Qualitative Organic Analysis – CH 351

Separation - Chromatography

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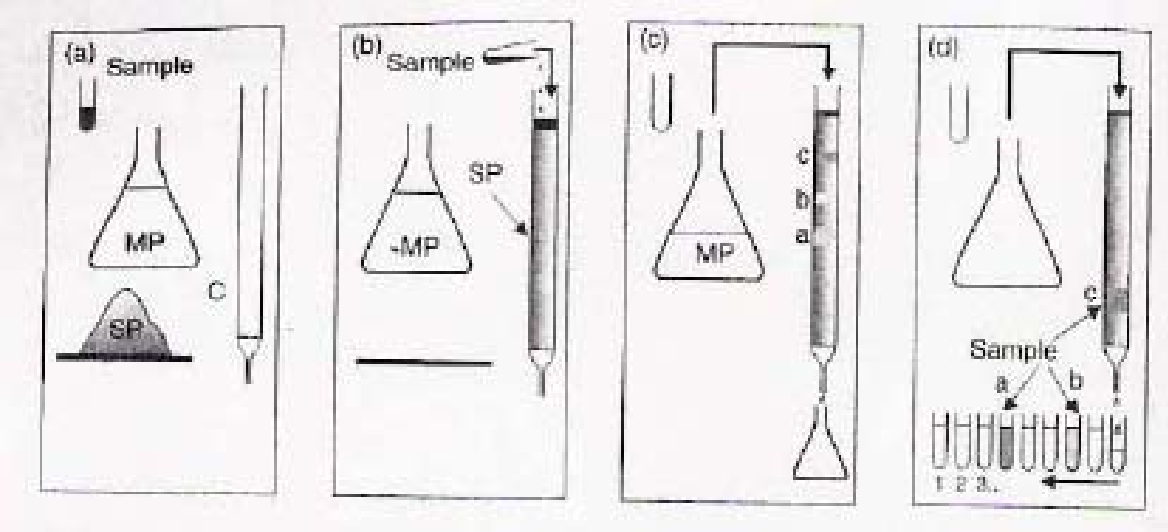
Chromatography – General Aspects

Basic experiment in chromatography

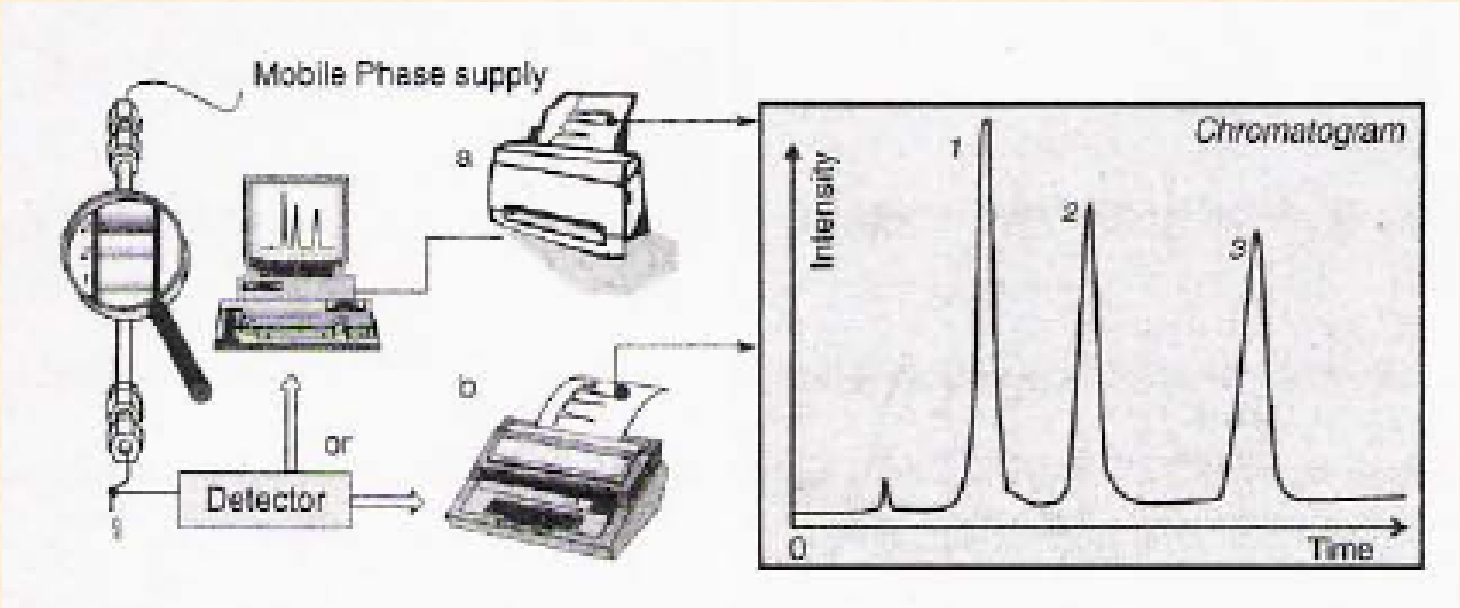


MP – mobile phase

SP – stationary phase

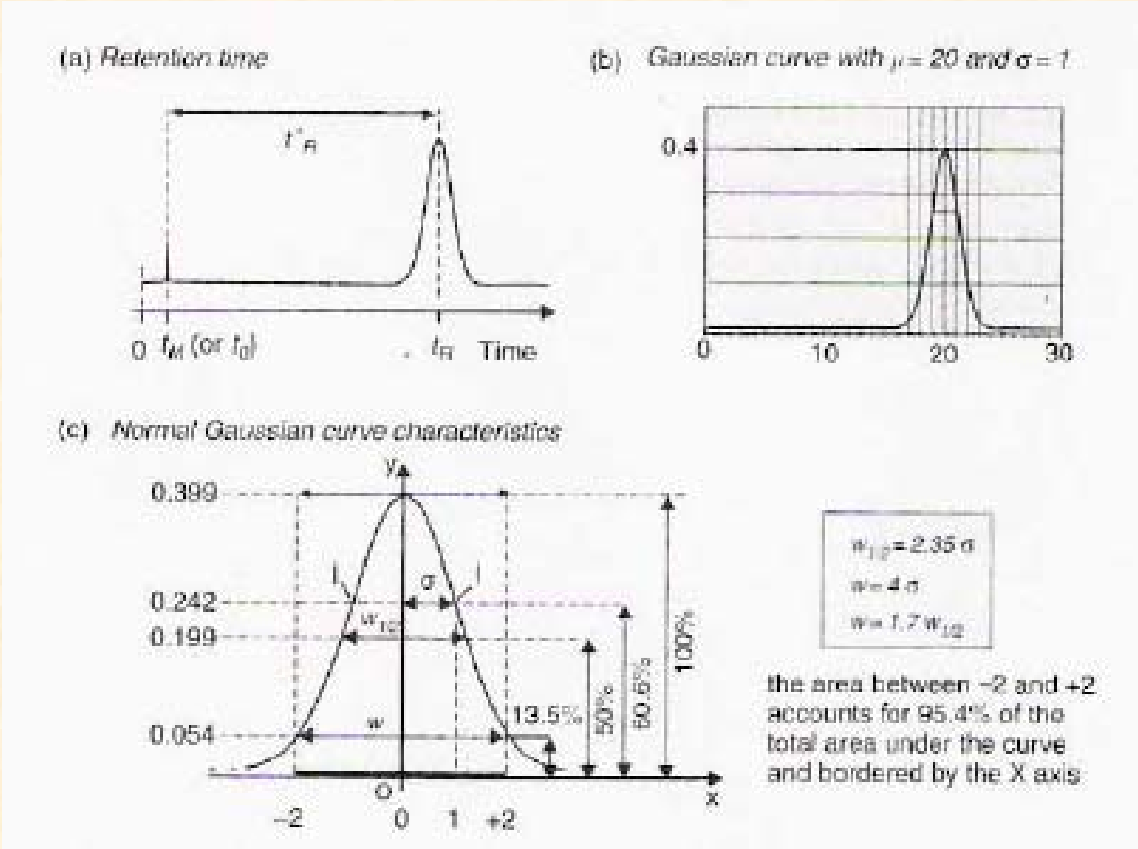
Chromatography – General Aspects

The principle of analysis by chromatography



Chromatography – General Aspects

The chromatogram

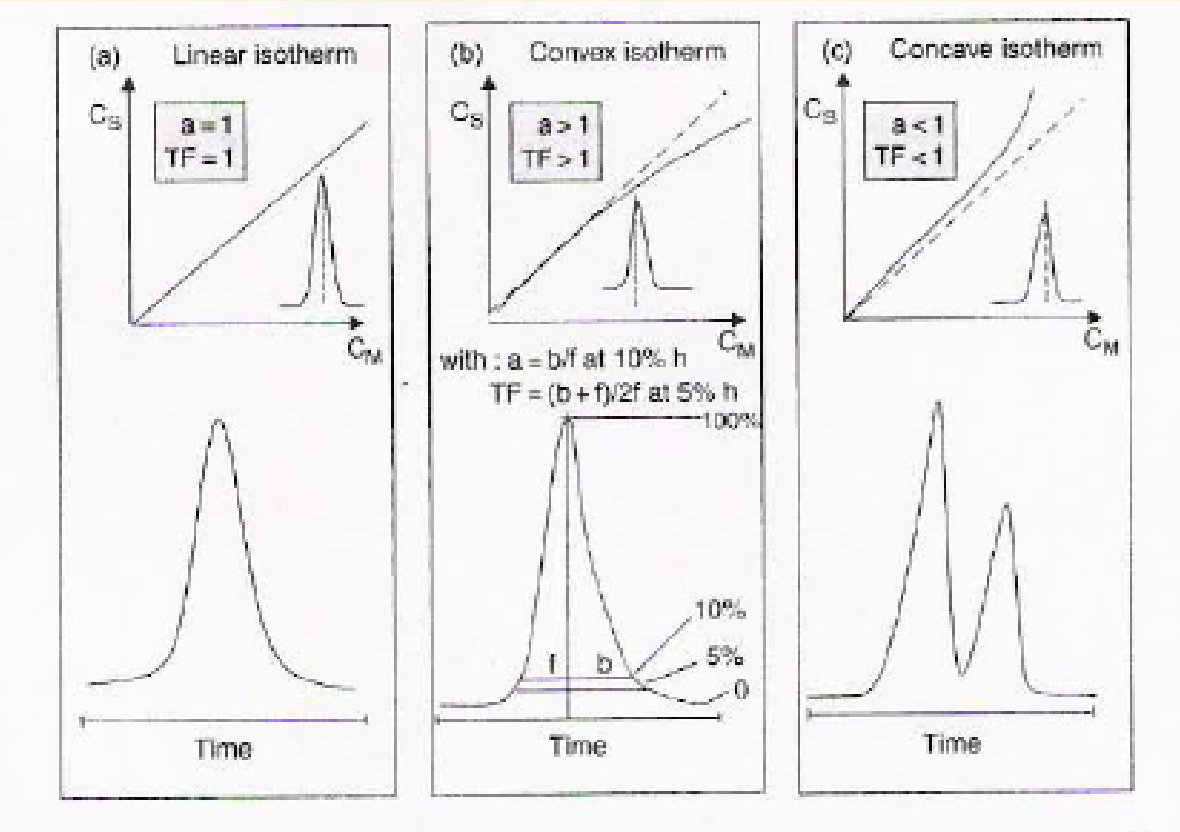


retention factor

$$k'_A = t_R - t_M / t_M$$

Chromatography – General Aspects

The chromatogram



Chromatography – General Aspects

The Plate Theory

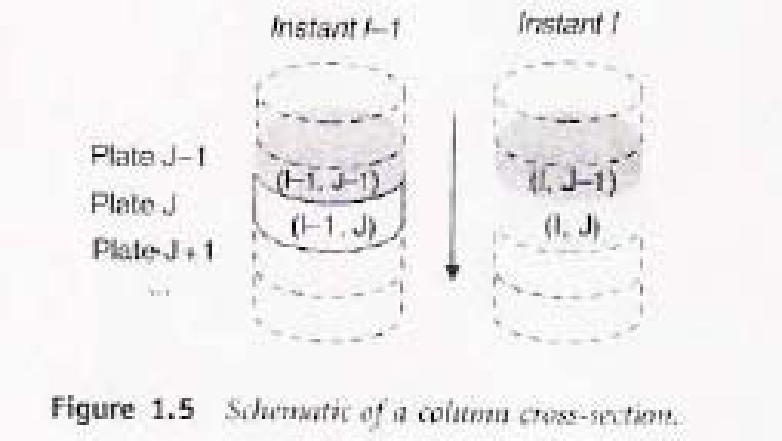
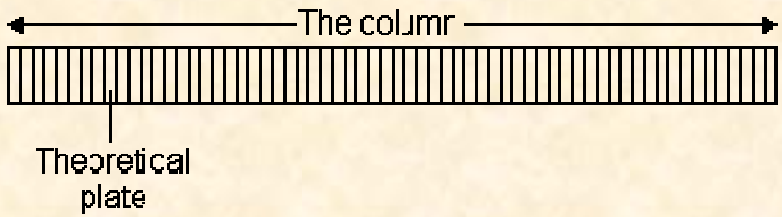


Figure 1.5 *Schematic of a column cross-section.*



If the length of the column is L, then the HETP is

HETP = L / N

$$N = \frac{5.55\, t_R^{\,2}}{w_{1/2}^{\,2}}$$

The number of theoretical plates that a real column possesses can be found by examining a chromatographic peak after elution; where $w_{1/2}$ is the peak width at half-height.

