules that can penetrate the gel particles are separated based on size and shape. Others pass straight through the column.



Prof. Dr. Khairia Al-Ahmary &
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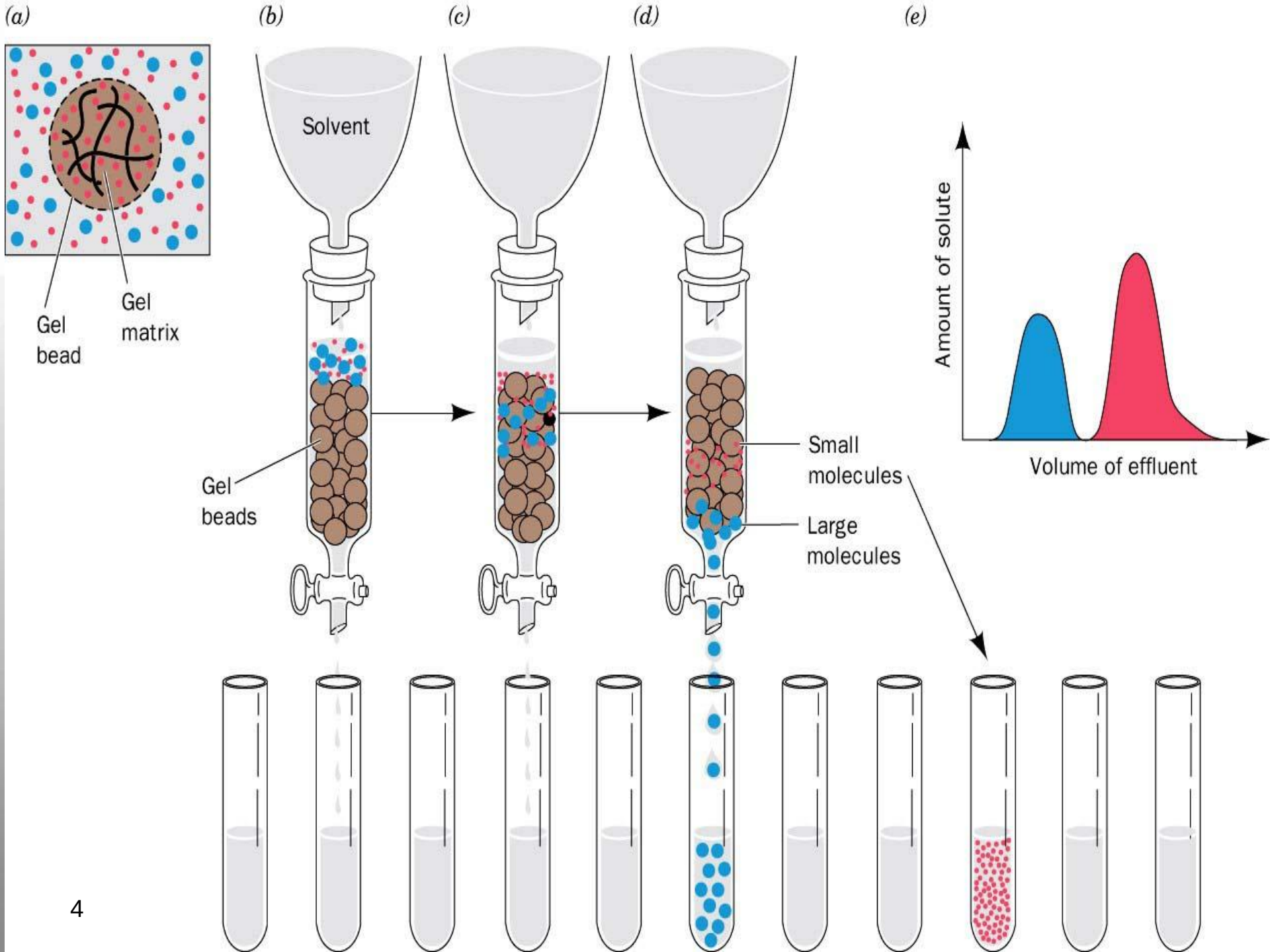

