CHE680
Advanced Analytical Chemistry
Lecture 8

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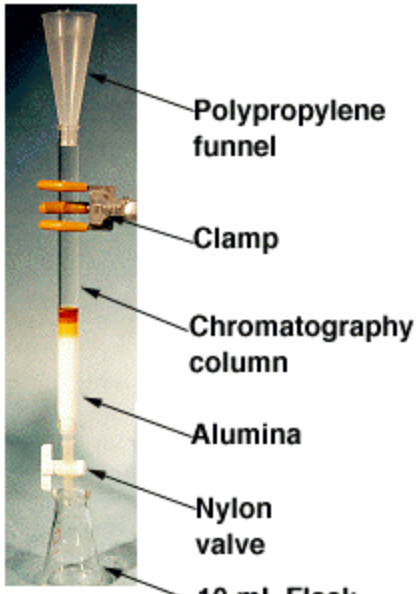
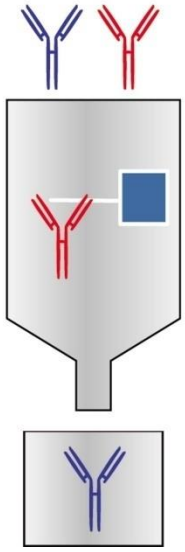
Separation and Chromatography

Paper chromatography

Thin layer chromatography (TLC)





Affinity chromatography

HPLC

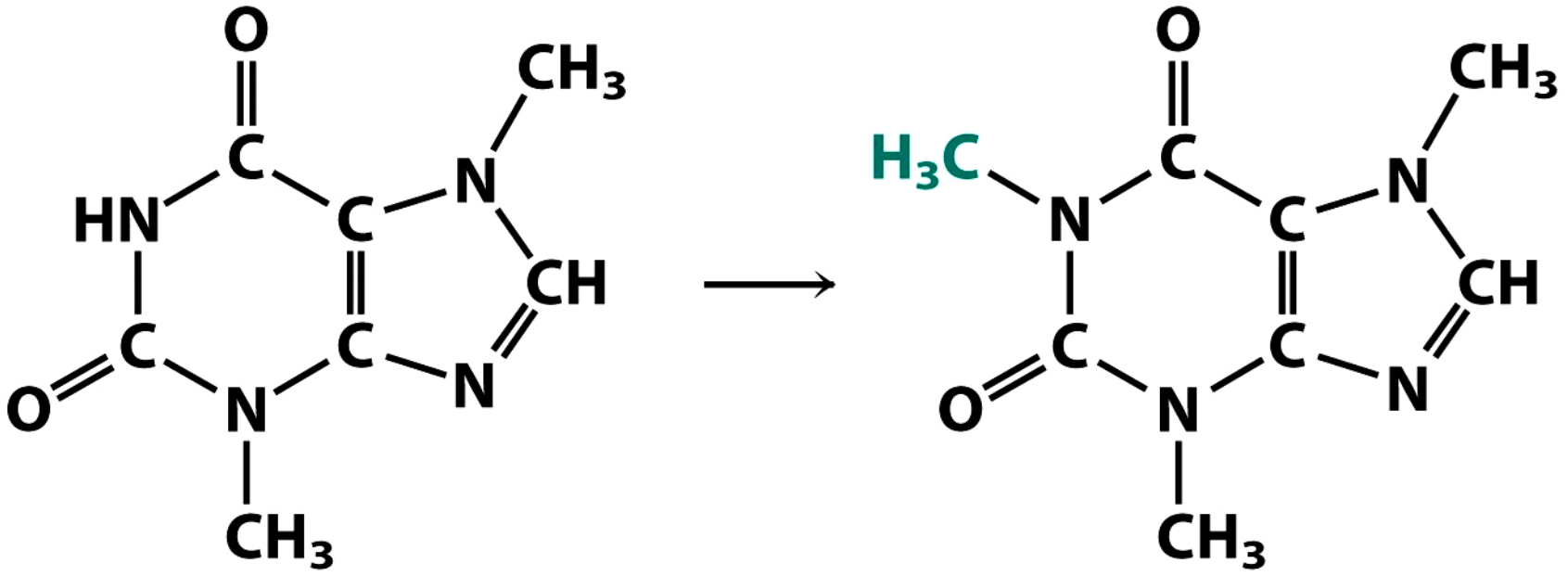
GC/MS

Separation and Chromatography

- Sample Preparation
- Basics in Chromatography

Problem:
How much caffeine in a chocolate bar?

Target Compounds: Caffeine and its Precursor



Theobromine

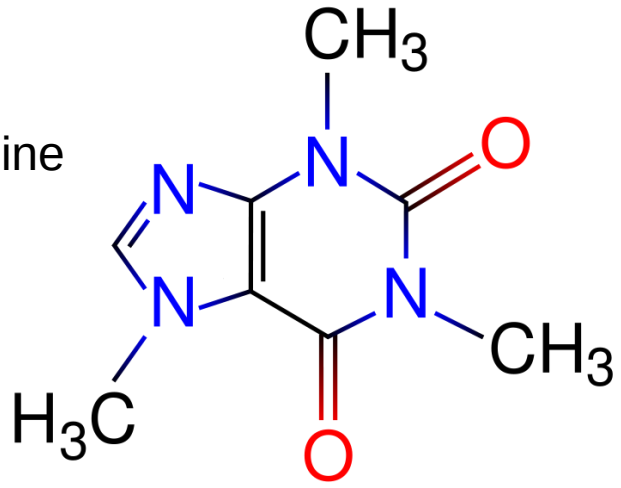
Diuretic, smooth muscle relaxant, cardiac stimulant, and vasodilator

Caffeine

Central nervous system stimulant and diuretic

Physical and Chemical Properties of Caffeine and Theobromine

Caffeine

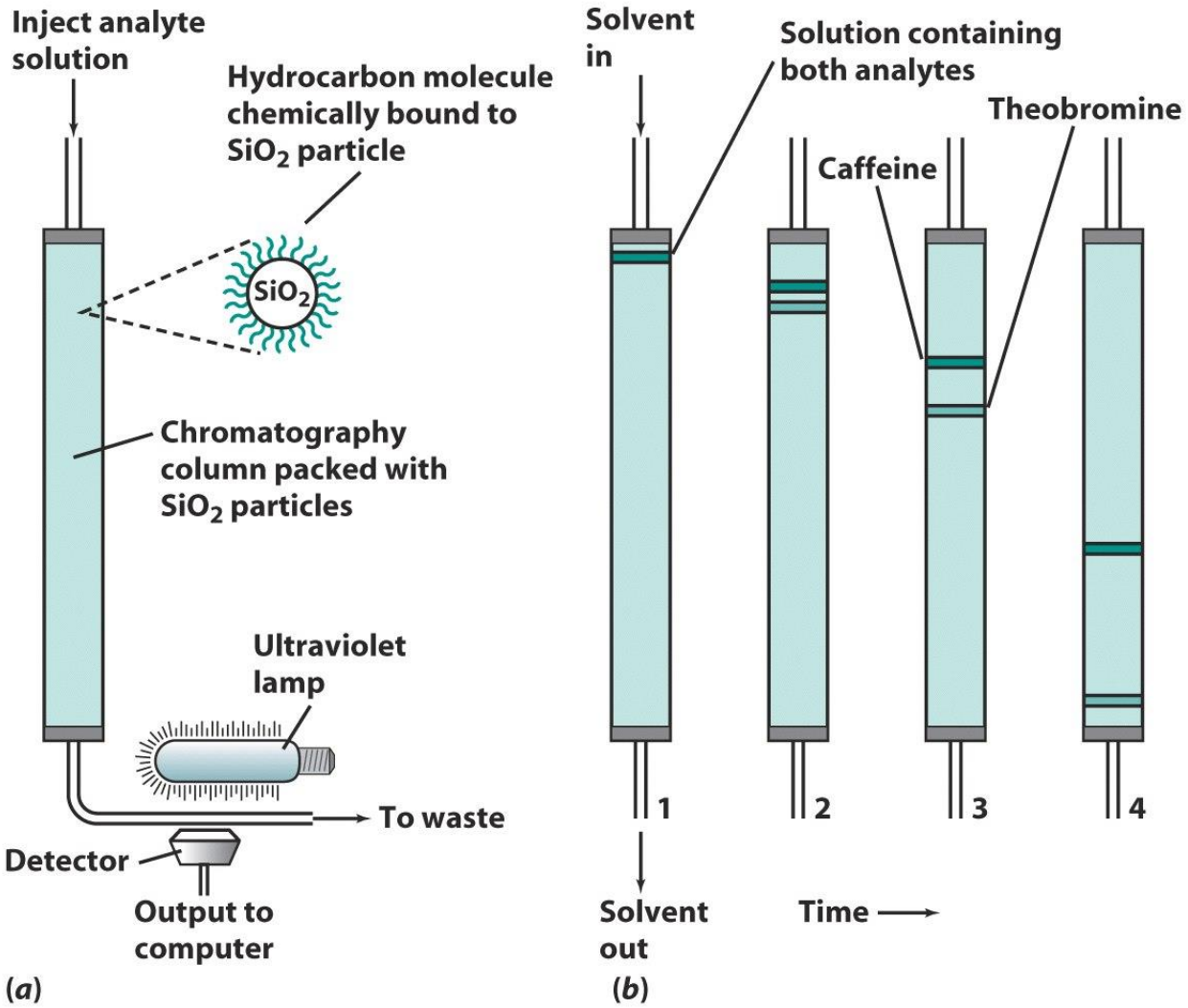


Molecular formula	$C_8H_{10}N_4O_2$
Molar mass	194.19 $g \cdot mol^{-1}$
Appearance	Odorless, white needles or powder
Density	1.2 $g \cdot cm^{-3}$, solid
Melting point	237 °C (non-equilibrium, superheated)
Boiling point	178 °C (sublimes)
Solubility in water	22 $mg \cdot mL^{-1}$ (25 °C) 180 $mg \cdot mL^{-1}$ (80 °C) 670 $mg \cdot mL^{-1}$ (100 °C)
Acidity (pK_a)	−0.13 – 1.22

What's the best analytical technique? and why?

- We know targets already
- We need to know quantity
- Chromatography: separation and quantification
- Gas Chromatography? Why?
- Liquid Chromatography? Why?
- Other Chromatographic Techniques?

Analysis of Caffeine and Theobromine by Liquid Chromatography



Sample Preparation

1. Samples are often complex **matrix** (e.g., alcohol in blood, cocaine in urine, caffeine in chocolate bar, pesticides in fruit).

2. Raw samples (e.g., blood, chocolate, urine, fruit, etc) can't be injected to instrument directly (damage to instrument, signal interference, signal reduction (matrix effect), etc).

3. Clean up is REQUIRED (sample preparation).

   - Target compound(s) can be separated or interference(s) can be removed from samples.

   - Separation via the differences in physical or chemical properties of the individual components.

   - Solubility, boiling point, pK_a, and vapor pressure, etc.

