Gel Permeation Chromatography - GPC

Separation and clean-up method

Group separation of compounds with similar molecular weight – fractionation

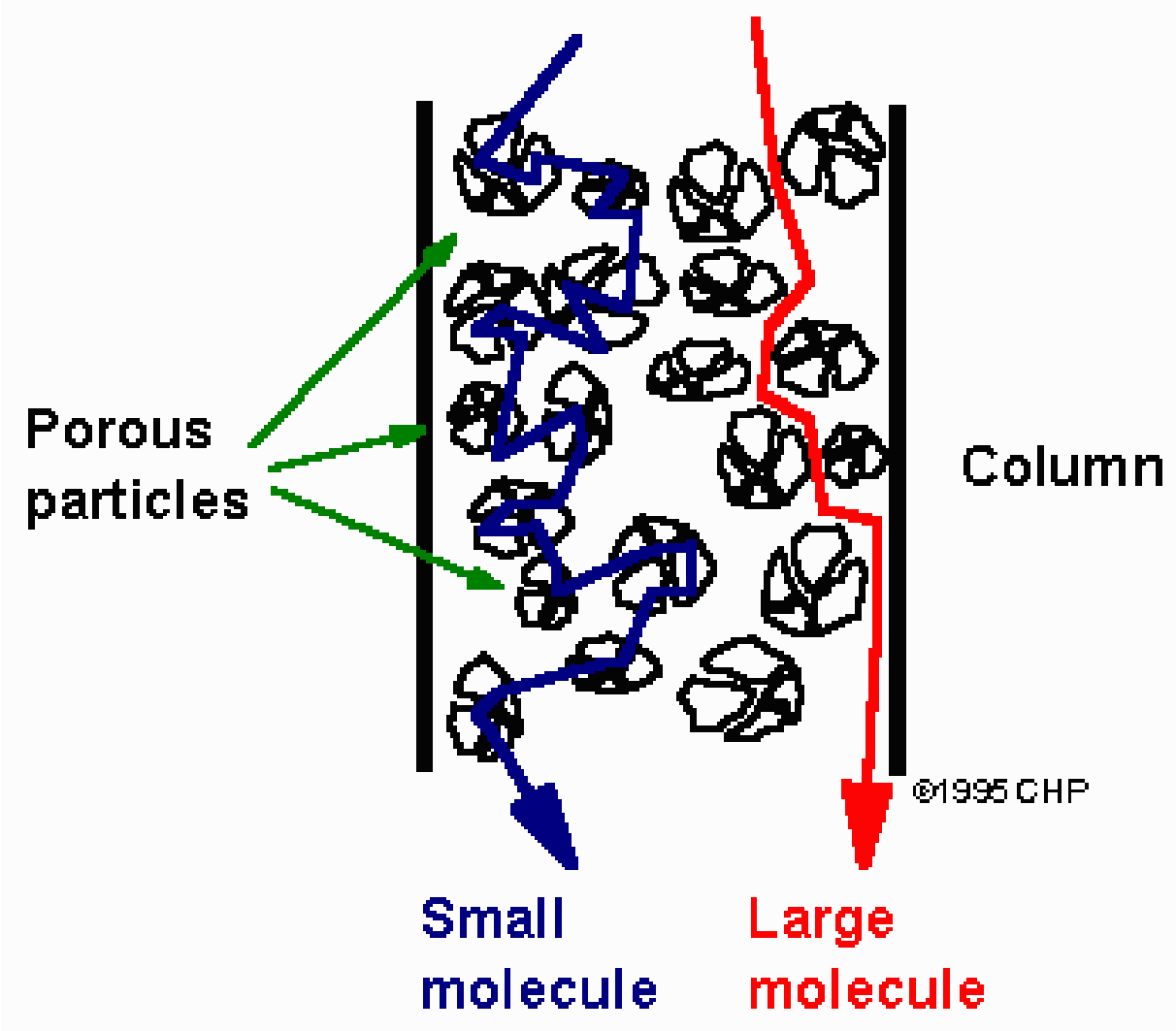
Analytes are diluted in eluate

$\Rightarrow$ necessary to concentrate (RE)

