Quantitative Methods in Chemistry

Week 8, Lecture 1

This week: Practice of Chromatography – HPLC, Gas Chromatography, Supercritical Fluid Chromatography, Detectors for analytes

This lecture: Practice of chromatography – HPLC

High Performance Liquid Chromatography

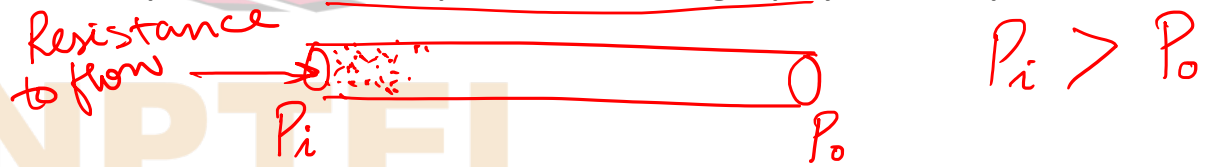
The work of Martin and Synge set the stage for high performance liquid chromatography.

Smaller particles of stationary phase lead to more efficient chromatography with increased resolution Rs and reduced time.

However, tight packing of small particles lead to generation of strong back-pressure and drop of pressure across the length column.

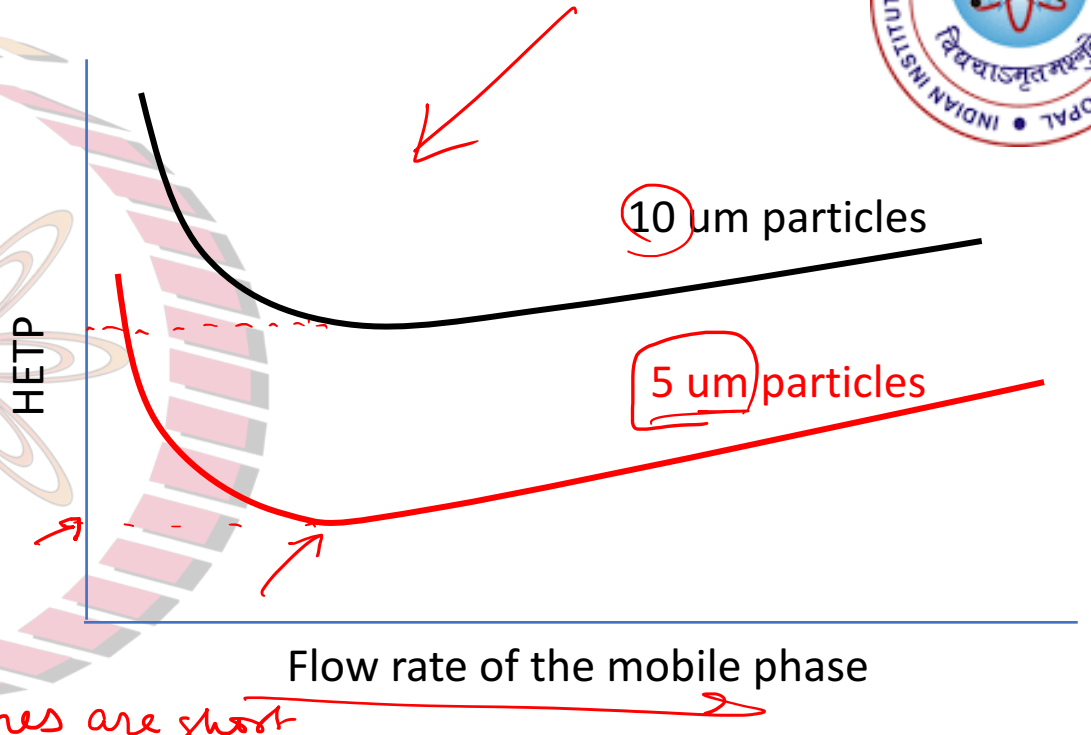
Another problem is related to column bleeding wherein disintegrated stationary phase material starts coming out of the column after repeated use.

Therefore HPLC necessitates pumps to flow could produce fast liquid chromatography techniques.