Extractions

1. Two immiscible phases (aqueous/organic or liquid/solid) to separate a solute from one phase into the other.

2. Boiling tea leaves in water extracts the tannins, theobromine, and caffeine out of the leaves and into the water (solid/liquid extraction).

3. Organic compounds out of an aqueous phase or vice versa.

4. Distribution of a solute (target) between two phases is an equilibrium condition described by **partition theory**.

Partition Theory

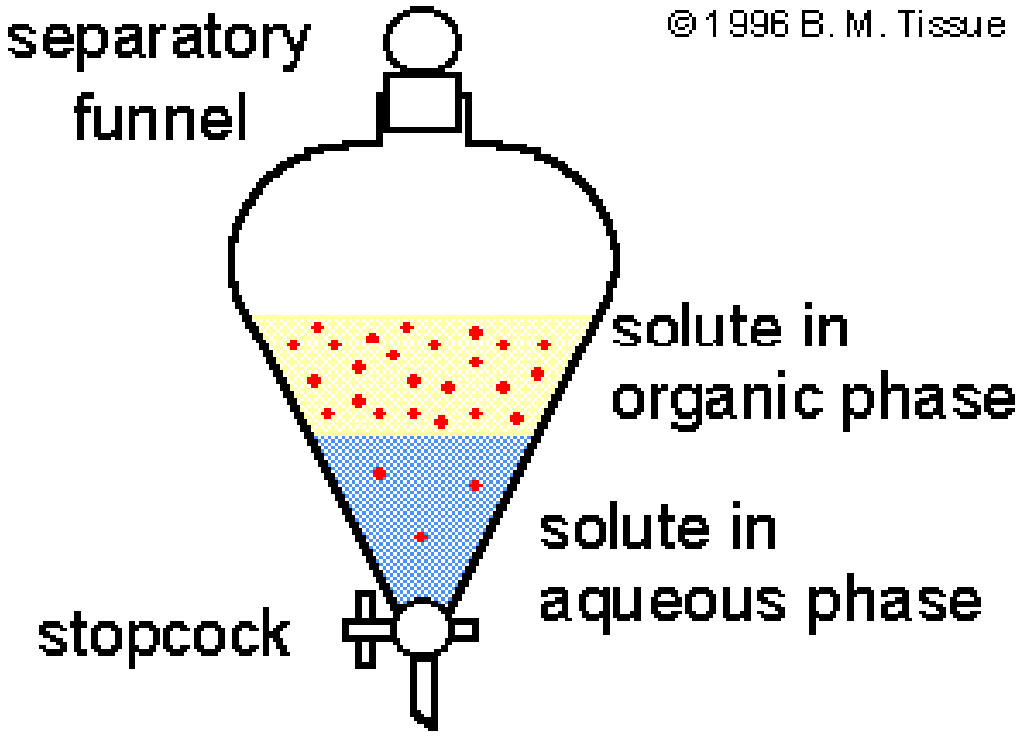
Distribution of a solute, *S*, between two immiscible solvents (such as aqueous and organic phases) is an equilibrium condition that is described by the following equation:

$S_{aq} <==> S_{org}$

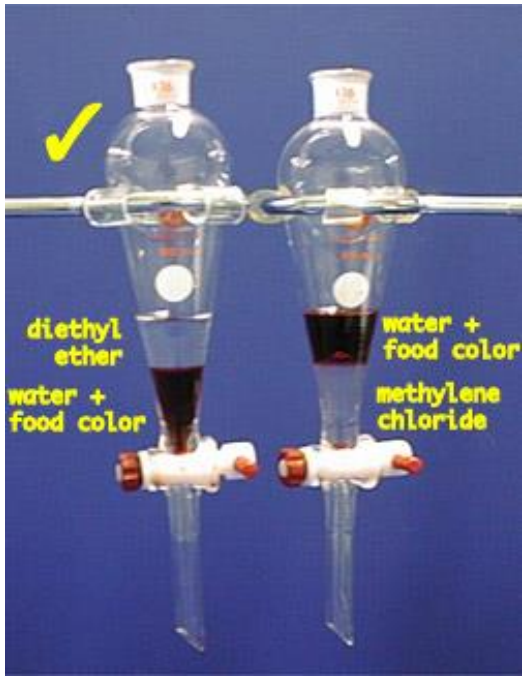
The equilibrium constant :

$K = [S_{org}]/[S_{aq}]$

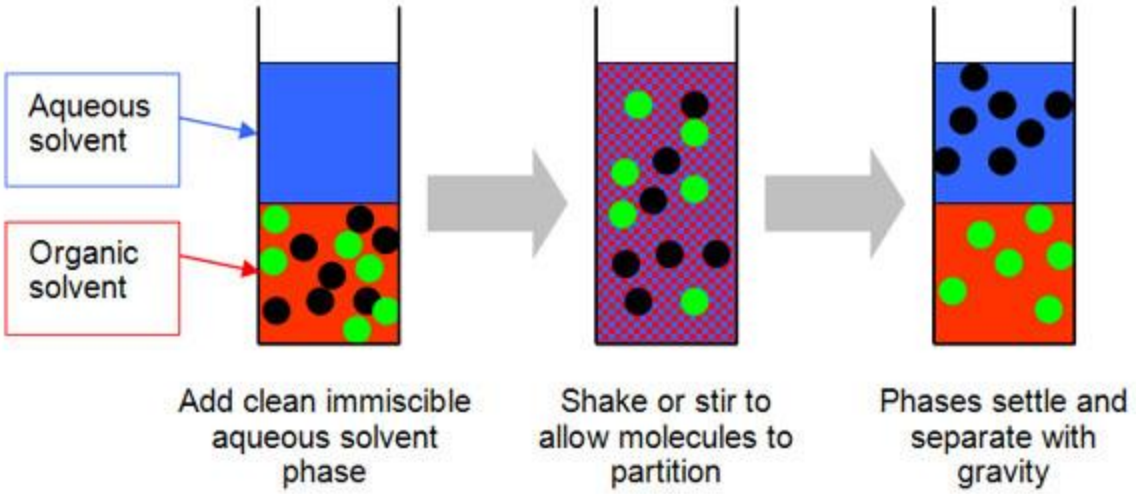
K: the partition
(or distribution) coefficient.



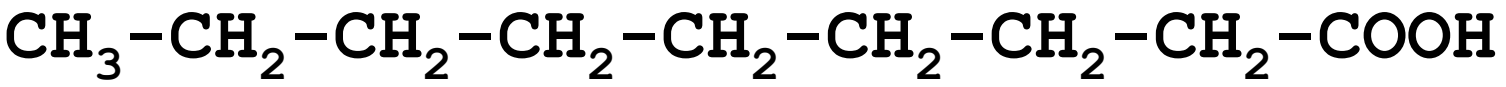
Liquid/Liquid Extractions



Solids are usually dissolved or digested in caustic solution and liquids are sometimes extracted to separate the analyte from interferences.



pH Controls Extraction



- At low pH: neutral, soluble in organic solvent
- At high pH: ionization of COOH group, soluble in water
- Effects of pK_a

pK_a vs. pH

$$HX(aq) \rightleftharpoons H^+(aq) + X^-(aq)$$

$$K_a = \frac{[H^+][X^-]}{[HX]}, then\ [H^+] = K_a \frac{[HX]}{[X^-]}$$

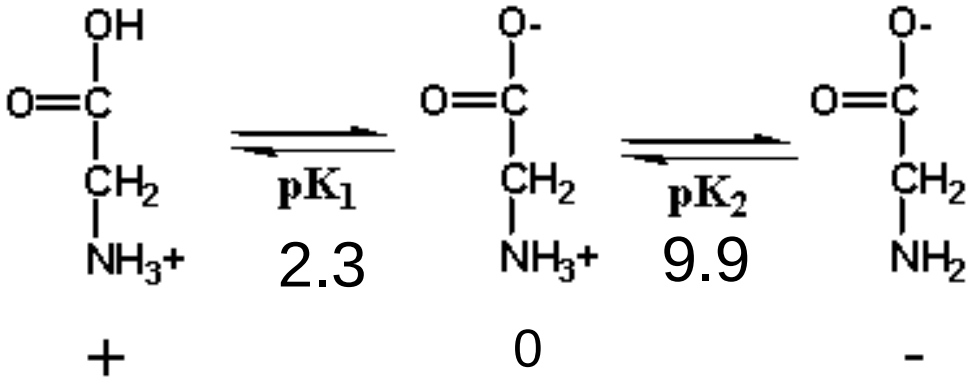
$$-log[H^+] = -log\ K_a - log \frac{[HX]}{[X^-]}$$

$$\therefore pH = pK_a + log \frac{[X^-]}{[HX]}$$

$$\therefore pH = pK_a, if\ [HX] = [X^-]$$

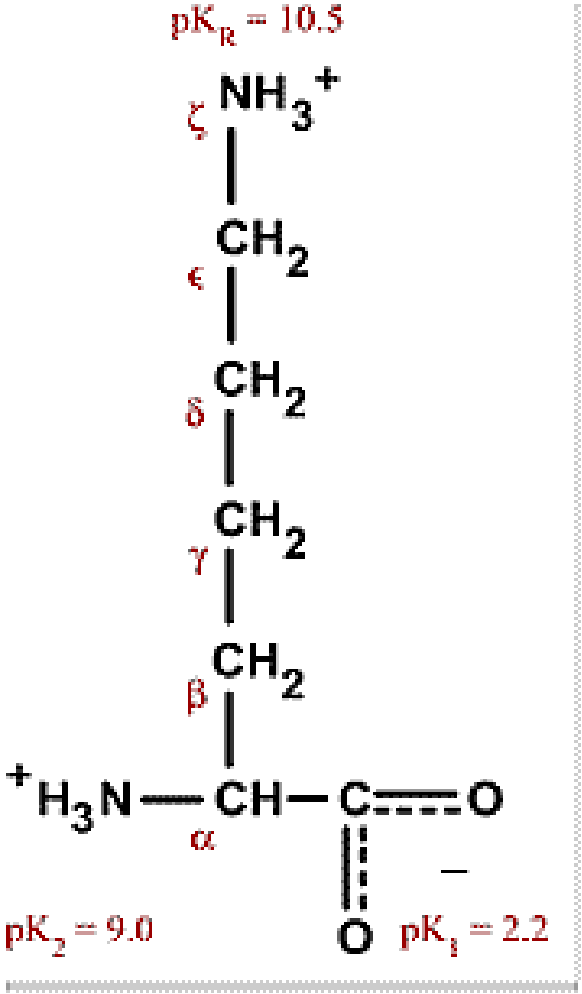
- pK_a is the pH where 50% of acids is deprotonated.
- If pH > pK_a, more than 50% of acids are deprotonated
- If pH < pK_a, less than 50% of acids are deprotonated

pH Effect on Net Charge



Glycine

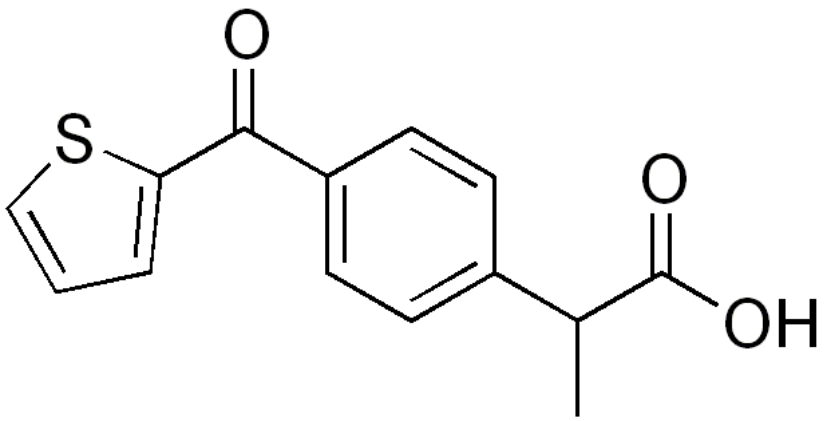
+2, pH < 2.2
+1, 2.2 < pH < 9.0
0, 9.0 < pH < 10.5
-1, 10.5 < pH



