

Chapter 23 Introduction to Analytical Separations

Homework Due Monday April 24

Problems 23-1, 23-2, 23-7, 23-15, 23-27, 23-29, 23-32

Analytical Separations: Universal approach to analyzing complex mixtures

- Most analytical problems involve the analysis of mixtures
- Mixtures present problems when determining what is present and how much.
- Analytical techniques are based on some physical or chemical characteristic that is common to a variety of compounds.
- Utilizing a specific characteristic to identify or quantify a specific analyte is difficult if many of the components present in the sample have that same characteristic (for example, similar masses, similar bonds, similar electronic structures).
- Analytical separations provides a way to isolate individual components of the mixture for further analysis.

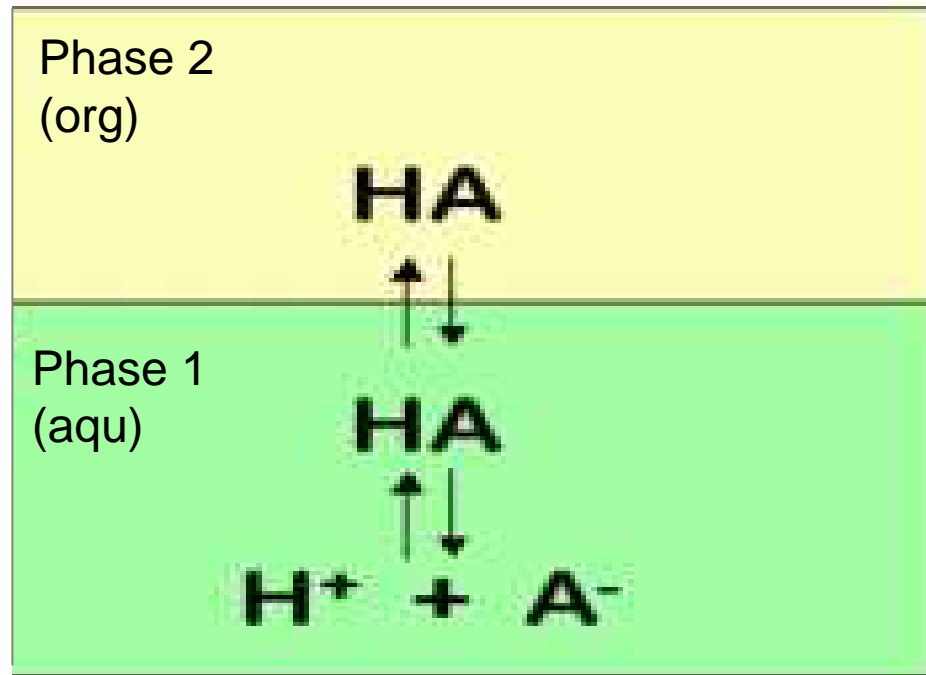
How would you determine the following:

- identity of accelerant at a suspected arson scene
- amount of caffeine in Coca Cola
- identifying active ingredient in an illicit drug preparation (LSD is heat sensitive)
- purification and characterization of novel thermophilic plant enzyme from South America
- identifying explosive materials used in Oklahoma bombing

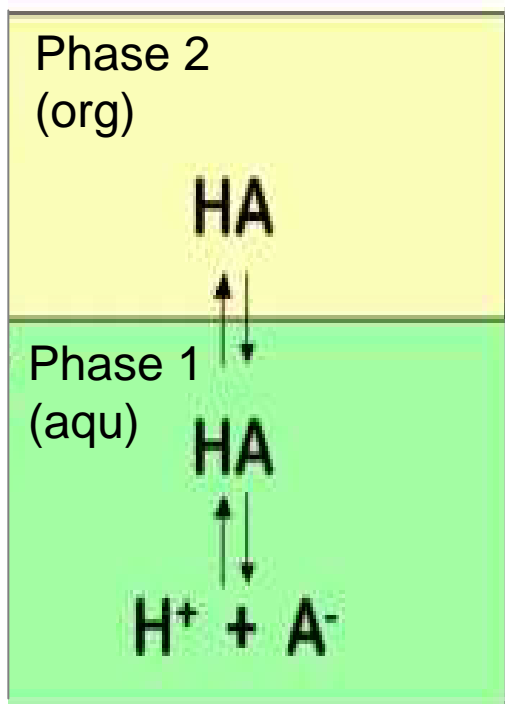
Simple Separation: Solvent Extraction

The basis of solvent extraction is the relative solubility of an analyte in two immiscible liquids.

For example: weak acid (HA)



Solvent extraction is used to remove interferences, concentrate analytes, produce measurable forms of specific analytes.



The distribution of the analyte between the 2 phases can be described by the partition coefficient:

$$K = \mathcal{A}_2 / \mathcal{A}_1 \approx [\text{HA}]_2 / [\text{HA}]_1$$

(where 1 is aqueous phase and 2 is organic phase)

Fraction of analyte partitioned in aqueous phase (1):

$$q = V_1 / (V_1 + KV_2)$$

where V_1 is the volume of the aqueous phase and V_2 is the volume of the organic phase.