(a)
(b)
(c)
(d)
(e)
Gel bead
Gel matrix
Solvent
Gel beads
Small molecules
Large molecules
Amount of solute
Volume of effluent

Types of size exclusion chromatography:

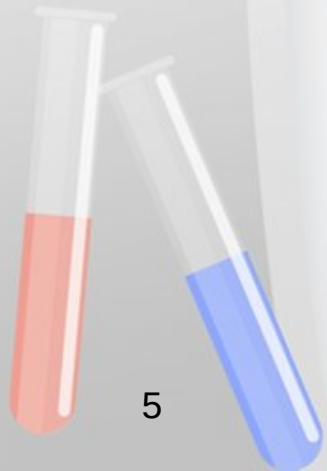
1- **Gel filtration chromatography** (mobile phase is water), used by biochemists.

2-**Gel permeation chromatography**

(mobile phase is an organic solvent) used by polymer chemists.

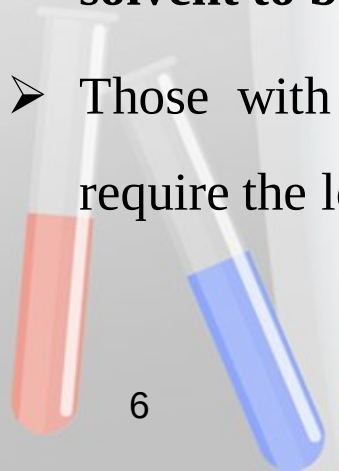
Application of size exclusion chromatography:

- In molecular weight distribution of polymers.
- In separation of protein, enzymes, peptides, nucleic acids, hormones.



Size exclusion chromatography technique:

- The **exclusion limit** is the molecular weight of that will just **permeate** the gel and be retarded.
- This can range from a molecular weight of **1000 to several million**, depending on the gel.
- It should be confirmed that separations are based on a molecule's **size and configuration** rather than just its **molecular weight**, but there is generally a correlation.
- **The gels must be equilibrated for a few hours to a day or more with the solvent to be used, depending on the solvent uptake**.
- Those with large cross-linking designed for high-molecular-weight substances require the longer periods of soaking.



Example of Gels

1- Sephadex is a popular molecular-sieve material for the separation of proteins.

- It is a **polymeric carbohydrate** material, because of **hydroxyl groups** along the polymer chain, fairly polar and so will adsorb water.
- The amount of **cross-linking in the preparation** can be carefully controlled to give **different pore sizes and exclusion limits.**
- Gels are characterized with respect to their swelling ability by their *"**water regain**"*.
- The type number of the Sephadex gels refer to the water-regaining values of the gels.

- Sephadex G-10, thus, has a water-regaining value of about 1 mL/g dry gel.
- Sephadex G-200 has a value of about 20 mL/g dry gel.
- Several types of sephadex gels and the fraction range of molecules are listed in Table 17.1.
- These gels are **insoluble in water** and **stable to mild redox agents as well as to bases and weak acids.**

2- Bio-Gel: is a more chemically **inert series** of molecular-sieve gels, consisting of **polyacrylamides.**

- These are **insoluble in water and common organic solvents and can be used in pH range from 2 to 11.**

3- Styra-Gel : is **a polystyrene gel** that is useful for purely **nonaqueous** separations in methylene chloride, toluene, trichlorobenzene, tetrahydrofuran, cresol, dimethylsulfoxide, and so on.

- It **cannot** be used with **water, acetone, or alcohols**.