Lysine

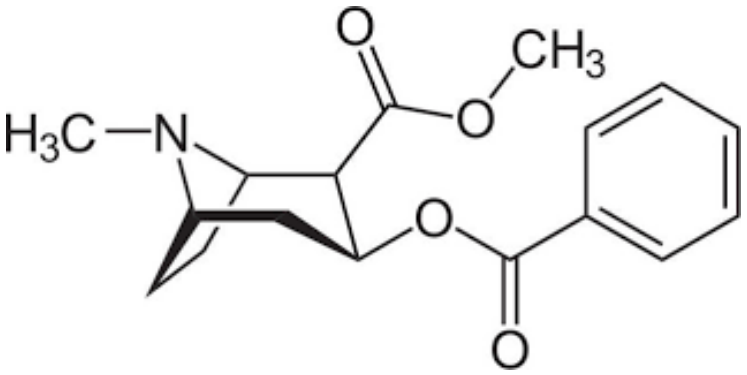
Many Drugs are Either Acids or Bases

suprofen



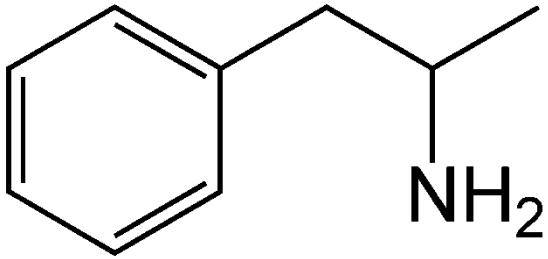
pK_a = 3.9

cocaine



pK_a = 8.6

amphetamine



pK_a = 9.8

composition at pH = 7.2?

Solid Phase Extractions (SPE)

1. Considered as a pre-liquid chromatographic process

2. SPE is an extraction method that uses a solid phase and a liquid phase to isolate one, or one type, of analyte from a solution.

3. The general procedure is to load a solution onto the SPE phase, wash away undesired components, and then wash off the desired analytes with another solvent into a collection tube.

4. SPEs use the same type of stationary phases as are used in liquid chromatography columns. The column might have a frit on top of the stationary phase and might also have a stopcock to control the flow of solvent through the column.

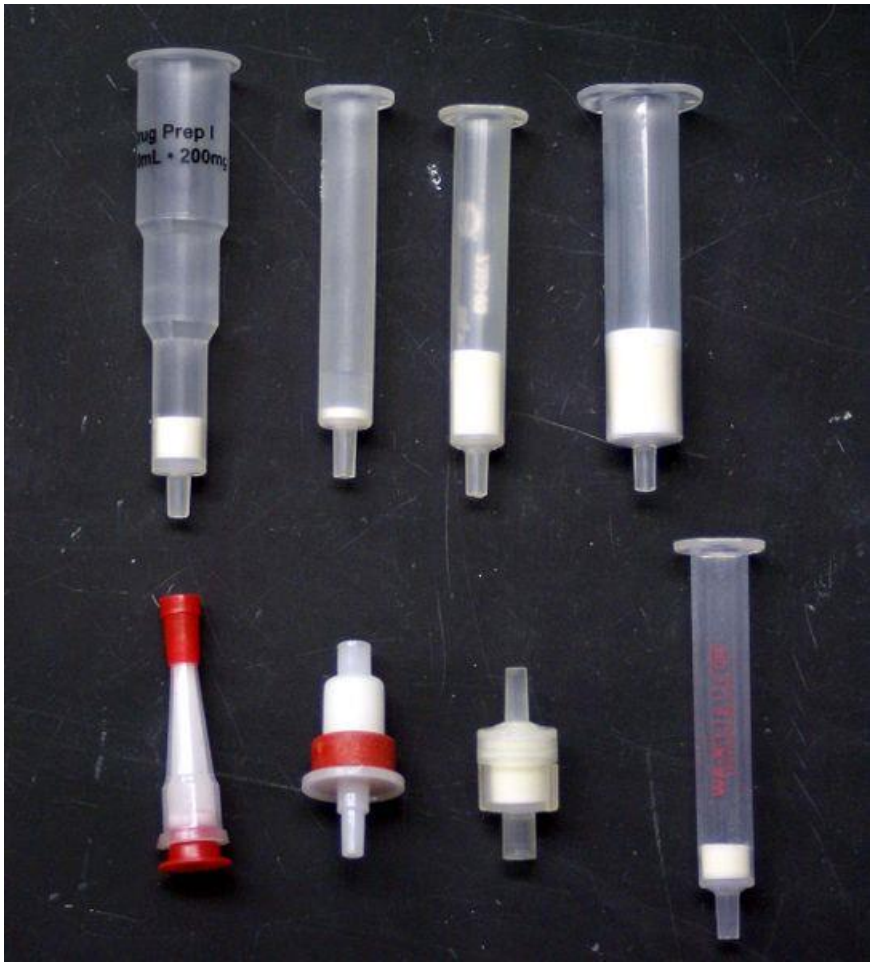
Why We Need Solid Phase Extractions

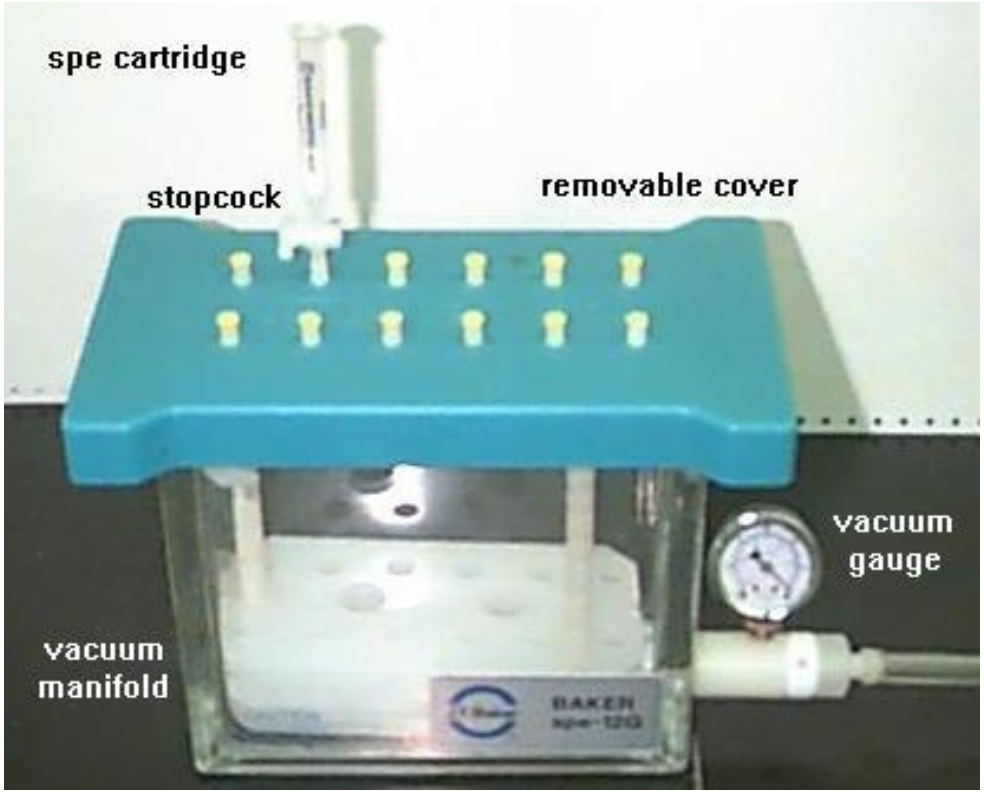
1. remove specific interferences (e.g., similar retention time, etc).

2. increase the concentration of the analyte of interest for easy and accurate detection.

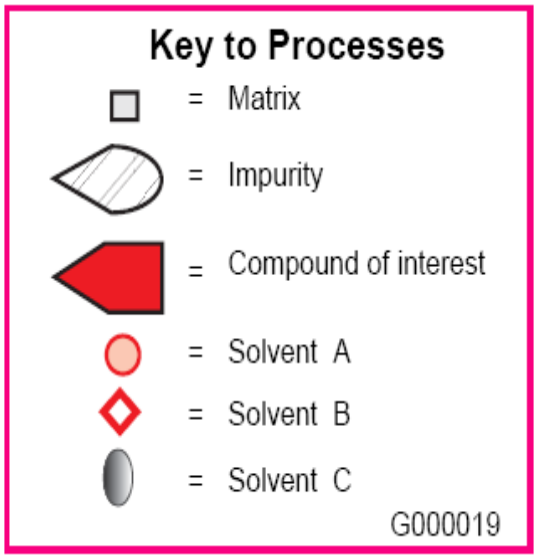
3. remove interferences in your sample that suppress the signal for the analyte of interest (matrix effect, mass spectrometry via reduced ionization)