Example

An analyte with a partition coefficient of 4.0 is extracted from 10 mL of the aqueous phase (phase 1) into an organic phase (phase 2). What volume of the organic phase is needed to extract 99% of the analyte in one extraction?

Fraction in aqueous phase $\Rightarrow q = V_1 / (V_1 + KV_2)$

Extracting 99% into organic phase means 1% in aqueous phase, so

$$0.01 = 10 \text{ mL} / (10 \text{ mL} + 4.0 \times V_2)$$

Volume of organic phase needed: $V_2 = 248 \text{ mL}$

Extraction Efficiency

What is the most efficient way to get the analyte extracted?

- Choices:
- a) one extraction with lots of volume
 - b) lots of extractions with smaller volumes

$$q = [V_1 / (V_1 + KV_2)]^n$$

where n is number of extractions

Example (similar to previous example)

An analyte with a partition coefficient of 4.0 is extracted from 10 mL of the aqueous phase (phase 1) into an organic phase (phase 2). What volume of the organic phase is needed to extract 99% of the analyte in three extractions using equal volumes?

Frac. in aqueous phase $\Rightarrow q = [V_1 / (V_1 + KV_2)]^n$

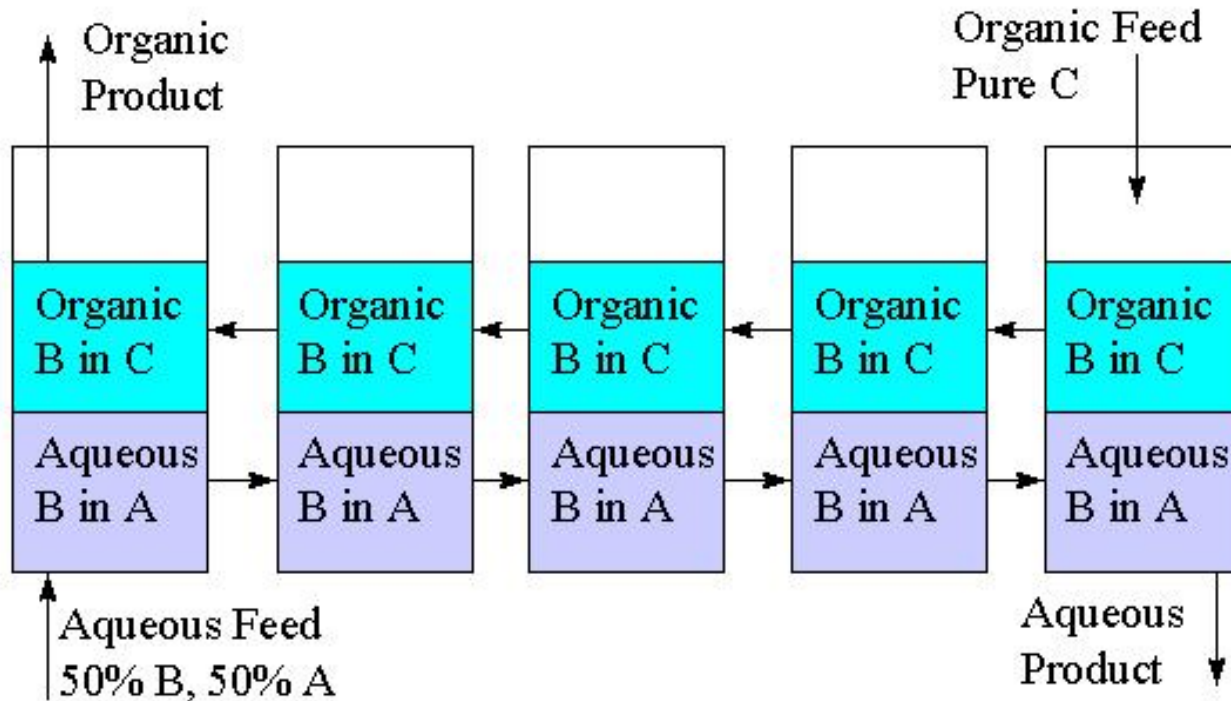
$$0.01 = [10 \text{ mL} / (10 \text{ mL} + (4.0) V_2)]^3$$

Volume of organic phase needed: 9.1 mL for each extraction or total volume of 27.3 mL

(compared to 248 mL for 1 extraction)

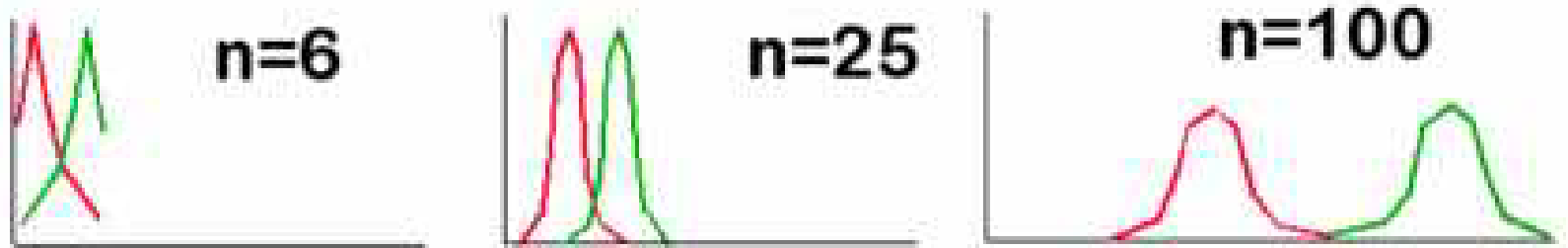
Multiple extractions can isolate many species from a mixture.