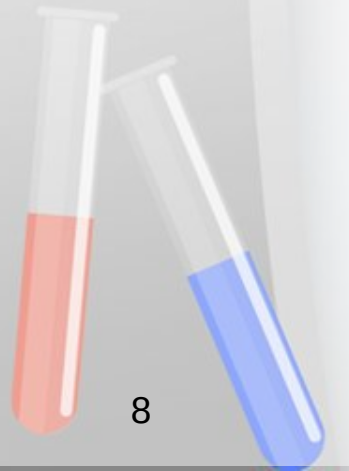


TABLE 17.1

Sephadex Gels[a] and Bio-Gels[b]

Sephadex Type	*Fractionation Range[c] for Peptides and Globular Proteins, MW*	*Bio-Gel Type*	*Fractionation Range, MW*
G-10	Up to 700	P-2	100–1800
G-15	Up to 1500	P-4	800–4000
G-25	1000 to 5000	P-6	1000–6000
G-50	1500 to 30,000	P-10	1500–20,000
G-75	3000 to 70,000	P-30	2500–40,000
G-100	4000 to 150,000	P-60	3000–60,000
G-150	5000 to 400,000	P-100	5000–100,000
G-200	5000 to 800,000	P-150	15,000–150,000
		P-200	30,000–200,000
		P-300	60,000–400,000

[a]Courtesy of Pharmacia Fine Chemicals Inc.

[b]Courtesy of Bio·Rad Laboratories.

[c]Upper limit is the exclusion limit.

17.6 ION EXCHANGE CHROMATOGRAPHY

- The most types of chromatography are used principally for separations of **complex organic substances**, while **ion exchange chromatography** is particularly well suited for the separation of **inorganic ions**, both **cations** and **anions**,
- It also useful for the separation of **amino acids.**
- The separation in ion exchange chromatography is based on exchange of ions in the stationary phase.
- The stationary phase in ion exchange chromatography consists of beads made of a **polystyrene polymer** cross-linked with **divinylbenzene (DVB).**
- The cross-linked polymer (**resin**) has free **phenyl groups** attached to the chain, which can easily be treated to add **ionic functional groups.**
- There are basically four types of ion exchange resins used in analytical chemistry, and these are summarized in Table 17.2.

TABLE 17.2

Types of Ion Exchange Resins

Type of Exchanger	*Functional Exchanger Group*	*Trade Name*
Cation		
Strong acid	Sulfonic acid	Dowex[a] 50; Amberlite[b] IR120; Ionac[c] CGC-240; Rexyn[d] 101;Permutit[e] Q
Weak acid	Carboxylic acid	Amberlite IRC 50; Ionac CGC-270; Rexyn 102; Permutit H-70
Anion		
Strong base	Quaternary ammonium ion	Dowex 1; Amberlite IRA 400; Ionac AGA-542; Rexyn 201; Permutit S-1
Weak base	Amine group	Dowex 3; Amberlite IR 45; Ionac AGA-316; Rexyn 203; Permutit W

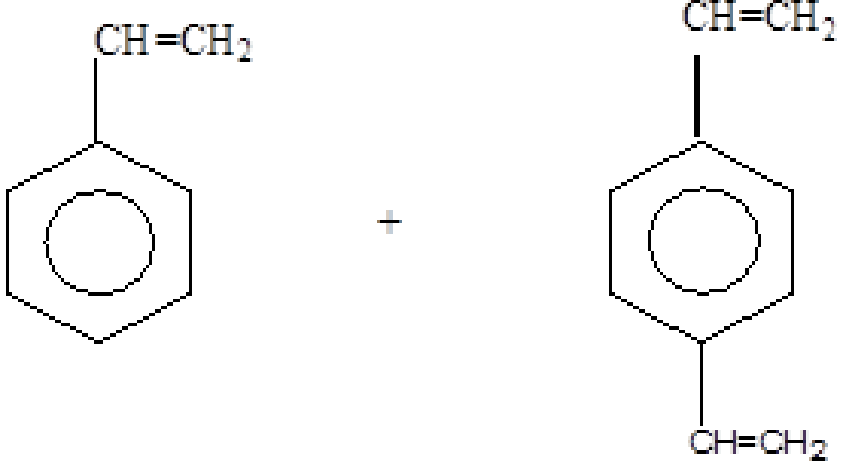
[a]Dow Chemical Company.

