Гельфильтрация в очистке белков
Принципы биохроматографии

Property	Technique
Size	Gel filtration (GF), also called size exclusion
Charge	Ion exchange chromatography (IEX)
Hydrophobicity	Hydrophobic interaction chromatography (HIC) Reversed phase chromatography (RPC)
Biorecognition (ligand specificity)	Affinity chromatography (AC)

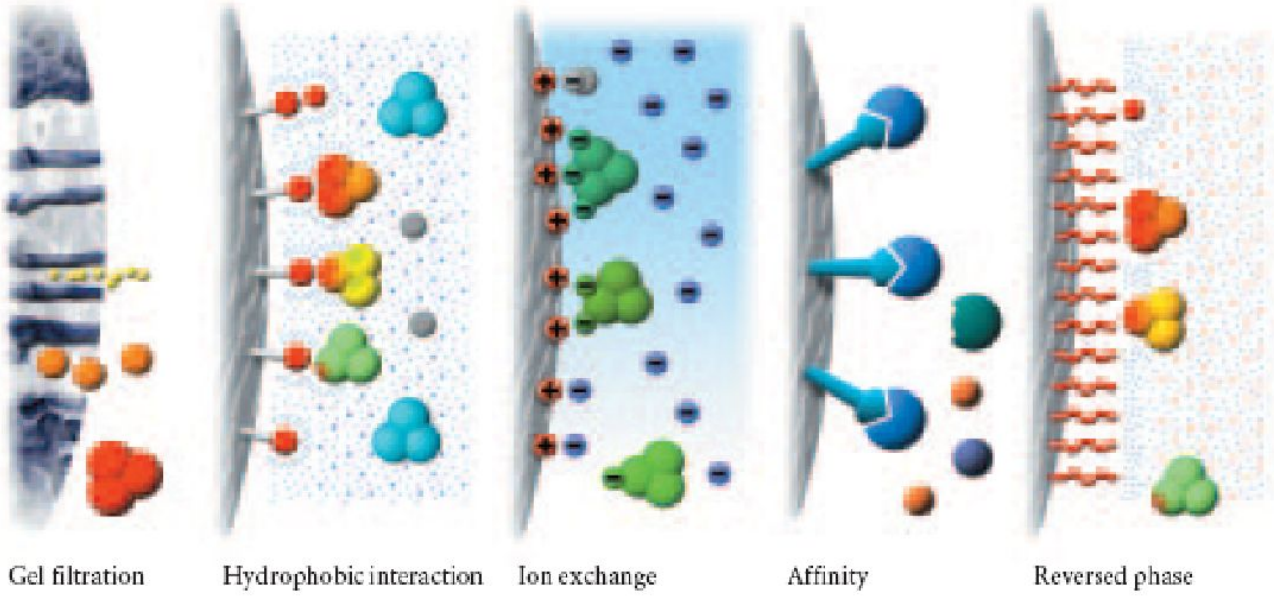


Fig. 1. Separation principles in chromatography purification.