- The two feeds contact each other counter currently.
- In each unit, the aqueous and organic phases are in equilibrium.
- The two phases are drawn off separately and each is sent on to the next unit.
- The mole fraction of B in the aqueous phase decreases progressively from left to right, as B is extracted in each unit.
- The organic phase enters as pure solvent, and as it proceeds from right to left, it becomes progressively richer in B.



“movement” of analytes with successive extractions

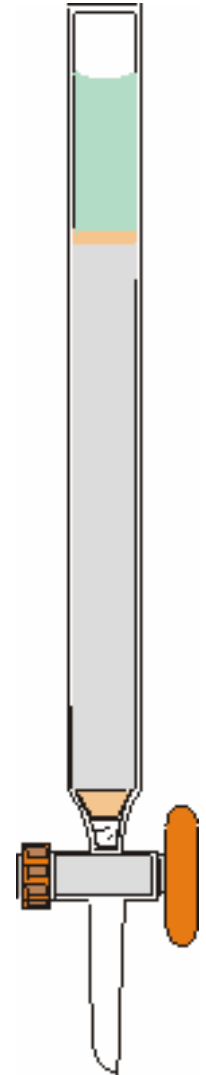
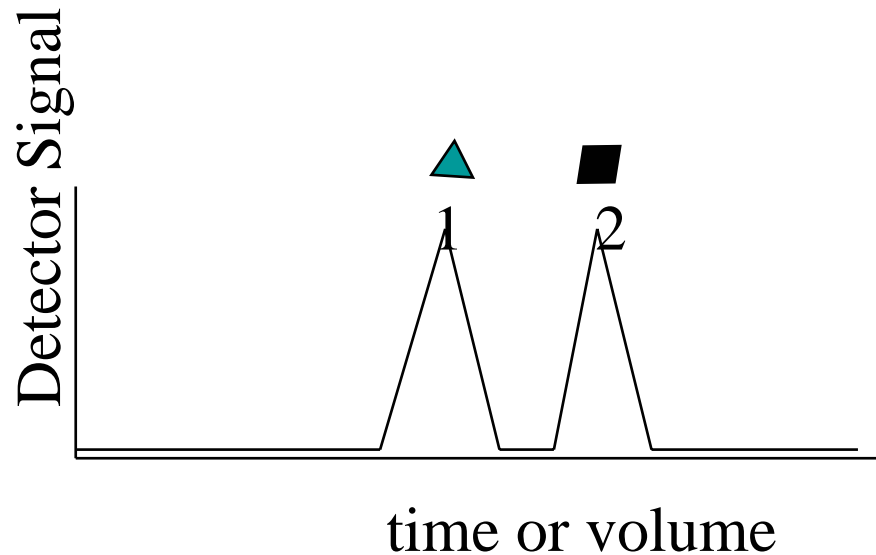
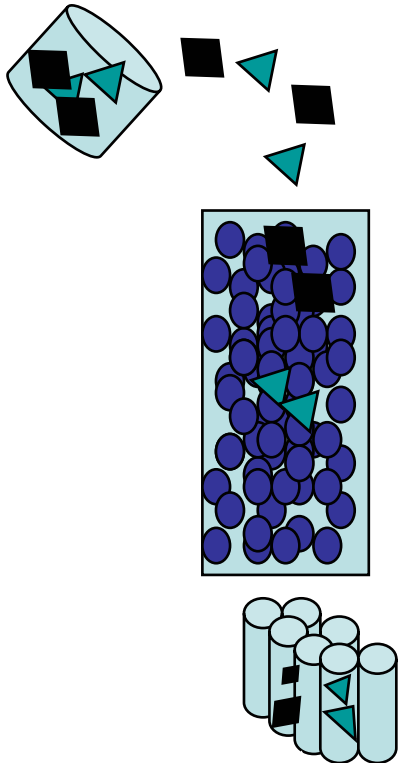
As the number of tubes are increased, the distribution of solutes appears more Gaussian. Ultimately, you can resolve them.



