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Liquid Chromatography: LC Basics and Separation Types

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DOI:10.15428/CCTC.2015.240044

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Outline

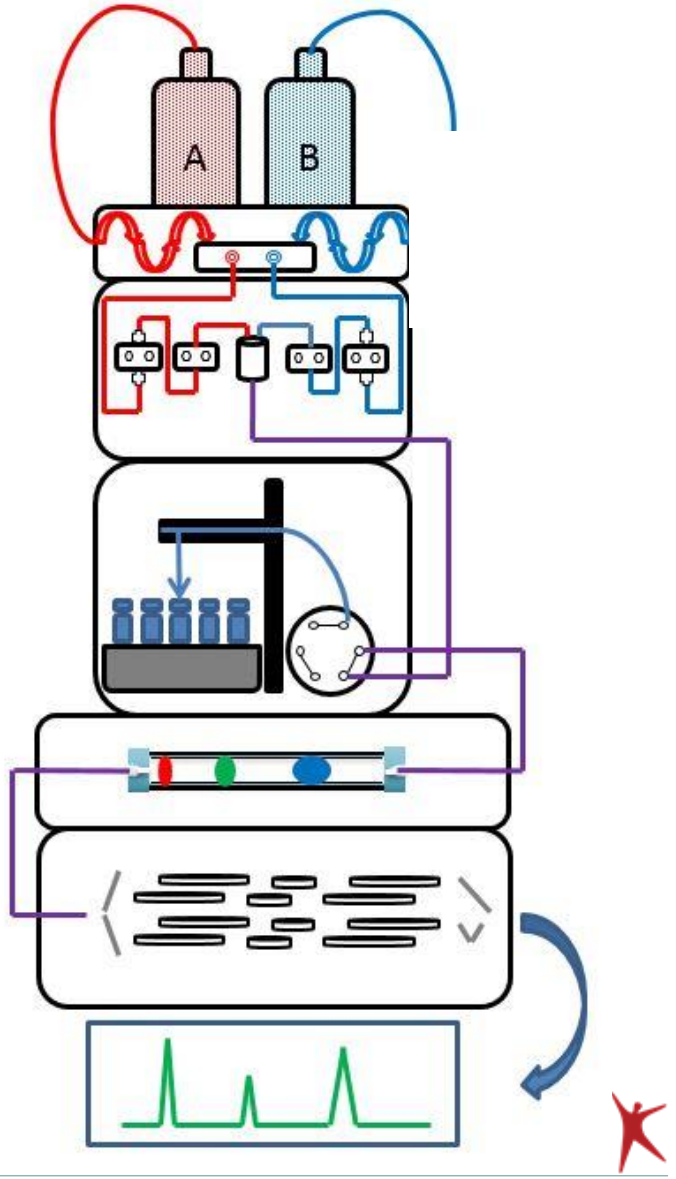
- **Definitions**
- **LC Components**
  - Columns; solvents/mobile phase; pumps; autosampler; detector
- **Columns**
  - Key parameters; stationary phase; dimensions; particle sizes; pressure regimes
- **Types of Separations**
  - Normal phase; reverse phase; HILIC; size exclusion; ion exchange; chiral chromatography

What is Chromatography?

- **Chromatography:**
  - Derived from the Greek words for “color writing”
  - Mikhail Tsvet
- **Types of Chromatography**
  - Based on mobile phase (GC, LC, SFC)
  - Based on separation type (IC, GPC/SEC)
- **Liquid:**
  - LC is a separation based on a liquid mobile phase
  - Other separations use gases or supercritical fluids as the mobile phase