Принципы гельфильтрации

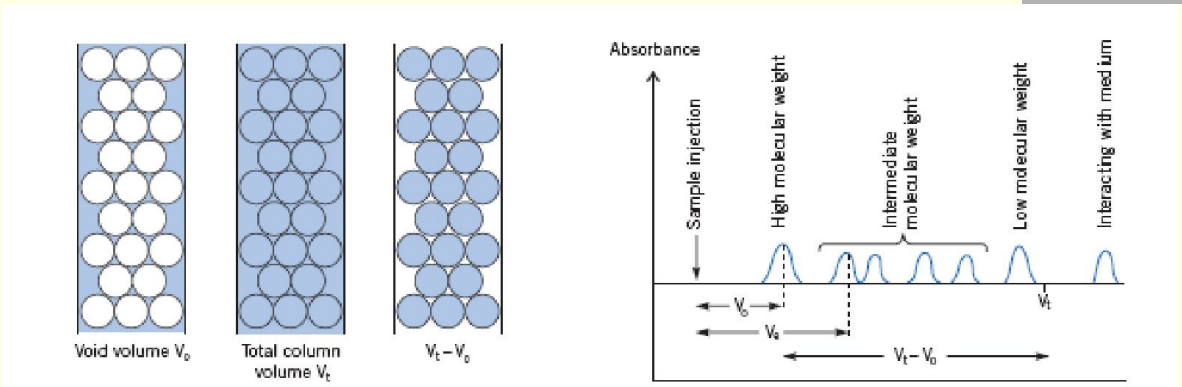
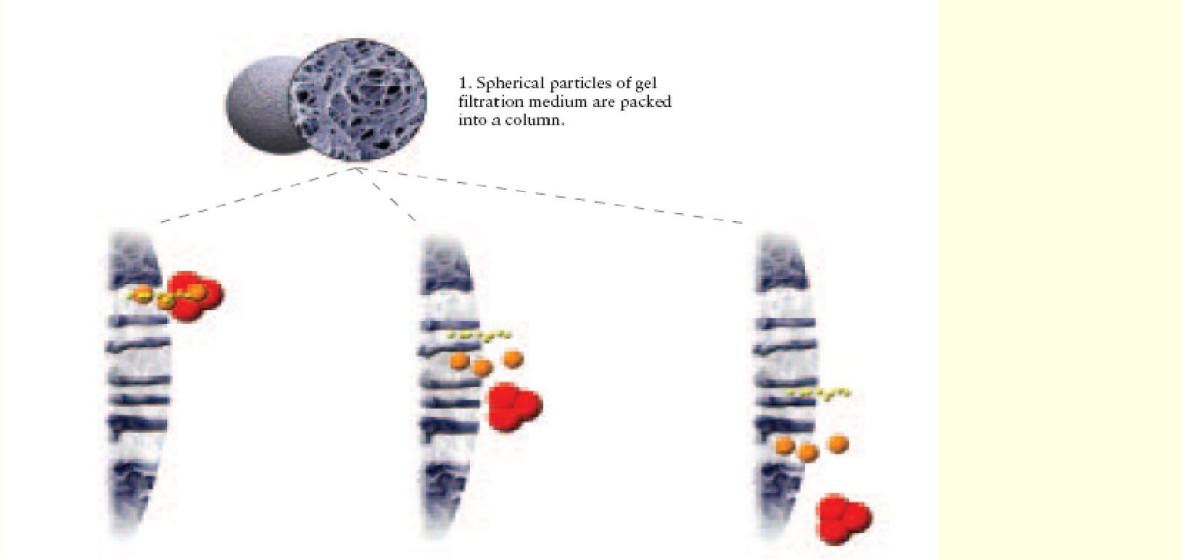


Fig. 2. Common terms in gel filtration.



5. Large molecules leave the column first followed by smaller molecules in order of their size. The entire separation process takes place as one total column volume (equivalent to the volume of the packed bed) of buffer passes through the gel filtration medium.

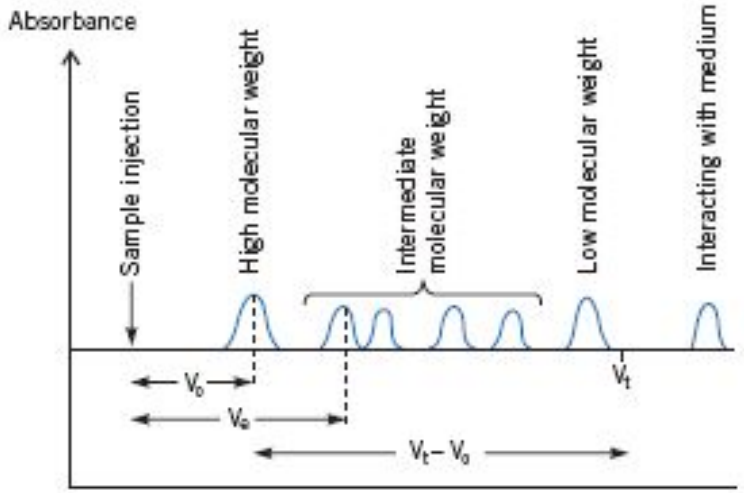


Fig. 3. Process of gel filtration.