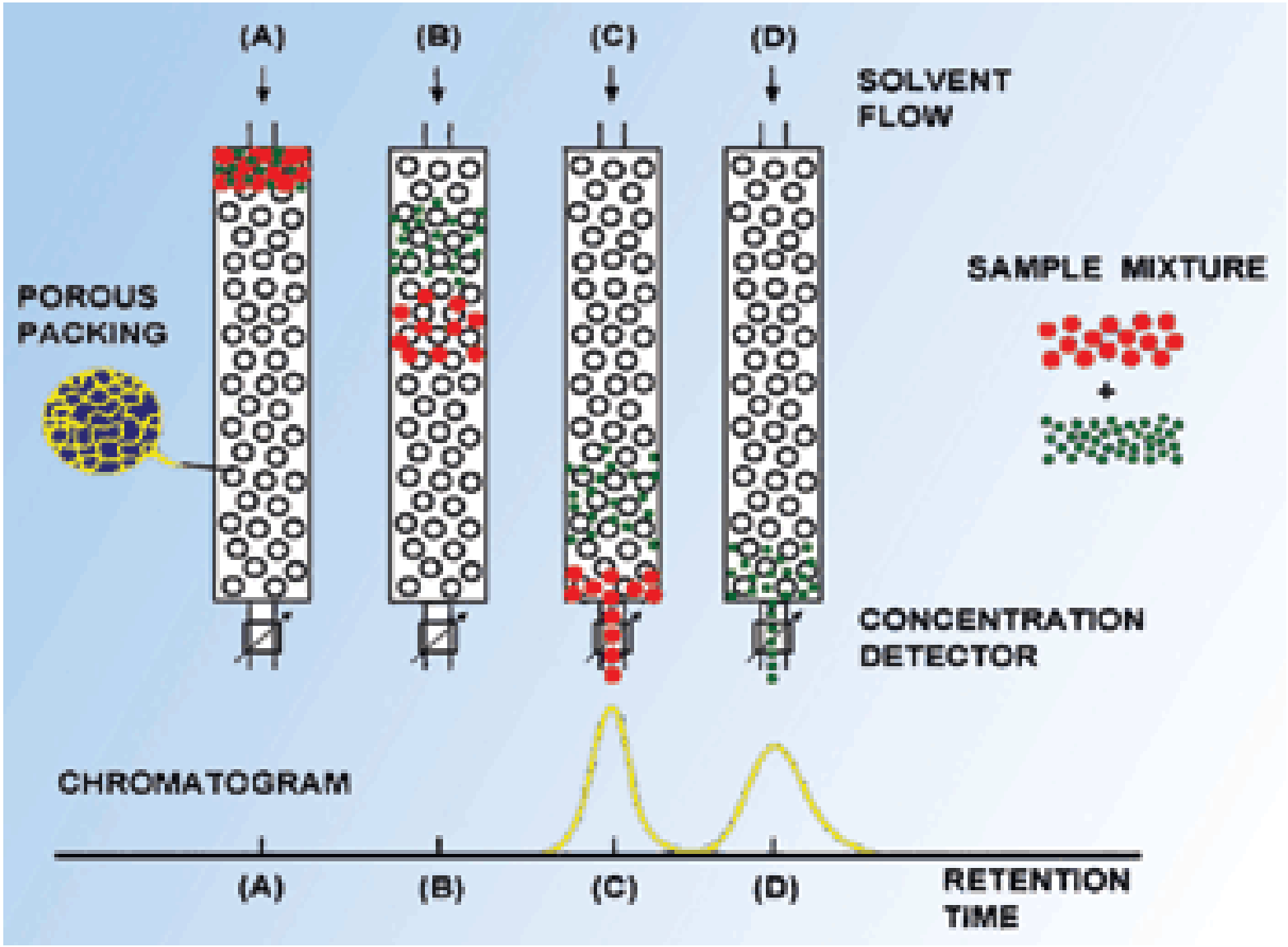
Optimisation for given combinations analyte / matrix