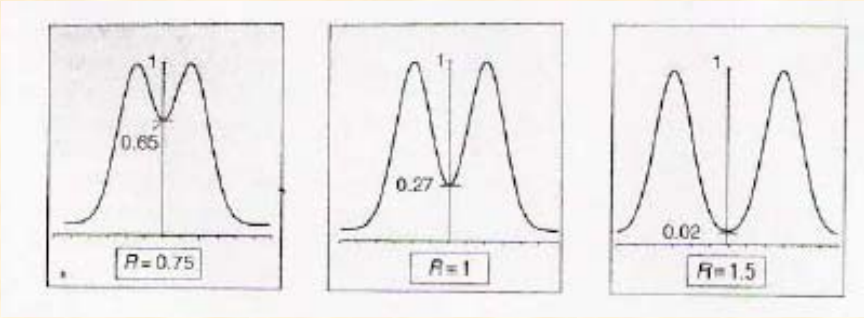
Chromatography – General Aspects

Nernst partition coefficient (K)

$$K = \frac{C_S}{C_M} = \frac{\text{Molar concentration of the solute in the stationary phase}}{\text{Molar concentration of the solute in the mobile phase}}$$

Chromatography – General Aspects

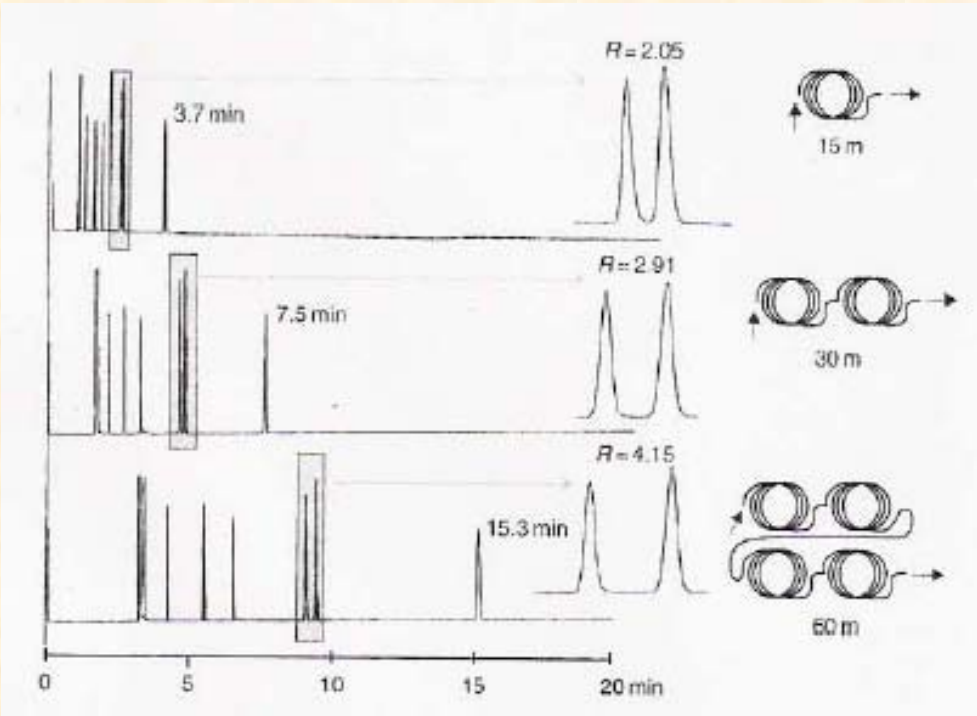
Column Efficiency – Resolution Factor



$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$

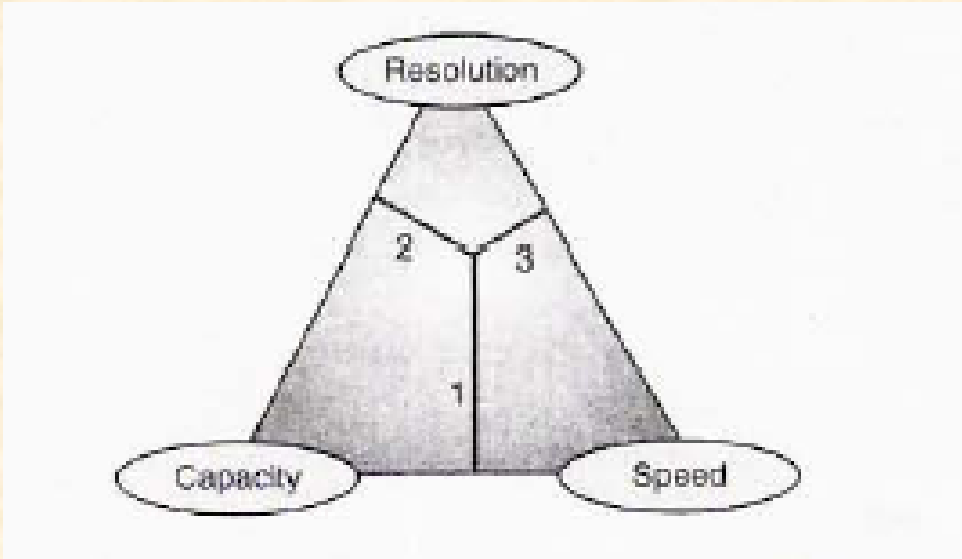
How to improve:

- mobile phase composition
- column temperature
- stationary phase
- special chemical effects



Chromatography – General Aspects

Optimization of Chromatographic Analysis



Chromatography – General Aspects

Classification of Chromatographic Techniques

Liquid phase Chromatography (LC)

- Liquid/Solid (or adsorption)
- Ion (IC)
- Size exclusion (SEC)
- Liquid/Liquid (or partition) (LLC)
- Liquid/bound phase

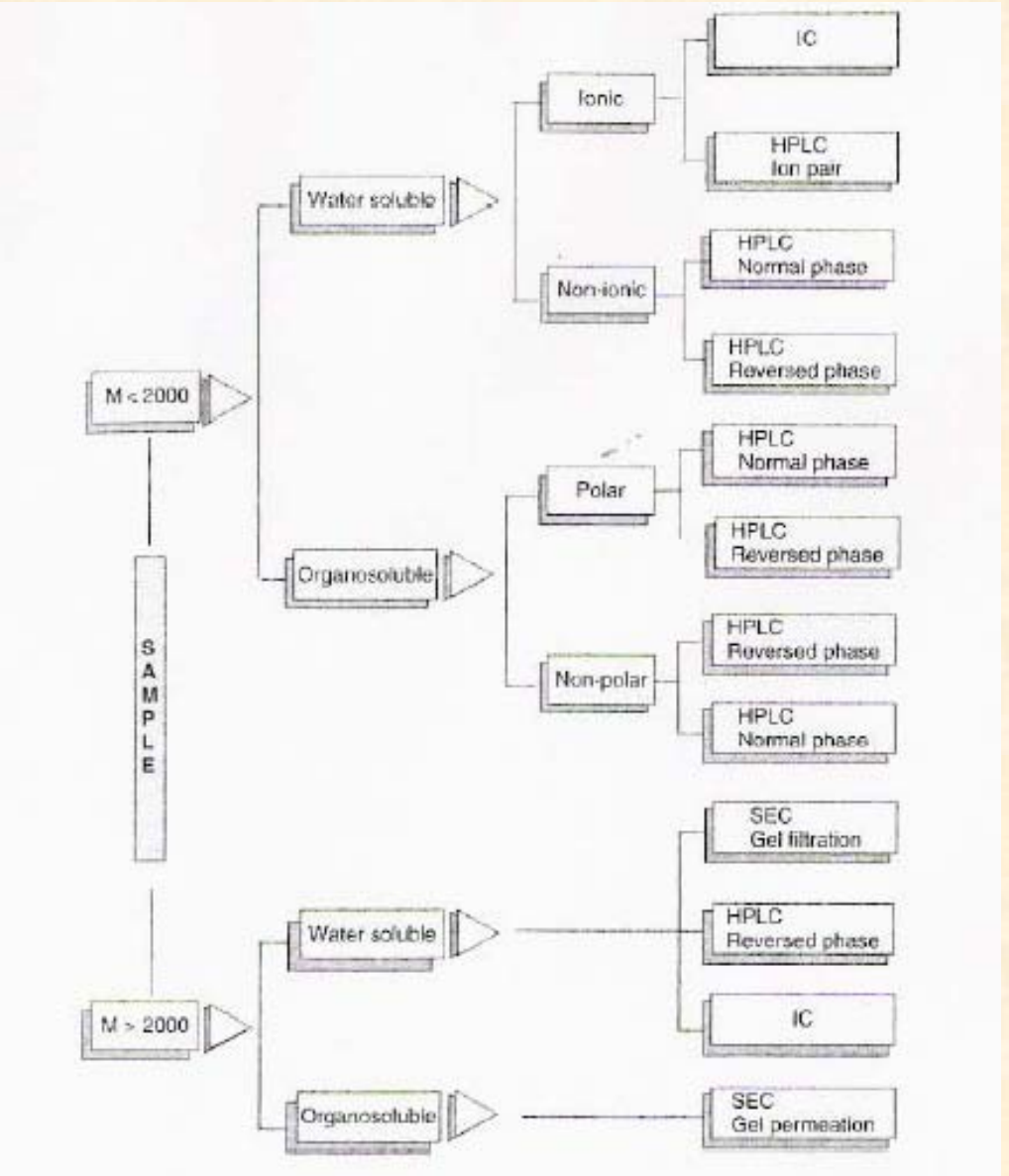
Gas phase Chromatography (GC)

- Gas/Liquid (GLC)
- Gas/Solid (GSC)

Supercritical Fluid Chromatography (SFC)

- Gas/Liquid (GLC)
- Gas/Solid (GSC)