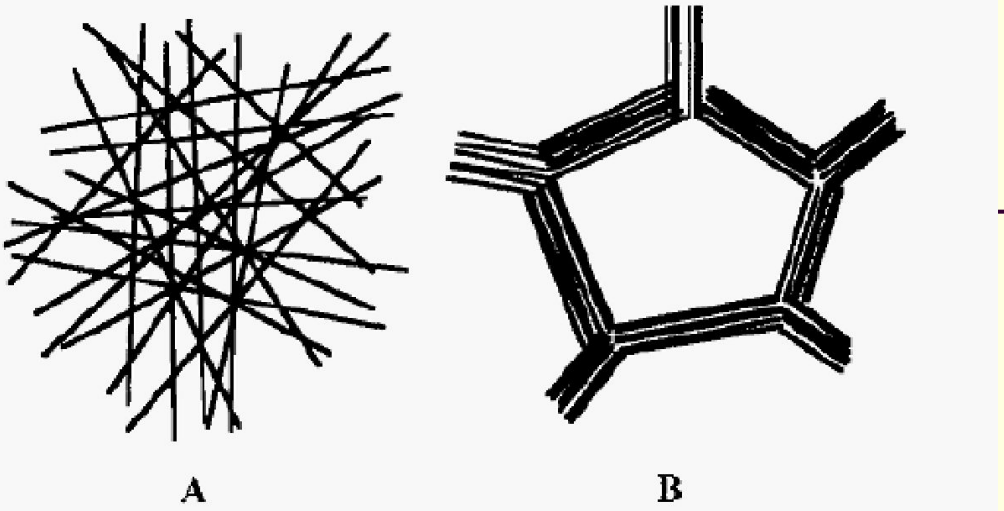


Figure 64. Comparison of micro- and macroreticular gels.

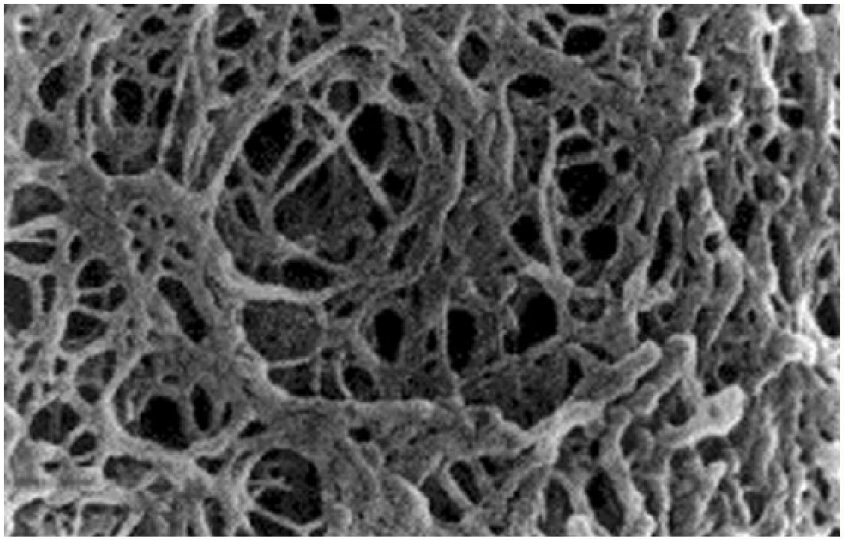


Figure 5.1

A scanning electron micrograph of a porous *agarose* gel (magnification: ×50 000).