Quick, rugged, easily automatable, almost universal

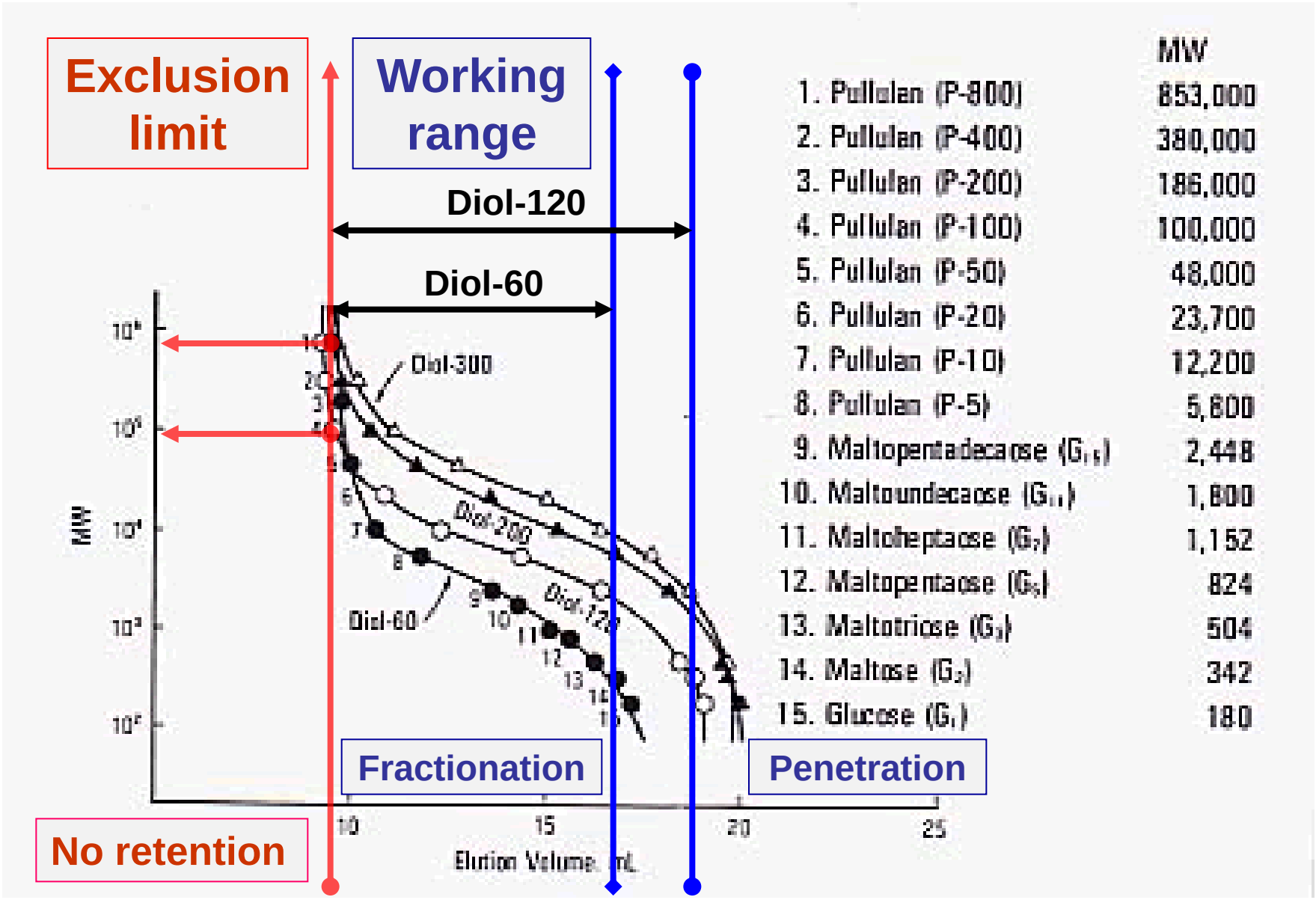
GPC principle



Fractionation according to MW



GPC column characterisation



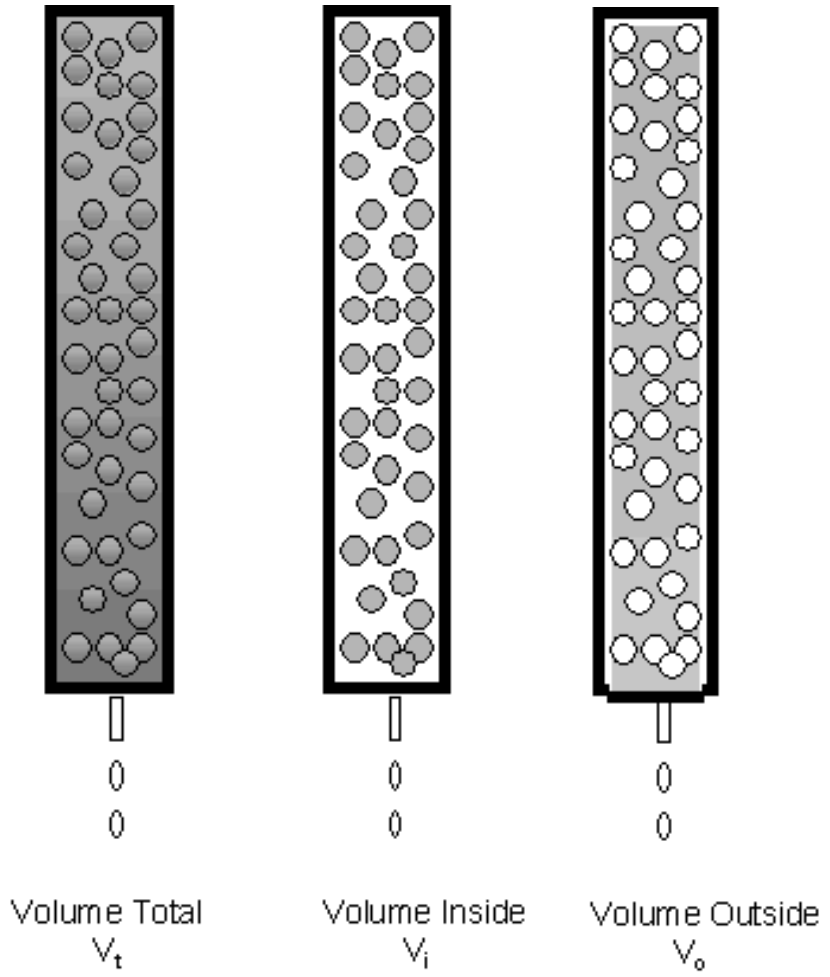
GPC theory

$$V_t = V_i + V_0$$

V_t – total volume of eluent in column

V_i – volume of eluent held in pores of gel (stationary phase)

V_0 – volume of eluent outside of gel particles – dead volume (mobile phase)



1. Size Exclusion Chromatography (SEC)

Separation based just on MW differences

$$K_{SEC} = (C_s / C_m)$$

$$V_e = V_0 + K_{SEC} V_i$$

V_e – analyte elution volume

C_s – analyte concentration in stationary phase

C_m – analyte concentration in mobile phase

$K_{SEC} = 0 \Rightarrow$ compound is excluded in dead volume

$K_{SEC} = 1 \Rightarrow$ compound is totally retained

2. Gel Permeation Chromatography (GPC)

Separation based on MW differences and also on other mechanisms – e.g. partition and adsorption

$$K_T = K_{SEC} + K_P + K_{AD}$$

$$V_e = V_0 + K_{SEC}\,V_i + K_P\,V_i + K_{AD}\,V_i$$

K_P – distribution constant of partition mechanism

K_{AD} – distribution constant of adsorption mechanism

Solubility Parameters (Cohesive Energy Density) (according to Hildebrand)

Cohesive energy density – c (ΔE)