Parameters typical of HPLC

Parameter	HPLC using 5 um particles
Column length L	~ 12 cm
No. of plates	~6000
Plate height (H)	~0.002 cm
Flow rates (u)	0.02 cm/s
No. of plates per meter	~48,000
Pump pressures	Upto 200 bars



Elution times are short
Elution volume are also low

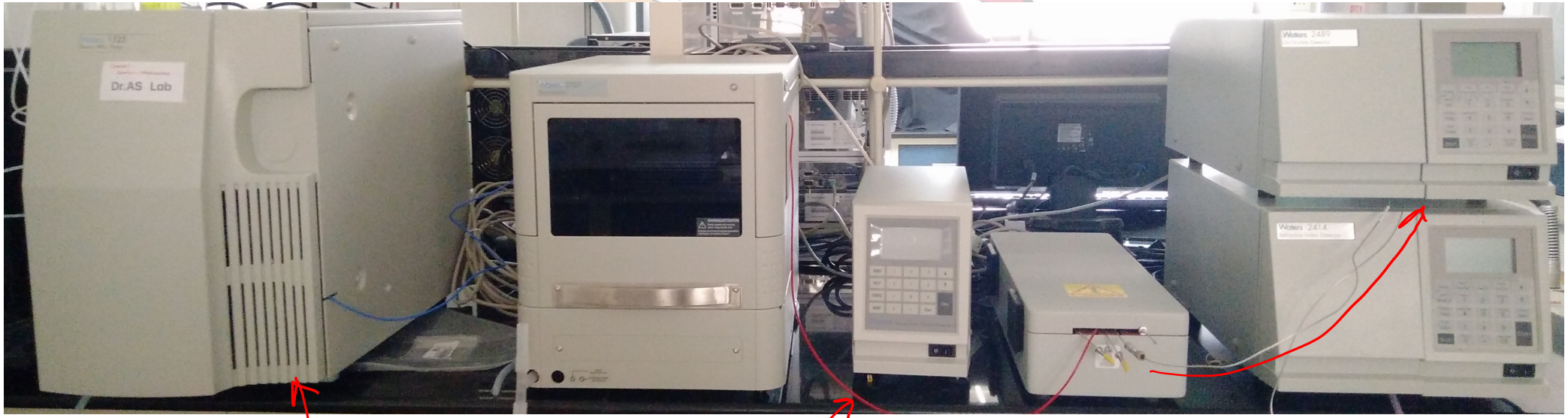
Ultraperformance Liquid Chromatography (UPLC) – Uses **1.8 um** particles in the column.

May need upto **1000 bar** pressures to move the solvent through the column.

Special pumps and connectors are therefore needed to achieve and sustain such high pressures.

Components of an HPLC

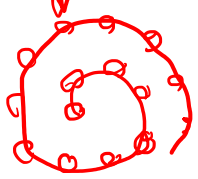
Pump, Injector, Column and Detector are the key component of HPLC systems