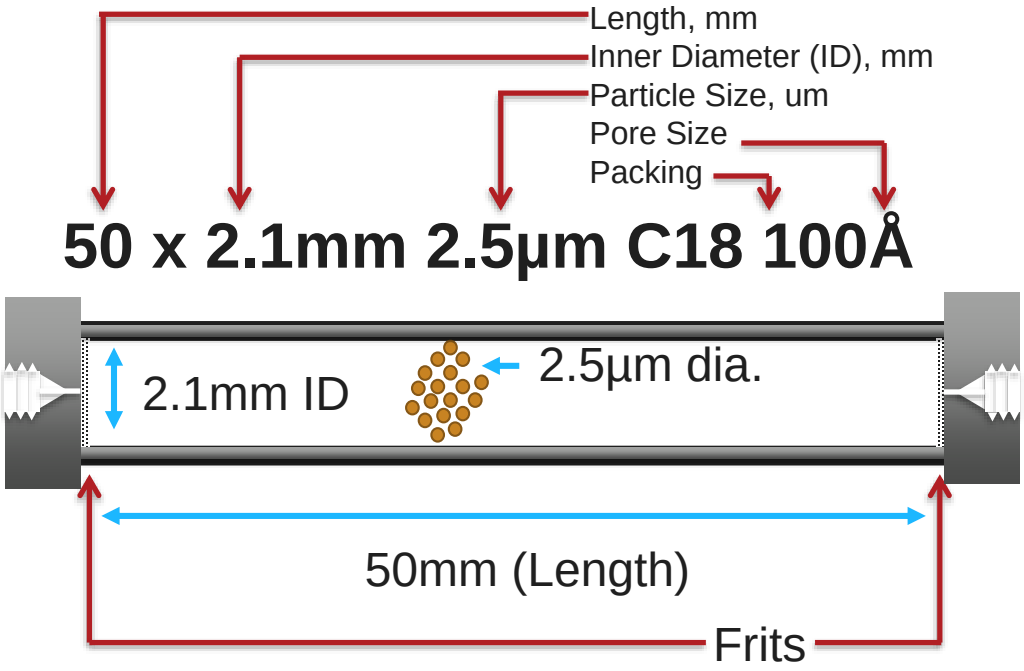
LC Components

- Solvents/ Mobile Phase
- Degasser
- Pumps
- Autosampler
- Column
- Detector: UV/Vis, MS, other
- Chromatogram



Columns: Key Parameters

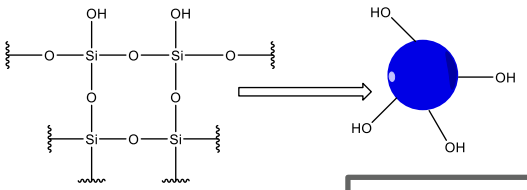
- Packing Material
- Dimensions
- Particle Size



Columns: Stationary Phase / Packing Material

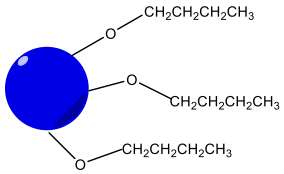
Column: where the separation happens
Separation: based on interaction between
- Analyte
- Mobile Phase
- Stationary Phase