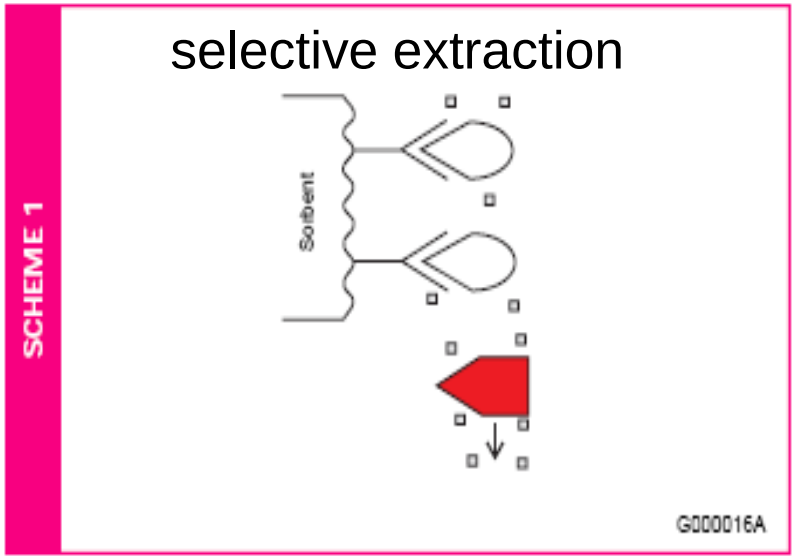
SPE Cartridges

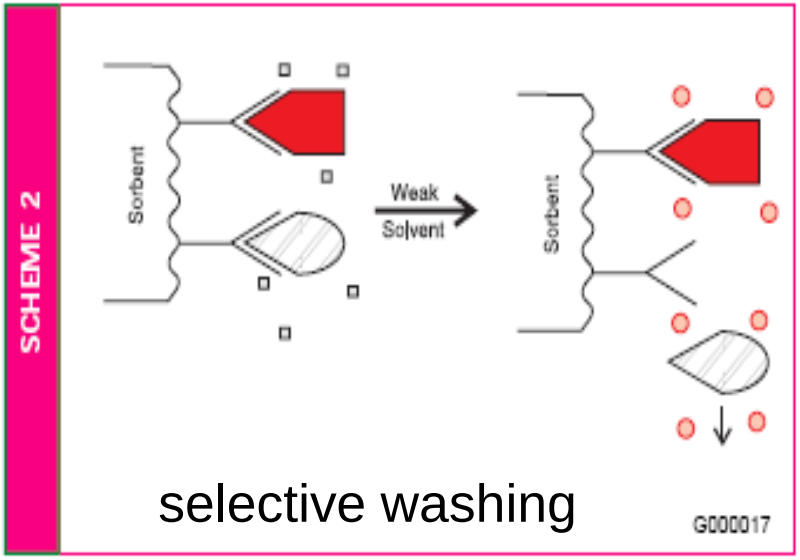


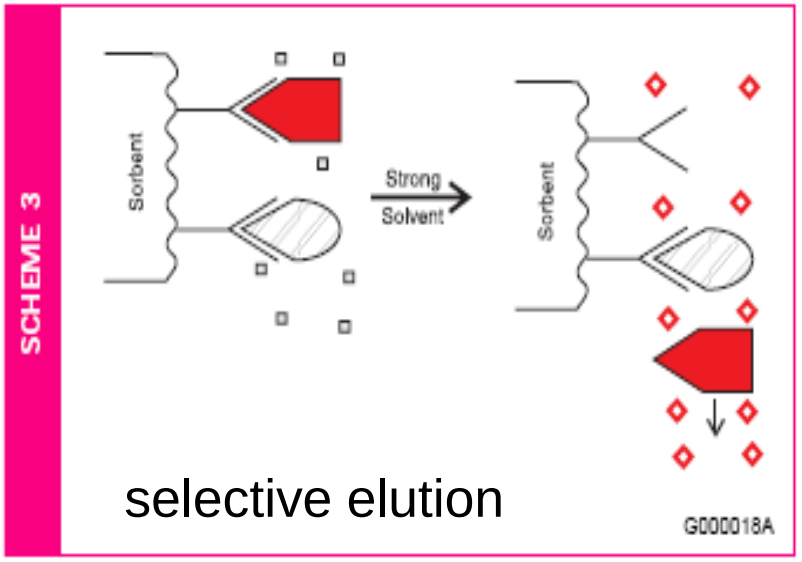


Three SPE Schemes



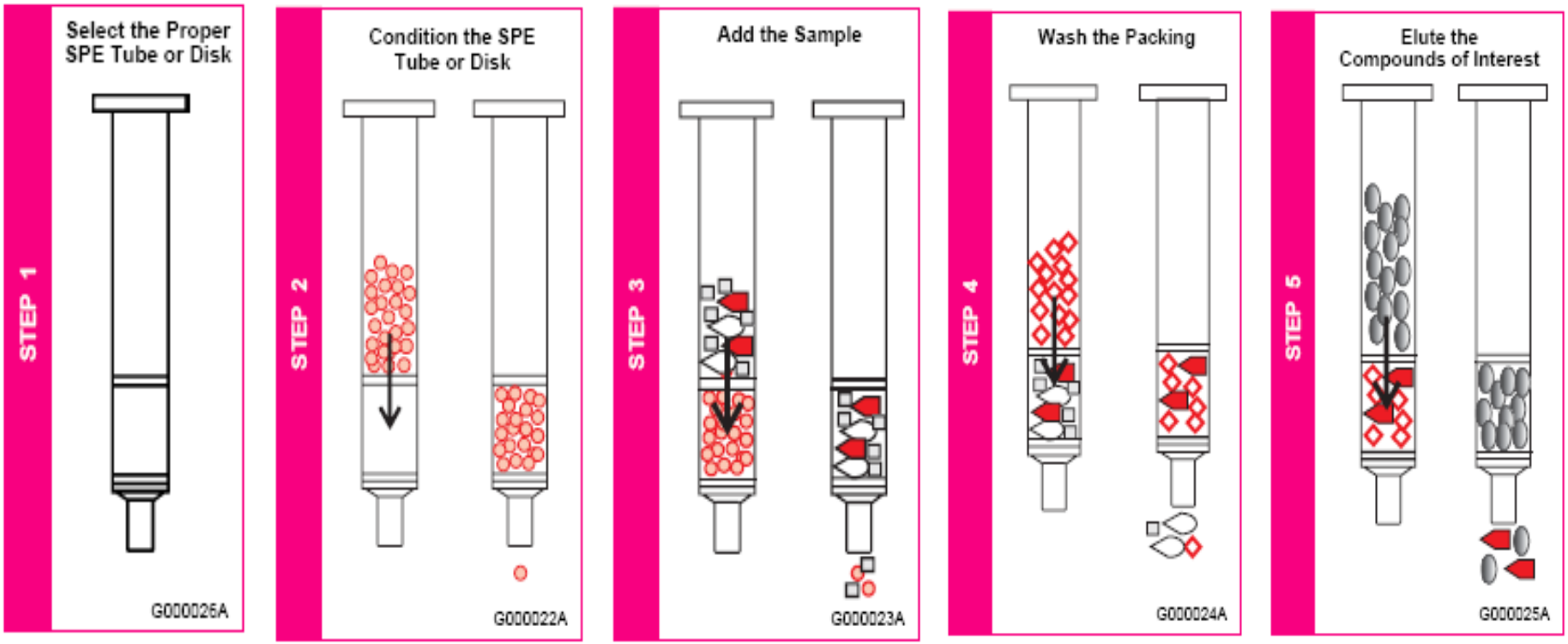






Example of SPE Procedures

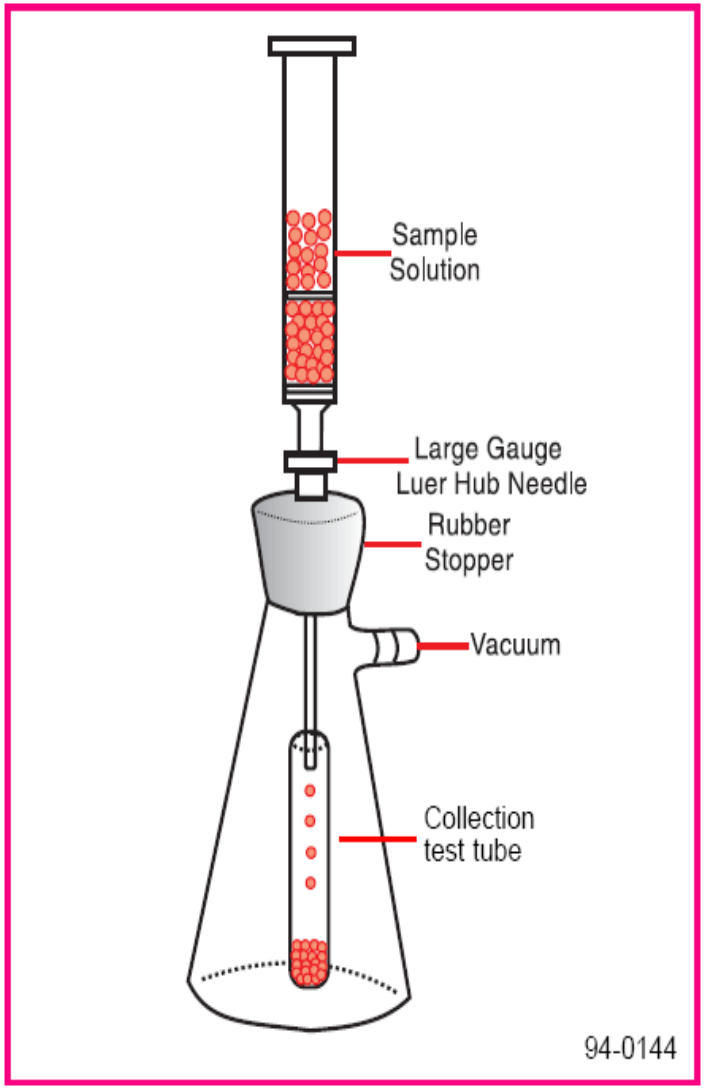
selection conditioning loading washing eluting