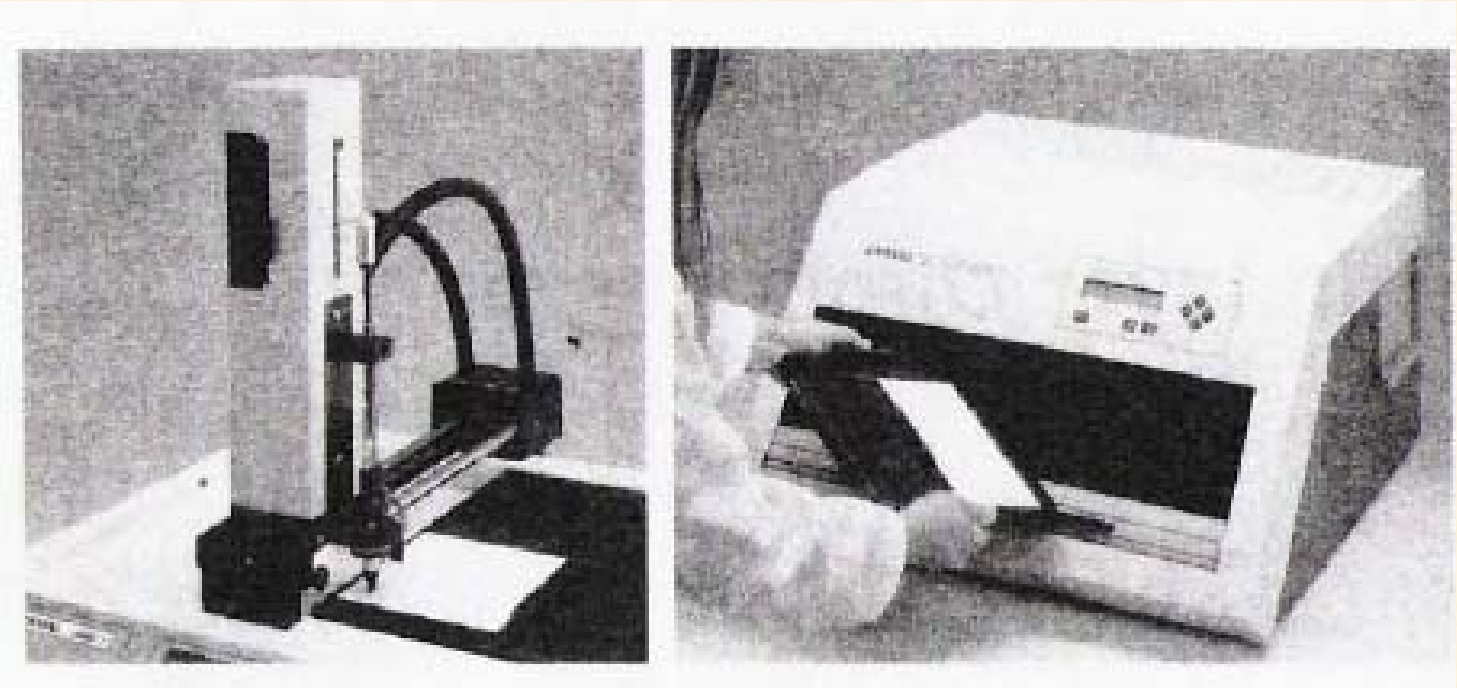
Chromatography – General Aspects



Chromatography – TLC

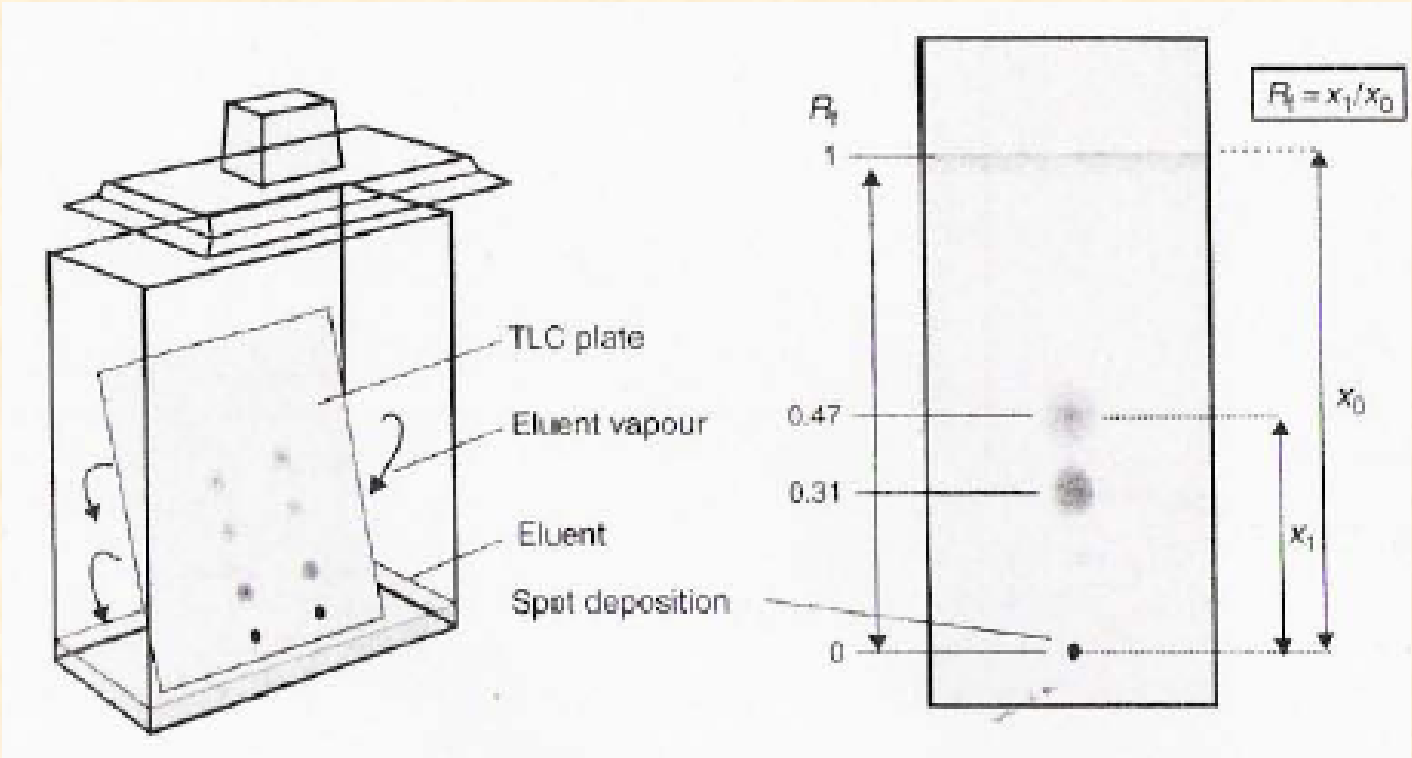
Principle of TLC

Deposition of the sample



Chromatography – TLC

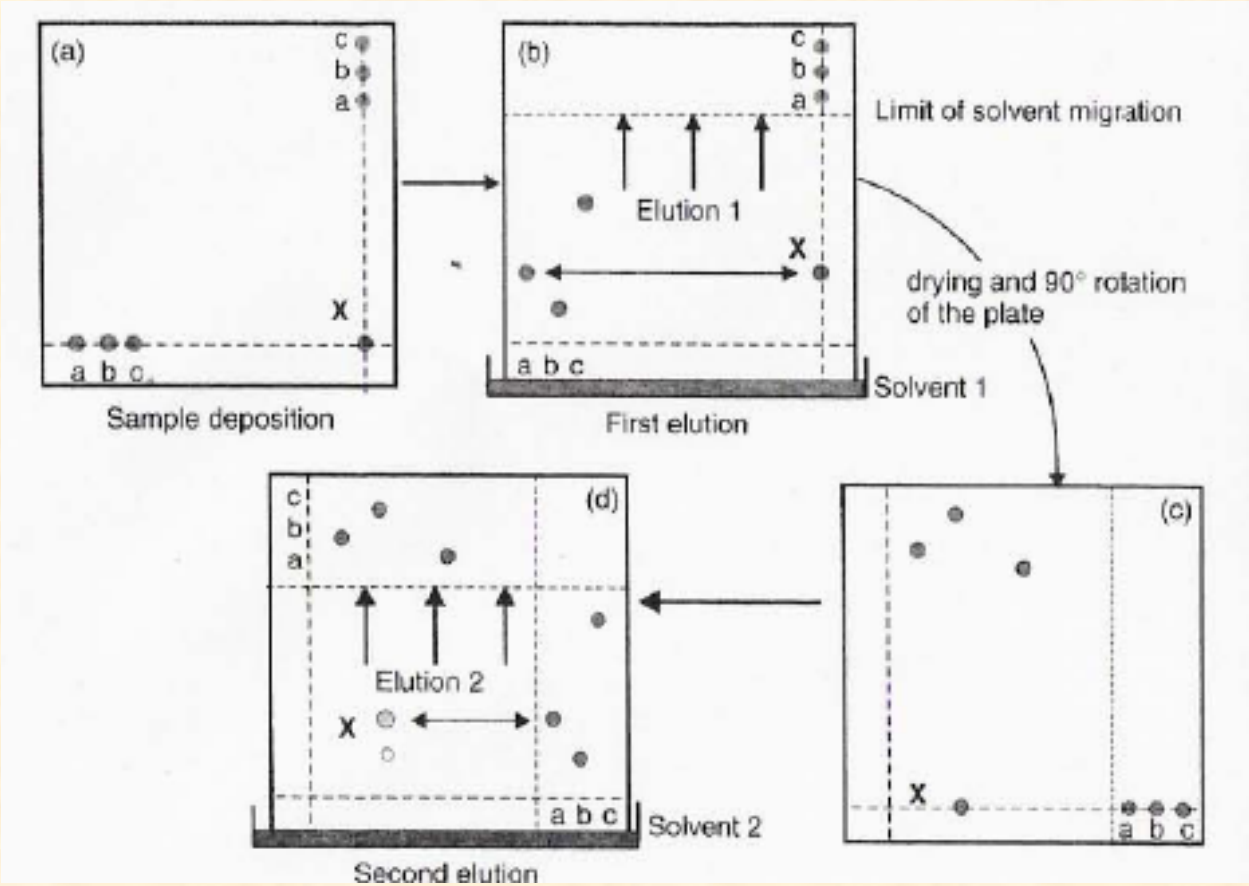
Developing the plate



Chromatography – TLC

Identifying the spots

Two dimensional TLC



Chromatography – TLC

Characteristics of TLC

- a three phase system

- only partial equilibrium (S/L or L/G)

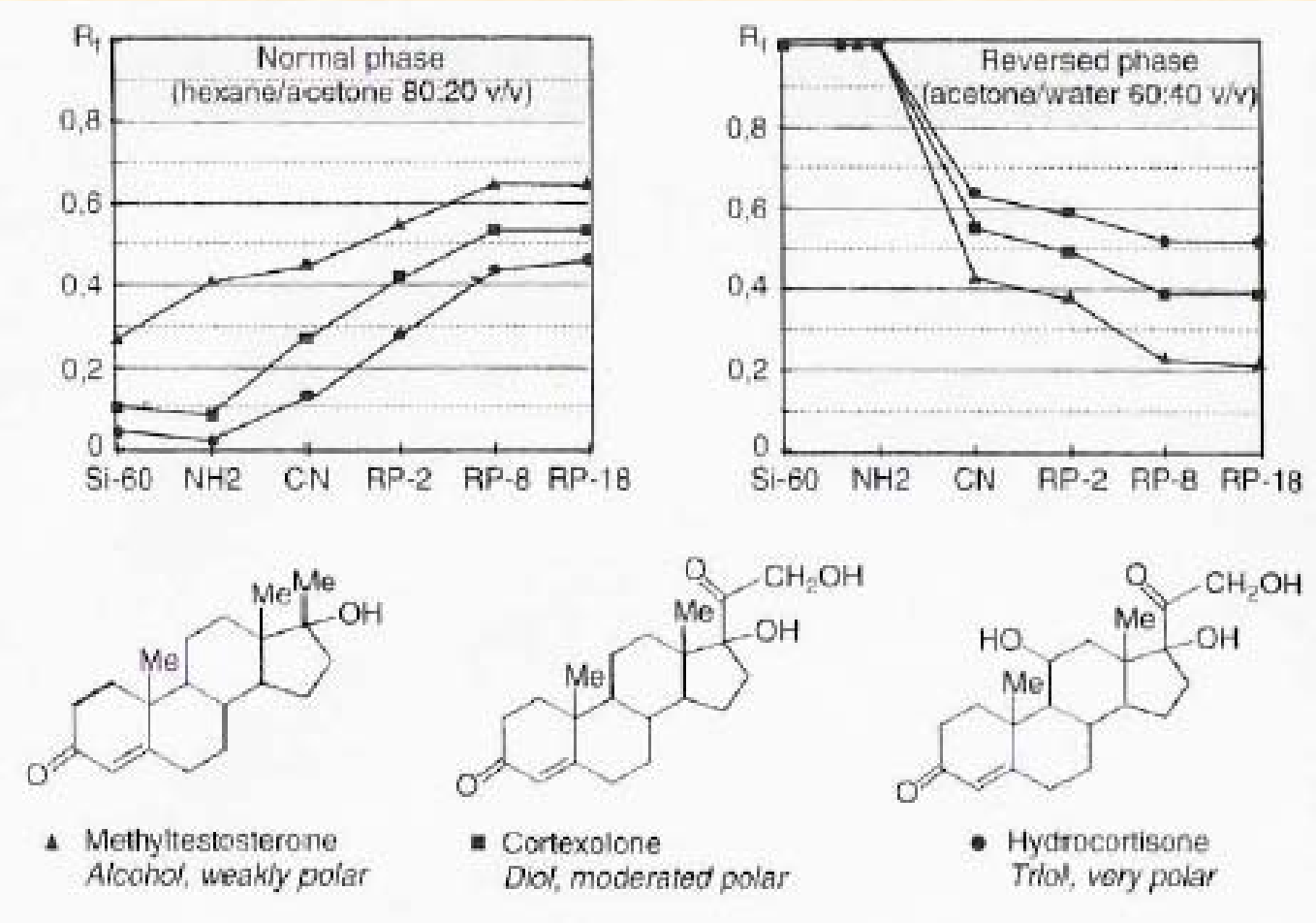
- adsorption on the stationary phase is reduced
  (different R_f in pure or mixture forms!)

-flow rate cannot be modified

- the migration of the solvent is not constant

Chromatography – TLC

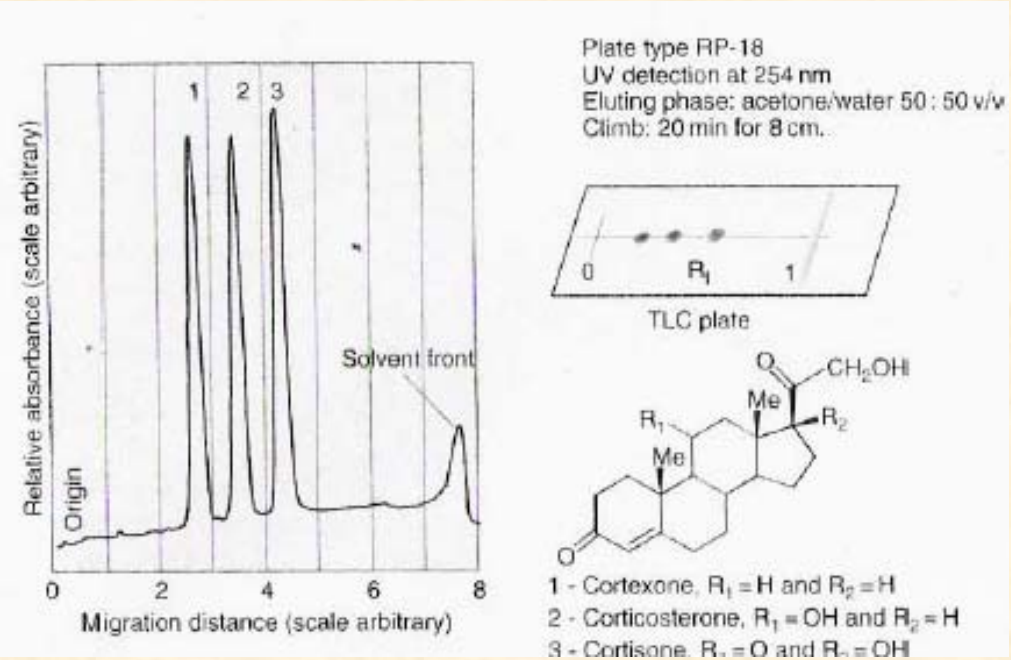
Stationary Phase



Chromatography – TLC

Quantitative TLC

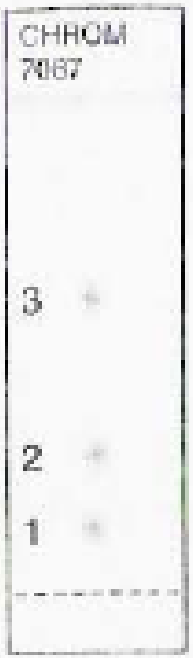
$$R_f = \frac{\text{Distance run by the solute}}{\text{Distance run by the solvent front}} = \frac{x}{x_0}$$



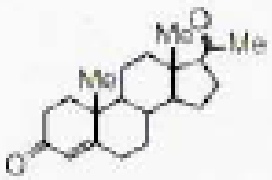
Chromatography – TLC

TLC as preHPLC

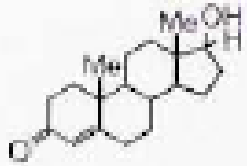
TLC Prep-Screen C18



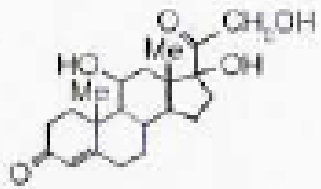
Mobile phase: Methanol : Water (70:30)