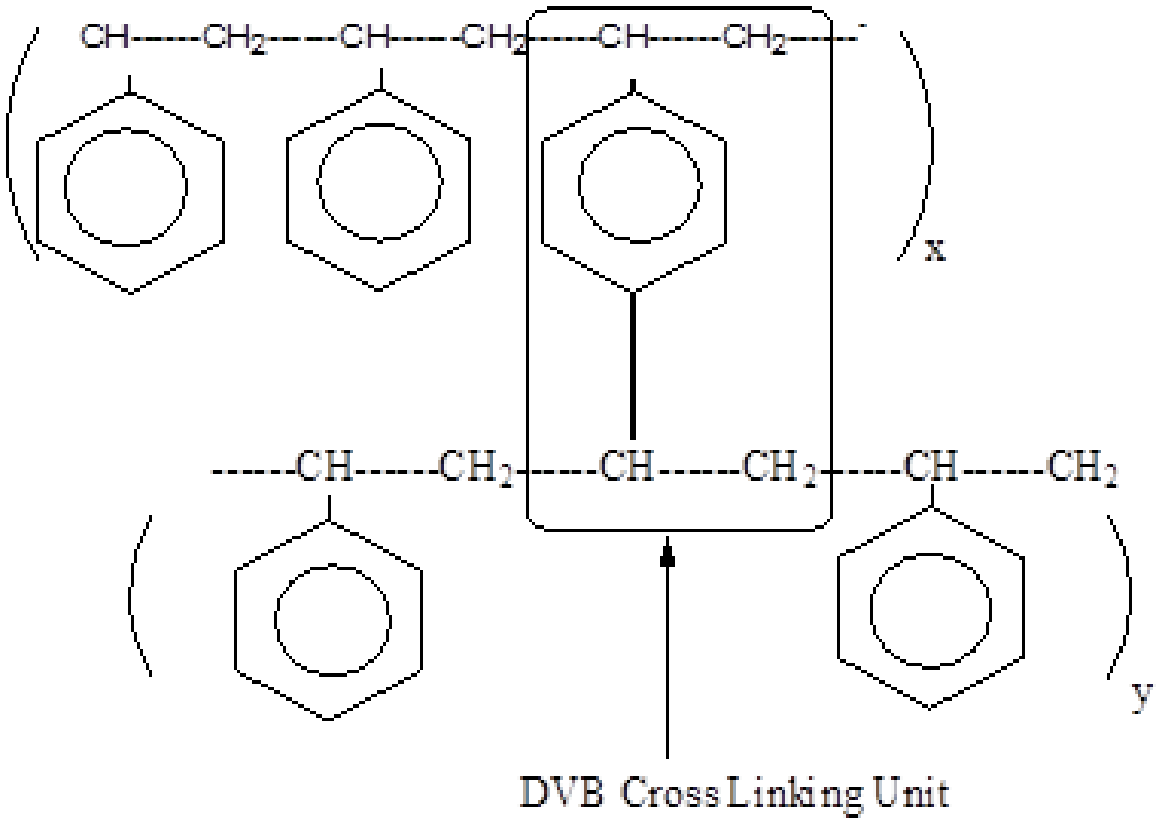
[b]Mallinckrodt Chemical Works.

[c]J. T. Baker Chemical Company

[d]Fisher Scientific Company.

[e]Matheson Coleman & Bell.