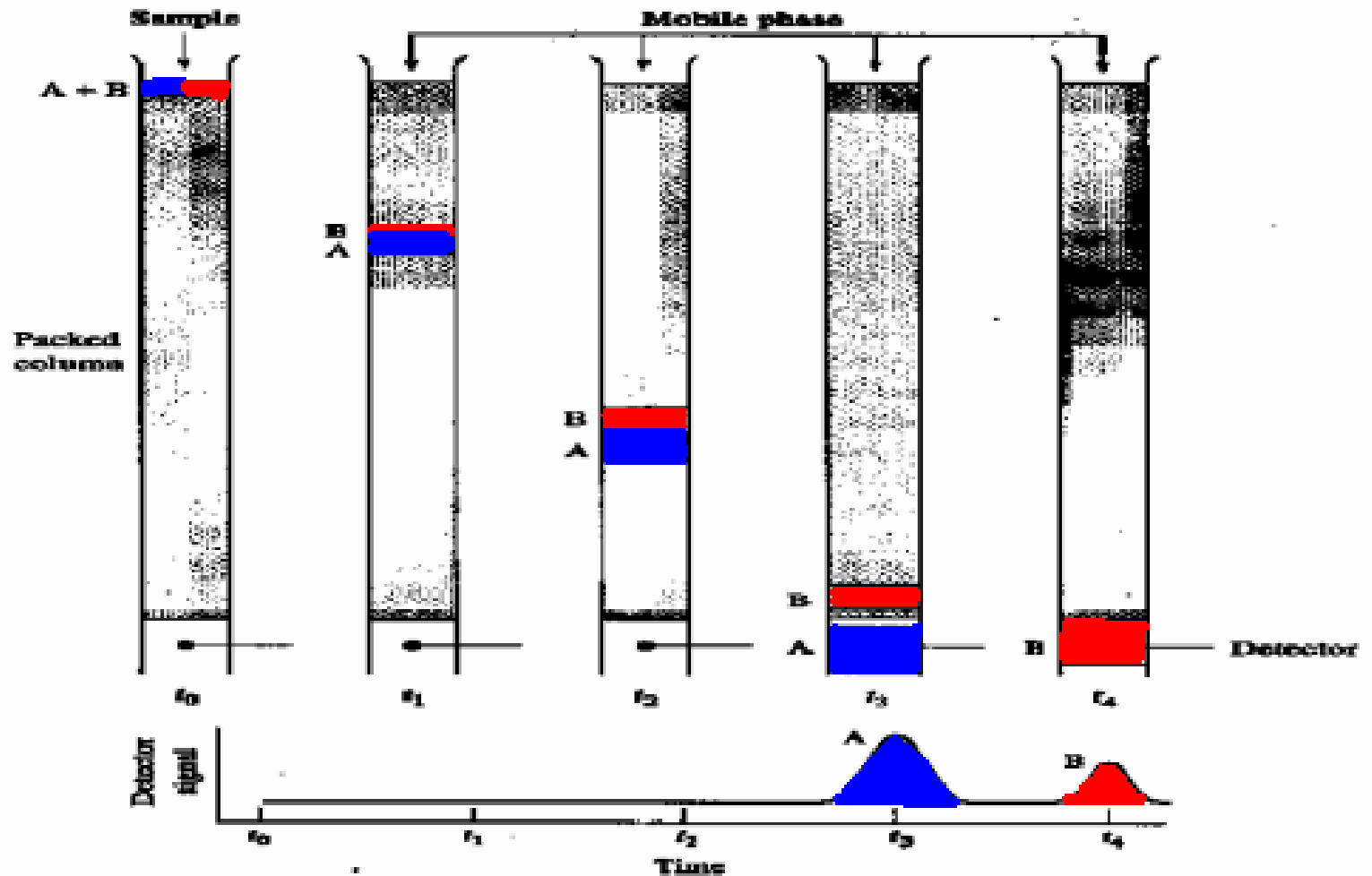
The peaks also become broader and shorter - they are distributed over a larger range of tubes.

What is chromatography?

Separation of components of a mixture by exploiting differences in partitioning between a stationary phase and a mobile phase.



Column Chromatography



Purpose of Chromatography

- **Analytical** - determine chemical composition of a sample
- **Preparative** - purify and collect one or more components of a sample