ΔH – vaporisation heat

R – universal gas constant

T – temperature

V_m – molar volume

$$c = \frac{\Delta H - RT}{V_m}$$

Solubility parameter - δ

Units: $cal^{1/2}cm^{-3/2}$ = 0,48888 $MPa^{1/2}$

$MPa^{1/2}$ = SI = 2,0455 $cal^{1/2}cm^{-3/2}$

$$\delta = \sqrt{c}$$

Non polar compounds – only dispersion forces

Polar compounds – other types of molecular interactions

Total solubility parameter: δ_t

$$\delta_t^2 = \delta_d^2 + \delta_o^2 + 2\delta_{ind}\delta_o + 2\delta_a\delta_b$$

δ_d **– dispersion interaction**

δ_o **- dipole interaction**

δ_{ind} **– induced dipole interaction**

δ_a **- proton donor character**

δ_b **- proton acceptor character**

Selected solubility parameters ($cal^{1/2}cm^{-3/2}$)

Liquid / gel	δ_d	δ_o	δ_{ind}	δ_a	δ_b	δ_t [a] *	δ [b] *
Hexane	7,3	-	-	-	-	7,3	7,3
Cyclohexane	8,2	-	-	-	-	8,2	8,2
Ethyl Acetate	7,0	4,0	1,0	-	2,7	8,5	8,9
Toluene	8,9	-	-	-	0,6	8,9	8,9
THF	7,6	3,5	0,8	-	3,7	8,7	9,1
Benzene	9,2	-	-	-	-	9,2	9,2
Chloroform	8,1	3,0	0,5	6,5	0,5	9,3	9,3
Acetone	6,8	5,1	1,5	-	3,0	9,4	9,6
Dichloromethane	8,9	< 2,8 >		< 3,9 >		9,9	9,7
Acetonitrile	6,5	8,2	2,8	-	3,8	12,5	12,7
Methanol	6,2	4,9	0,8	8,3	8,3	14,4	14,5
Water	-	-	-	-	-	-	23,5
Polystyrene	-	-	-	-	-	9,1-9,4 [c] *	-
Polyacrylamide	-	-	-	-	-	15 [c] *	-

* a – value calculated using equation for δ_t ; b – value determined by evaporation ; c – estimation

Application of solubility parameters for GPC optimisation

Estimation of δ_{MX} for solvent mixtures:

$$\delta_{MX} = \Sigma \Phi_i \delta_i$$

Φ_i = solvent fraction in mixture

The same δ values ...X... different molecular interactions

Equation for capacity ratio: - K_i

$$\ln K_i = \frac{V_{m,i}}{RT}(\delta_m + \delta_s - 2\delta_i) \cdot (\delta_m - \delta_s) + \ln \frac{n_s}{n_m}$$

$$K_i = \frac{n_s}{n_m}$$

δ_m – mobile phase solubility parameter
δ_s – stationary phase solubility parameter
n_m - number of moles in mobile phase
n_s – number of moles in stationary phase

Mobile and stationary phase selection

Analyte solubility (polarity)

⇒ solvent selection

⇒ gel selection

GELS

HYDROPHILIC (POLAR) ...X... HYDROPHOBIC (NONPOLAR)

SOFT (SWELLING) ...X... RIGID (NONSWELLING)

Soft gels (SEC, GPC, GF)

Working range and exclusion limit

– corresponds to gel cross linking (producer) and swelling (mobile phase selection – analyst)

Compressible column ⇒ limited applicable pressure (risk of gel structure collapse)

Usually higher capacity

Less rugged systems – many possibilities for optimisations

Sephadex: dextran gel, hydrophilic

Sepharose: agarose gel, hydrophilic

Bio-Gel P: acrylamide gel, hydrophilic

Bio-Beads: styrene-divinylbenzene copolymer, hydrophobic

Rigid gels (HPSEC, HPGPC)

Working range and exclusion limit – practical invariable

Possibility of mobile phase changes – without column refilling

Stability – higher pressure (higher flow rate)