CH=CH2
+
CH=CH2
CH=CH2


CH
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x
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CH
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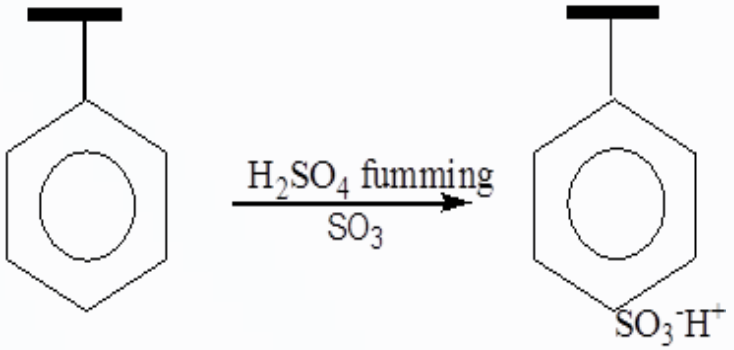
y
DVB Cross Linking Unit

Cation Exchange Resins

- These resins contain **acidic functional groups** added to the **aromatic ring of the resin.**
- The **strong-acid cation** exchangers have **sulfuric acid groups**, - **SO_3H**,which are strong acids much like sulfuric acid.



- The **weak-acid cation** exchangers have **carboxylic acid groups**, - **CO_2H**, which are only partially ionized.

- The protons on these groups can exchange with other cations:

$$nRzSO_3^-H^+ + M^{n+} \Leftrightarrow (RzSO_3)_n M + nH^+$$

And

$$nRzCO_2^-H^+ + M^{n+} \Leftrightarrow (RzCO_2)_n M + nH^+$$

where Rz represents the resin.

- The equilibrium can be shifted to the left or right by increasing $[H^+]$ or $[M^{n+}]$ or decreasing one with respect to the amount of resin present.
- Cation exchange resins are usually supplied in the **hydrogen ion** form, but they can easily be converted to the **sodium ion** form by treating with a sodium salt.
- The sodium ions then undergo exchange with other cations.
- **The exchange capacity** of a resin is the **total number of equivalents of replaceable hydrogen per unit volume or per unit weight of resin**, and it is determined by the number and strength of fixed ionic groups on the resin.
- The ion exchange **capacity affects solute retention**, and exchangers of **high capacity** are most often used for separating **complex mixtures**, where **increased retention improves resolution**.

- **Weak acid cation** exchanger resins are more restricted in the pH range in which they can be used, from **5 to 14**, while the **strong acid** resins can be used from pH **1 to 14**.
- At low pH values, the weak acid exchangers will hold on to the protons too strongly for exchange to occur.
- Also, the weak acid cation exchangers will **not completely remove** the **cations of very weak bases**, while strong acid resins will.
- Weak-acid resins are generally used for separating strongly basic or multifunctional ionic substances such as proteins or peptides that are often firmly retained on strong-acid exchangers, while strong-acid resins are more generally preferred, especially for complex mixtures.

Strong-acid resins are used for most separations. Weak-acid resins are preferred for proteins and peptieds that are retained too strongly by the strong acids.

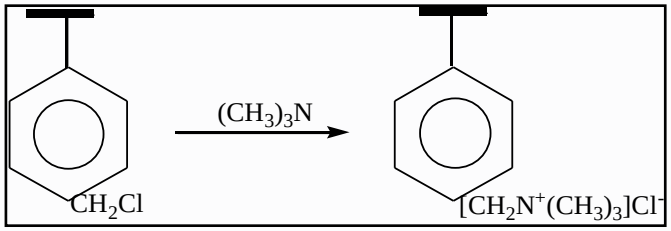
Anion Exchange Resins

- Basic groups in the resin in the **hydroxyl anion** can be exchanged with other anions make up the anion exchange resins.
- There are strong-base groups (quaternary ammonium groups) and weak-base groups (amine groups).
- The exchange reactions can be represented by

$$nRzNR_3^+OH^- + A^{n-} \Leftrightarrow (RzNR_3)_n A + nOH^-$$

and

$$nRzNH_3^+OH^- + A^{n-} \Leftrightarrow (RzNH_3)_n A + nOH^-$$



where R represents organic groups, usually methyl.

- The **strong-base** exchangers can be used over the pH range **0 to 12**, but the **weak-base** exchangers only over the range of **0 to 9**.
- The **weak-base** exchangers will not remove very weak acids, but they are preferred for strong acids that may be retained by strong-base resins, such as sulfonates.

