Ch. 23 Fundamentals of Analytical Separations

Separation

- Samples are usually complex mixtures. In order to identify and quantify the components of a mixture, we have to separate the components in the mixture.
- Separation methods
  - Extraction
  - Chromatography
  - Electrophoresis

Solvent Extraction

- The transfer of an analyte from one phase to a second based on the relative solubility of the analyte in two immiscible liquids.

\[ K = \frac{[S]_2}{[S]_1} = \frac{(1-q)m/V_2}{qm/V_1} \]

- \( K \): the partition coefficient for distribution of S between the two phases at equilibrium;
- \( m \): the moles of S in the system
- \( q \): the fraction of S remaining in phase 1;

Extraction Efficiency

- A solute S has a partition coefficient of 3 between toluene and water. If you have 100 mL of a 0.010 M solution of S in water.
  (1) What fraction of the solute remains in H₂O after a 500 mL extraction with toluene?
  \[ q = \frac{100}{100 + (3)(500)} = 0.062 \approx 6\% \]
  (2) What fraction of the solute remains in H₂O after a 5-100 mL extractions with toluene?
  \[ q = \left( \frac{100}{100 + (3)(100)} \right)^n \approx 0.00098 = 0.1\% \]
- It is more efficient to do several small extractions than one big extraction.

\[ q^n = \frac{V_1}{V_1 + KV_2} \]

\[ K = \frac{[S]_2}{[S]_1} = \frac{(1-q)m/V_2}{qm/V_1} \]

\[ q^n = \frac{V_1}{V_1 + KV_2} \]

If \( q = 1/4 \), then 1/4 remains in phase 1 after one extraction

\[ K \]: the partition coefficient; \( q \): the fraction of S remaining in phase 1; \( n \): the # of extractions.

pH Effects

- The charge changes of an acid or base as the pH is changed.
- Distribute coefficient (D): an alternate form of the partition coefficient.

\[ D = \frac{\text{Total conc. in phase 2}}{\text{Total conc. in phase 1}} = \frac{C_2}{C_1} \]

- A basic amine whose neutral form, B, has partition coefficient, \( K \), between phases 1 and 2. The conjugated acid BH⁺ is soluble only in phase 1 and the acid dissociation constant is \( K_a \).

\[ K = \frac{[B]}{[BH^+]}, \quad K_a = \frac{[B][H^+]}{[BH^+]} \]
**pH Effects**

\[
D = \frac{\text{Total conc. in phase 2}}{\text{Total conc. in phase 1}} = \frac{C_2}{C_1} \quad Ka = \frac{[B][H^+]}{[BH^+]}, \quad K = \frac{[B][H^+]}{[B]^+}
\]

\[
D = \frac{[B]}{[B] + [BH^+]} = \frac{K_a}{K_a + [H^+]}
\]

\[
D = \frac{[HA]^+}{[HA] + [A^-]} = K = \frac{[HA]^+}{[HA]}
\]

\[
K_a = \frac{[A^-][H^+]}{[HA]}
\]

\[
\alpha = \text{fraction of the species (P.191)}
\]

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**pH Effects**

• K for an amine B is 3.0 and the Ka for BH+ is 1.0×10^-9. If 50.00 mL of 0.010 M aqueous amine is extracted with 100 mL of solvent, calculate the % remaining in the aqueous phase in M at (1) pH 10.00; (2) pH 8.00.

\[
pH = 10.00: D = \frac{K_a}{K_a + [H^+]} = \frac{3.0 \times 10^{-6}}{1.0 \times 10^{-9} + 1.0 \times 10^{-9}} = 2.73
\]

\[
q = \frac{V}{V' + DV'} = \frac{50}{50 + 2.73 \times 100} = 0.15 \Rightarrow 15\%
\]

\[
pH = 8.00: D = \frac{K_a}{K_a + [H^+]} = \frac{3.0 \times 10^{-6}}{1.0 \times 10^{-9} + 1.0 \times 10^{-9}} = 0.273
\]

\[
q = \frac{V}{V' + DV'} = \frac{50}{50 + 0.273 \times 100} = 0.65 \Rightarrow 65\%
\]

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**pH Effects**

• The weak acid HA with Ka = 1.0×10^-5 is to be quantitatively extracted from an aqueous sample (100 mL sample volume) using 100 mL of a suitable organic solvent, by doing a single extraction. The partition coefficient is 30.0. What is the maximum pH allowed in order that no more than 5% of the acid remains in the aqueous solution?

\[
q = \frac{V}{V' + DV'} = \frac{100}{100 + D \times 100} = 5\%
\]

\[
\Rightarrow D = 19
\]

\[
D = \frac{[H^+]}{K_a + [H^+]} = \frac{30 \times [H^+]}{1.0 \times 10^{-5} + [H^+]} = 19
\]

\[
\Rightarrow [H^+] = 1.73 \times 10^{-4} M
\]

\[
\Rightarrow pH = 4.76
\]

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**Extraction with a Metal Chelator**

• Usually neutral complexes can be extracted into organic solvents. Charged complexes (e.g. MEDTA^2-) are not very soluble in organic solvents.

• Commonly used: dithizone, 8-hydroquinoline, and cupferron.

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**Extraction with a Metal Chelator**

• Each ligand can be presented as a weak acid, HL.

• Mn^2+ is in the aqueous phase and MLn is in the organic phase.

• The distribution coefficient (D) for metal ion extraction depends on pH and [ligand].

• By select a pH, you can bring the metal into either phase.
Chromatography

- A separation process based on the various partitioning coefficients of different solutes between the two phases.
- Involving the interaction of solute(s) and two phases; operates on the same principle as extraction, but one phase is held in place while the other moves past it.
- **Mobile phase**: A gas or liquid that moves through the column.
- **Stationary phase**: A solid or liquid that remains in place.

**Type of Chromatography**

- Based on the mechanism of interaction of the solute with the stationary phase

1. **Adsorption chromatography**
   - Solute is adsorbed on the surface of the stationary phase (solid).
   - The stronger a solute adsorbs, the longer it takes to travel through the chromatography column.

2. **Partition chromatography**
   - GC
   - The partitioning of solutes between a mobile phase (gas) and bonded liquid stationary phase.

3. **Ion-exchange chromatography**
   - Retention is based on the attraction between solute ions and charged sites bound to the stationary phase (ionic interactions to separate ions).
   - A stationary phase of cations will separate anions and vice versa.

4. **Molecular Size exclusion chromatography**
   - size exclusion, gel filtration, or gel permeation chromatography
   - separate molecules by size
   - large molecules pass through faster (they do not get caught up in pores)

5. **Affinity chromatography**
   - Specific interactions of one kind of solute molecule to a second molecular that is covalently attached to the stationary phase
   - Most selective (e.g., use antibodies to select out one protein from a mixture of hundreds)