Chemistry 3403 Exp: UPLC

Goals:

1. **Familiarization with Ultra Performance Liquid Chromatography(UPLC).**

2. **Compare UPLC-MSMS. UPLC-PDA. Comparing Linearity. Plot of Peak area vs. ng on column.**

Liquid Chromatography(LC):

LC used for compounds not volatile enough for GC.

Equilibration slower in LC. Use packed columns.

HPLC vs. UPLC:

HPLC:

Column 1.7 – 5 µm diameter bead size.

Pressures: 7-40 MPa

UPLC:

Column 1.5 – 2 µm diameter bead size.

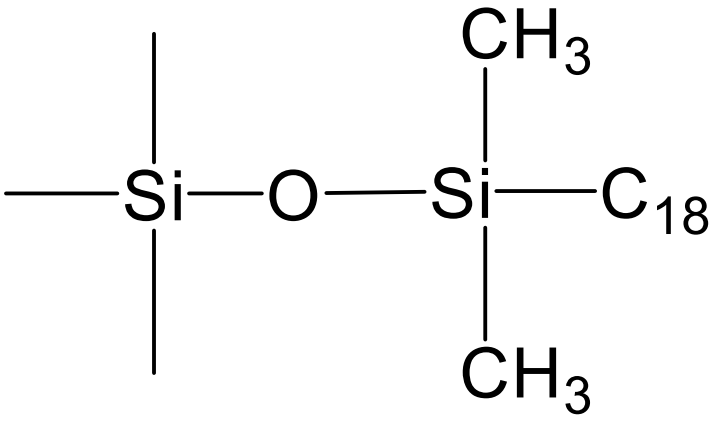
Pressures: Up to 100 MPa.

UPLC: Normal vs. Reverse Phase

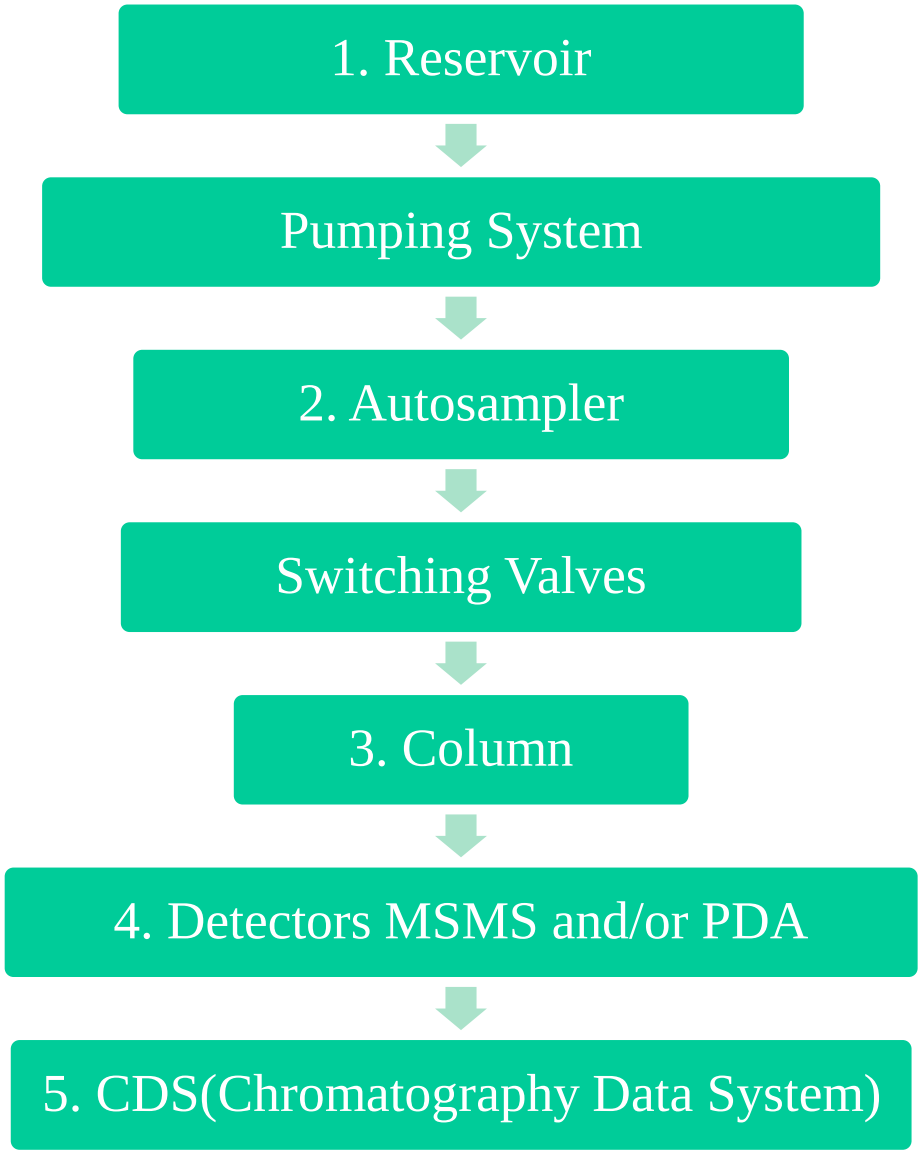
Normal: Polar Si column/Nonpolar mobile phase.