Classification of Methods

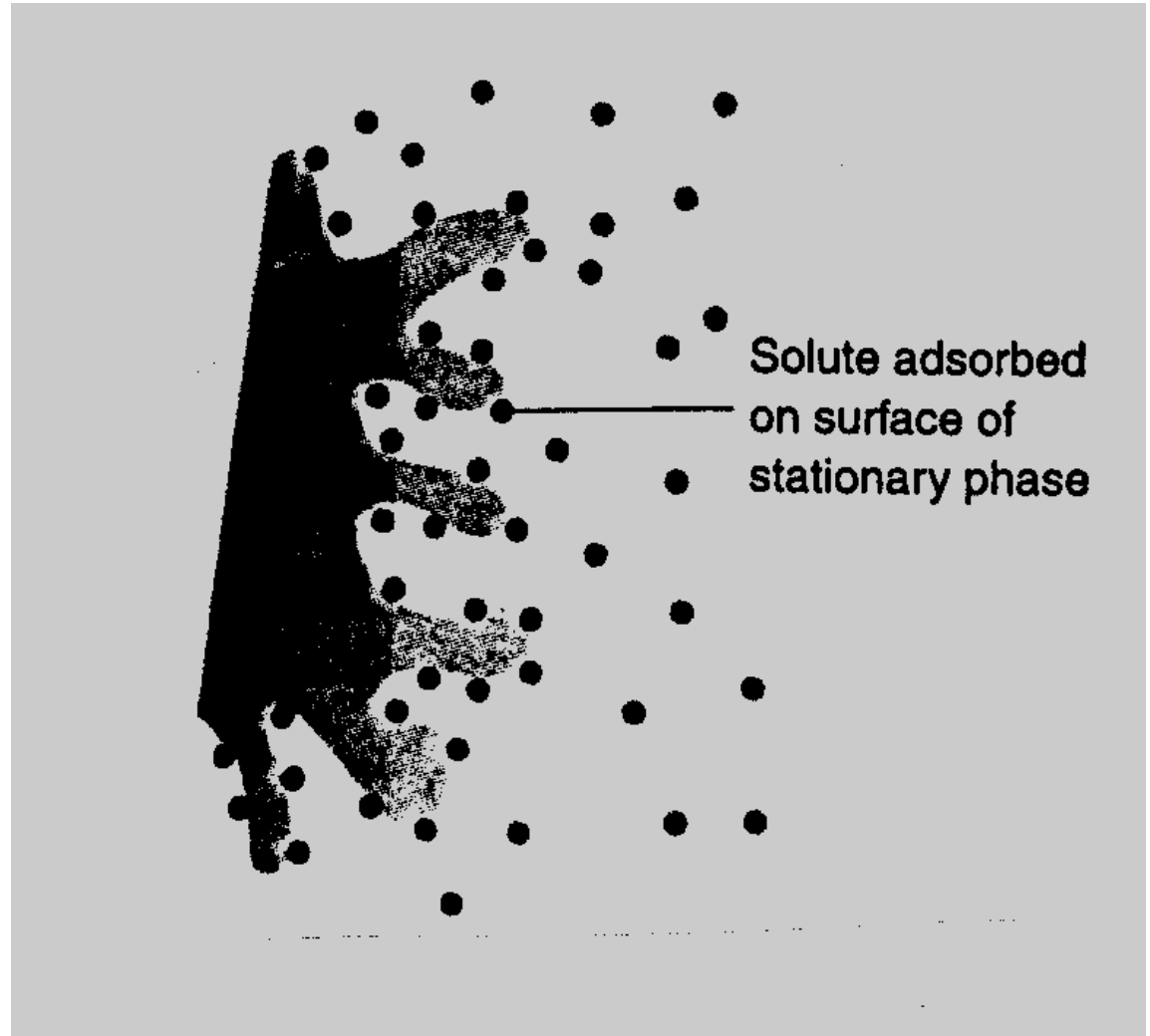
- There are two classification schemes:
 - mobile phase
 - attractive forces (stationary phase)

Classification Based on Attractive Forces

- Adsorption - for polar non-ionic compounds
- Ion Exchange - for ionic compounds
 - Anion - analyte is anion; bonded phase has positive charge
 - Cation – analyte is cation; bonded phase has negative charge
- Partition - based on the relative solubility of analyte in mobile and stationary phases
 - Normal – analyte is nonpolar organic; stationary phase MORE polar than the mobile phase
 - Reverse – analyte is polar organic; stationary phase LESS polar than the mobile phase
- Size Exclusion - stationary phase is a porous matrix; sieving

