12th Week

25-1 Ion-Exchange Chromatography

In ion-exchange chromatography, retention is based on the attraction between solute ions and charged sites bound to the stationary phase (Figure 22-6). In anion exchangers, positively charged groups on the stationary phase attract solute <u>anions</u>. Cation exchangers contain covalently bound, negatively charged sites that attract solute <u>cations</u>.

Anion exchangers contain bound *positive* groups.

Cation exchangers contain bound *negative* groups.

Ion Exchangers

Resins are amorphous (noncrystalline) particles of organic material. *Polystyrene resins* for ion exchange are made by the copolymerization of styrene and divinylbenzene (Figure 25-1). Divinylbenzene content is varied from 1 to 16% to increase the extent of **cross-linking** of the insoluble hydrocarbon polymer. Benzene rings can be modified to produce a cation-exchange resin, containing sulfonate groups ($-SO_3^-$), or an anion-exchange resin, containing ammonium groups ($-NR_3^+$). If methacrylic acid is used in place of styrene, a polymer with carboxyl groups results.
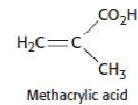
Table 25-1 classifies ion exchangers as strongly or weakly acidic or basic. Sulfonate groups ($-SO_3^-$) of strongly acidic resins remain ionized even in strongly acidic solutions. Carboxyl groups ($-CO_2^-$) of the weakly acidic resins are protonated near pH 4 and lose their cation-exchange capacity. "Strongly basic" quaternary ammonium groups ($-CH_2NR_3^+$) (which are not really basic at all) remain cationic at all values of pH. Weakly basic tertiary

Methacrylic acid

Strongly acidic cation exchangers: RSO_3^-
Weakly acidic cation exchangers: RCO_2^-
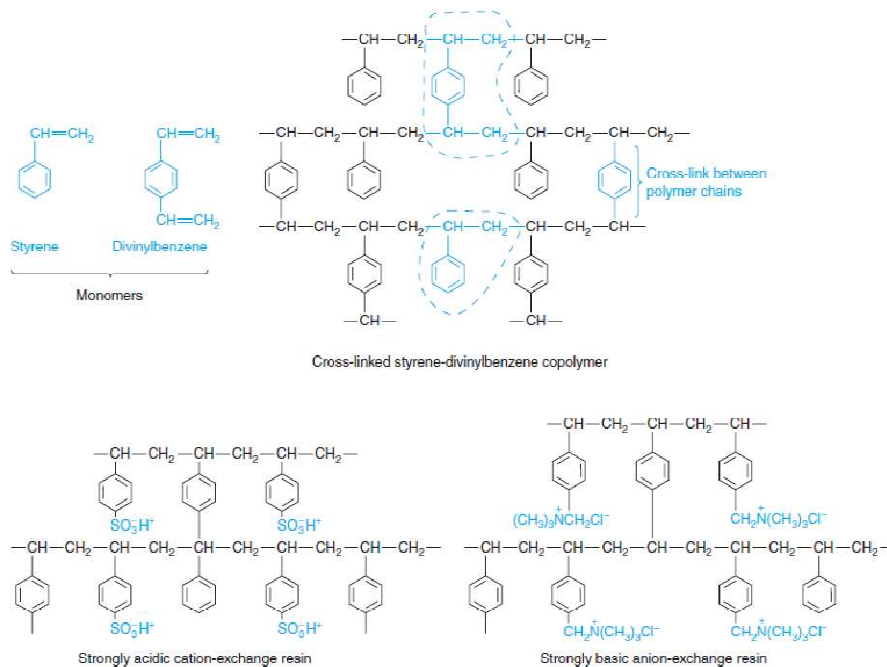"Strongly basic" anion exchangers: $RNR_3'^+$
Weakly basic anion exchangers: $RNR_2'H^+$

FIGURE 25-1 Structures of styrene-divinylbenzene cross-linked ion-exchange resins.

ammonium ($—CH_2NHR_2^+$) anion exchangers are deprotonated in moderately basic solution and lose their ability to bind anions.