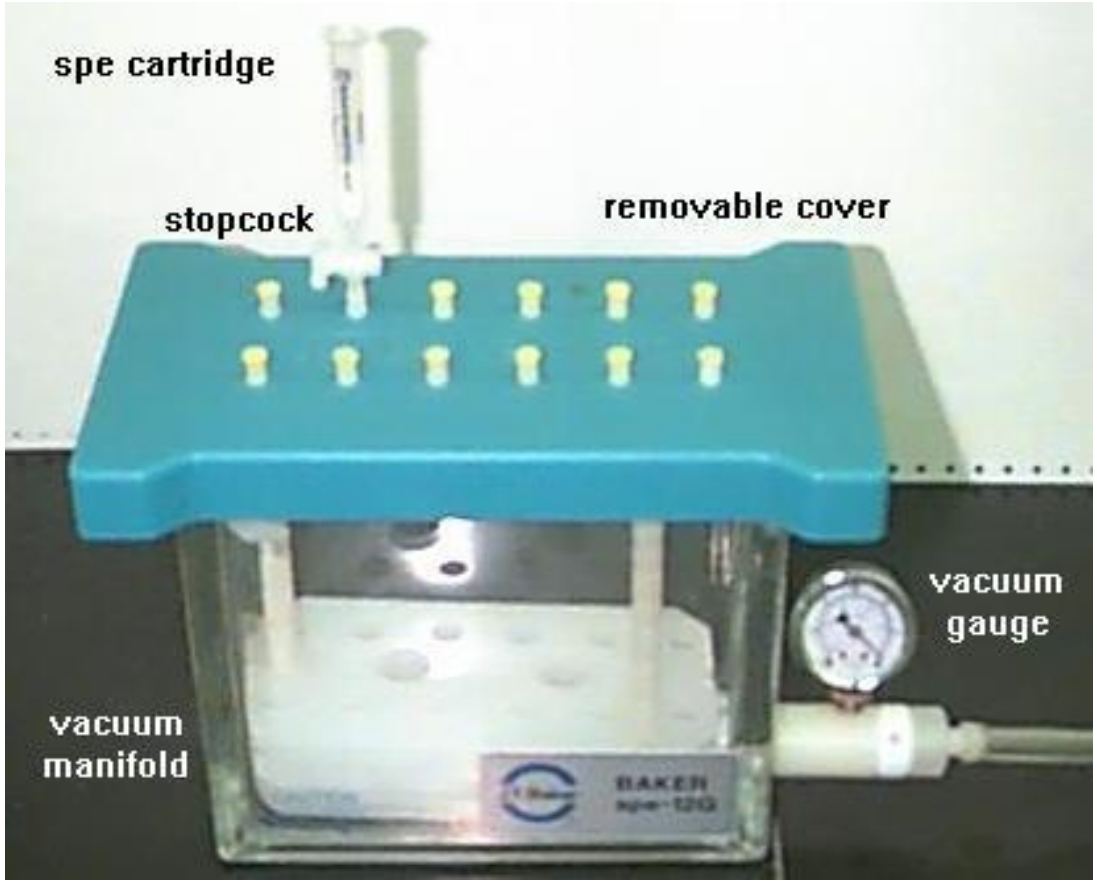


Characteristic of Solvents Commonly Used in SPE

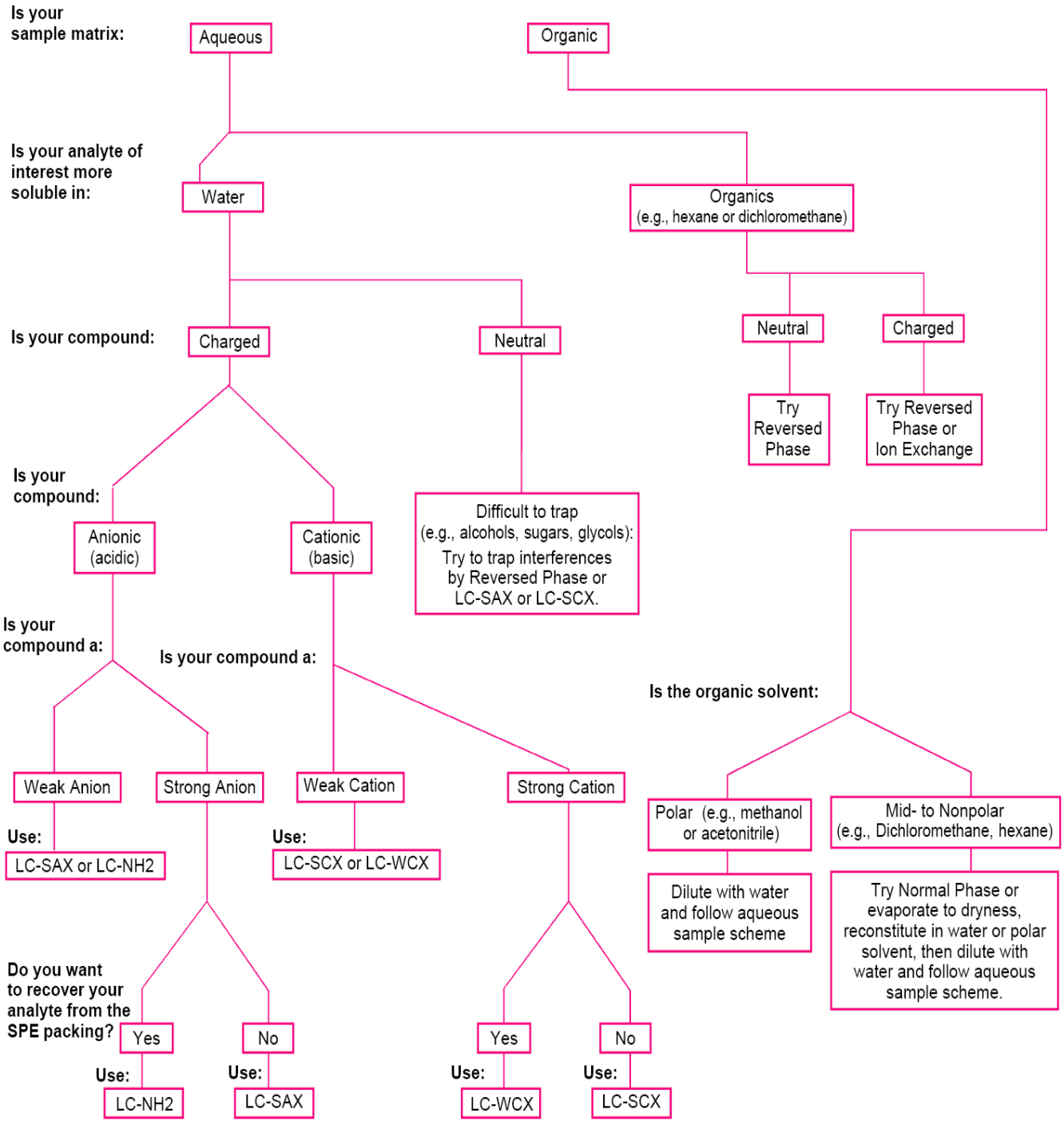
Polarity			Solvent	Miscible in Water?
Nonpolar	Strong Reversed Phase	Weak Normal Phase	Hexane	No
			Isooctane	No
			Carbon tetrachloride	No
			Chloroform	No
			Methylene chloride (dichloromethane)	No
			Tetrahydrofuran	Yes
			Diethyl ether	No
			Ethyl acetate	Poorly
			Acetone	Yes
			Acetonitrile	Yes
			Isopropanol	Yes
	Weak Reversed Phase	Strong Normal Phase	Methanol	Yes
			Water	Yes
Polar			Acetic acid	Yes

SPE Procedures





process using a vacuum


Is your sample matrix:
Aqueous
Organic
Is your analyte of interest more soluble in:
Water
Organics (e.g., hexane or dichloromethane)
Is your compound:
Charged
Neutral
Neutral
Charged
Try Reversed Phase
Try Reversed Phase or Ion Exchange
Is your compound:
Anionic (acidic)
Cationic (basic)
Difficult to trap (e.g., alcohols, sugars, glycols): Try to trap interferences by Reversed Phase or LC-SAX or LC-SCX.
Is your compound a:
Is your compound a:
Is the organic solvent:
Weak Anion
Strong Anion
Weak Cation
Strong Cation
Polar (e.g., methanol or acetonitrile)
Mid- to Nonpolar (e.g., Dichloromethane, hexane)
Use:
LC-SAX or LC-NH2
Use:
LC-SCX or LC-WCX
Dilute with water and follow aqueous sample scheme
Try Normal Phase or evaporate to dryness, reconstitute in water or polar solvent, then dilute with water and follow aqueous sample scheme.
Do you want to recover your analyte from the SPE packing?
Yes
No
Yes
No
Use:
LC-NH2
Use:
LC-SAX
Use:
LC-WCX
Use:
LC-SCX

Issues in SPE Procedures

- Is the separation perfect?
- Or are all target compounds separated (or collected)?
- Or all interference (matrix) removed?
- Any loss of target compounds?
- If not, how do you know the real amount of target compounds in a sample?

Recovery Test and Standard Addition

- Recovery test:
  1. Prepare sample using a known amount of target and same (or similar) matrix.
  2. Repeat same SPE and estimate how much target was recovered