Normal Phase

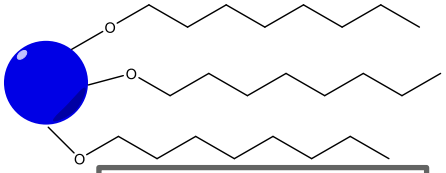


Silica Gel

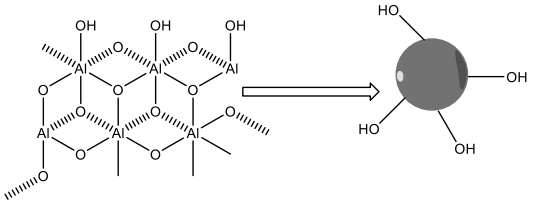
Reverse Phase



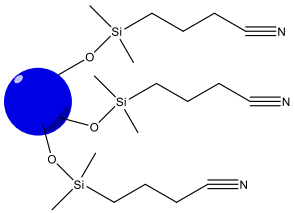
C4 on Silica



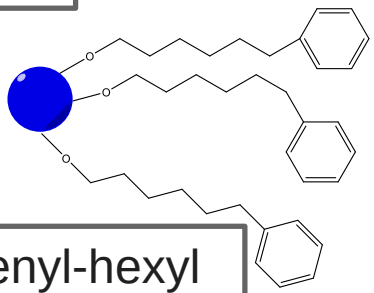
C8 on Silica



Alumina



Cyanopropyl



Phenyl-hexyl

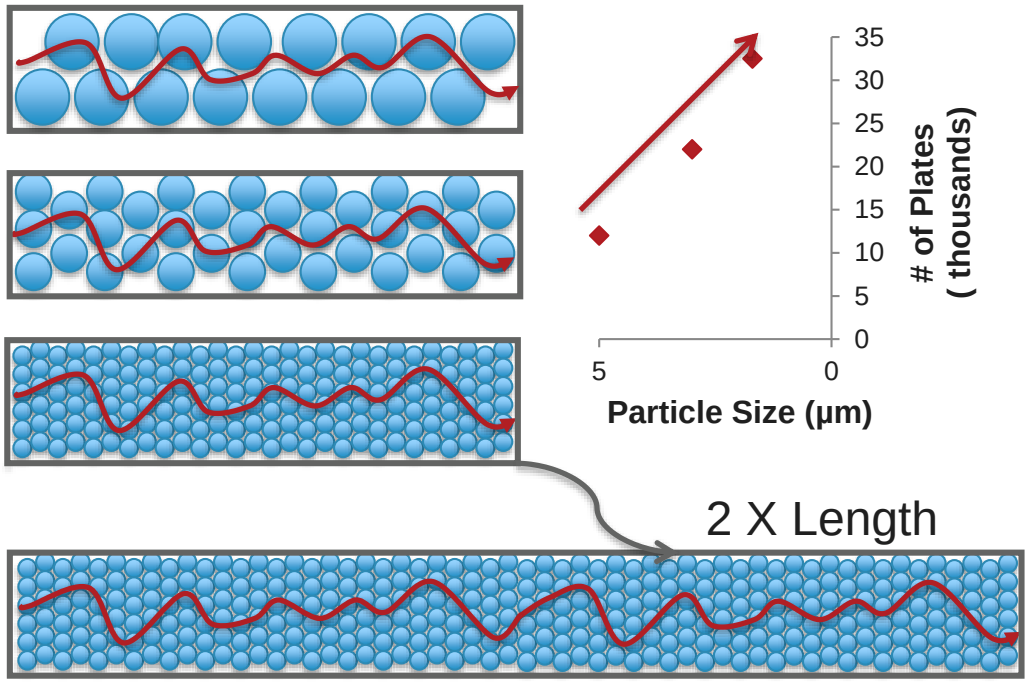
Columns: Dimensions

- Available in a variety of lengths and internal diameters (IDs)
- Variability in particle and pore sizes
- Packing is determined by the type of separation desired

Common IDs (mm)	Common Lengths (mm)	Common Particle Sizes (um)	Common Pore Sizes (Å)
(smaller)	2	1.3	20
0.2	4	1.7	25
0.5	5	1.8	50
1	10	2.5	60
2	15	2.6	65
3	20	3	70
4	30	3.5	80
4.6	35	3.6	90
5	50	4	100
6	60	5	110
10	75	6	120
(larger)	100	7	125
	150	8	(larger)
	200	10	
	250	(larger)	
	300		
	(longer)		

Columns: Particle Sizes

Particle Size	# of Theoretical Plates	Back Pressure
5µm	12000	1100psi
3µm	22000	2900psi
1.7µm	32500	8600psi
1.7µm	65000	17200psi

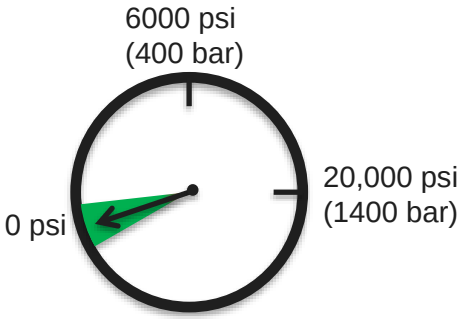


Compromise is to go to shorter, narrower columns with smaller particle sizes

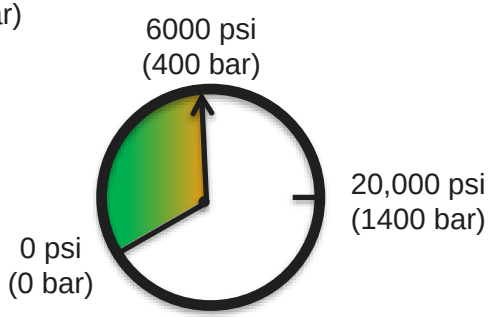
Figure courtesy of Mike Wright with modifications

