Analytical Separations

Rarely is a sample collected in a form ready for analysis

- need sample preparation
- must deal with sample matrix and possible interferents

Possible solutions

- Do wet-chemical methods (precipitation, complexation, etc.) to "purify" sample
- Design analysis that discriminates against interferents
  - find the "magic" wavelength for spectroscopy, etc.
- Design a method that allows you to look at the sample one component at a time
  - Separations:
    - Chromatography
    - Electrophoresis

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Chromatography

Chromatographic separations rely on varying equilibria for the distribution of analyte between a *mobile phase* and a *stationary phase*.

$$A_{m.p.} = A_{s.p.} \qquad K = \frac{[A_{s.p.}]}{[A_{m.p.}]}$$

- K =*partition coefficient*

- Different "types" of chromatography result from different mobile phase:stationary phase combinations

Often in chromatography, the equilibrium condition is not achieved, but tendency still drives separation

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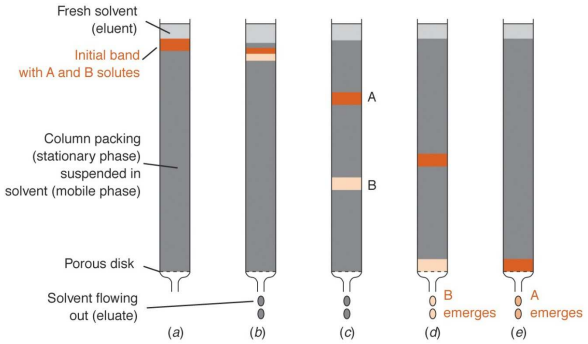
Generic Separations Experiment

- Mixture introduced as narrow “band”
- Differing partition coeff. result varying migration times
- On elution, bands are broader than initial band



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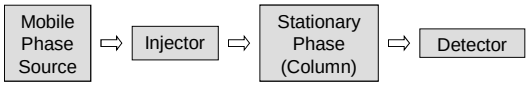
Generic Separations Experiment

Basic Instrument Components



Individual components vary depending on the “Type” of separation

- “Type” of interaction/physical property that separation is driven by.
- Hydrophobic/hydrophyllic, ionic, size, boiling point…

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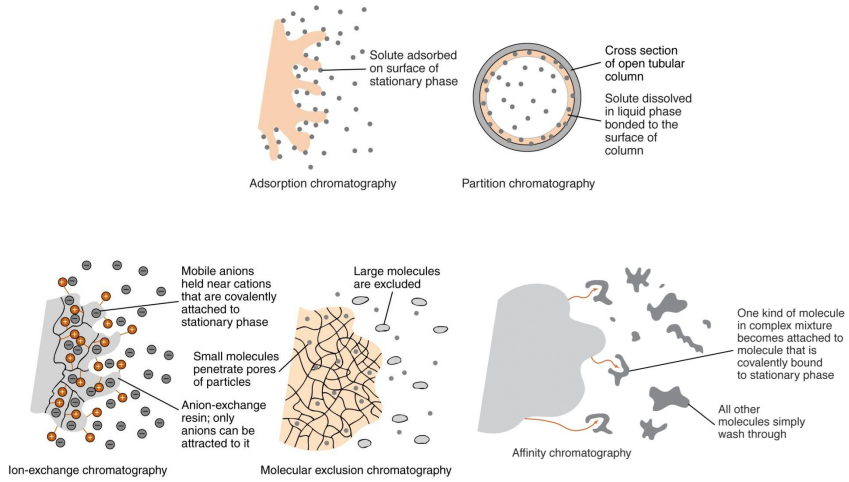
Types of Separation Modes

Analytes may be in the gas or liquid phase



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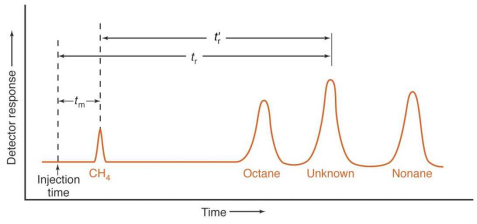
Quantitative Descriptions of a Separation



Retention Time:

Adjusted Retention Time:

Dead Time:

Capacity Factor (retention factor): Tendency of compound to remain on the column.

$$k_A' = \frac{t_R - t_M}{t_M}$$

Selectivity Factor: Tendency of compound to remain on the column (compared to other compounds).

$$\alpha = \frac{K_A}{K_B} = \frac{k_A'}{k_B'} =$$

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Describing the Efficiency of a Separation

Describing column efficiency using *theoretical plates*.

- What is a theoretical plate???
- The more theoretical plates we can stuff into a column, the better a separation should be.

$$N = \frac{t_R^2}{\sigma^2} \approx \frac{16 t_R^2}{w^2} = \frac{5.55 t_R^2}{w_{1/2}^{\ 2}} \qquad H = \frac{L}{N}$$

Resolution

- What determines resolution?

$$R = \frac{\Delta t_R}{\overline{w}} \approx \frac{0.589 \Delta t_R}{\overline{w}_{1/2}} \qquad R = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_B'}{1 + \overline{k'}}\right)$$

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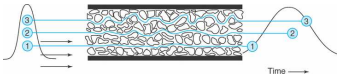
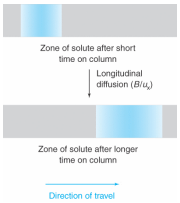
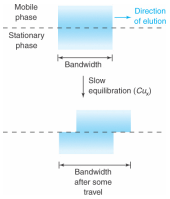
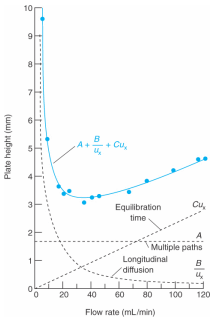
Factors that Influence Peak Shape and Efficiency

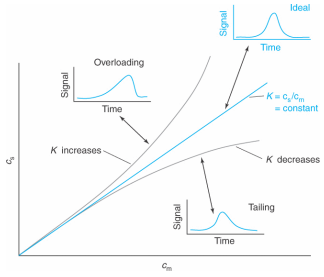
$$H \approx A + \frac{B}{u} + Cu$$











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Separations Odds and Ends

Important realization: ***REAL ≠ IDEAL***

- Peaks aren't perfectly shaped
- Equilibria aren't always fast
- Diffusion plays a role
- General Elution Problem: Optimum conditions for two compounds won't be optimum for others

GC vs. LC

- Requirements
- Benefits and challenges

Qualitative and Quantitative Analysis with Separations

Peak Height vs. Peak Area for Quantitative Analysis

- Peak area is more reliable
- Peak height is easier to measure!

The smaller plug you inject, the narrower the peaks will be.

Dead Volume

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So You Think You Understand?

- You are comparing chromatograms of two samples run under the same set of conditions; both show peaks at the same retention time. Does this mean that both samples contain the same compound?

- In one chromatogram, peaks corresponding to two components have the same area. Does this mean that the two components are present at the same concentration?

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