1. Progesterone

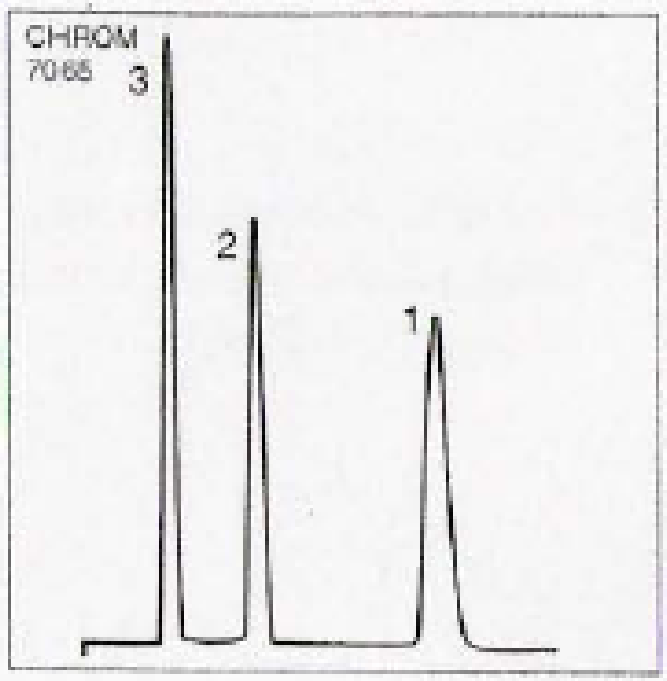


2. Testosterone



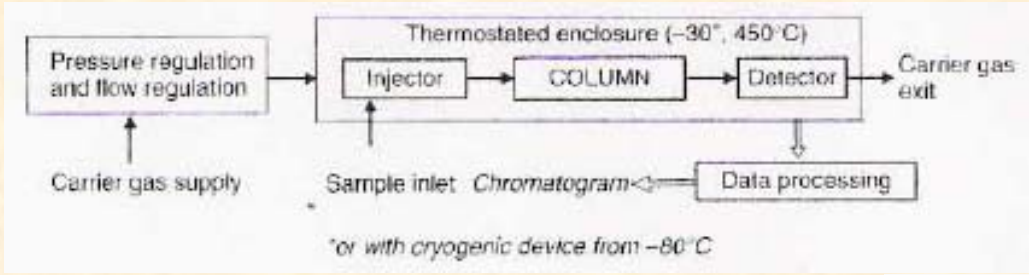
3. Hydrocortisone

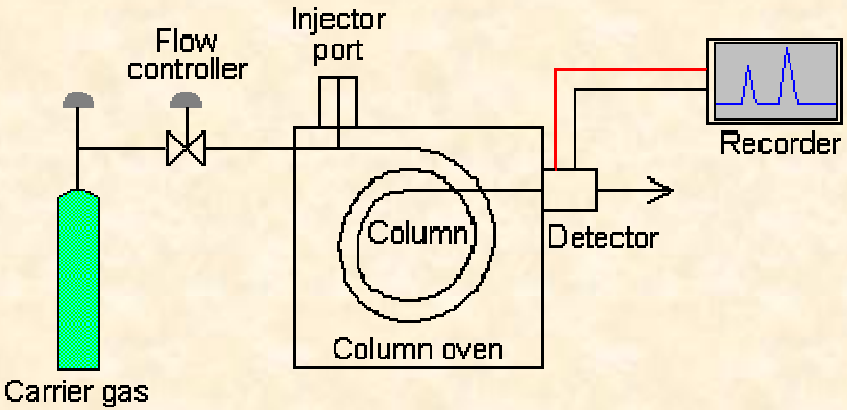
Econo-Prep HPLC

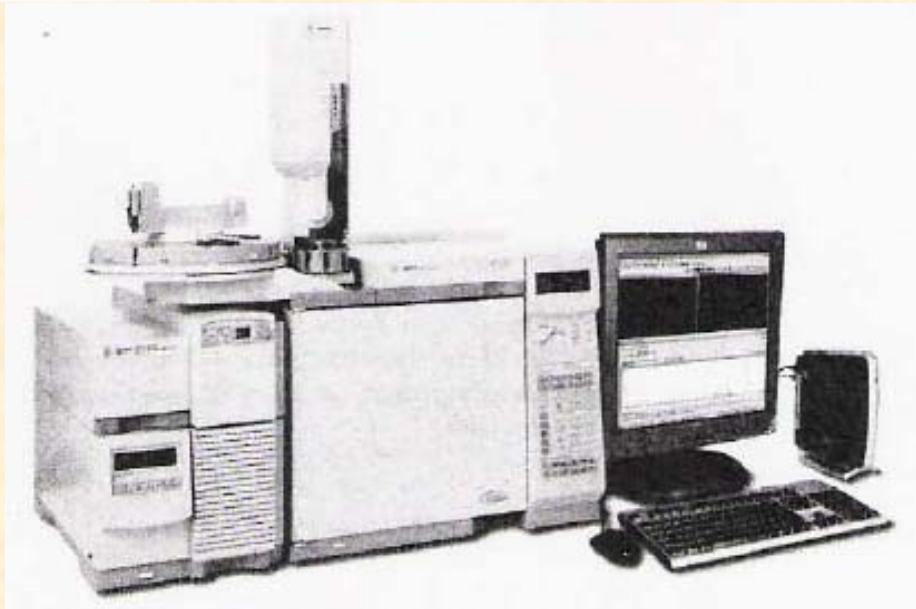


Chromatography – GC

Components of a GC

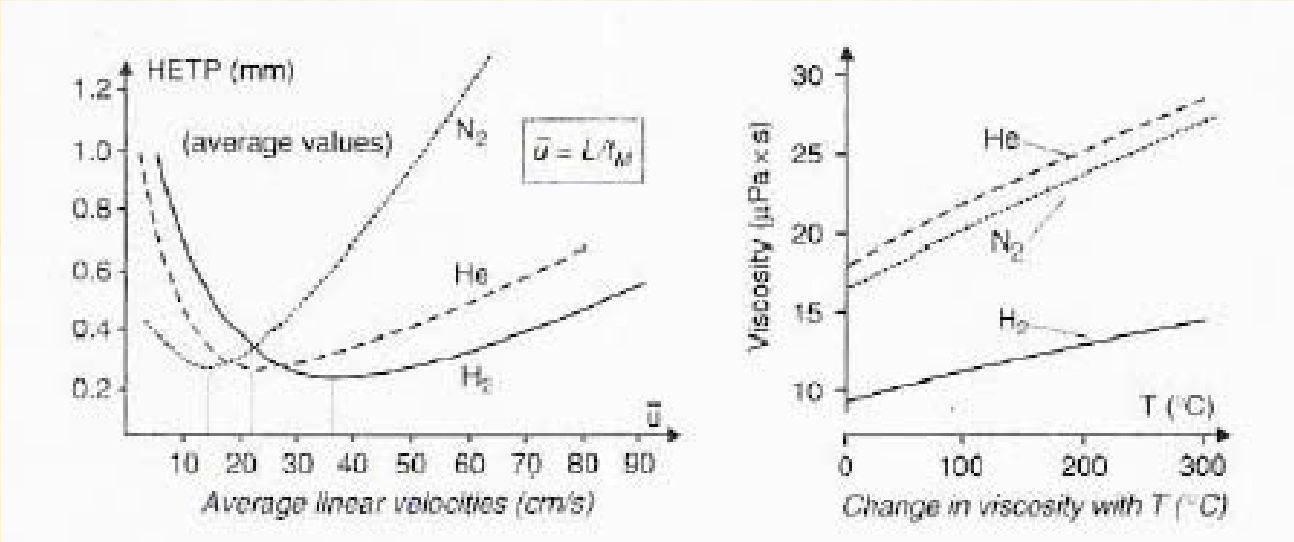






Chromatography – GC

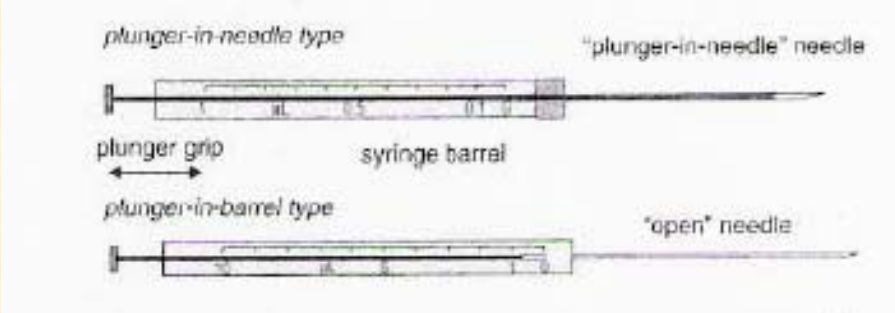
Carrier gas H_2, N_2, He, Ar, CO_2



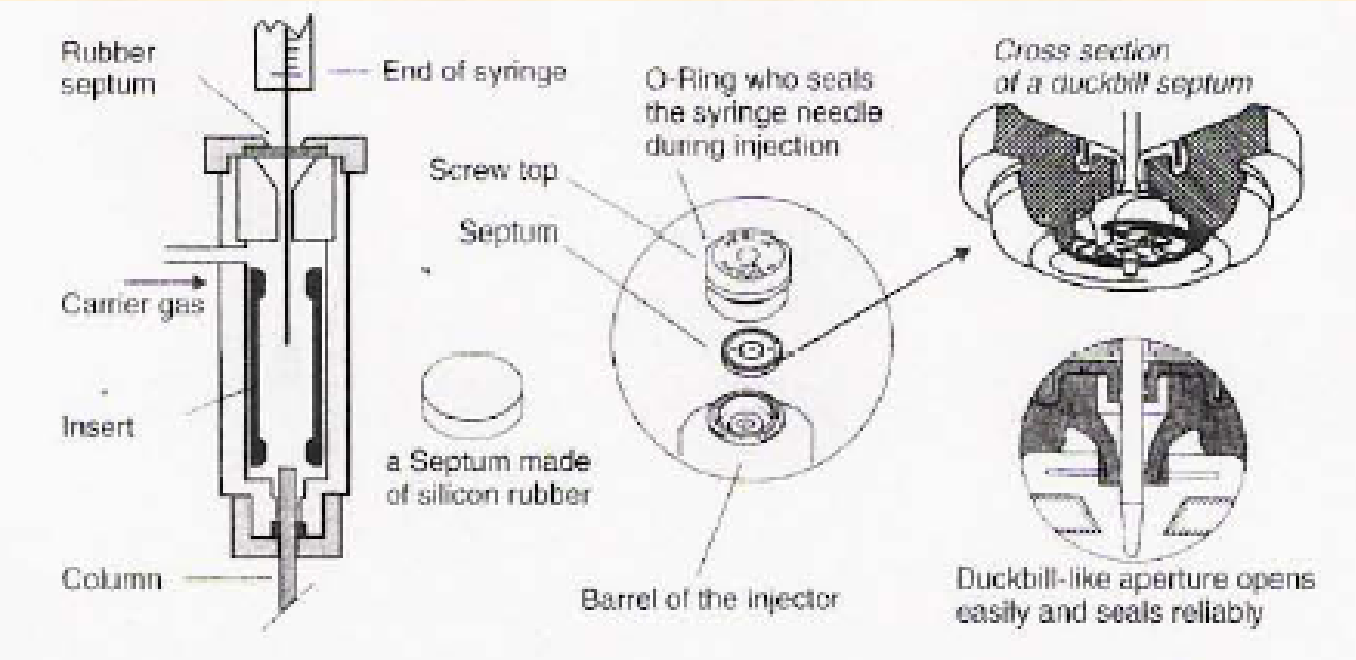
Detector !

Chromatography – GC

Sample introduction



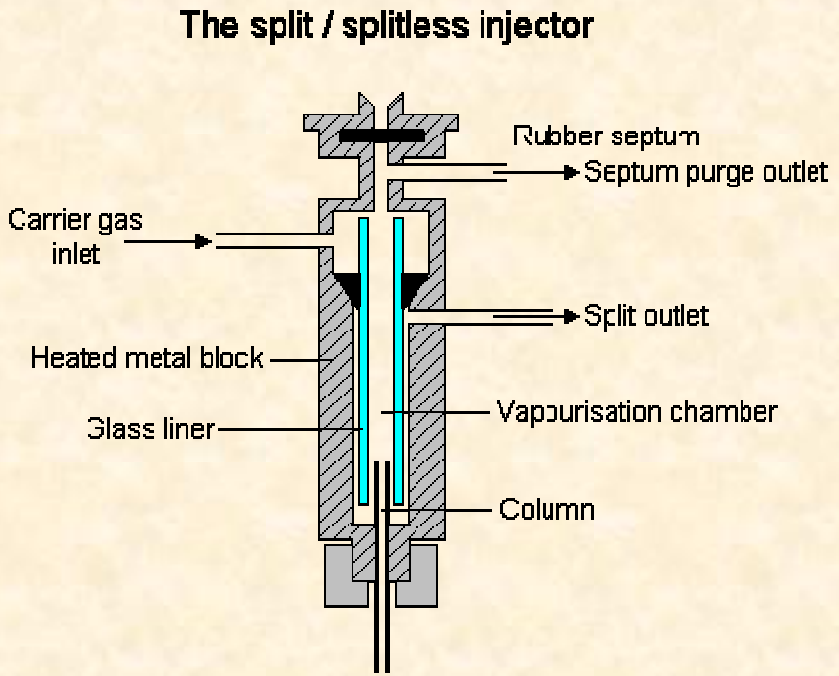
Direct vaporization injector



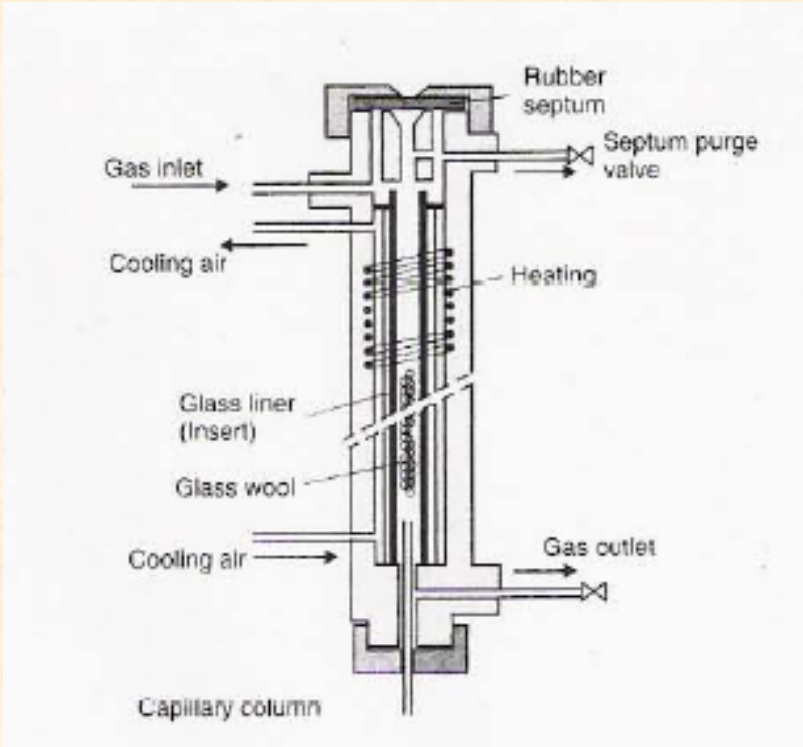
Chromatography – TLC

Sample introduction

Split-splitless injector



Programmed Temp. Vaporization Inj.