Chromatography Pressure Regimes

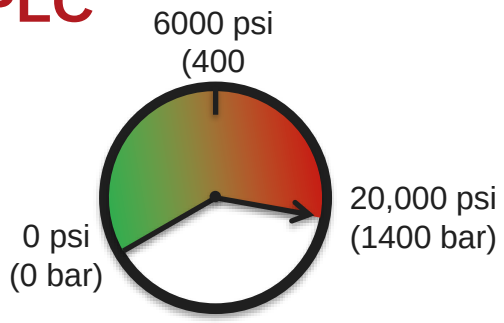
- **Low Pressure**
  - Gravity is the “Pump”
  - Used for sample prep
  - Used for synthesis purification



- **High Pressure – HPLC**
  - Traditional pressure
  - Routine



- **Ultra High Pressure – UHPLC or UPLC**
  - Newest Technology
  - Better Performance
  - Plumbing Concerns

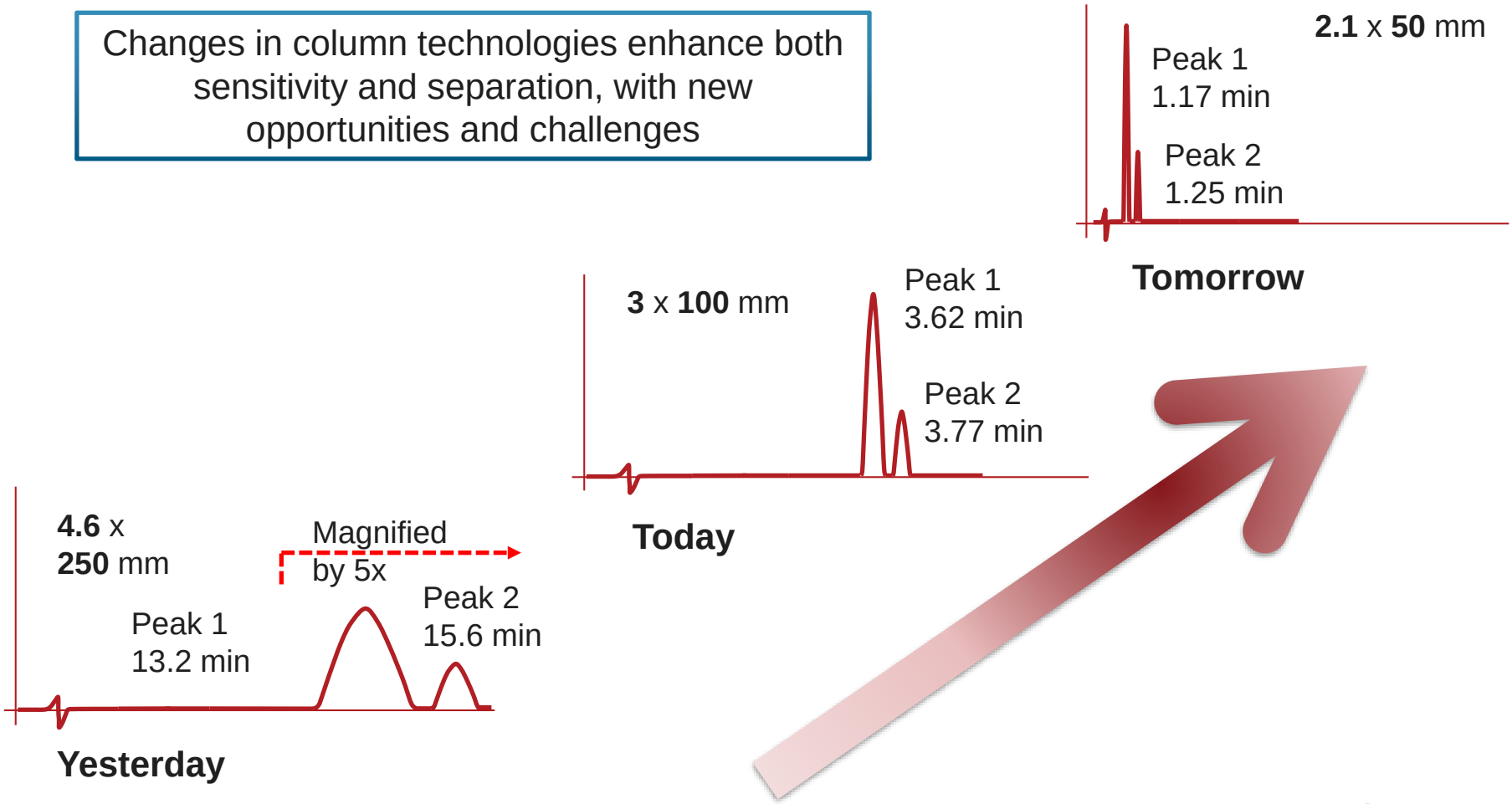


- Two pressure units – bar and psi

To convert from bar to psi, multiply by 14.50 (or 15 for quick calculation)
To convert from psi to bar, divide by 14.50 (or 15 for quick calculation)

Progress in Column Technology

Changes in column technologies enhance both sensitivity and separation, with new opportunities and challenges

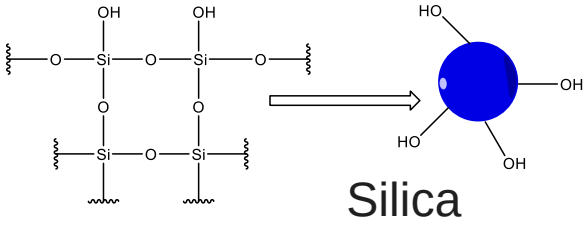


Types of Separation

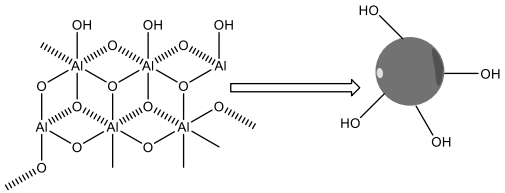