The extent of cross-linking is indicated by the notation "-XN" after the name of the resin. For example, Dowex 1-X4 contains 4% divinylbenzene, and Bio-Rad AG 50W-X12 contains 12% divinylbenzene. The resin becomes more rigid and less porous as cross-linking increases. Lightly cross-linked resins permit rapid equilibration of solute between the inside and the outside of the particle. However, resins with little cross-linking swell in water. Hydration decreases both the density of ion-exchange sites and the selectivity of the resin for different ions. More heavily cross-linked resins exhibit less swelling and higher exchange capacity and selectivity, but they have longer equilibration times. The charge density of polystyrene ion exchangers is so great that highly charged macromolecules such as proteins may be irreversibly bound.
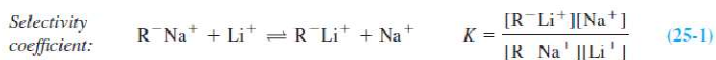
Cellulose and dextran ion exchangers, which are polymers of the sugar glucose, possess larger pore sizes and lower charge densities than those of polystyrene resins. They are well suited to ion exchange of macromolecules, such as proteins. Dextran, cross-linked by glycerin, is sold under the name Sephadex (Figure 25-2). Other macroporous ion exchangers are based on the polysaccharide agarose and on polyacrylamide. Table 25-2 lists charged functional groups used to derivatize polysaccharide hydroxyl groups. DEAE-Sephadex, for example, is an anion-exchange Sephadex containing diethylaminoethyl groups.

Because they are much softer than polystyrene *resins*, dextran and its relatives are called **gels**.

Figure 25-15 shows the structure of polyacrylamide.

Ion-Exchange Selectivity

Consider the competition of Na^+ and Li^+ for sites on the cation-exchange resin R^-:

Selectivity coefficient:
$$R^-Na^+ + Li^+ \rightleftharpoons R^-Li^+ + Na^+ \qquad K = \frac{[R^-Li^+][Na^+]}{[R^-Na^+][Li^+]} \qquad (25\text{-}1)$$

The equilibrium constant is called the **selectivity coefficient**, because it describes the relative selectivity of the resin for Li^+ and Na^+. Selectivities of polystyrene resins in Table 25-3 tend to increase with the extent of cross-linking, because the pore size of the resin shrinks as cross-linking increases. Ions such as Li^+, with a large *hydrated radius* (Chapter 7 opener), do not have as much access to the resin as smaller ions, such as Cs^+, do.
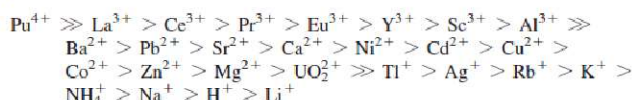
TABLE 25-3 Relative selectivity coefficients of ion-exchange resins

| Sulfonic acid cation-exchange resin | | | | Quaternary ammonium anion-exchange resin | |
| Cation | Relative selectivity for divinylbenzene content | | | Anion | Relative selectivity |
	4%	8%	10%		
Li^+	1.00	1.00	1.00	F^-	0.09
H^+	1.30	1.26	1.45	OH^-	0.09
Na^+	1.49	1.88	2.23	Cl^-	1.0
NH_4^+	1.75	2.22	3.07	Br^-	2.8
K^+	2.09	2.63	4.15	NO_3^-	3.8
Rb^+	2.22	2.89	4.19	I^-	8.7
Cs^+	2.37	2.91	4.15	ClO_4^-	10.0
Ag^+	4.00	7.36	19.4		
Tl^+	5.20	9.66	22.2		

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

Polarizability measures the ability of an ion's electron cloud to be deformed by nearby charges. Deformation of the cloud induces a dipole in the ion. The attraction between the induced dipole and the nearby charge increases the binding of the ion to the resin.