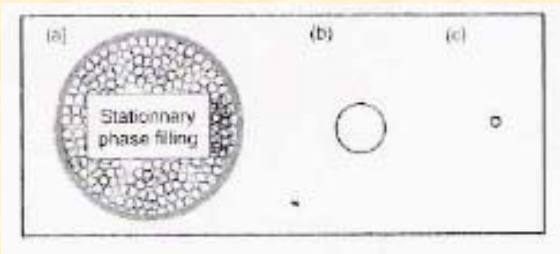


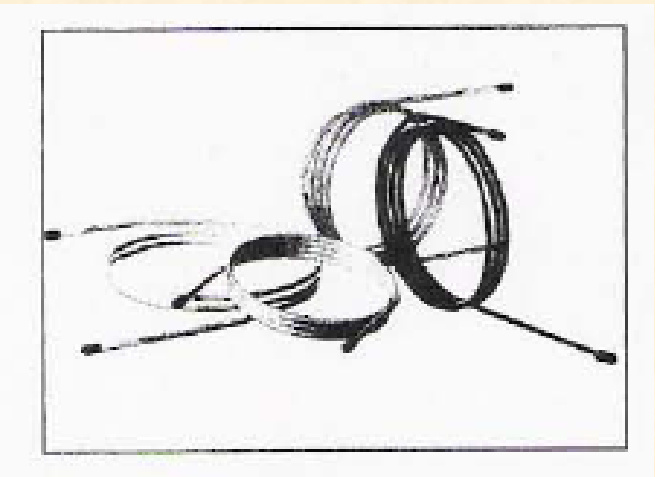
Chromatography – GC

Oven

Columns

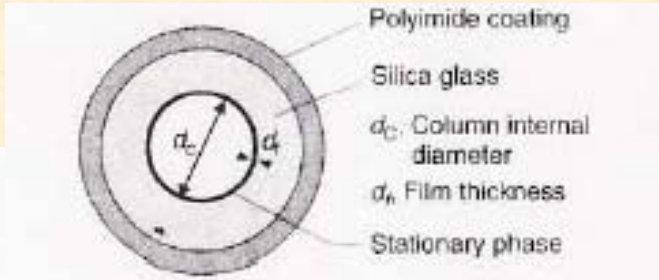
Packed





Capillary

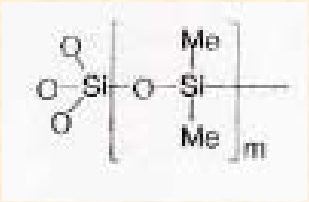
"530 μm" Columns

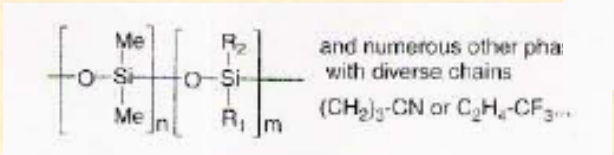


Chromatography – GC

Stationary Phases

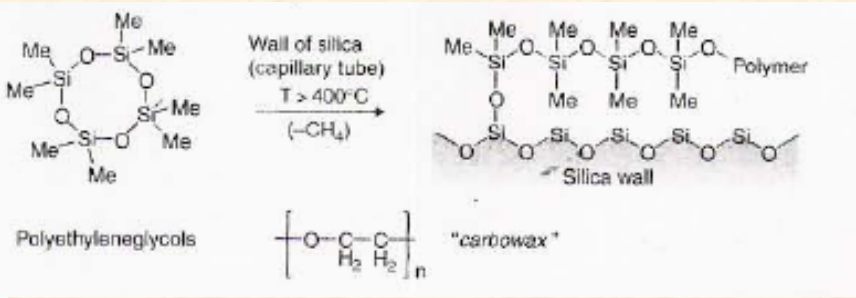
Polysiloxanes





ex. R_1 and R_2 = Ph m = 95% and n = 5%

poly(ethylene)glycols



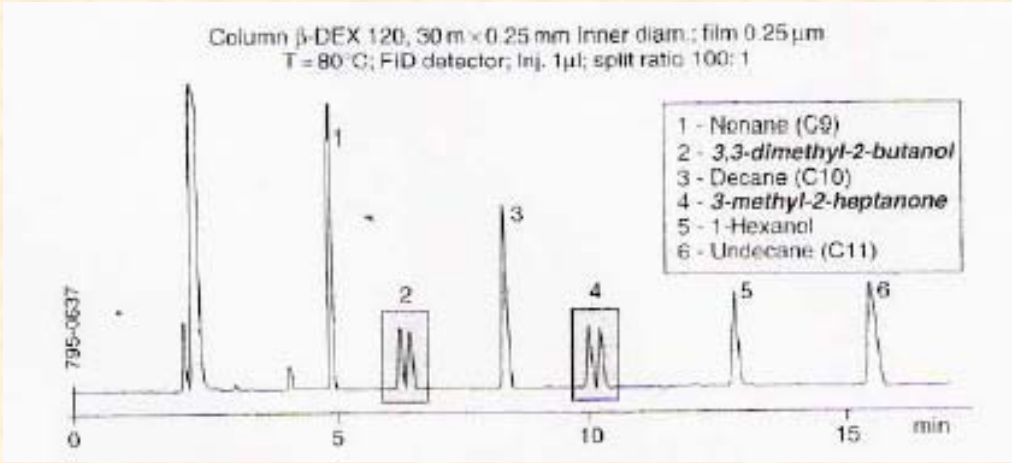
solid SPs

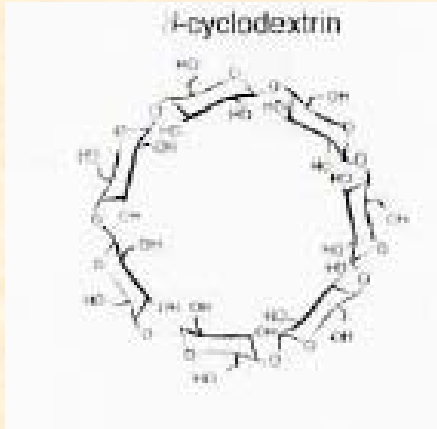
Alumina, silica, metal oxides etc.

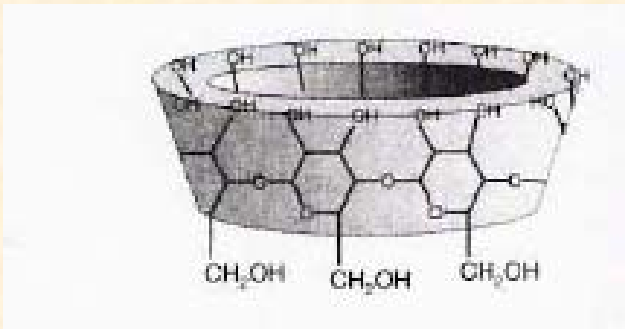
Chromatography – GC

Stationary Phases

Chiral stationary phases



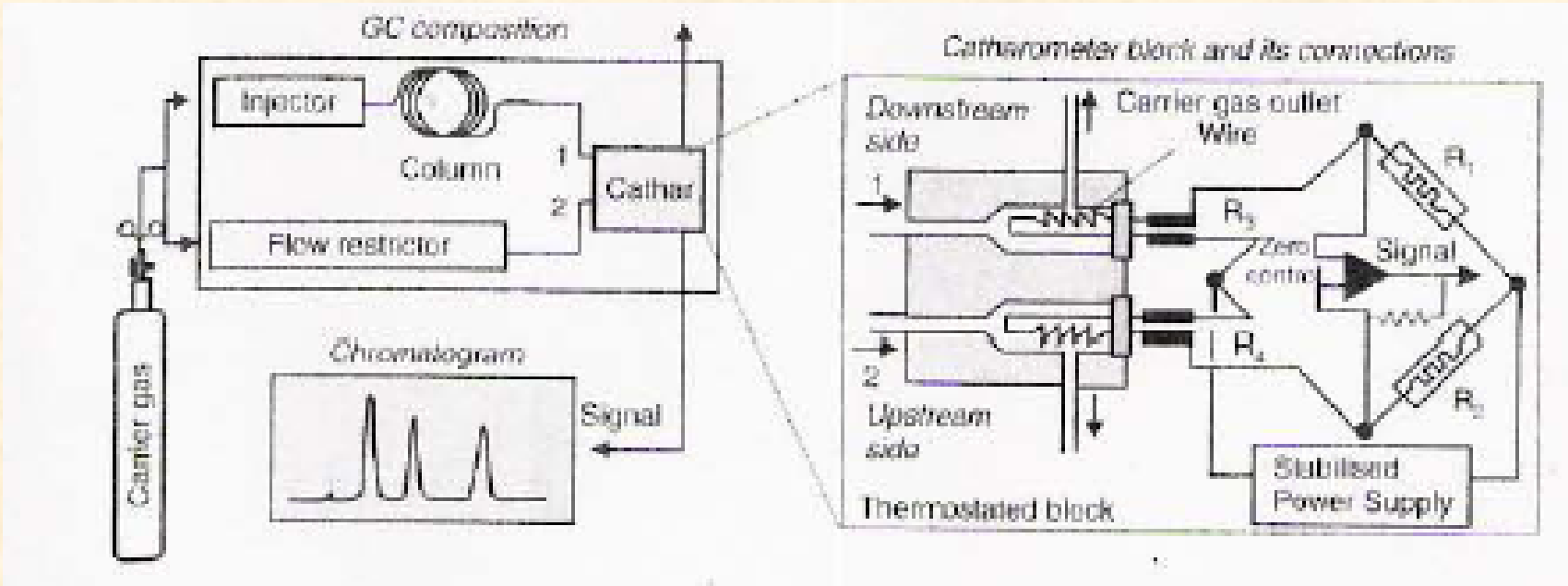




Chromatography – GC

Detectors

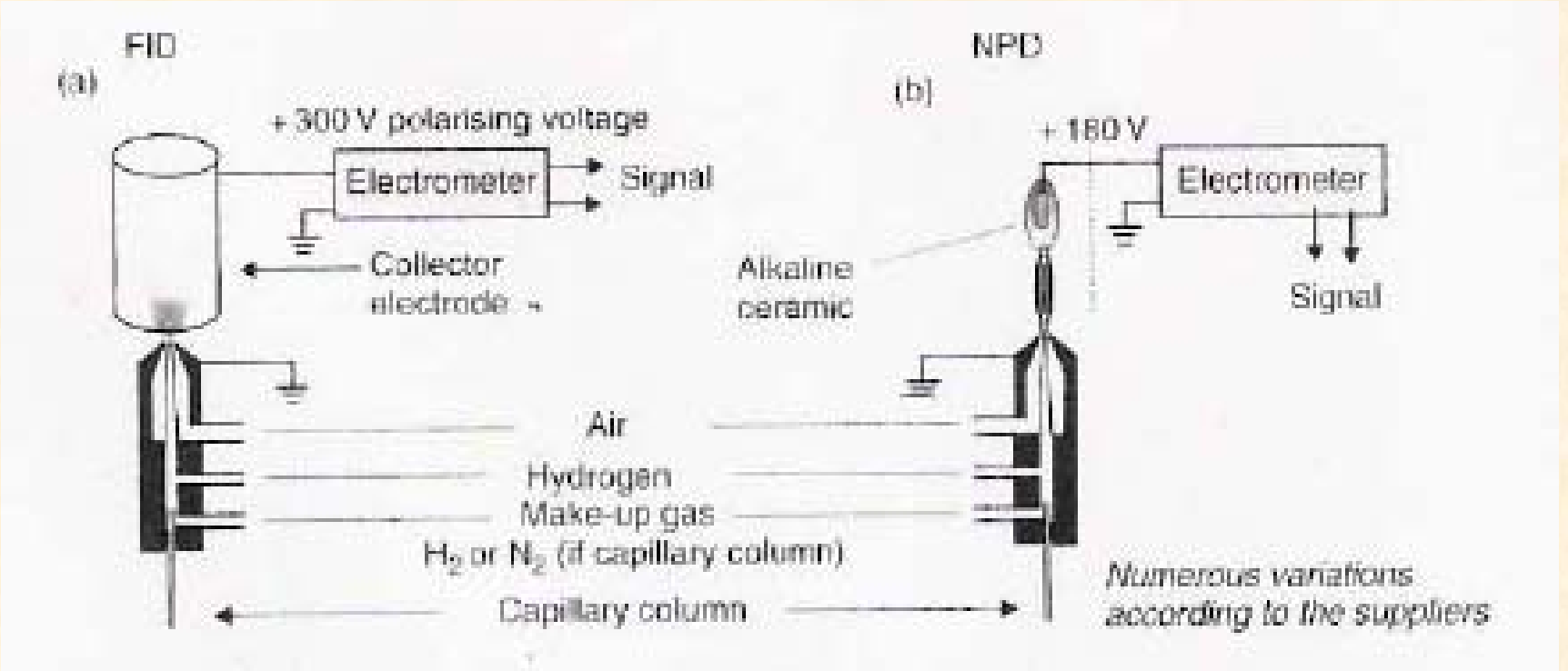
TCD (thermal conductivity detector)



Chromatography – GC

Detectors

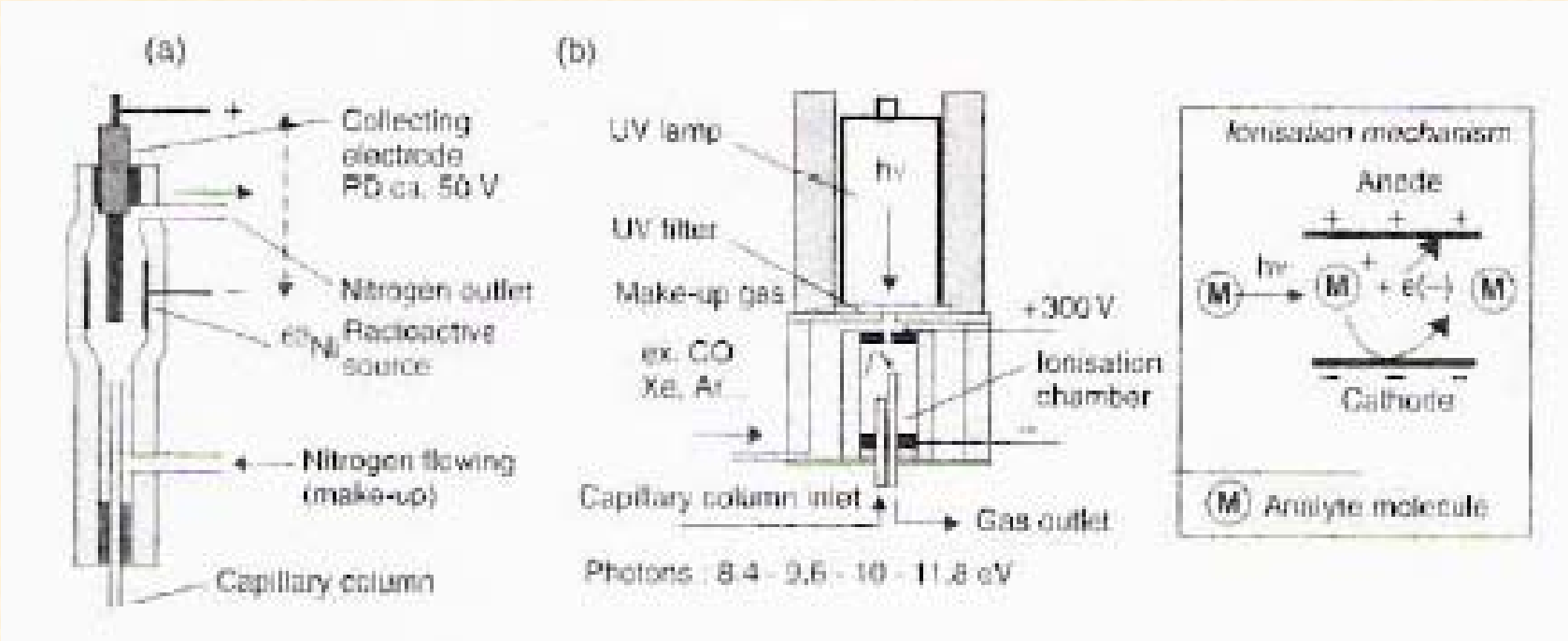
FID (flame ionization detector) + NPD



Chromatography – GC

Detectors

ECD (electron capture detector) + PID (photo ionization detector)



Chromatography – GC

Detectors

Detector	Type	Support gases	Selectivity	Detectability	Dynamic range
Flame ionization (FID)	Mass flow	Hydrogen and air	Most organic cpds.	100 pg	10^7
Thermal conductivity (TCD)	Concentration	Reference	Universal	1 ng	10^7
Electron capture (ECD)	Concentration	Make-up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallics	50 fg	10^5
Nitrogen-phosphorus	Mass flow	Hydrogen and air	Nitrogen, phosphorus	10 pg	10^6
Flame photometric (FPD)	Mass flow	Hydrogen and air possibly oxygen	Sulphur, phosphorus, tin, boron, arsenic, germanium, selenium, chromium	100 pg	10^3
Photo-ionization (PID)	Concentration	Make-up	Aliphatics, aromatics, ketones, esters, aldehydes, amines, heterocyclics, organosulphurs, some organometallics	2 pg	10^7
Hall electrolytic conductivity	Mass flow	Hydrogen, oxygen	Halide, nitrogen, nitrosamine, sulphur		