Reverse: Nonpolar C-18 Si column/polar mobile phase.

More common.

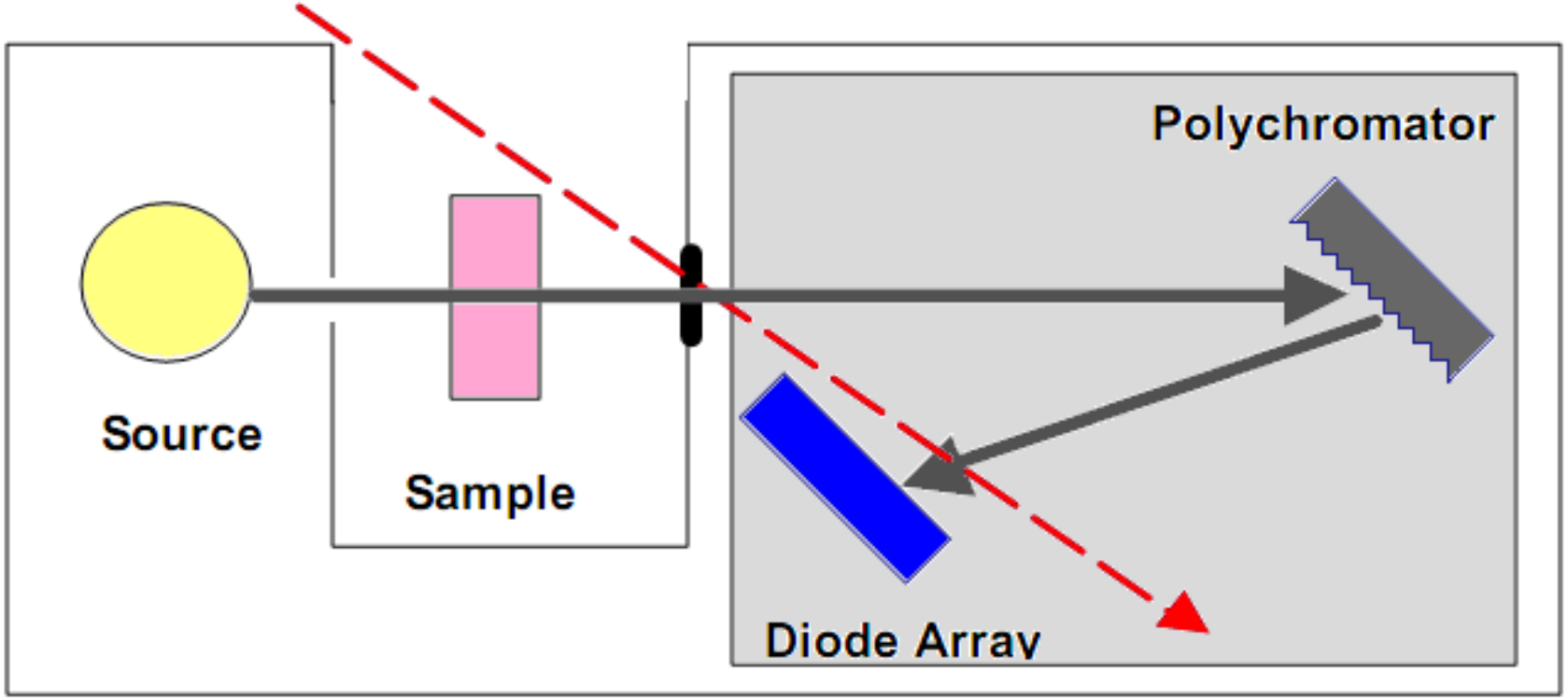


UPLC:


1. Reservoir
Pumping System
2. Autosampler
Switching Valves
3. Column
4. Detectors MSMS and/or PDA
5. CDS(Chromatography Data System)

Detectors: PDA

Photodiode Array(UV).