- Standard addition: Addition of a known amount of standard to samples

SPE Procedure Tips

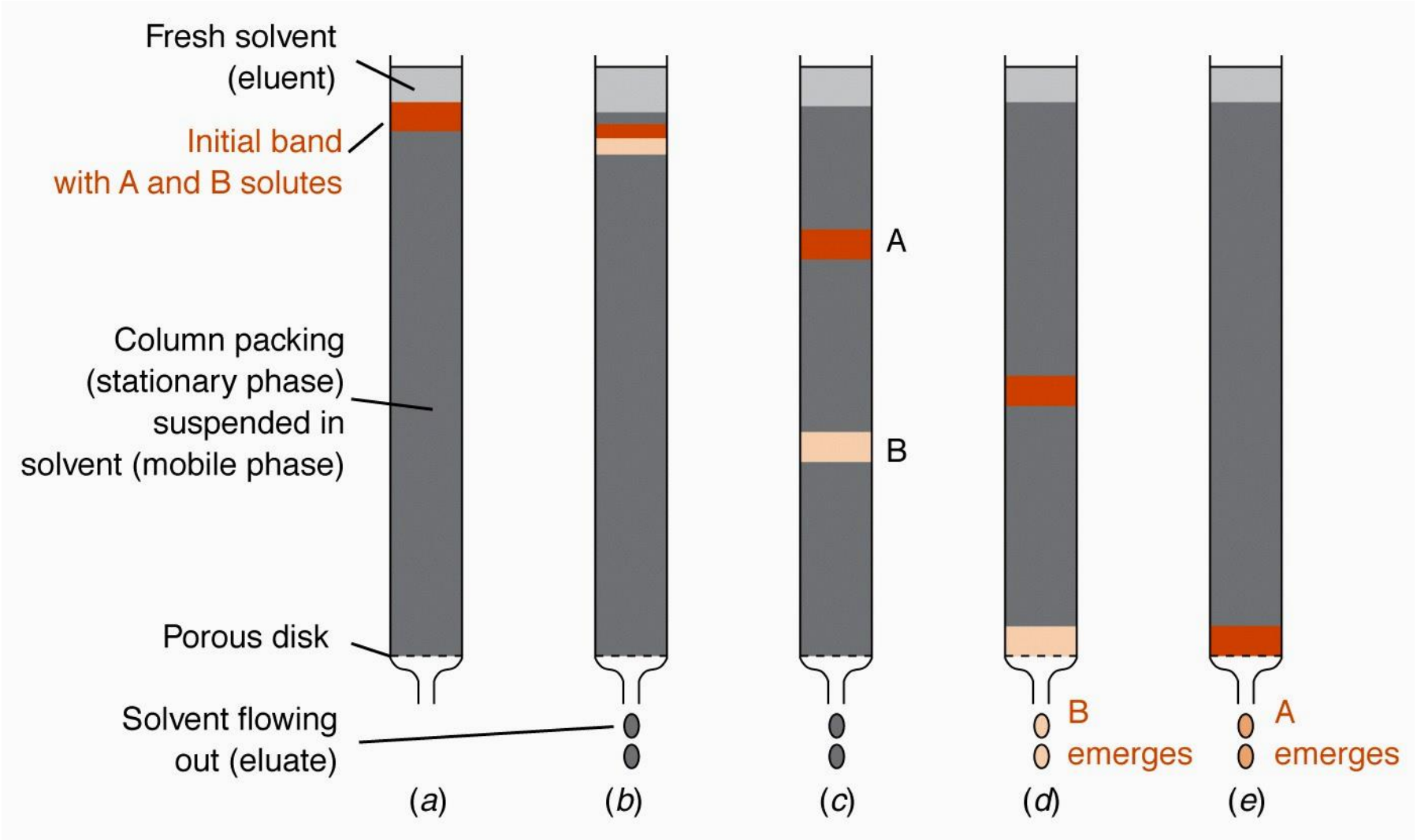
1. Find appropriate literatures

2. Compare results and tune the experimental conditions by changing solvent compositions (polarity of solvents), pH, etc

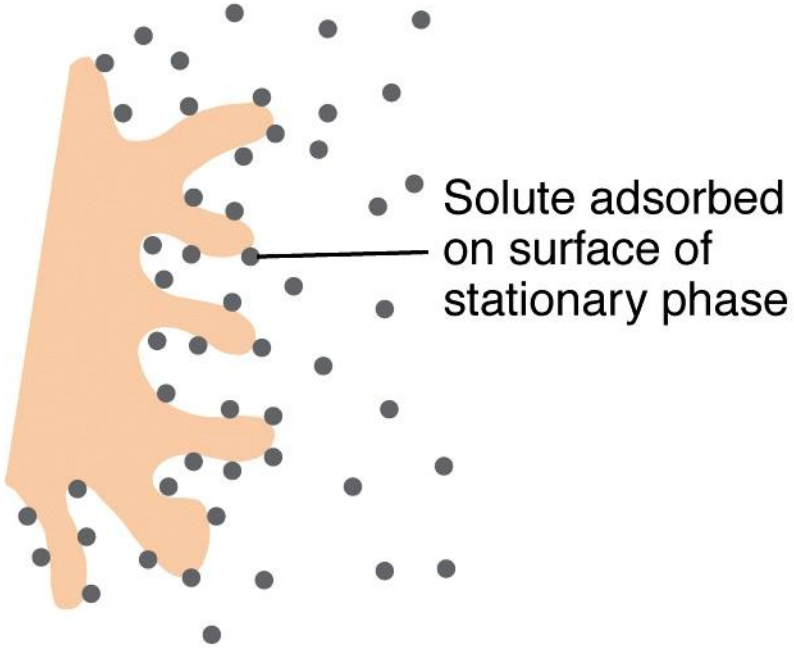
Basic Principles of Chromatography

1. Chromatography involves a sample (or sample extract) being dissolved in a *mobile phase* (gas or liquid), which is then forced through an immobile, immiscible *stationary (solid) phase*.
2. The stationary phases are chosen such that components of the sample have different solubilities (or affinity or interaction, etc) in each phase.
3. A component which is quite soluble (more interactions) in the stationary phase will take longer to travel through it than a component which is not very soluble in the stationary phase but very soluble in the mobile phase.
4. As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase.
5. Techniques such as HPLC (High Performance Liquid Chromatography) and GC (gas ghromatography) use *columns* - narrow tubes packed with stationary phase, through which the mobile phase is forced.

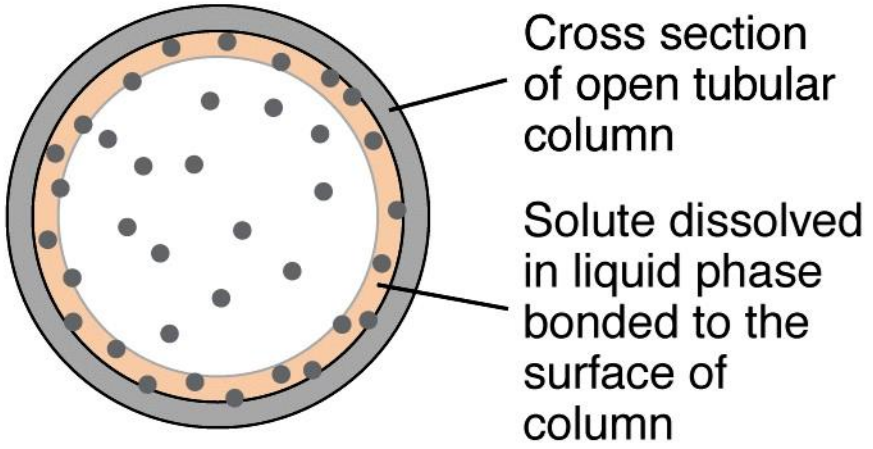
Chromatography (e.g. LC)



Why Separation Is Possible?



Adsorption chromatography



Partition chromatography

Compounds in a sample are dissolved in solid stationary phase solute

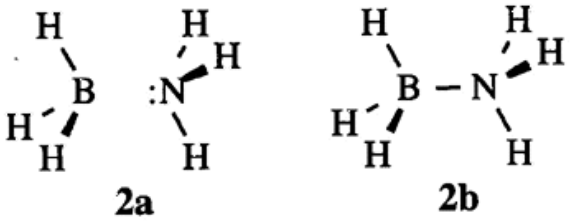
Interactions Between Surfaces and Molecules

1. Charge-charge interactions (electrostatic interactions): These are interactions between cations and anions with formal charge. The electrostatic force between two point charges is given by $F = k\,[q1 \cdot q2]/r^2$

2. Acid-base interaction: electron pair donor Lewis bases "react with" or "complex with" or "interact with" electron pair acceptor Lewis acids.

3. Dipole-dipole interactions: Water and methanol

4. Dipole-ion interaction: NaCl (Na^+ and Cl^-) in water



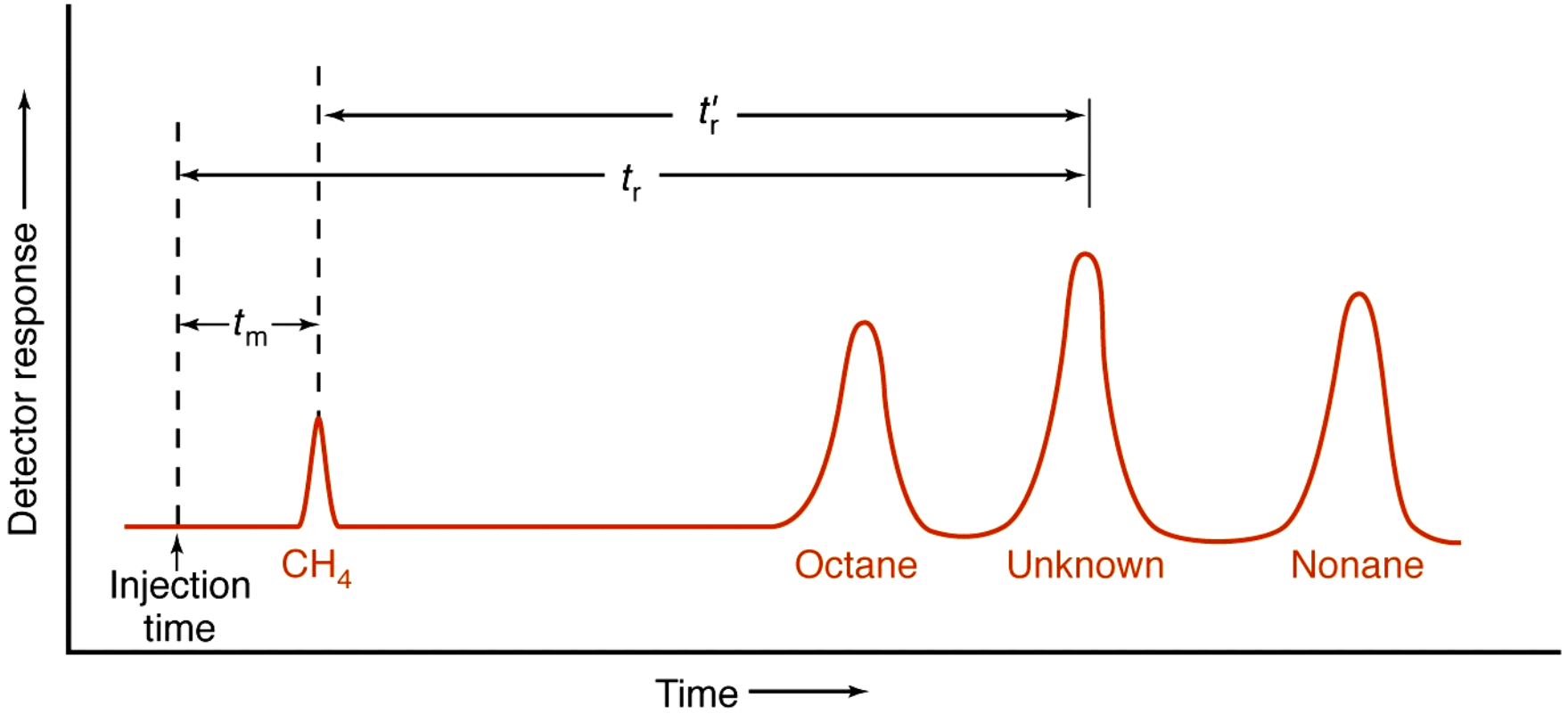
2a 2b

5. van der Waals interactions

6. Hydrogen bonding

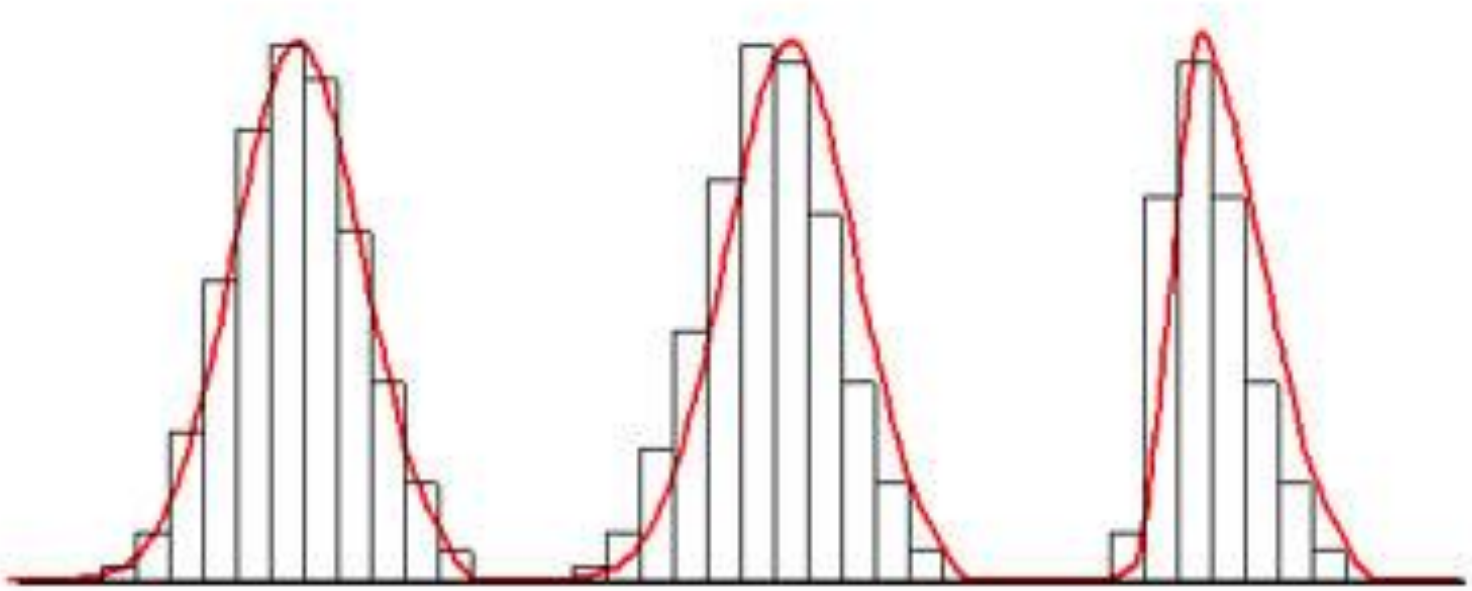
7. Hydrophobic interaction: Two areas of hydrophobic substances will encounter one another, combine and form one larger hydrophobic region that is excluded from the water matrix.