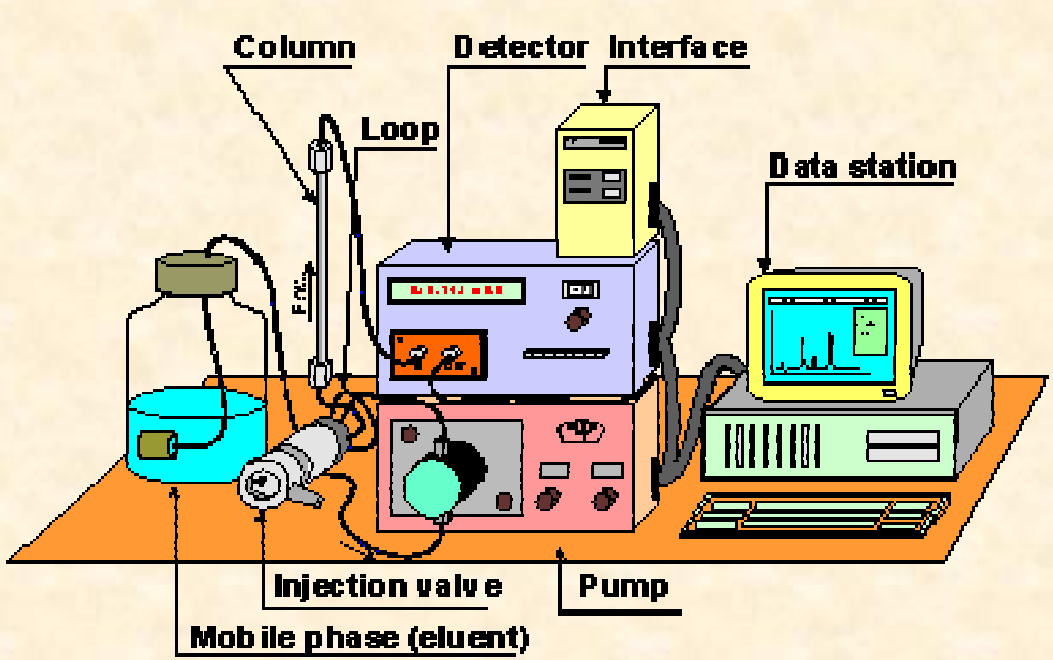
Chromatography – GC

Kovats' index

$$I_x = 100n + 100\frac{\log t'_{R(X)} - \log t'_{R(n)}}{\log t'_{R(n+1)} - \log t'_{R(n)}} \quad [0, 1]$$