Reference: http://www.intechopen.com/source/html/18835/media/image5.png

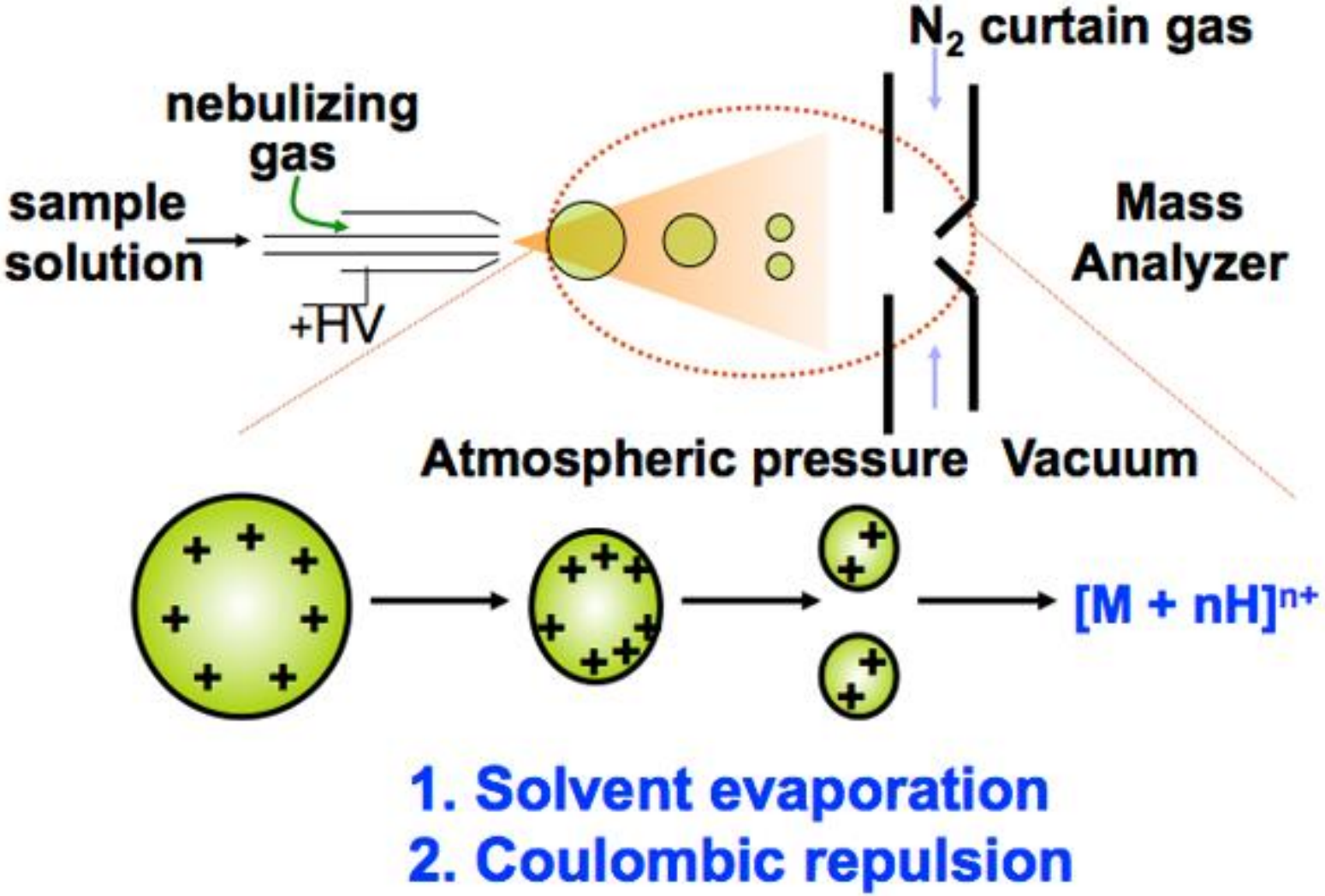
Detectors: MSMS

ESI: Electrospray Ionization

Uses a voltage to convert liquid from column into a charged aerosol.

Soft ionization technique. Results in charged macromolecules with very little fragmentation.

ESI:



Reference: http://www.uab.edu/proteomics/massspec/education/esi.php

Detectors: MSMS

MSMS: Quadrupole and Time of Flight(QTof)