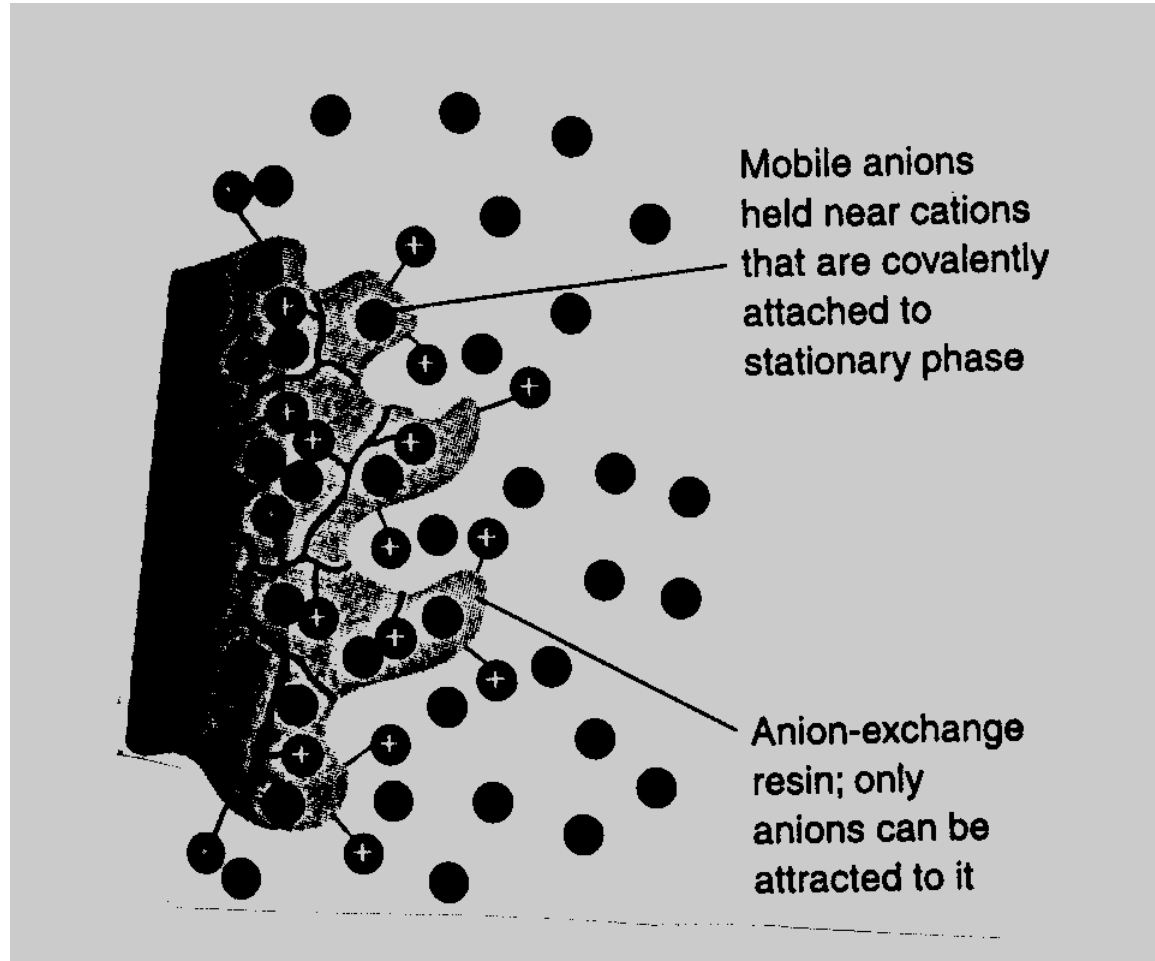
Adsorption chromatography

- Interactions are via hydrogen bonding, dipole-dipole interactions
- Examples: silica gel, activated alumina