In general, ion exchangers favor the binding of ions of higher charge, decreased hydrated radius, and increased *polarizability*. A fairly general order of selectivity for cations is

$$Pu^{4+} \gg La^{3+} > Ce^{3+} > Pr^{3+} > Eu^{3+} > Y^{3+} > Sc^{3+} > Al^{3+} \gg$$
$$Ba^{2+} > Pb^{2+} > Sr^{2+} > Ca^{2+} > Ni^{2+} > Cd^{2+} > Cu^{2+} >$$
$$Co^{2+} > Zn^{2+} > Mg^{2+} > UO_2^{2+} \gg Tl^+ > Ag^+ > Rb^+ > K^+ >$$
$$NH_4^+ > Na^+ > H^+ > Li^+$$

Reaction 25-1 can be driven in either direction, even though Na^+ is bound more tightly than Li^+. Washing a column containing Na^+ with a substantial excess of Li^+ will replace Na^+ with Li^+. Washing a column in the Li^+ form with Na^+ will convert it into the Na^+ form.

Na^+ has a *smaller* hydrated radius than Li^+.

Ion exchangers loaded with one kind of ion bind small amounts of a different ion nearly quantitatively. Na^+-loaded resin will bind small amounts of Li^+ nearly quantitatively, even though the selectivity is greater for Na^+. The same column binds large quantities of Ni^{2+} or Fe^{3+} because the resin has greater selectivity for these ions than for Na^+. Even though Fe^{3+} is bound more tightly than H^+, Fe^{3+} is removed from the resin by washing with excess acid.

Donnan Equilibrium

A phase containing bound charges tends to exclude electrolyte.

When an ion exchanger is placed in an electrolyte solution, *the concentration of electrolyte is higher outside the resin than inside it.* The equilibrium between ions in solution and ions inside the resin is called the **Donnan equilibrium.**

Consider a quaternary ammonium anion-exchange resin (R^+) in its Cl^- form immersed in a solution of KCl. Let the concentration of an ion inside the resin be $[X]_i$ and the concentration outside the resin be $[X]_o$. It can be shown from thermodynamics that the ion product inside the resin is approximately equal to the product outside the resin:

$$[K^+]_i[Cl^-]_i = [K^+]_o[Cl^-]_o \qquad (25\text{-}2)$$

From considerations of charge balance, we know that

We are ignoring H^+ and OH^-, which are assumed to be negligible.

$$[K^+]_o = [Cl^-]_o \qquad (25\text{-}3)$$

Inside the resin, there are three charged species, and the charge balance is

$$[R^+]_i + [K^+]_i = [Cl^-]_i \qquad (25\text{-}4)$$

where $[R^+]$ is the concentration of quaternary ammonium ions attached to the resin. Substituting Equations 25-3 and 25-4 into Equation 25-2 gives

$$[K^+]_i([K^+]_i + [R^+]_i) = [K^+]_o^2 \qquad (25\text{-}5)$$

which says that $[K^+]_o$ must be greater than $[K^+]_i$.

Conducting Ion-Exchange Chromatography

Ion-exchange *resins* are used for applications involving small molecules (FM $\lesssim$ 500), which can penetrate the small pores of the resin. A mesh size (Table 27-2) of 100/200 is suitable for most work. Higher mesh numbers (smaller particle size) lead to finer separations but slower column operation. For preparative separations, the sample may occupy 10 to 20% of the column volume. Ion-exchange *gels* are used for large molecules (such as proteins and nucleic acids), which cannot penetrate the pores of resins. Separations involving harsh chemical conditions (high temperature, high radiation levels, strongly basic solution, or powerful oxidizing agents) employ *inorganic ion exchangers*, such as hydrous oxides of Zr, Ti, Sn, and W.

Gradient elution with increasing ionic strength or changing pH is common in ion-exchange chromatography. Consider a column to which anion A^- is bound more tightly than anion B^-. We separate A^- from B^- by elution with C^-, which is less tightly bound than either A^- or B^-. As the concentration of C^- is increased, B^- is eventually displaced and moves down the column. At a still higher concentration of C^-, the anion A^- also is eluted.