Binary Pump

Sample
Auto-injector

Manual injector



Column oven with columns inside

accommodate upto 3 columns

L → N

Detectors:
UV-vis and refractive index

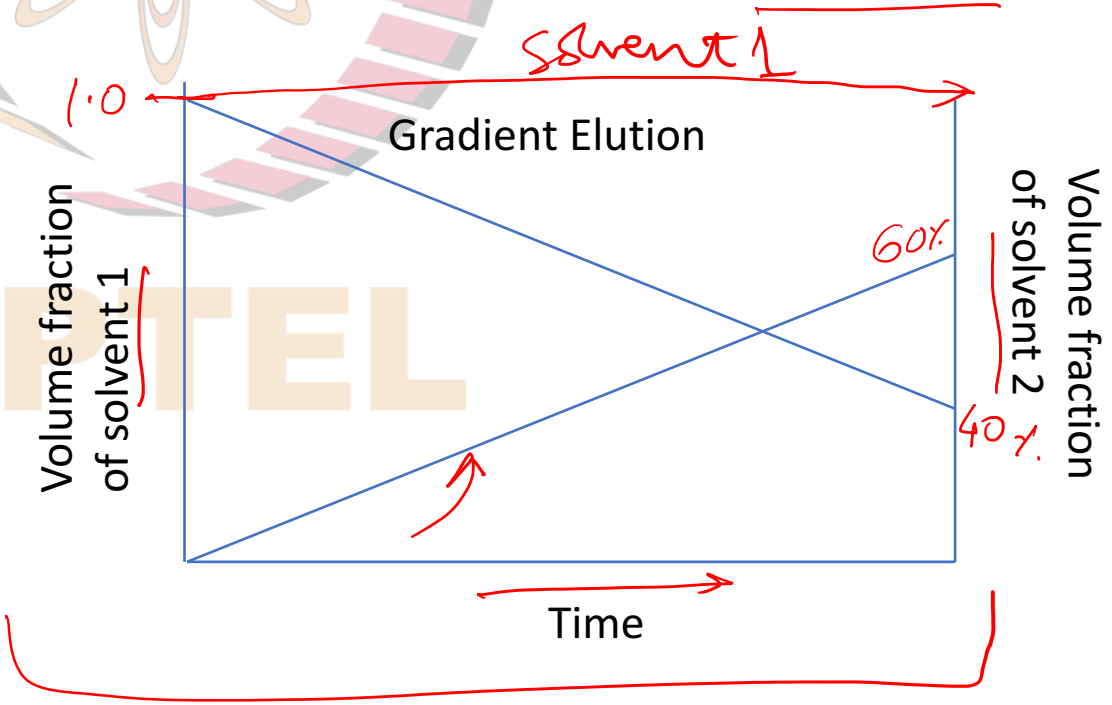
Isocratic elution vs. Gradient elution

The initial HPLC profiles were all isocratic i.e. had only one solvent eluting out the analyte.

Refractive index (RI) detector was used in early HPLCs also required Isocratic Elutions.

Slowly, the isocratic elution was replaced by Gradient Elution.

Pumping systems, mixing chambers and detectors were developed to accommodate Gradient Elution.