Strong-base resins are generally applicable.
Weak-base resins are used for separating strong acids.

Cross-linkage

- The greater the cross-linkage of the resin, the greater the difference in **selectivities.**
- Generally, cross-linkage also increases the rigidity of the resin, reduces swelling, reduces porosity, and reduces the solubility of the resin.
- In general, medium-porosity materials are used for low-molecular-weight ionic species and high-porosity materials are used for high-molecular-weight ionic species.
- **The degrees of the cross-linkage** is expressed by manufactures as **percent of divinylbenzene**.
- Generally, cross-linkage of **8 to 10 %** is used.

Effect of pH-Separation of Amino Acids

- The ionic forms of **many substances will be affected by the pH** of the effluent solution.
- **Hydrolysis** of metal ions and of salt of weak acids and bases is controlled by adjusting the pH.
- **Weak acids** will not dissociate in **high acid concentrations** and will not exchange, and the same is true for **weak bases** in **high alkaline** concentrations.
- Control of **pH** is especially **important** in the separation of **amino acids,** which are amphoteric (can act as acids or bases).
- There are three possible forms:

$$\underset{\underset{A}{\downarrow}}{\underset{NH_3^+}{R-CH-CO_2H}} \xleftrightarrow{+H^+} \underset{\underset{B}{\downarrow}}{\underset{NH_3^+}{R-CH-CO_2^-}} \xleftrightarrow{-H^+} \underset{\underset{C}{\downarrow}}{\underset{NH_2}{R-CH-CO_2^-}}$$

- Form B, called **a zwitterion,** is the dominat form at the pH corresponding to the **isoelectric point** of the amino acid.

- The **isoelectric point** is the pH at which the net charge on the molecule is zero.
- In more acid solutions than this, the $-CO^-_2$ group is prontonated to form a cation (form A),
- While in more alkaline solutions, the $-NH^+_3$ group loses a proton to form an anion (form C).
- The isoelectric point will vary from one amino acid to another, depending on the relative acidity and basicity of the carboxylic acid and amino group.
- Thus, group separations based on the isoelectric points are possible by pH control.

$$\underset{\underset{NH_3^+}{\downarrow}}{R-CH-CO_2H} \xleftrightarrow{+H^+} \underset{\underset{NH_3^+}{\downarrow}}{R-CH-CO_2^-} \xleftrightarrow{-H^+} \underset{\underset{NH_2}{\downarrow}}{R-CH-CO_2^-}$$

$$A \qquad\qquad B \qquad\qquad C$$

- At a given pH, **the amino acids** can be separated **into three groups** by being passed successively through an anion and a cation exchange column.

- The uncharged zwitterions (isoelectric point) will **pass** through both columns, while the positively and negatively charged amino acids will each be retained by one of the columns.
- **Moore and Stein** successfully separated up to **50 amino acids** and related compounds on a single **Dowex-50 cation exchange column** by a combination of **pH and temperature control.**
- **Automatic amino acids analyzers** based on ion exchange separation are commercially available.
- **The elution** of each amino acid is **automatically recorded** by **measuring the color formed** between **the amino acid** and **ninhydrin** as it is eluted.

Effect of Complexing Agents -Separation of Metal Ions on Anion Exchange Columns

- Many **metals** can be separated on **anion exchange column** by being **converted to anions by complexation**.
- The **complexing agent** is an anion such as chloride, bromide or fluoride.
- Some of the most successful separation of metals have been on anion exchange columns.
- **A complexing acid is added in high concentration to form anionic complexes of the metals.**
- Concentrated hydrochloric acid forms **anionic chloro-complexes** with all the common metals, with the **exception of the alkali and alkaline earth metals and Al(III), Ni(II) and Cr(III),** and so all of these can be adsorbed on **a quaternary ammonium anion exchange column**.
- Distribution coefficients of metals on Dowex-1 anion exchange resin as a function of hydrochloric acid concentration are summarized in fig. 17.8 for different valences of the metals.