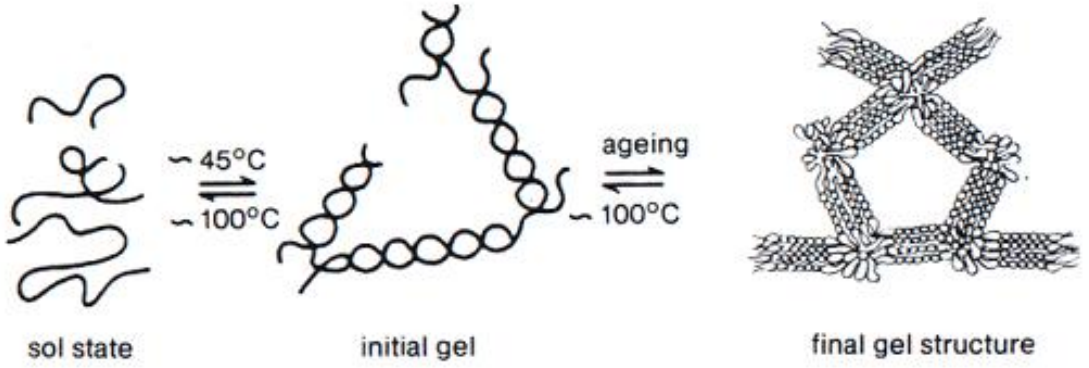
$\Rightarrow$ higher speed, efficiency

Rugged systems – limited optimisation

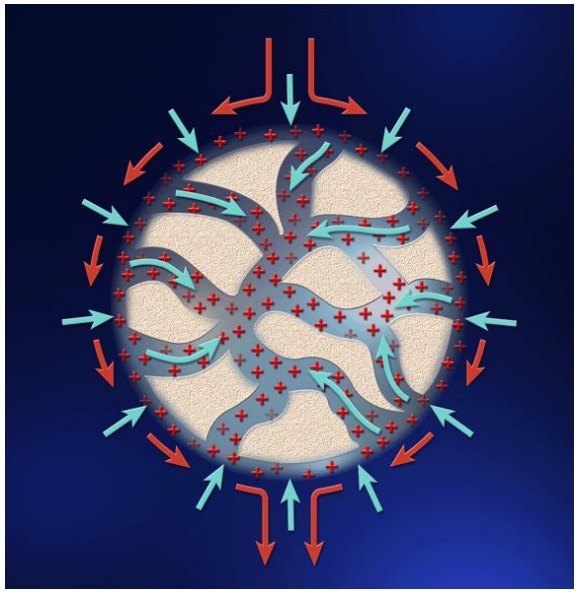
PL gel: styrene based

BioSepra – ceramic core

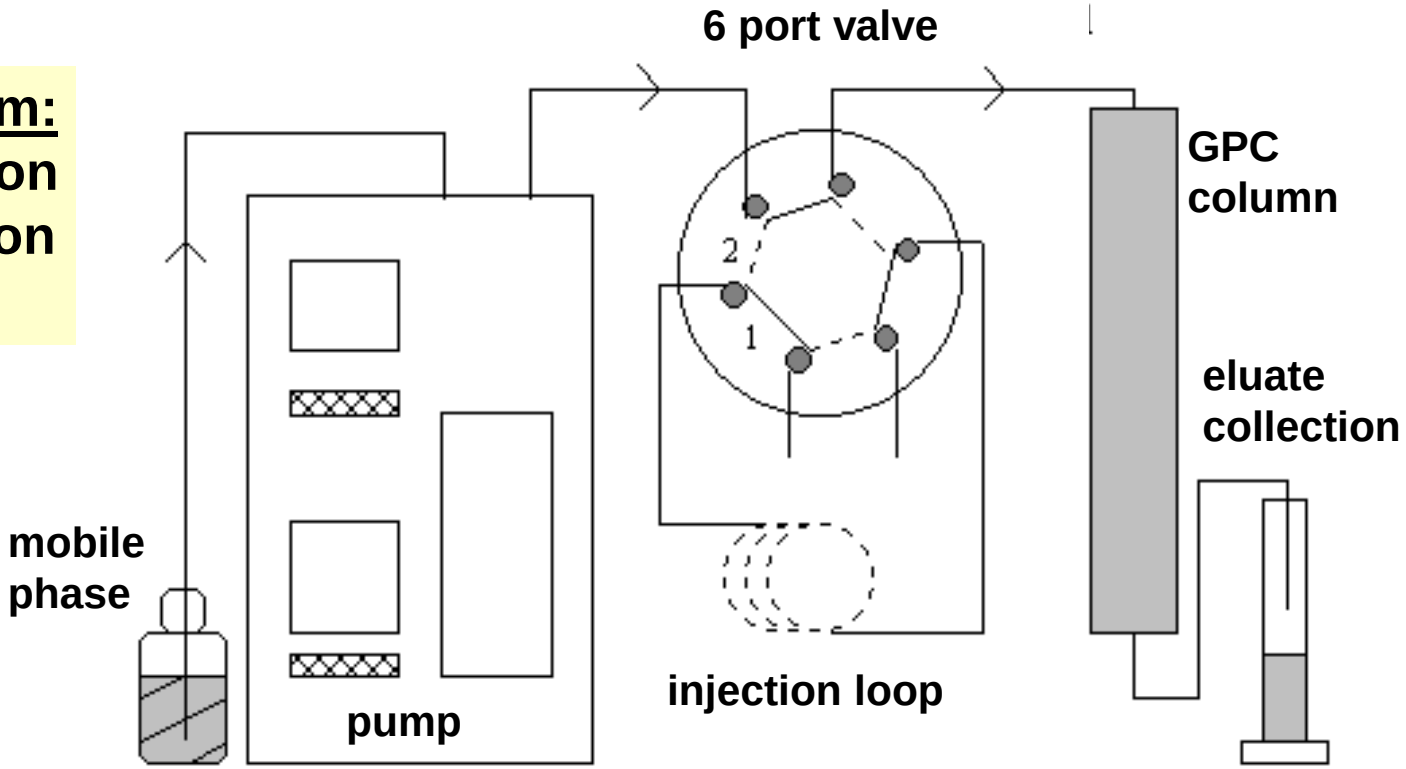
Soft gel - agarose



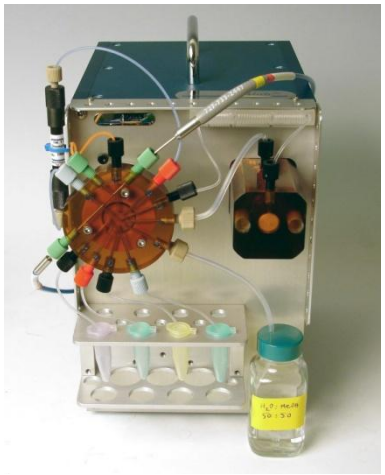
Rigid gel - BioSepra



Simple system:
hand injection and collection of fractions



Automatic system:
programmable injection and collection of fractions



Technical parameters:

Columns: steel - stainless, titanium, glass, plastic
from *10 x 0.5 cm* to *60 x 5 cm*