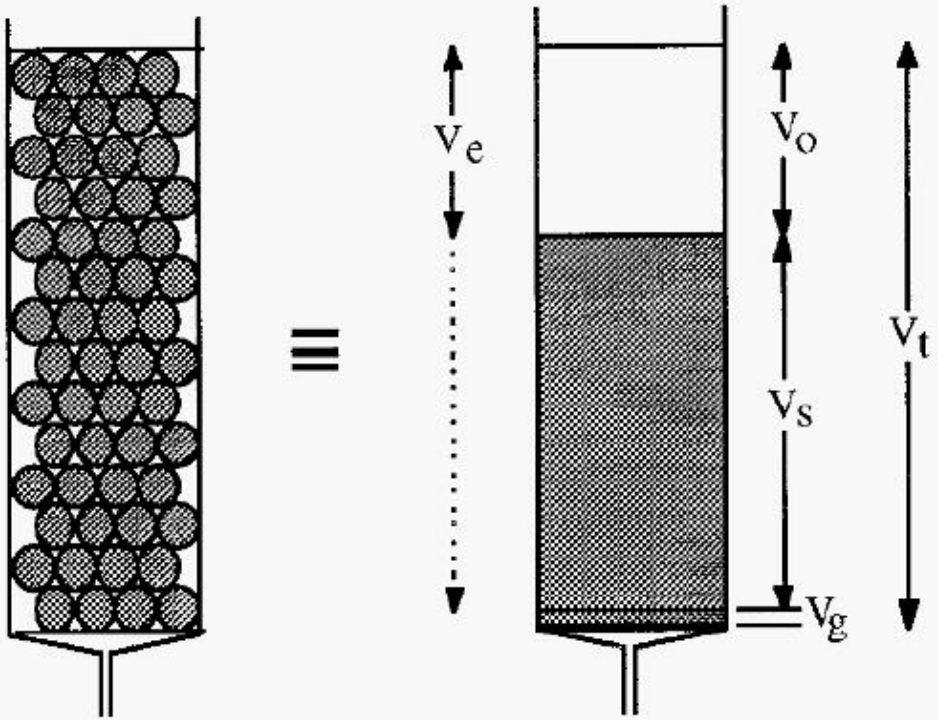
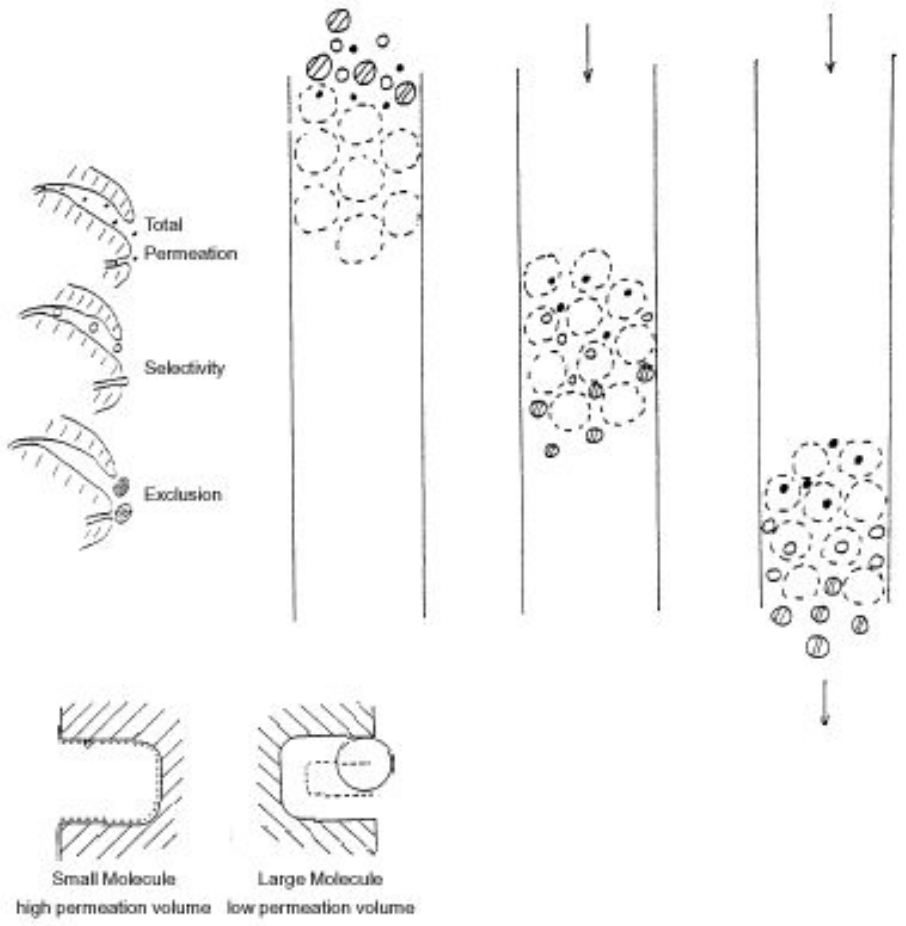