- Normal Phase
- Reverse Phase
- Hydrophilic Interaction Liquid Chromatography (HILIC)
- Size Exclusion Chromatography (SEC)
  - Gel Permeation Chromatography (GPC)
- Ion Exchange
- Chiral Separation

Normal Phase

- Polar stationary phase: silica or alumina
  - (many exposed hydroxyl groups)
- Non-polar mobile phase
- Largely supplemented by other techniques
- Good for polar analytes
- Reproducibility can be difficult



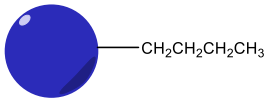
Silica



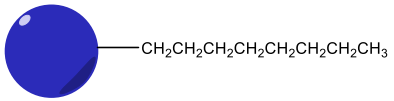
Alumina

Reverse Phase

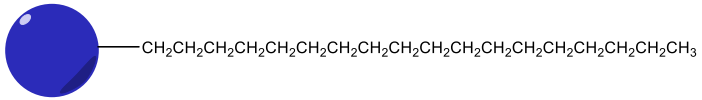
- Most common
- Non-polar stationary phase
- Aqueous or moderately polar mobile phase
- MANY different stationary phases available
  - C4, C8, C18
  - Cyano, Phenyl, Fluorophenyl, PFP
  - Amide, Amino
- Excellent for “normal” organic compounds