Retention Time: Qualitative Analysis



Accuracy of Quantification: Peak Shape

Effect of summation algorithm on recorded peak area

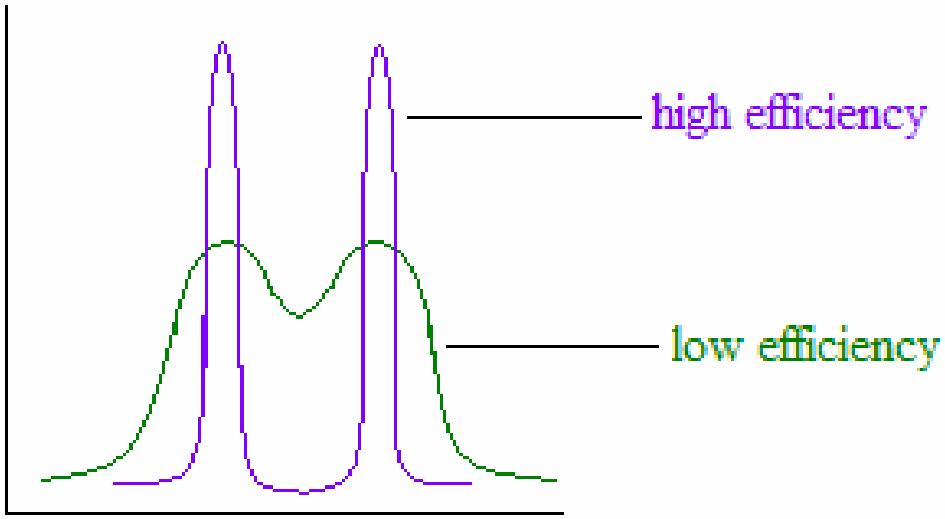


rectangles symmetrical around data points optimal estimate of true peak area (left)

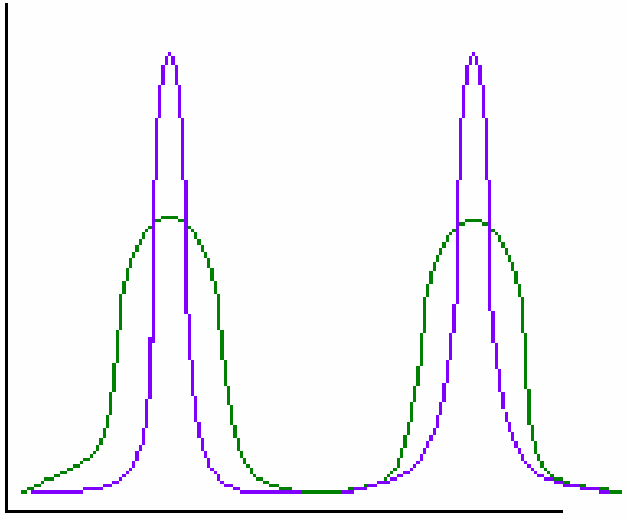
rectangles asymmetrical with respect to curve front overestimate compensated by tail underestimate for symmetrical peaks (center), but not for asymmetrical peaks (right)

Resolution and Peak Width

low selectivity



high selectivity



Ideally, the goal is to achieve both high efficiency and high selectivity so that the samples are adequately separated.

This is achieved in the blue peaks in the chromatogram on the left.

Broadening

- Individual molecule undergoes "random walk"
- Many thousands of adsorption/desorption processes
- Average time for each step with some variations
  - Gaussian peak
    - → like random errors
- Breadth of band increases down column because more time
- Efficient separations have minimal broadening

Zone broadening:
The van Deemter equation

$$H = A + \frac{B}{u} + C \times u$$

H - **plate height**

u - **flow rate**

Details of Broadening Factors

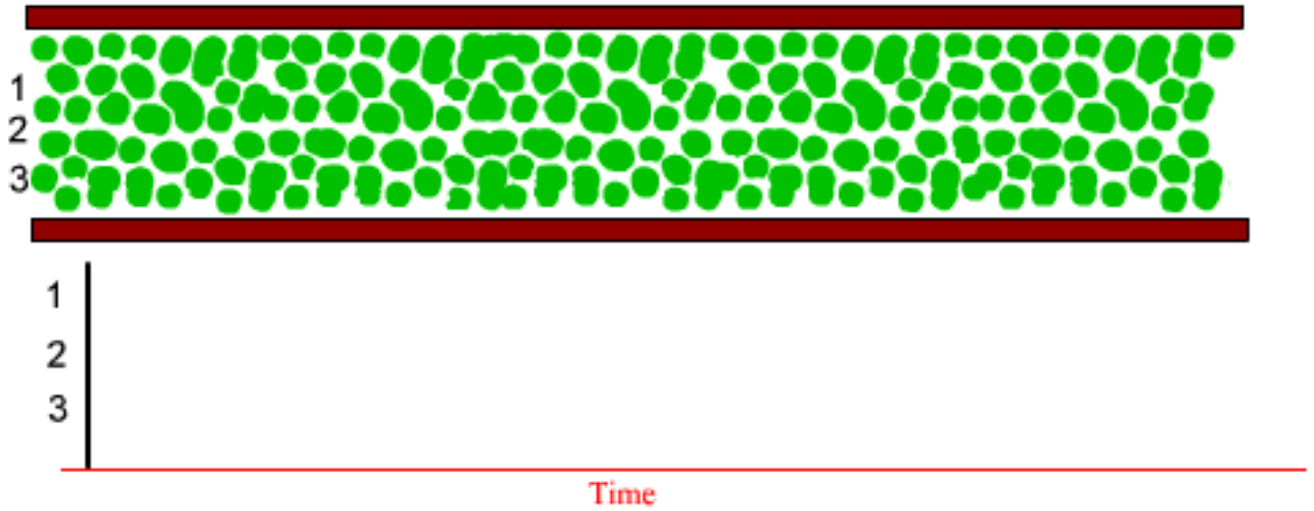
$$H = A + B/u + Cu$$

***A* (Eddy diffusion)**: Solute molecules will take different paths through the stationary phase at random. This will cause broadening of the solute band, because different paths are of different lengths.

***B* (Longitudinal diffusion)**: The concentration of analyte is less at the edges of the band than at the center. Analyte diffuses out from the center to the edges. This causes band broadening. If the velocity of the mobile phase is high then the analyte spends less time on the column, which decreases the effects of longitudinal diffusion.

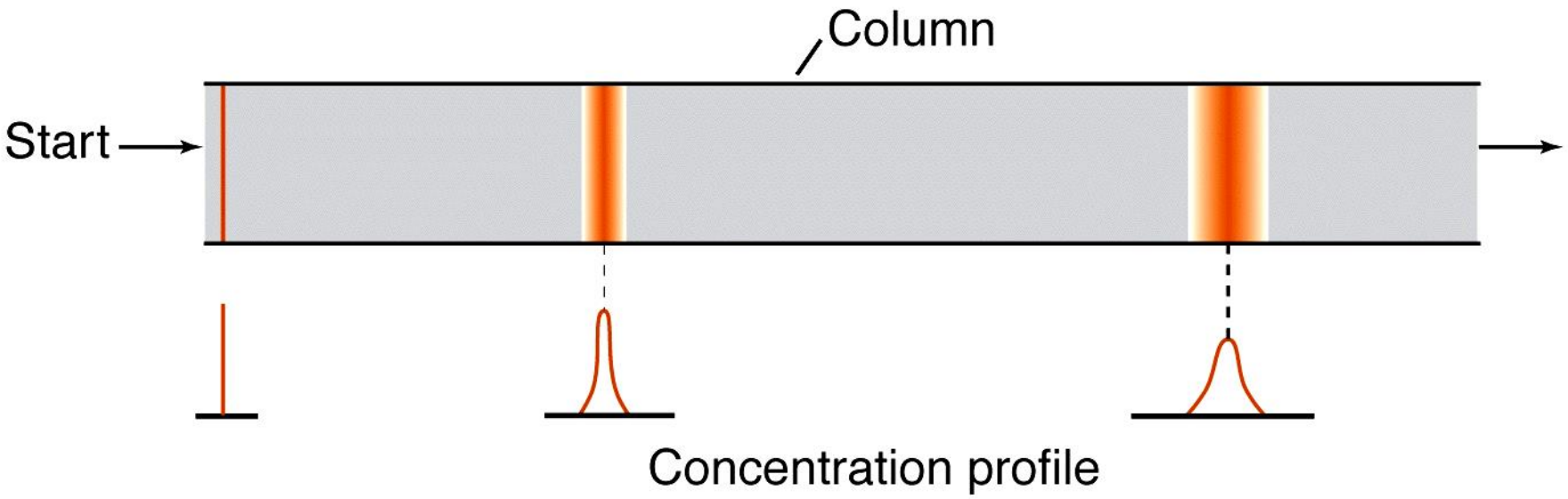
***C* (Resistance to mass transfer)**: The analyte takes a certain amount of time to equilibrate between the stationary and mobile phase. If the velocity of the mobile phase is high and the analyte has a strong affinity for the stationary phase, then the analyte in the mobile phase will move ahead of the analyte in the stationary phase. The band of analyte is broadened. The higher the velocity of mobile phase, the worse the broadening becomes