Binary Pump HPLC in a nut-shell

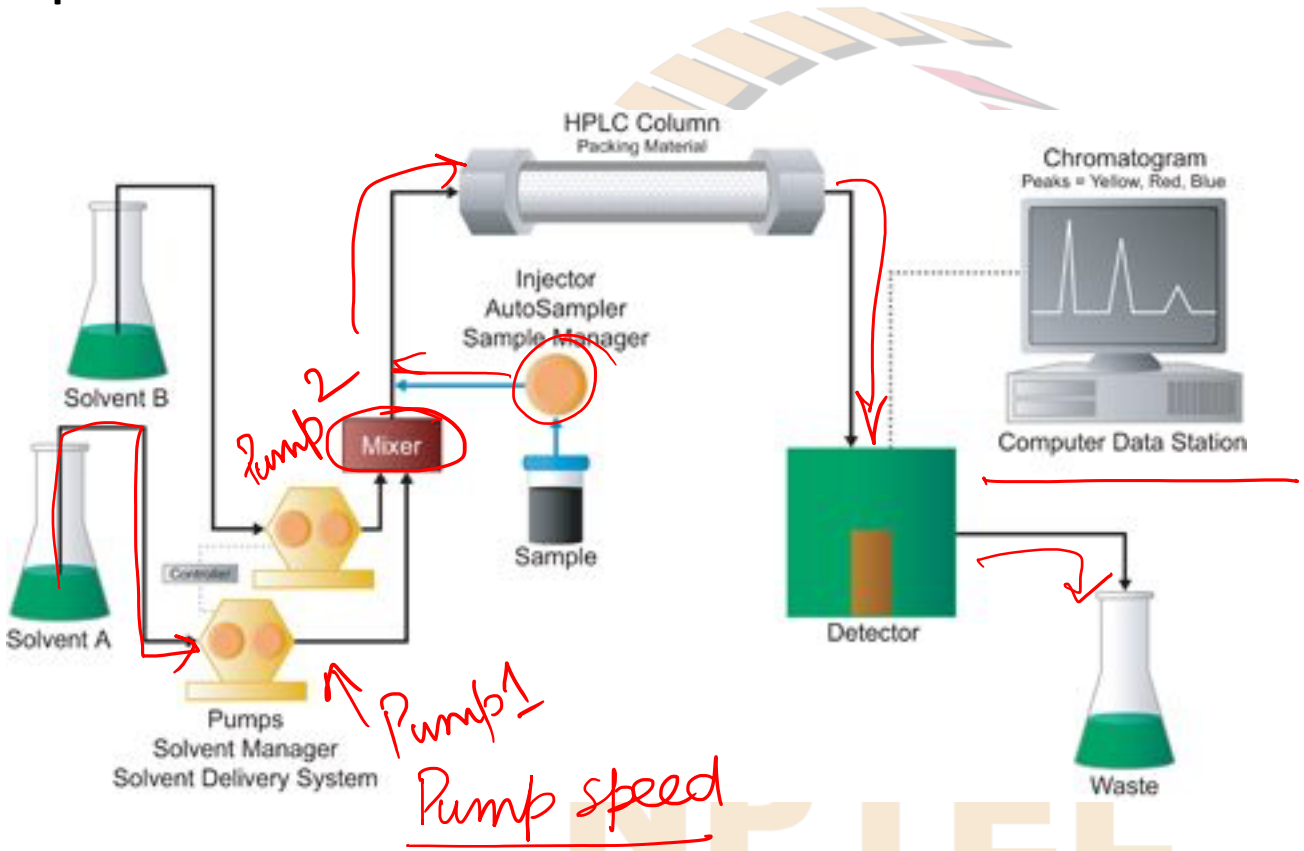
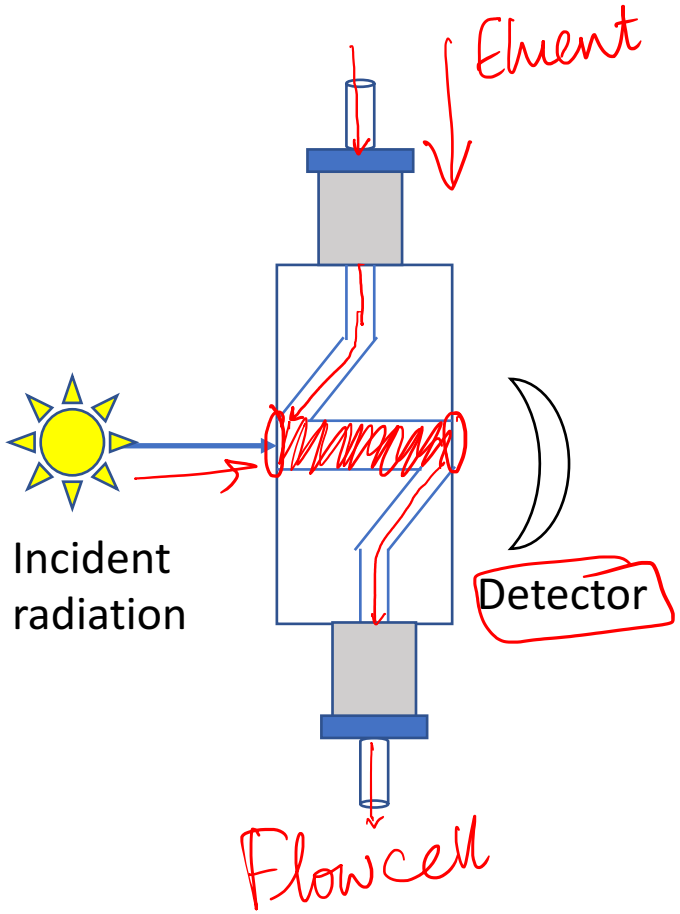


Image courtesy Waters.com

Flow cell for analyzing small volumes



Types of detectors employed in HPLC:

- Refractive Index Detector (For non-absorbing compounds, absorbing solvents, non-selective, low sensitivity, isocratic elution)
- UV/vis detector (change in absorbance at particle wavelength, sensitivity depends on the analyte)
- Photodiode array detector (absorption spectrum of analyte)
- Fluorescence detector (highly sensitive, very few organic compounds fluoresce)
- Evaporative Light Scattering Detector (Can detect non-absorbing compounds with high sensitivity, low MW compounds more difficult to detect)
- Mass analyzers (Highly sensitive but quite costly. May have mass limits)