In Figure 25-3, a gradient of ammonium acetate was used for an anion-exchange separation of differently charged forms of the protein transferrin at pH 6.0. Transferrin (Figure 17-7)
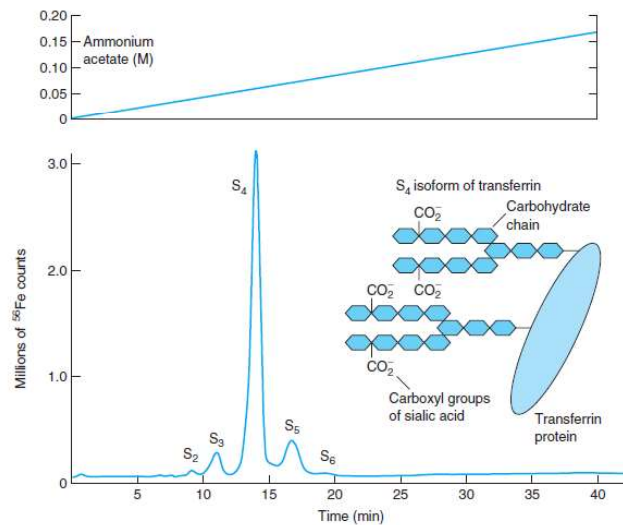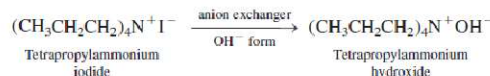
An ionic strength gradient is analogous to a solvent or temperature gradient.

FIGURE 25-3 Ionic-strength-gradient anion-exchange separation of *isoforms* S_2 to S_6 of the protein transferrin bearing two to six sialic acid groups. The 5 × 50 mm column contained Pharmacia Mono-Q resin, which consists of 10-μm-diameter beads with quaternary ammonium anion-exchange sites. [From S. A. Rodríguez, E. B. Gonzalez, G. A. Llamas, M. Montes-Bayón, and A. Sanz-Medel, "Detection of Transferrin Isoforms in Human Serum: Comparison of UV and ICP–MS Detection after CZE and HPLC Separations," *Anal. Bioanal. Chem.* 2005, *383*, 390.]

has a molecular mass near 81 000 and can bind two Fe^{3+} ions for transport through the blood. Attached to the protein are two carbohydrate chains, each terminated in one to four negatively charged sialic acid sugars. The most common form, designated S_4 in Figure 25-3, contains four sialic acid groups. Transferrin molecules containing different numbers of sialic acid sugars are called *isoforms* and are labeled S_2 through S_6 in Figure 25-3. Relative amounts of different isoforms vary with certain pathological disorders. In Figure 25-3, transferrin was detected by directing eluate into an inductively coupled plasma–mass spectrometer to detect ^{56}Fe (Section 20-6). Serum species that do not contain iron are invisible to this detector.

Applications of Ion Exchange

Ion exchange can be used to convert one salt into another. For example, we can prepare tetrapropylammonium hydroxide from a tetrapropylammonium salt of some other anion:

$$(CH_3CH_2CH_2)_4N^+I^- \xrightarrow[\text{OH}^-\ \text{form}]{\text{anion exchanger}} (CH_3CH_2CH_2)_4N^+OH^-$$

Tetrapropylammonium iodide   Tetrapropylammonium hydroxide

Ion exchange is used for **preconcentration** of trace components of a solution to obtain enough for analysis. For example, the GEOTRACES program (Box 20-2) is measuring Al, Mn, Fe, Cu, Zn, and Cd at part per trillion levels in the presence of orders-of-magnitude higher concentrations of Na^+, Ca^{2+}, and Mg^{2+} in the ocean. Trace elements are preconcentrated and separated from alkali (Group 1) and alkaline earth (Group 2) elements by passage through the Chelate-PA1 ion-exchange resin shown in Figure 25-4. Ethylenediaminetriacetic acid groups in the resin bind analyte elements quantitatively at pH 6 and above. Alkali and alkaline earth elements are weakly bound at pH 6. The procedure is to pass 125 g of seawater (adjusted to pH 6 with ammonium acetate buffer) through 0.5 g of resin and wash away loosely bound metals with 40 mL of pH 6 buffer. Strongly retained metal ions are then quantitatively eluted in the reverse direction with 15 mL of 1 M HNO_3. Trace metals are concentrated by a factor of 8 from 125 g of seawater into ~15 g of eluate, and >99.9% of alkali and alkaline earth metals are removed. Eluate is analyzed by inductively coupled plasma–mass spectrometry.
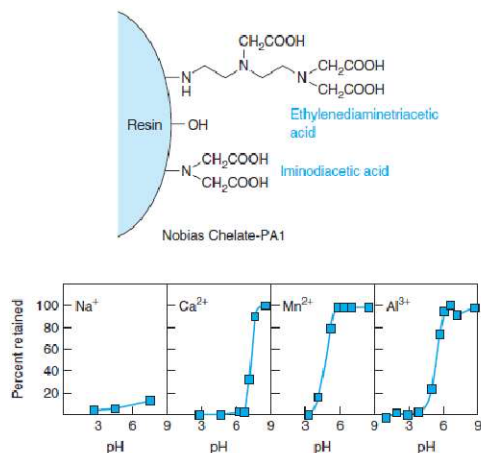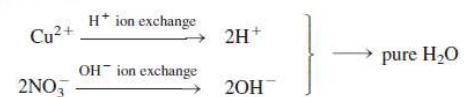