Eddy Diffusion



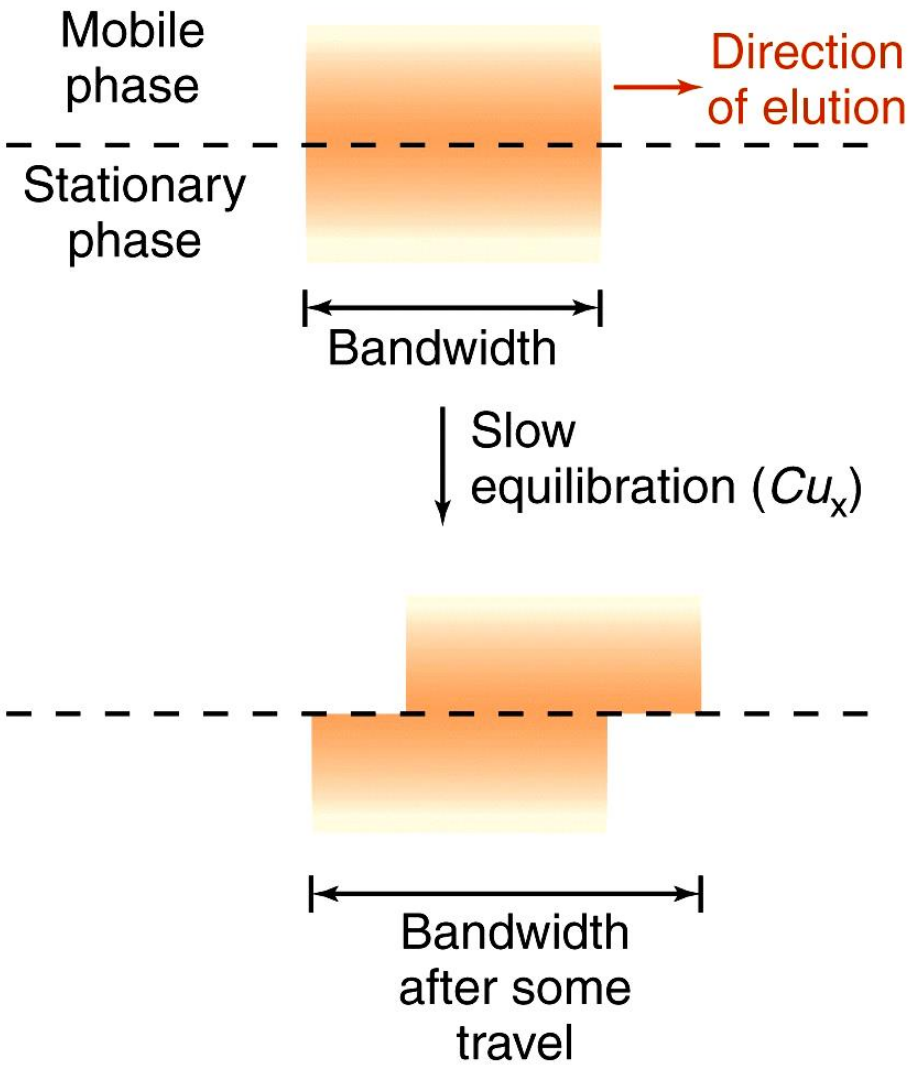
A = 0

Longitudinal diffusion



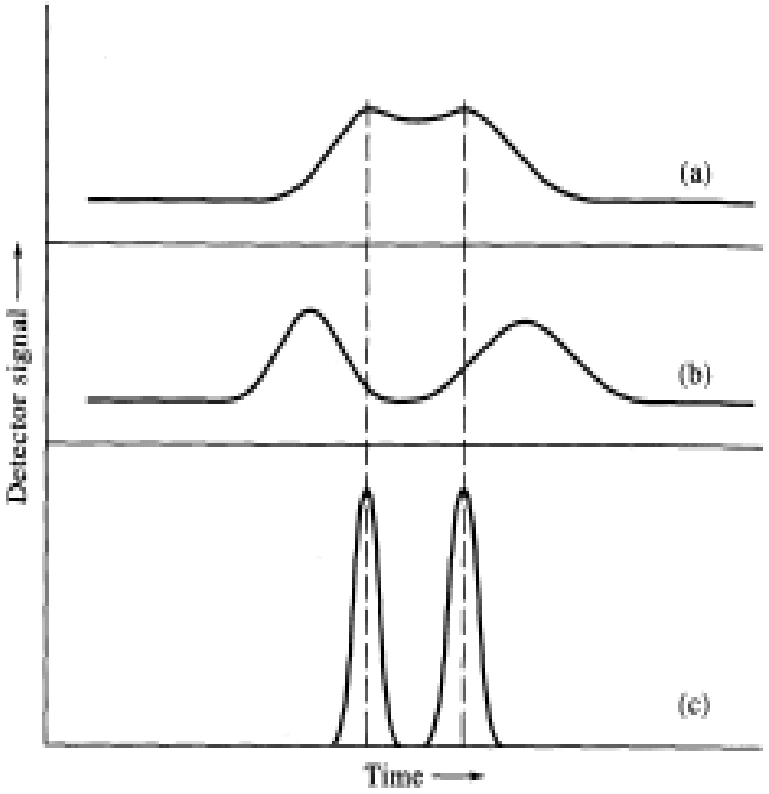
Longitudinal diffusion

Resistance to Mass Transfer



How To Improve Separation

- Separations enhanced by varying experimental conditions
  - adjust migration rates for A and B
    - increase band separation
  - adjust zone broadening
    - decrease band spread

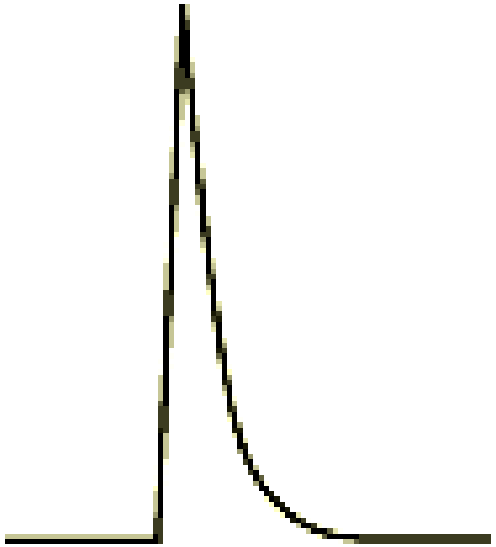


$$A_{mobile} \leftrightarrow A_{stationary}$$

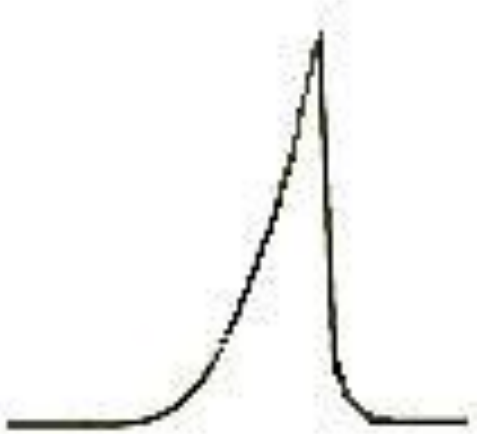
$$K = \frac{c_{stationary}}{c_{mobile}}$$

partition ratio

Bad Peak Shapes

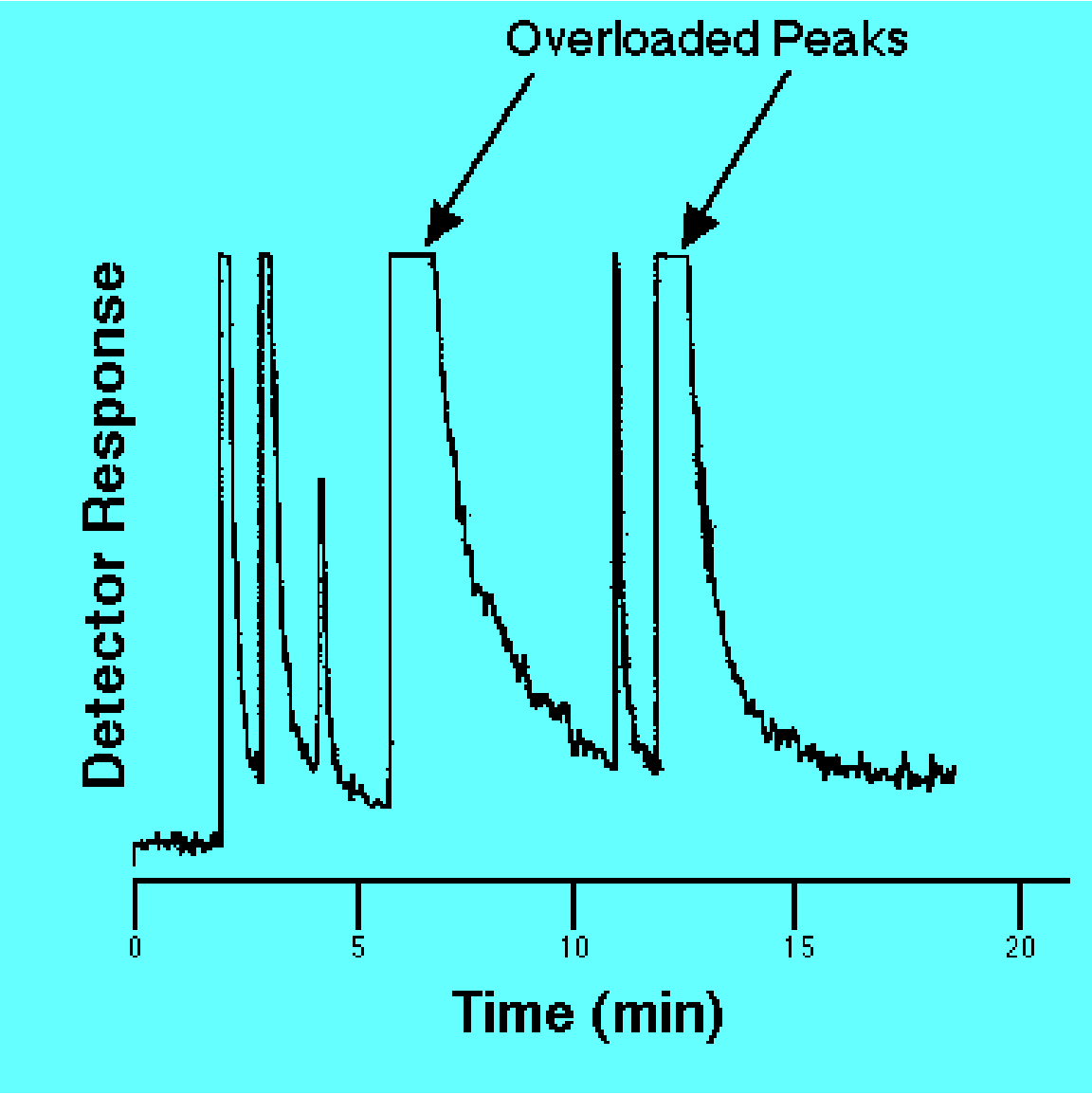


tailing



fronting

Peak Tailing



Tips for Obtaining Best Results

1. Injection of optimized amount of sample (LC GC)
2. Check leaking (LC GC)
3. Adjust temperatures of injection port, oven, and detector (GC)
4. Adjust flow rate of carrier (LC GC)
5. Change column (LC GC)
6. Make sample cleaner (LC GC)
7. Dead volume (LC GC)