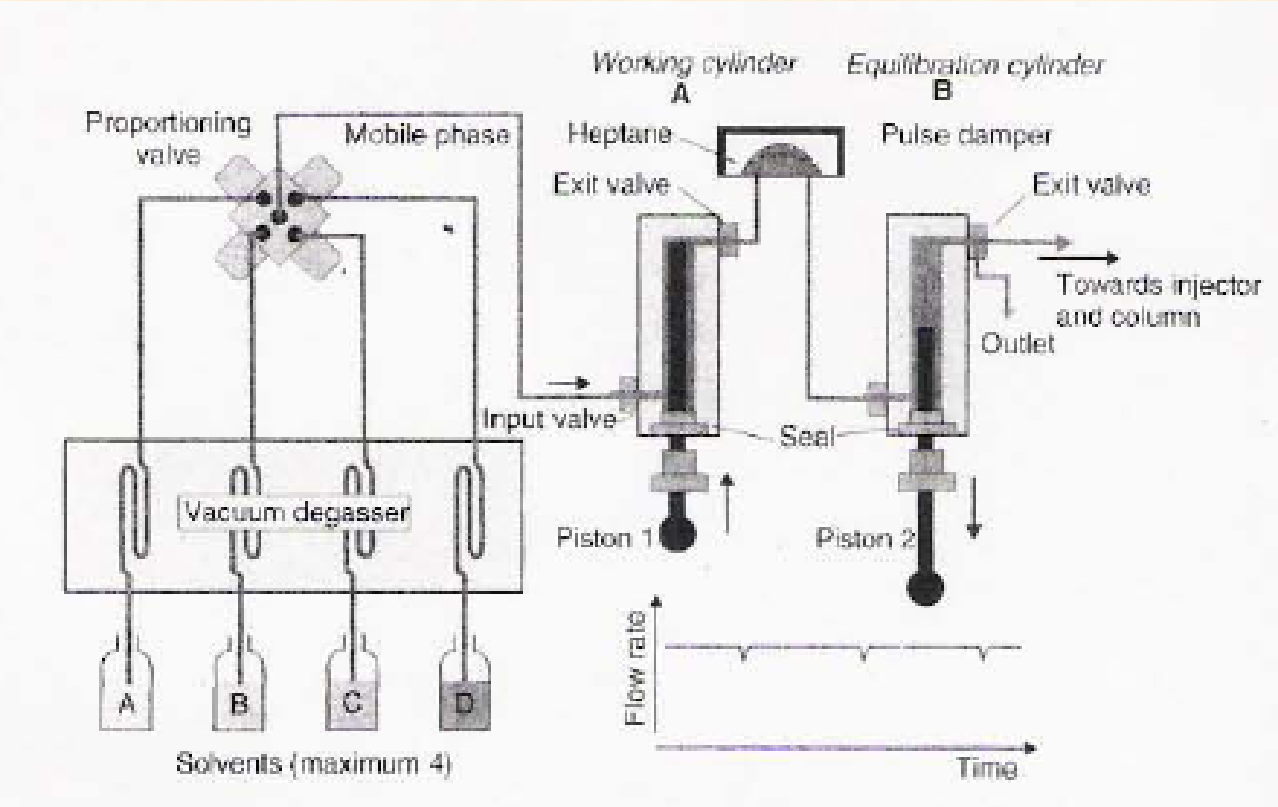
Chromatography – HPLC

General Concept of HPLC



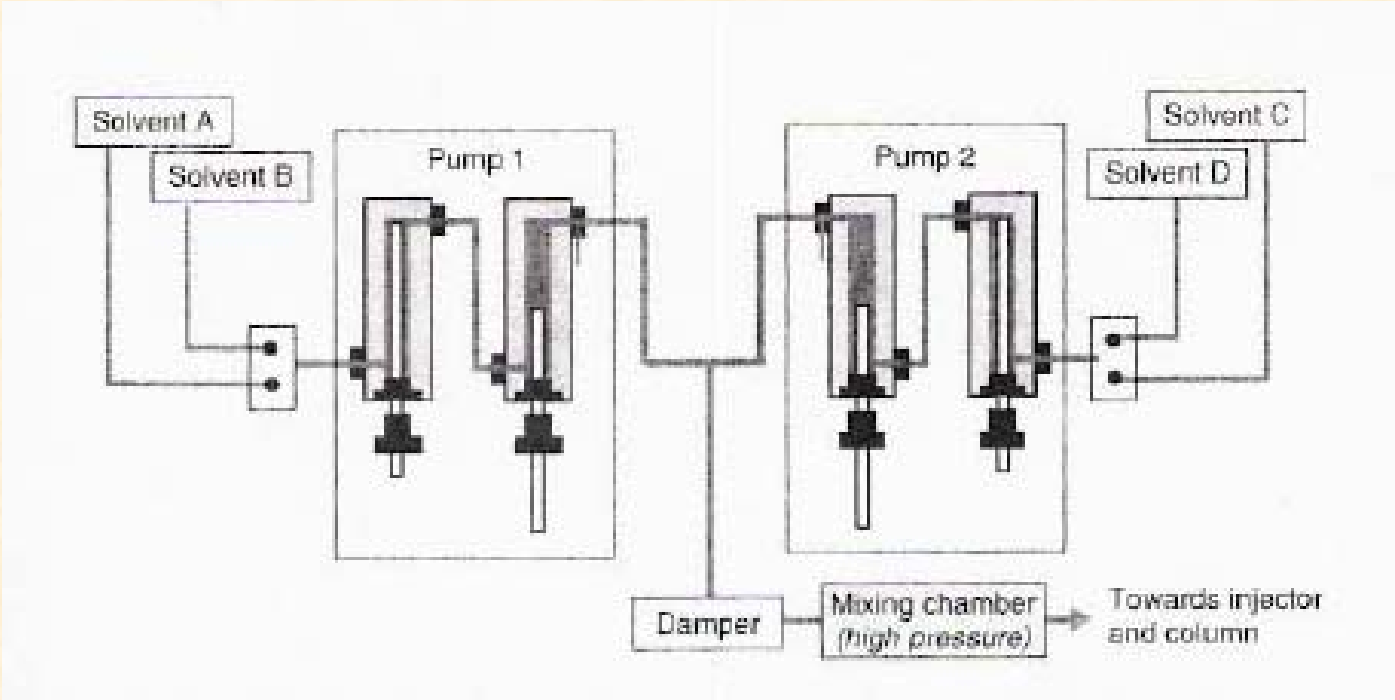
Chromatography – HPLC

Pumps



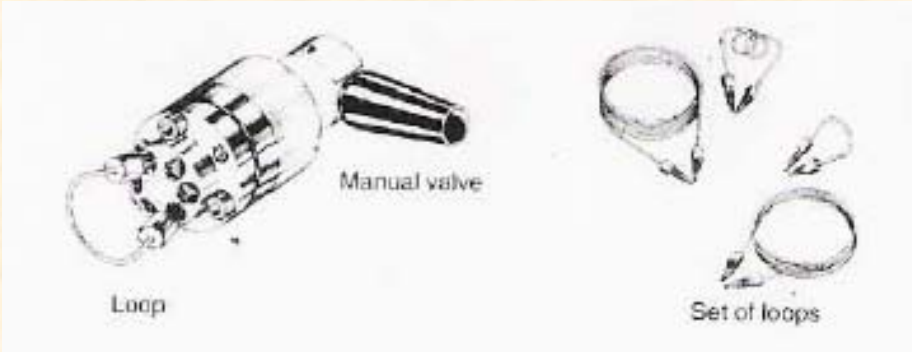
Chromatography – HPLC

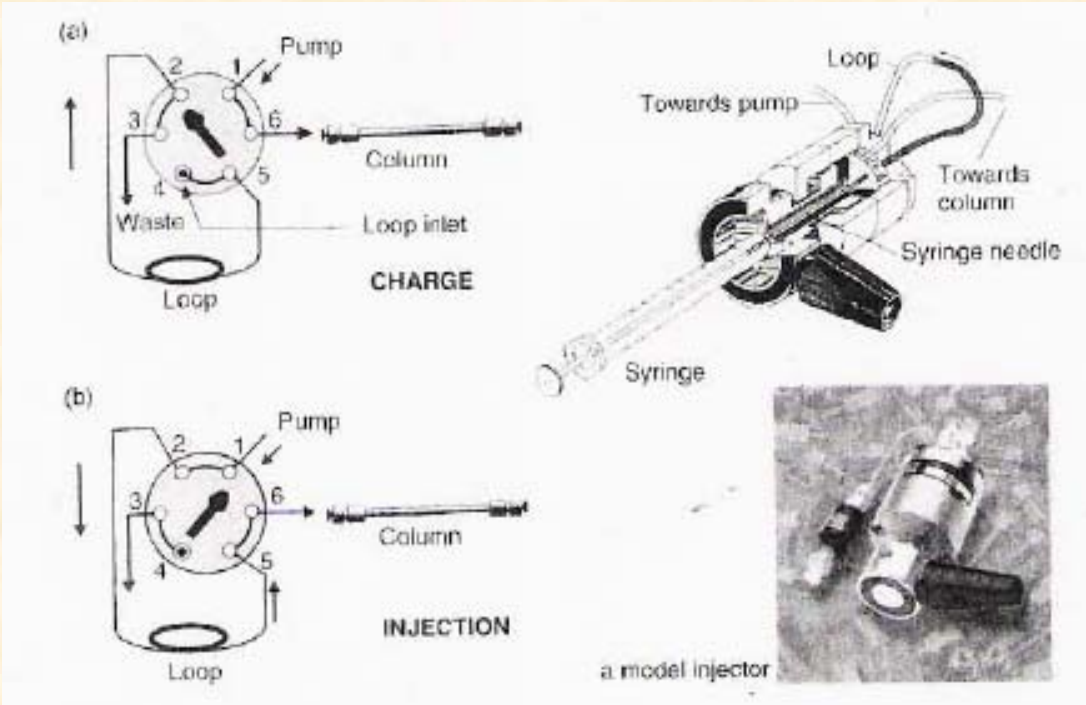
Pumps



Chromatography – HPLC

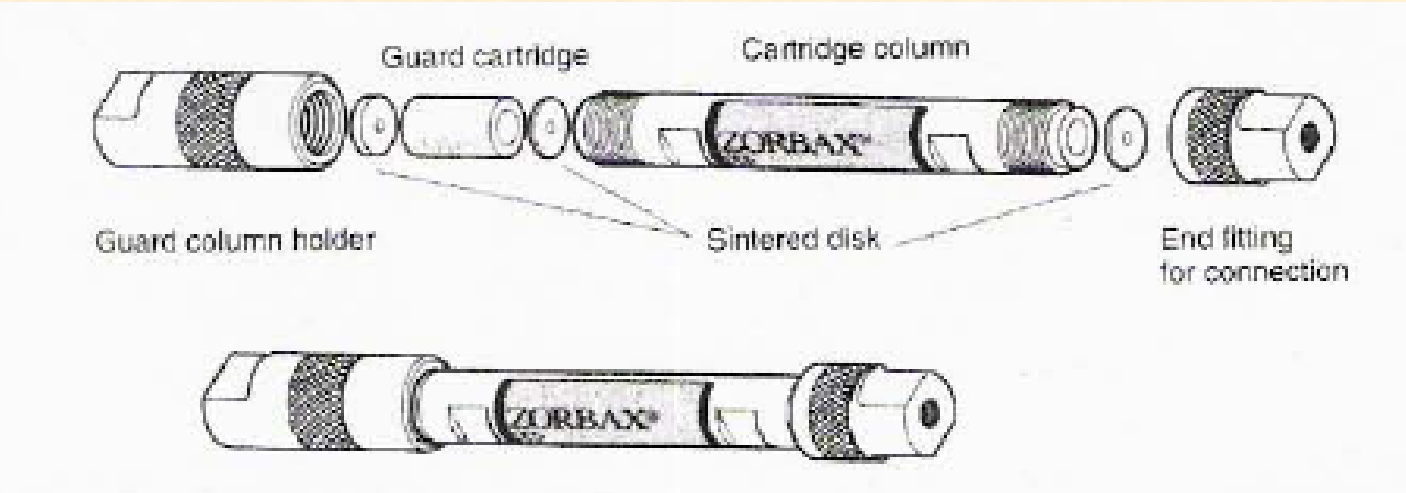
Injectors





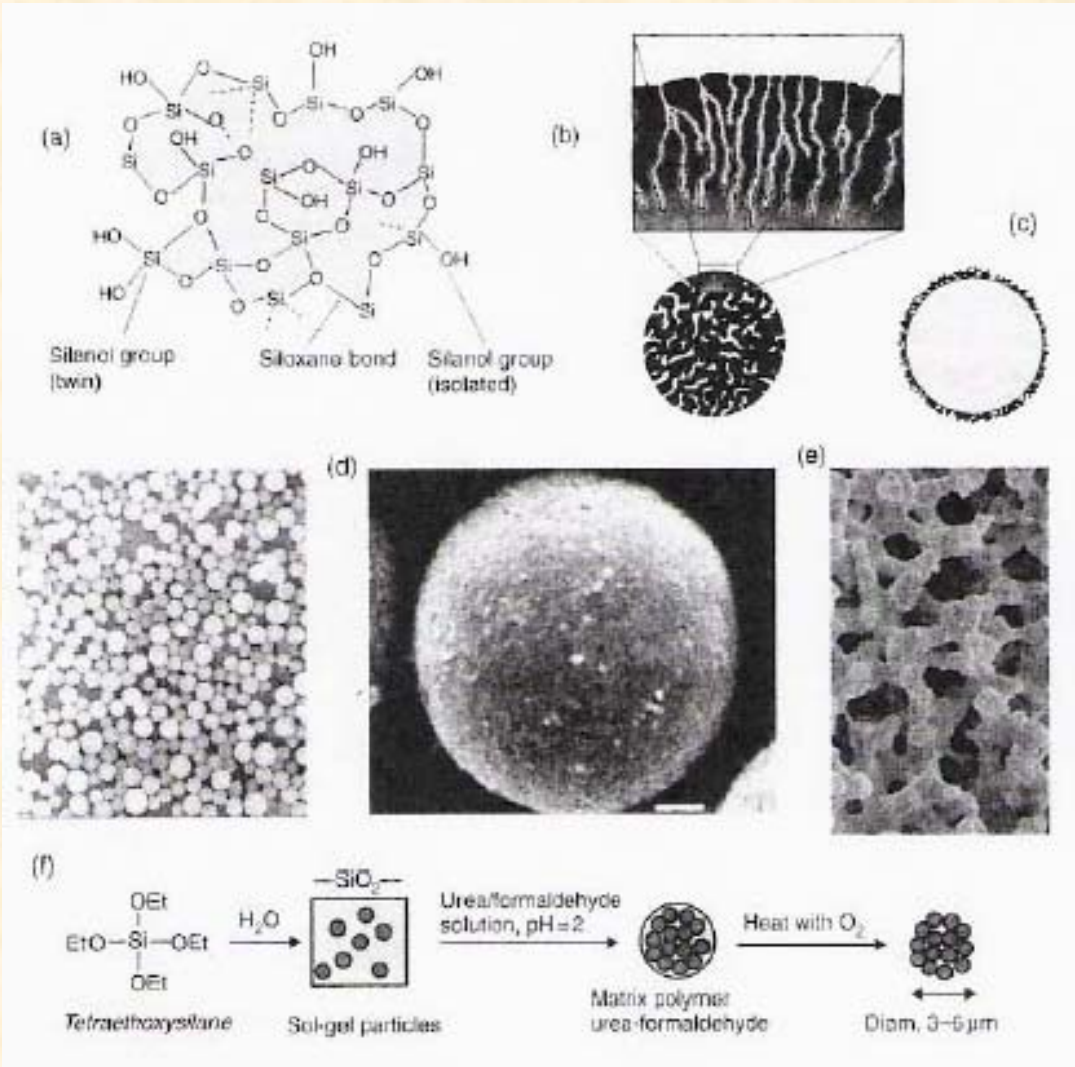
Chromatography – HPLC

Columns



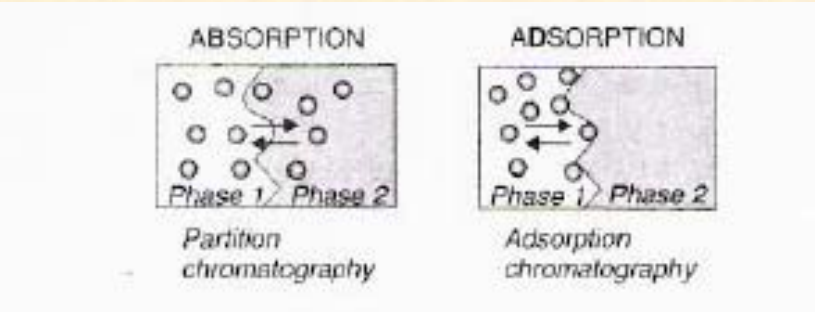
Chromatography – HPLC

Stationary Phases



Chromatography – HPLC

Stationary Phases

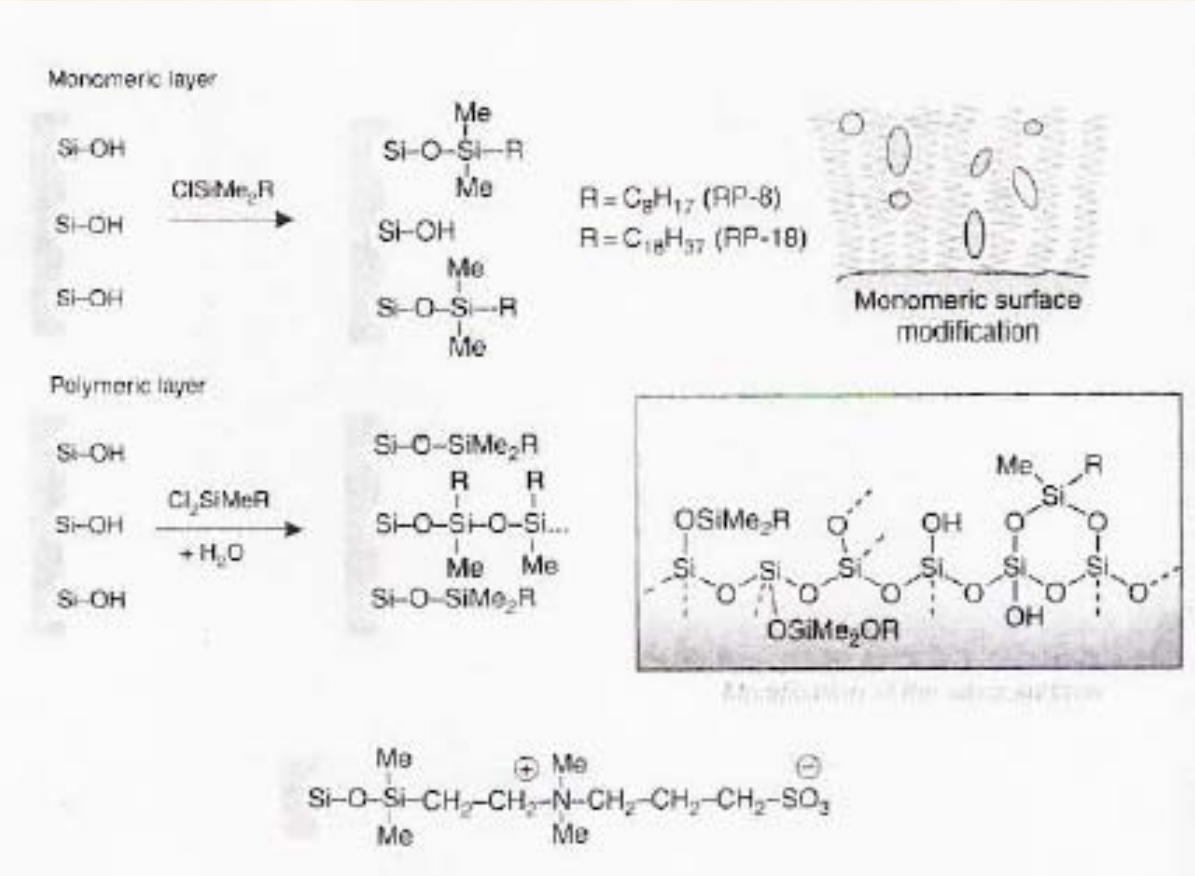


$$SiO_2 + NaOH \longrightarrow Na_2SiO_3 + H_2O$$

$$Na_2SiO_3 \xrightarrow{H_3O^+} \underset{\text{orthosilicic acid1}}{[Si(OH)_4]} \xrightarrow{-H_2O} \underset{\text{orthosilicic acid2}}{[(HO)_3SiOSi(OH)_3]} \xrightarrow{-nH_2O} \underset{\text{silica gel}}{(SiO_2)_n \cdot (H_2O)_r}$$