C4 bonded to Silica particle

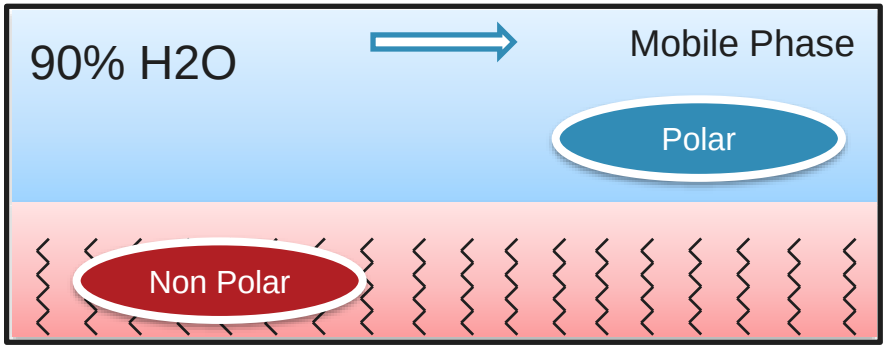


C8



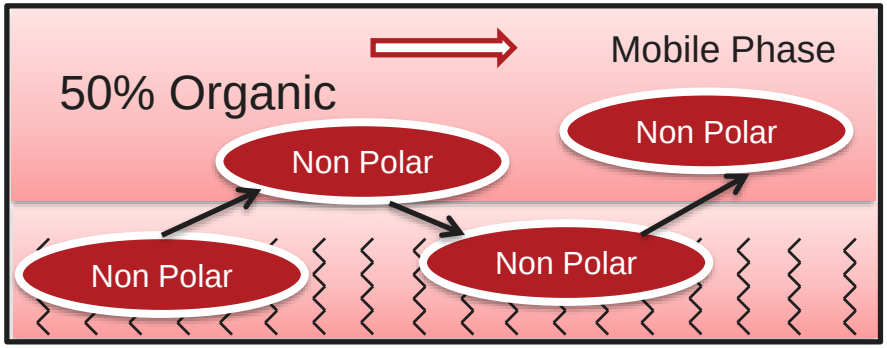
C18

Reverse Phase: Partition and Separation



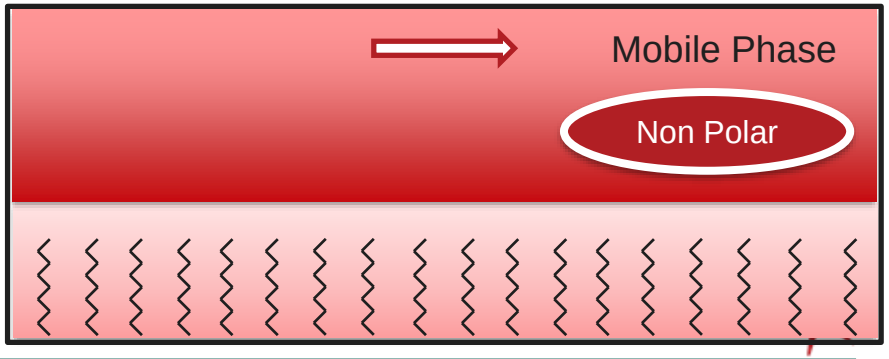
Retention

More mechanisms in Part 2 of this series



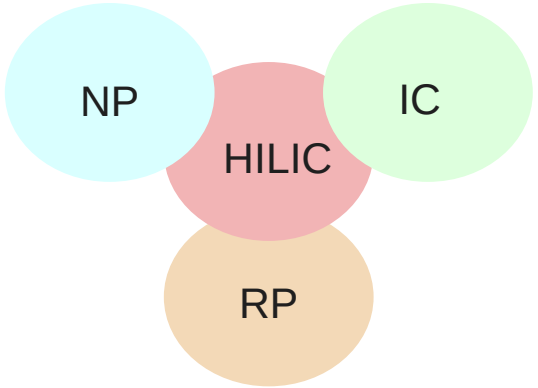
Partition

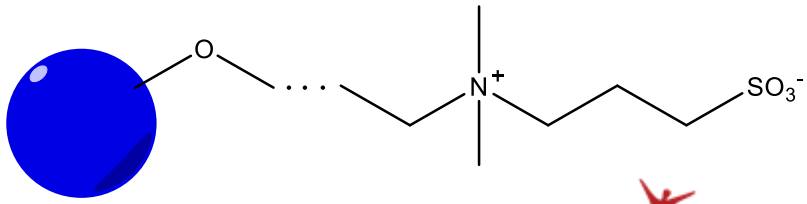
Elution



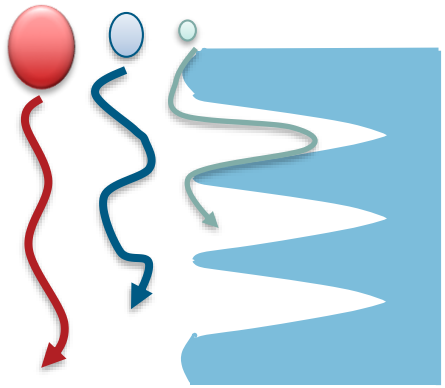
HILIC (Hydrophilic Interaction Liquid Chromatography)