Effect of Complexing Agents -Separation of Metal Ions on Anion Exchange Columns

- **Separations can be achieved by choosing a hydrochloric acid concentration at which one element has a high distribution coefficient and the other a low distribution coefficient.**
- The former will then be strongly retained by the resin, while the other will be eluted.
- **Then, by decreasing the hydrochloric acid concentration to a level at which the distribution coefficient of the second metal is low, the metal can be eluted.**
- Several metals can be successively eluted in this manner.
- The sample is usually placed on the column in **10 M or 11 M HCl**.

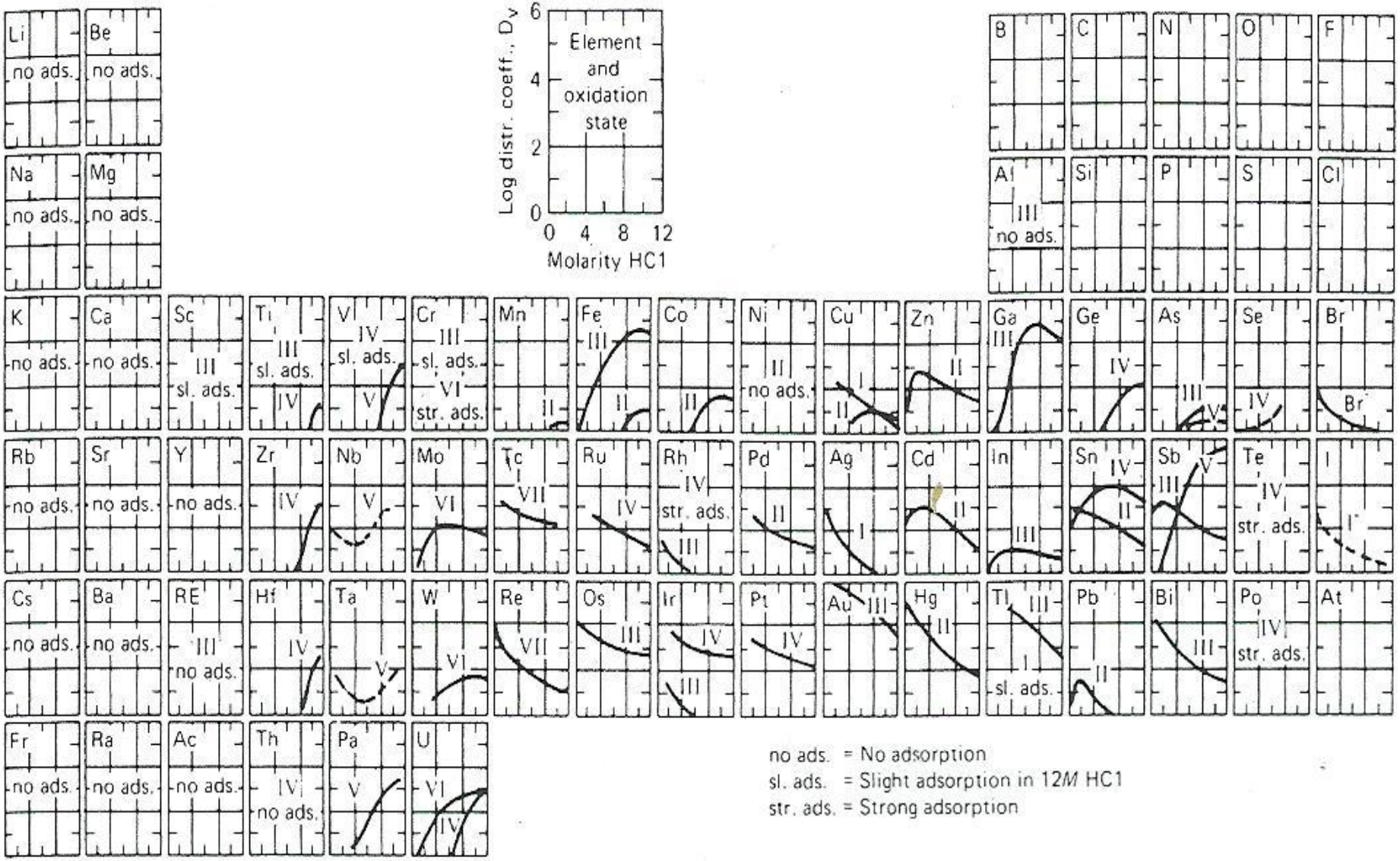


FIGURE 17.8 Distribution coefficients of the elements on Dowex-1 anion exchange resin as a function of the hydrochloric acid concentration. From K. A. Kraus and F. Nelson, *Proceedings of the First United Nations International Conference on the Peaceful Uses of Atomic Energy*, **7** (1956) 113.

- **As example**, a mixture of **Ni^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+} and Zn^{2+}** can be separated as follows:
- The **Ni^{2+}** is not anionic and is eluted in the first column volume of **12 M HCl.**
- **Mn^{2+}** is essentially not adsorbed in **6 M HCl.**
- While the other metals have rather large distribution coefficients (remember that the values in fig. 17.8 are log D, and so a value of 2 means D is equal to 100).
- Thus, manganese is eluted with 6 M HCl.
- At 4 M HCl, D for **Co^{2+}** is 1 (log D=0), and it is eluted.
- The **Cu^{2+}** is only partially eluted in the volume required to elute the Mn^{2+}, but it is completely eluted in **2.5 M HCl**.
- Note that **Fe^{3+}** and **Zn^{2+}** still have large D values at this concentrations.
- At **0.5 M HCl**, the **Fe^{3+}** D value is near unity, and it becomes eluted.
- The **Zn^{2+}** is so strongly adsorbed that the acid concentration must be decreased to **0.005 M HCl,** and even then it requires several column volumes for complete elution.
- It can be more easily eluted with **2 M HNO_3** (after the others are eluted).