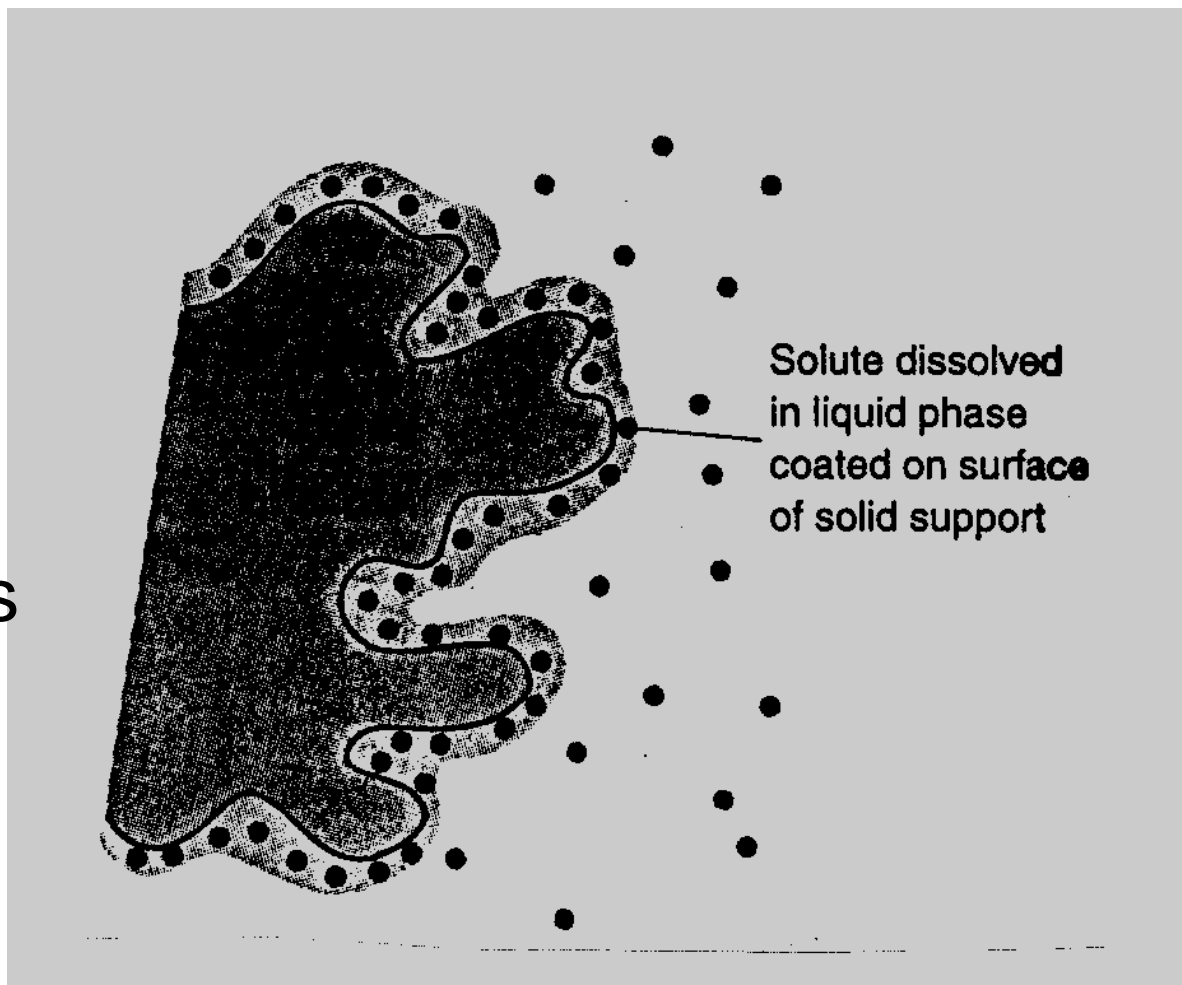
Ion exchange chromatography

- Stationary phase has fixed ions - must be associated with “exchange-able” ions of opposite charge
- Very important in water purification, preparative separations



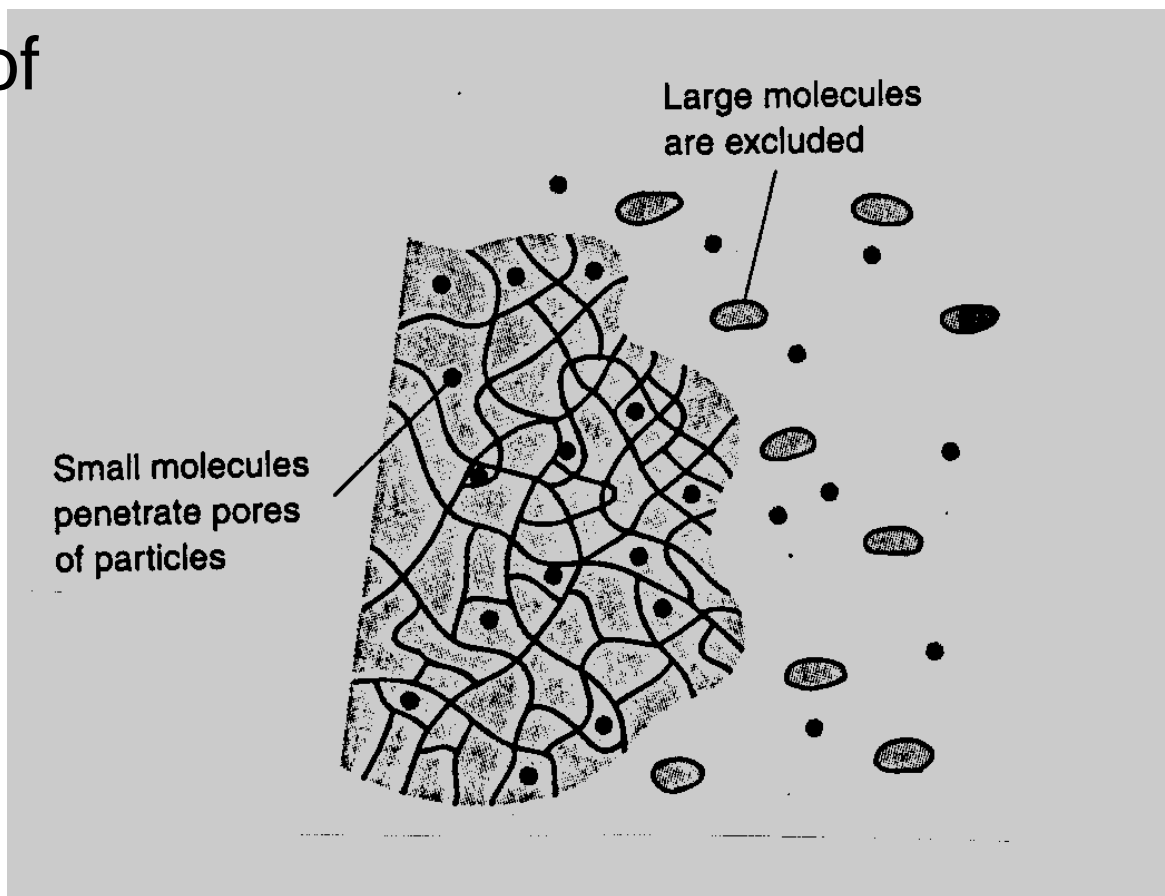
Partition chromatography

- Molecules are retained by dissolution in the coated or bonded liquid phase
- All types of solute-solvent interactions possible
- Examples: C_{18} HPLC phases, many common GC stationary phases