- Modern adaptation of normal phase chromatography
- Well defined polar stationary phase
- Acetonitrile + water mobile phase
  - (or other aqueous-miscible solvent)
- Works well for very polar compounds
  - Acids, Bases, Zwiterions
  - Glycosylates, Metabolites





SEC / GPC / GFC



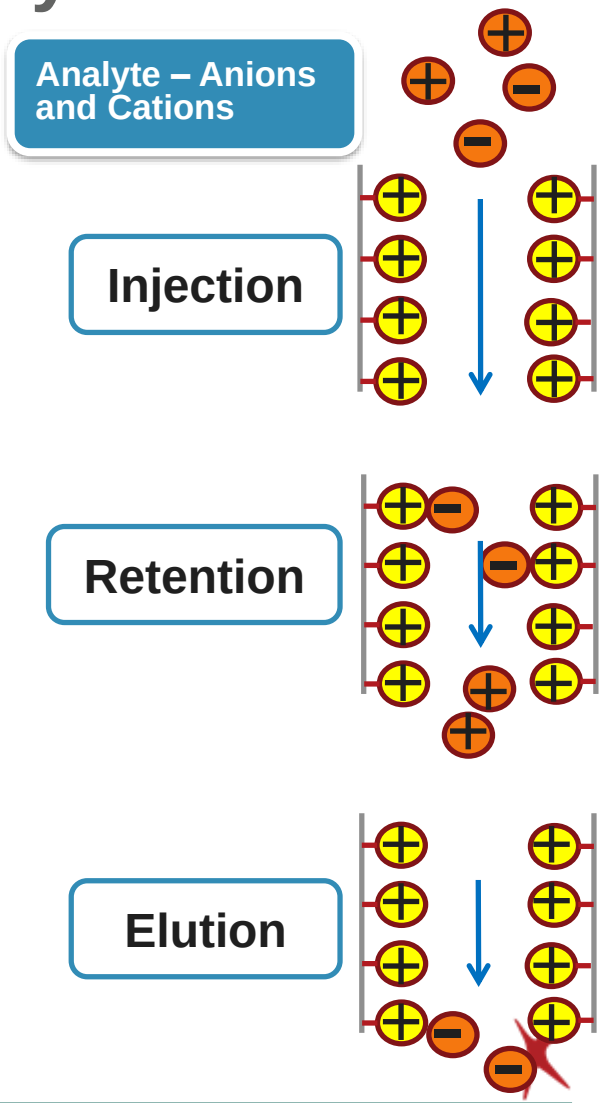
- Size Exclusion Chromatography / Gel Permeation Chromatography / Gel Filtration Chromatography
- Separation of polymers (SEC) or biopolymers (GPC/GFC)
- Separation based on molecular size (Stokes radius)
- Used for large molecule separations: protein MW, polymer MW, and polymer distributions

Stationary phase: gel or polymer with tightly controlled pore size

Separation Mechanism:
– based on molecules path through the column
– small molecules travel easier into pores of the stationary phase, “retained” longer than larger molecules

Ion Exchange Chromatography

- Stationary phase: resin with covalently bound charged functional groups
  - Different columns for analyzing anions and cations
- Used for separating ionic species
  - F^-, Cl^-, Br^-, NO_3^-, SO_4^{2-} etc.
    - Anions in physiological fluids
  - Ammonia, Methylamine, etc.
    - Cations in physiological fluids
    - Transition metal ions in plasma and blood
  - Proteins
  - Carbohydrates
  - Oligosaccharides



Chiral Chromatography

- Separation of enantiomers
- Chiral stationary phase
  - Cellulose
  - β-cyclodextrin
- Imidizole antifungals
- NSAIDS

Summary

LC - Basics

LC – Separation Mechanisms

LC – Method Development

References

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Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**: None declared
- **Consultant or Advisory Role:** None declared
- **Stock Ownership:** None declared
- **Honoraria:** None declared
- **Research Funding:** None declared
- **Expert Testimony:** None declared
- **Patents:** None declared

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