- We attempt to choose conditions so that each given metal is eluted quickly in essentially one or two column volumes ($D \leq 1$).

Some Applications of Ion Exchange Chromatography

1- Purification

- One of the most important applications of ion exchange is the deionization of water, which, although non analytical, offers great advantage for the analytical chemist.
- The water is passed through a mixed-bed ion exchange resin (commercially available) that contains both a strong-acid cation and a strong-base anion exchange resin.
- When water containing a salt as $CaCl_2$ is passed through the column, the Ca^{2+} ion is exchanged for two H^+ ions and two Cl^- ions are exchanged for two OH^- ions.
- The net result is that the salt is exchanged from H_2O.
- Water with resistance of several μohms can be obtained in this way.
- Organic constituents, however, are not removed, and sometimes the water is passed through a column of activated charcoal for removal of organic matter.
- If these interfere with a particular analysis, it might be preferable to distill the water.

A mixed-bed cation/anion exchanger removes salts from water by exchnging them for H_2O.
This is how we prepare "deionized" water.

2- Concentration

- Ionic materials that exist at very low concentrations can often be concentrated by collecting them on an ion exchange column and then eluting them with a much smaller volume of an appropriate eluting agent.
- In this manner, the ions are also often removed from the bulk matrix so they may be obtained in purer form.
- The concentration of trace elements in seawater by ion exchange is commonly employed.

3- Analytical Separations

- We have mentioned some representative analytical separations earlier of metal ions and amino acids.
- Halide ions can be separated on a Dowex 2 column in the order F^-, Cl^-, Br^-, I^-, with 1 M $NaNO_3$ at pH 10.4 (adjusted with NaOH).
- The alkali metals can be separated on Dowex 50 or Amberlite IR 120 sulfonated cation exchange resin in the order Li^+, Na^+, K^+ by eluting with 0.7 M HCl.
- The alkaline earth metals Ca^{2+}, Sr^{2+} and Ba^{2+} can be eluted from a Dowex 50 column in that order by 1.2 M ammonium lactate.