FIGURE 25-4 Nobias Chelate-PA1 resin used to preconcentrate trace metals from seawater contains ethylenediaminetriacetic acid and iminodiacetic acid groups covalently attached to hydrophilic methacrylate polymer resin. Graphs show fraction of metal ions retained by resin as a function of pH. Mn^{2+} and Al^{3+} are well retained at pH 6. Na^+ and Ca^{2+} are poorly retained at pH 6. [Data from Y. Sohrin, S. Urushihara, S. Nakatsuka, T. Kono, E. Higo, T. Minami, K. Norisuye, and S. Umetani, "Multielemental Determination of GEOTRACES Key Trace Metals in Seawater by ICPMS after Preconcentration Using an Ethylenediaminetriacetic Acid Chelating Resin," *Anal. Chem.* 2008, *80*, 6267.]

Ion exchange is used to purify water. **Deionized water** is prepared by passing water through an anion-exchange resin in its OH^- form and a cation-exchange resin in its H^+ form. Suppose, for example, that $Cu(NO_3)_2$ is present in the solution. The cation-exchange resin binds Cu^{2+} and replaces it with $2H^+$. The anion-exchange resin binds NO_3^- and replaces it with OH^-. The eluate is pure water:

$$Cu^{2+} \xrightarrow{\ H^+ \ \text{ion exchange}\ } 2H^+$$
$$2NO_3^- \xrightarrow{\ OH^- \ \text{ion exchange}\ } 2OH^-$$
$$\left. \right\} \longrightarrow \text{pure } H_2O$$

In many laboratory buildings, tap water is purified, first, by passage through activated carbon to adsorb organic material, and then by *reverse osmosis*. In the latter process, water is forced by pressure through a membrane containing pores through which few molecules larger than H_2O can pass. Ions do not pass through the pores because their hydrated radius is too large. Reverse osmosis removes 95–99% of ions, organic molecules, bacteria, and particles from water.

"Water polishing" equipment is used in many laboratories after reverse osmosis. The water is passed through activated carbon and then through several ion-exchange cartridges that convert ions into H^+ and OH^-. The resulting high-purity water has a resistivity (Chapter 14, note 37) of 180 000 ohm · m (18 Mohm · cm), with concentrations of individual ions below 1 ng/mL (1 ppb).[2]

Cation-exchange chromatography has been used to separate enantiomers (mirror image isomers) of cationic metal complexes by elution with one enantiomer of tartrate anion.[3] Tartrate has a different ion-pair formation constant with each cation enantiomer and therefore elutes one cation enantiomer from the column before the other.

In pharmaceuticals, ion-exchange resins are used for drug stabilization and as aids for tablet disintegration. Ion exchangers are also used for taste masking, for sustained-release products, as topical products for application to skin, and for ophthalmic or nasal delivery.[4]

Mirror plane

$$\begin{array}{c} CO_2^- \\ | \\ H \blacktriangleright C \blacktriangleleft OH \\ | \\ HO \blacktriangleright C \blacktriangleleft H \\ | \\ CO_2^- \end{array} \qquad \begin{array}{c} CO_2^- \\ | \\ HO \blacktriangleright C \blacktriangleleft H \\ | \\ H \blacktriangleright C \blacktriangleleft OH \\ | \\ CO_2^- \end{array}$$

(+)-Tartrate   (−)-Tartrate