Exclusion chromatography

- Stationary phase has pores ~ size of molecular dimensions
- Large molecules excluded, elute quickly; smaller molecules “retained”
- Common in separations of macromolecules by HPLC



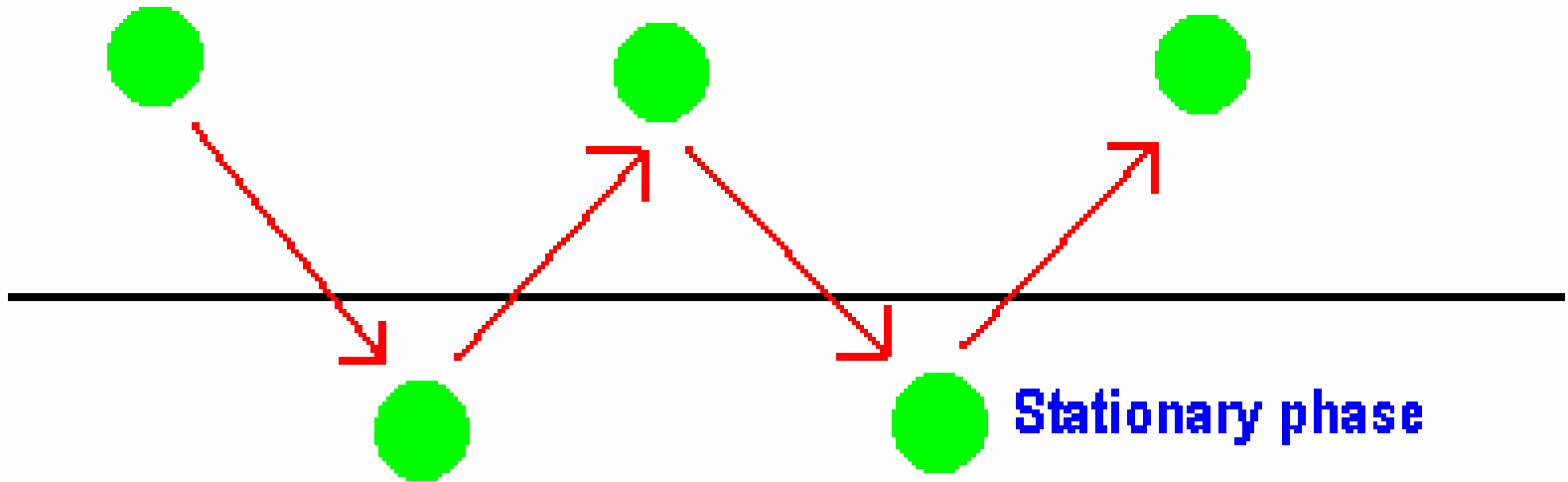
What is chromatography?

The separation of a mixture by distribution of its components between a mobile and stationary phase over time

- Mobile phase - phase that moves through chromatograph
 - gas (GC)
 - water (LC)
 - organic solvent (LC)
 - supercritical fluid (SCFC)
- Stationary phase - column; phase that is stationary in chromatograph that has a bonded phase with reactive groups imparted to stationary phase in order to achieve selectivity

A simple conceptual model of dynamic interactions in chromatographic separations

Mobile phase =====>



Degree of retention is determined by the relative amounts of time the analyte spends in each phase

Mobile phase - phase that moves analyte along the solid phase and through the chromatograph

Mobile phases:

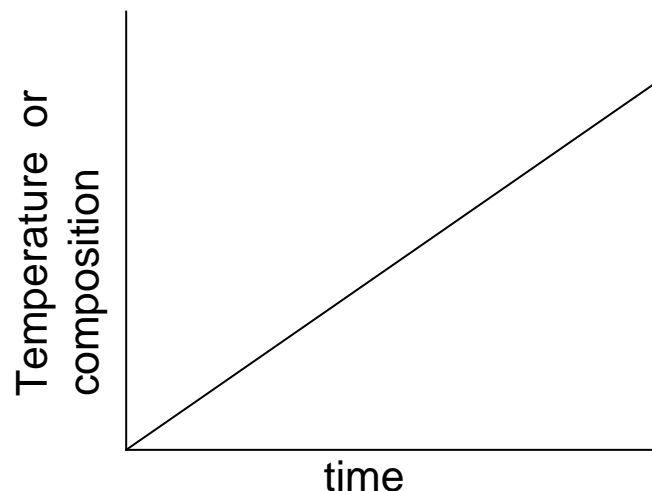
- gas (GC)
- water (LC)
- organic solvent (LC)
- supercritical fluid (SCFC)

How to affect the partitioning (interactions) in the mobile phase:

- temperature (GC)
- gradient (mix) of solvents (LC)

Gas mobile phase:

- Temperature is the most conveniently manipulated variable that controls/affects retention
- Use temperature programming to optimize chromatography for solutes of varying boiling points



Liquid mobile phase:

- Single solvent used as mobile phase (isocratic elution)
- Two or more solvents used as the mobile phase by continuously changing the solvent composition to improve the interaction with the mobile phase (gradient elution)