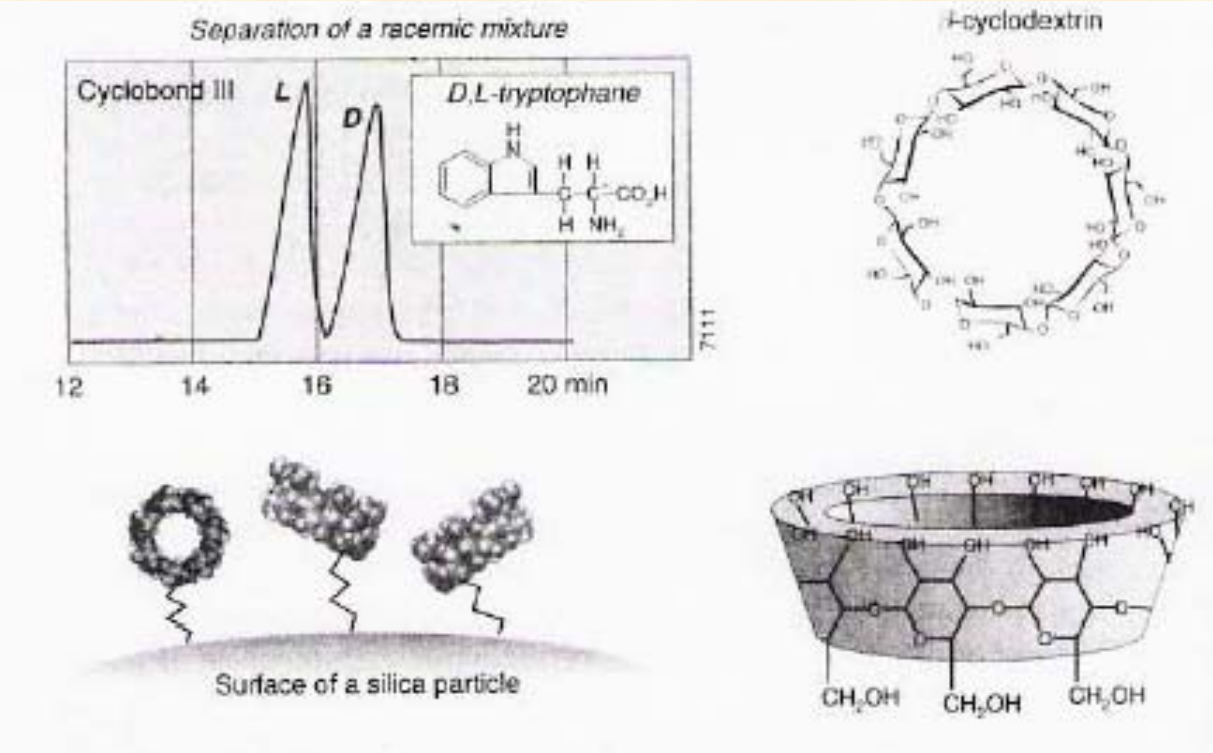
Chromatography – HPLC

Stationary Phases



Chromatography – HPLC

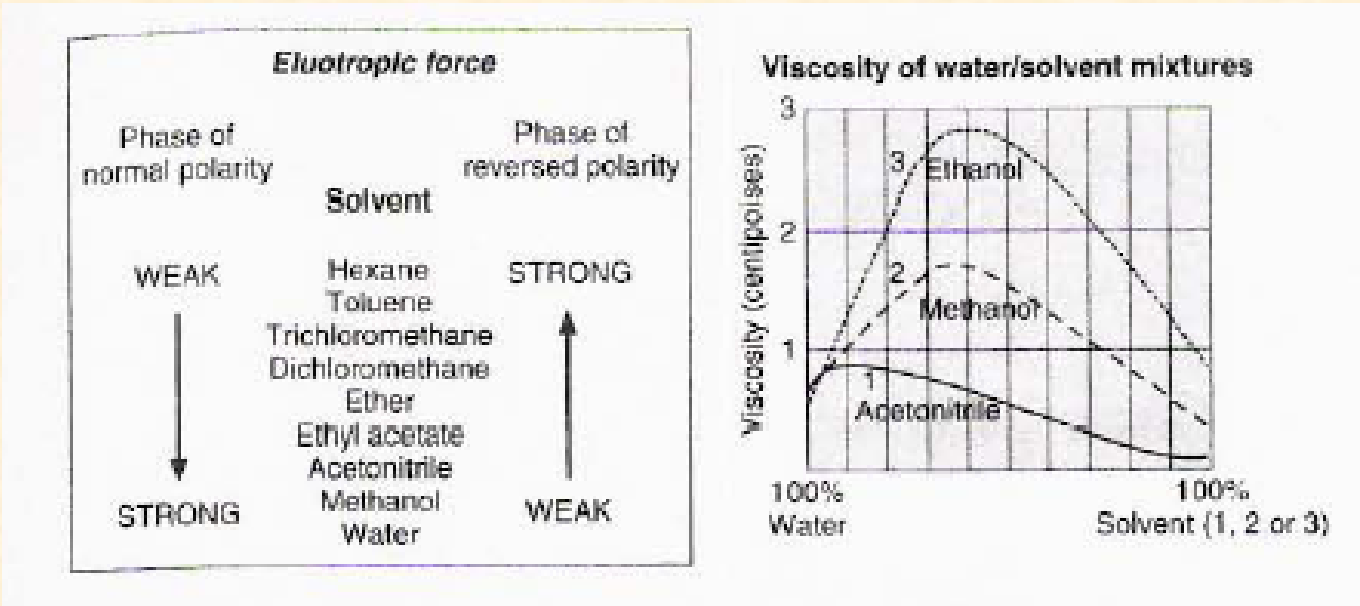
Stationary Phases



$$\text{optical purity (e.e.\%)} = 100\frac{|A_R - A_S|}{A_R + A_S}$$

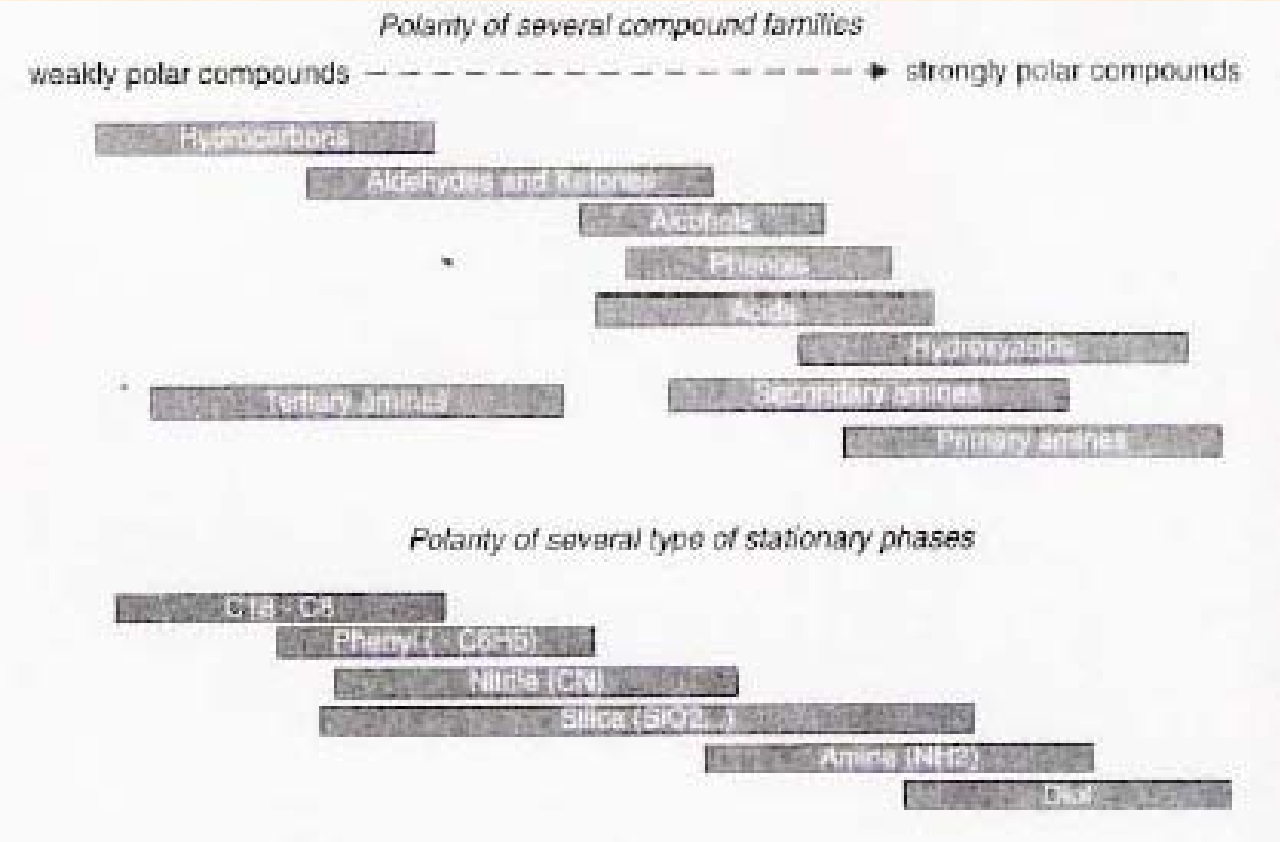
Chromatography – HPLC

Mobile Phases



Chromatography – HPLC

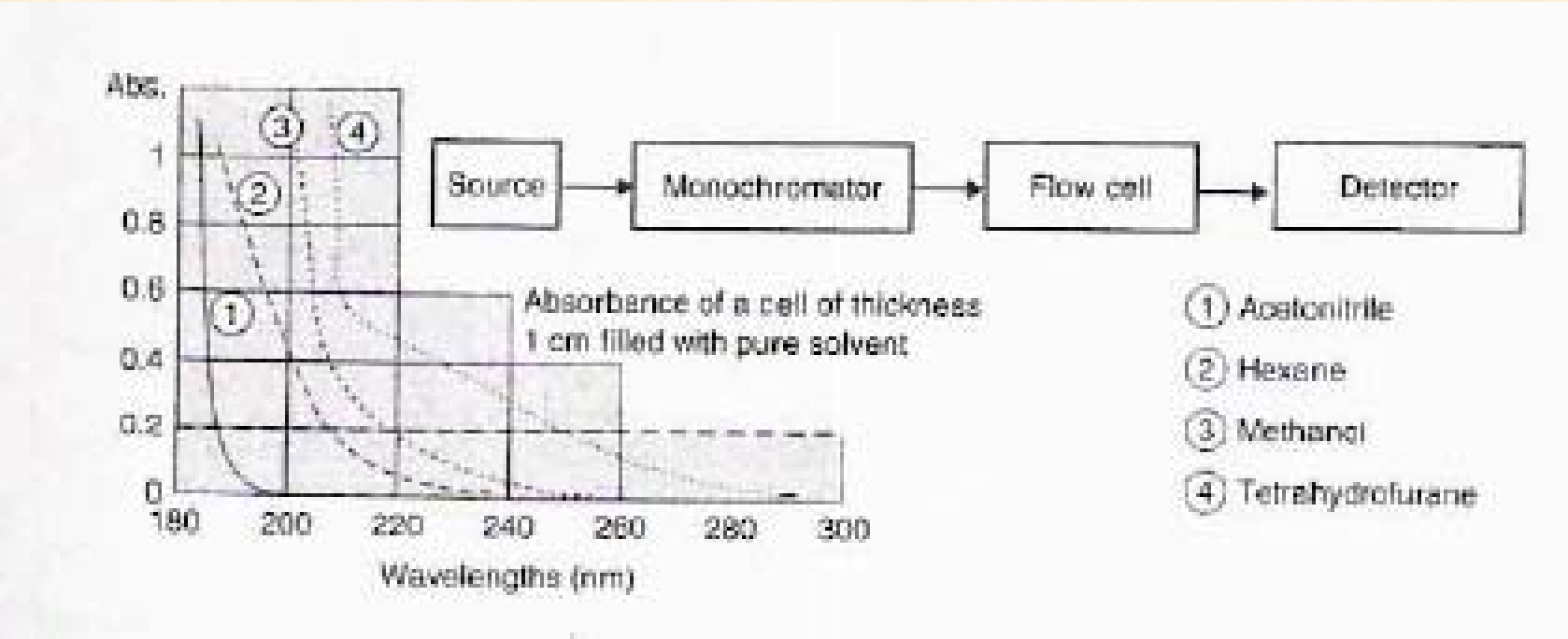
Mobile Phases



Chromatography – HPLC

Detectors

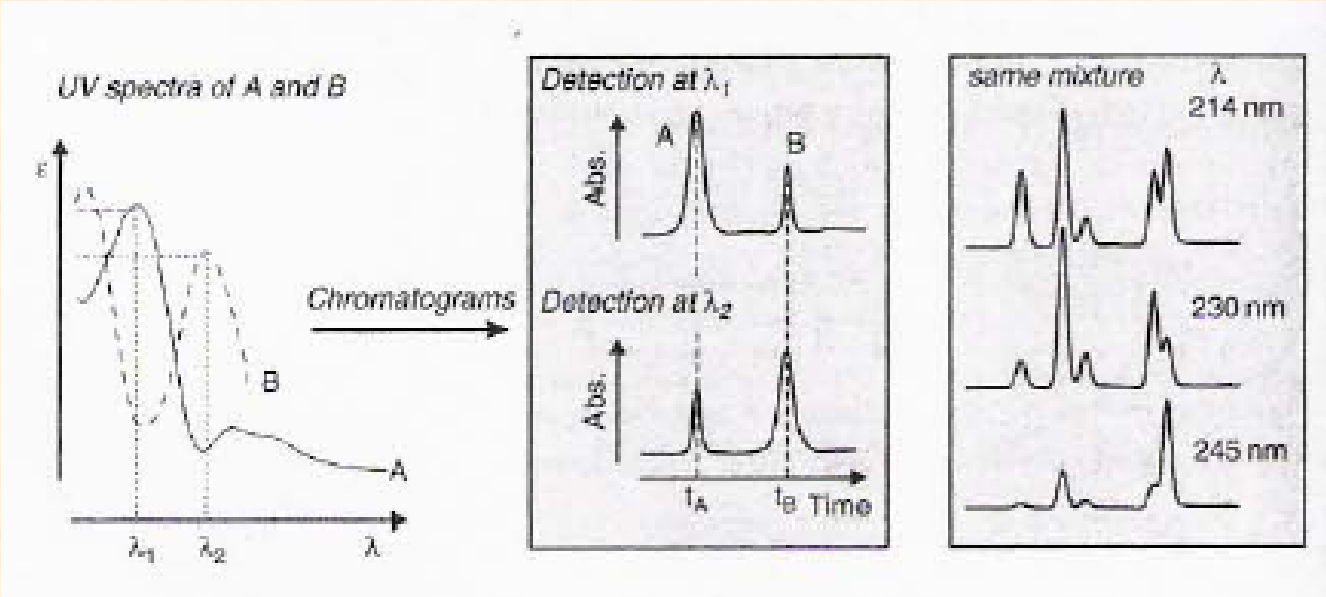
Spectrophotometric detectors



Chromatography – HPLC

Detectors

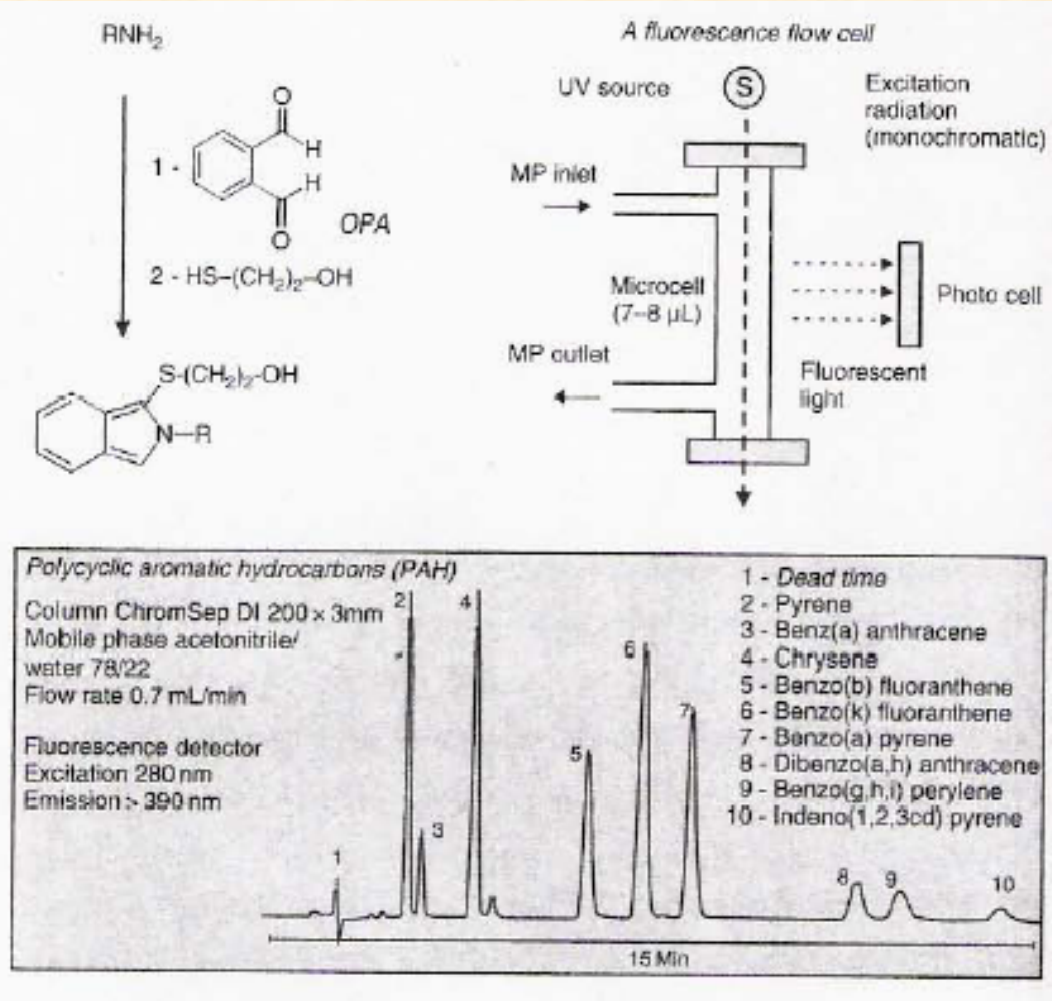
Spectrophotometric detectors



Chromatography – HPLC

Detectors

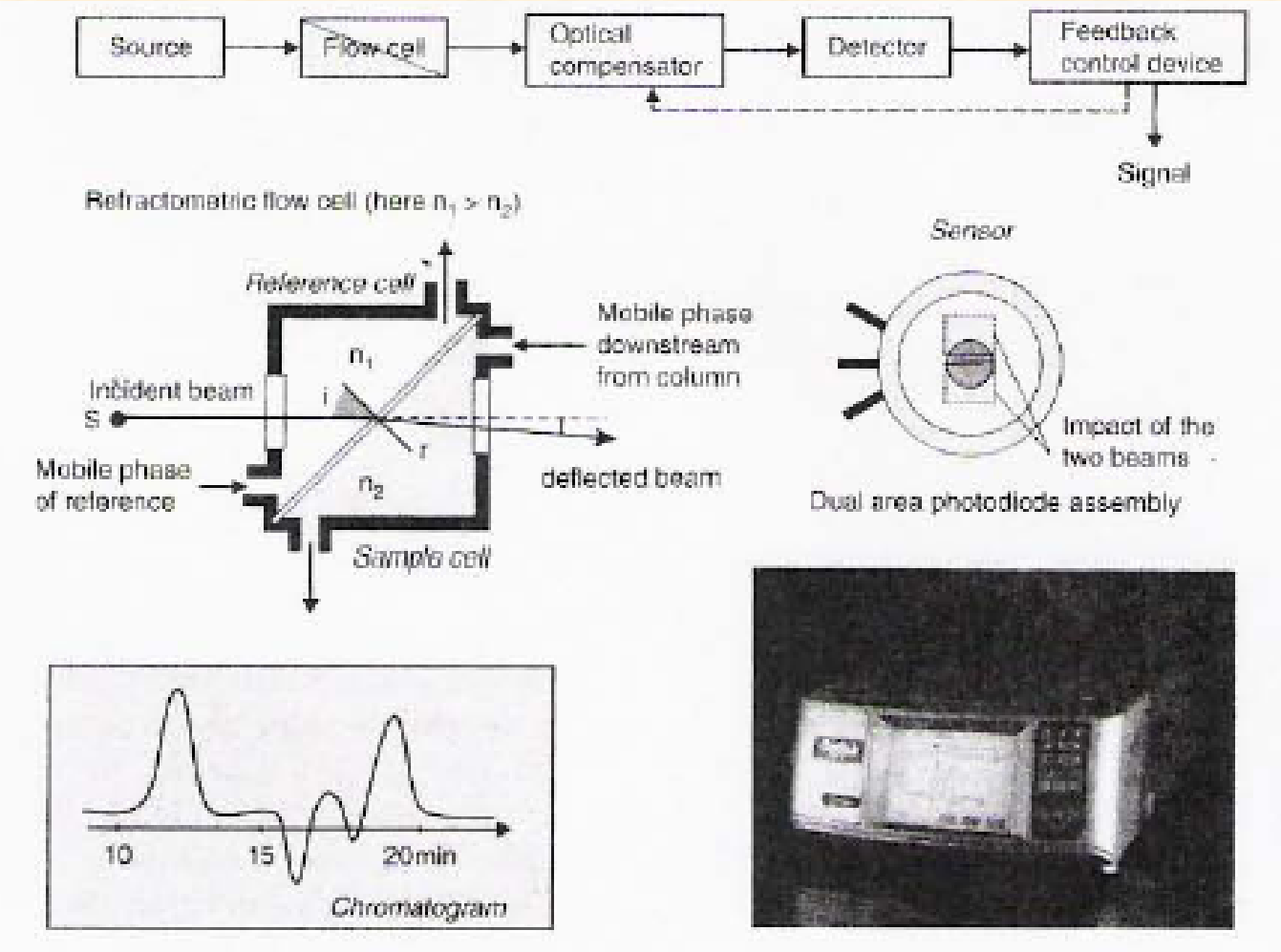
Fluorescence detectors



Chromatography – HPLC

Detectors

Refractive Index detectors



Chromatography – References

- F. Rouessac, A. Rouessac; Chemical Analysis, Wiley, 2007