Gel bed: firm – defined by column size
variable – e.g. movable frits

Injection: 0.1 - 10 ml

Sample capacity: 0.1 - 10 g

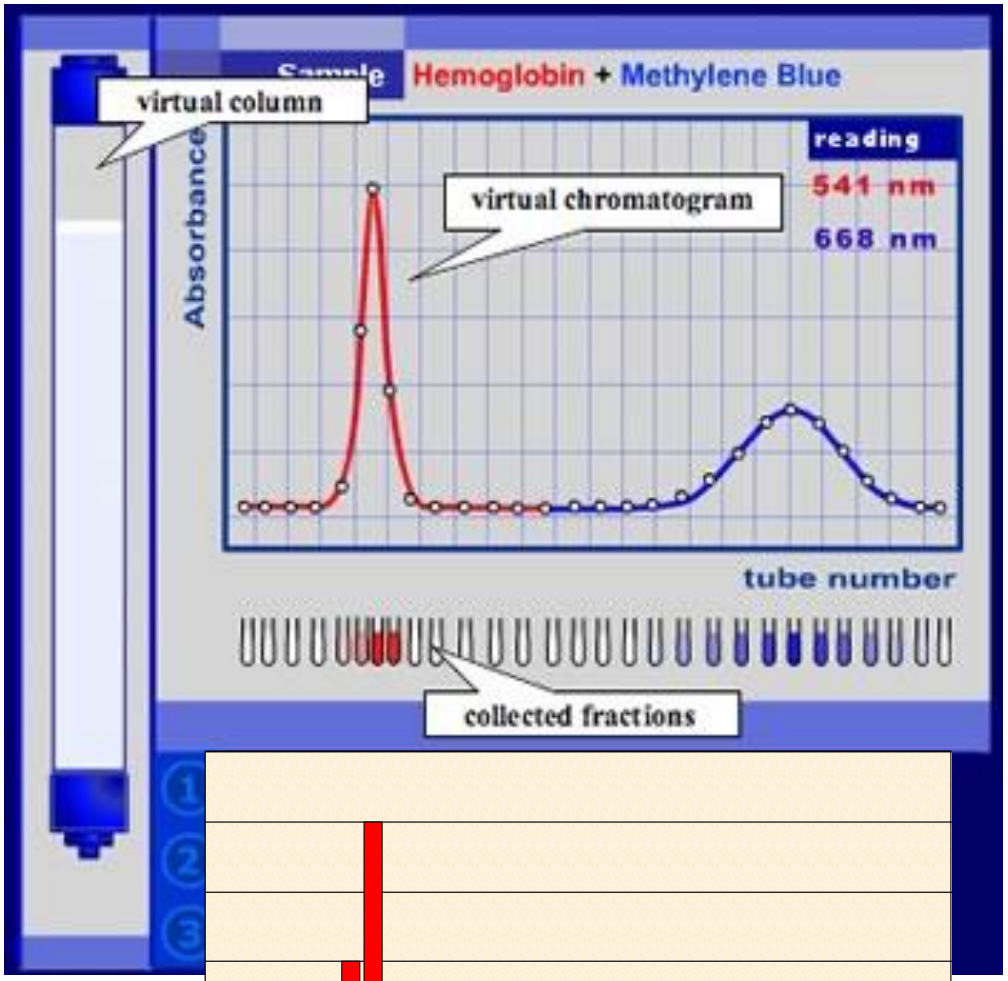
Flow rate: 0.1 - 10 ml/min

Elution volumes (fractions): 20 - 300 ml (0.5 - 50 ml)

Model example:

Graphic output

Interpretation



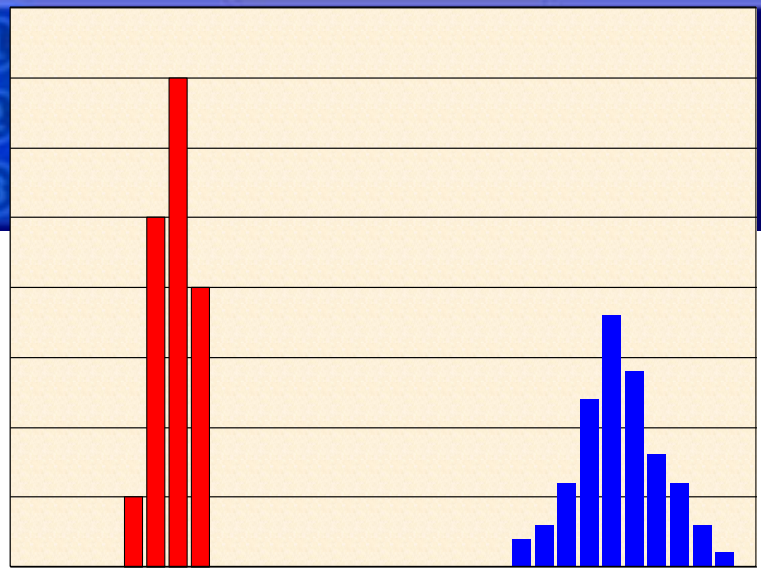
Fractions range:

FROM – TO

(in *ml* or in *min*)

Alternatively:

Retention volume (time)



GPC applicability

1. Fractionation – separation

- amino acids, peptides, proteins, nucleic acids, carbohydrates

2. Clean-up

- removal of higher molecular weight undesirable compounds
  (often in over-abundance = waste)
  from lower molecular weight compounds (collected fraction)

- trace analysis (pesticides, industrial contaminants)