Figure 71. The volumes comprising the total volume of a molecular exclusion column. V_t is the total column volume, V_s is the volume of the water immobilised within the gel beads, V_o is the volume of the mobile water between the gel beads, V_g is the volume of the gel polymer strands and V_e is the elution volume of a solute. Depending upon the size of the solute, V_e may vary between V_o and $V_o + V_s$.

TB005: Size Exclusion Mechanism

Keywords
GPC, Theory, Size Exclusion Mechanism



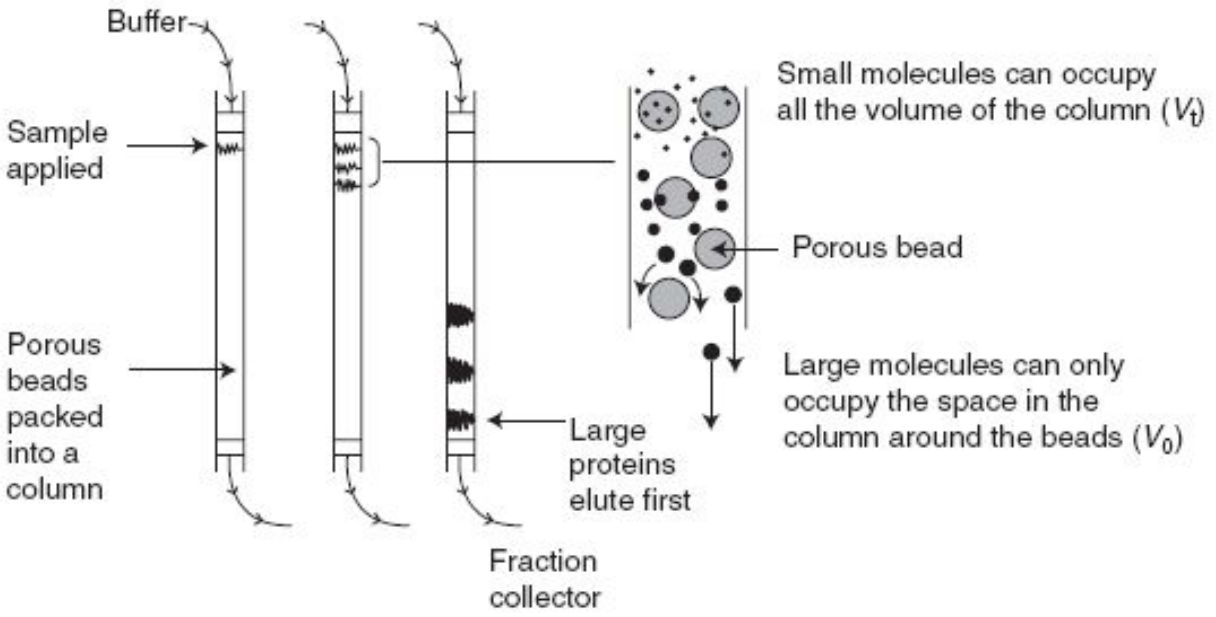
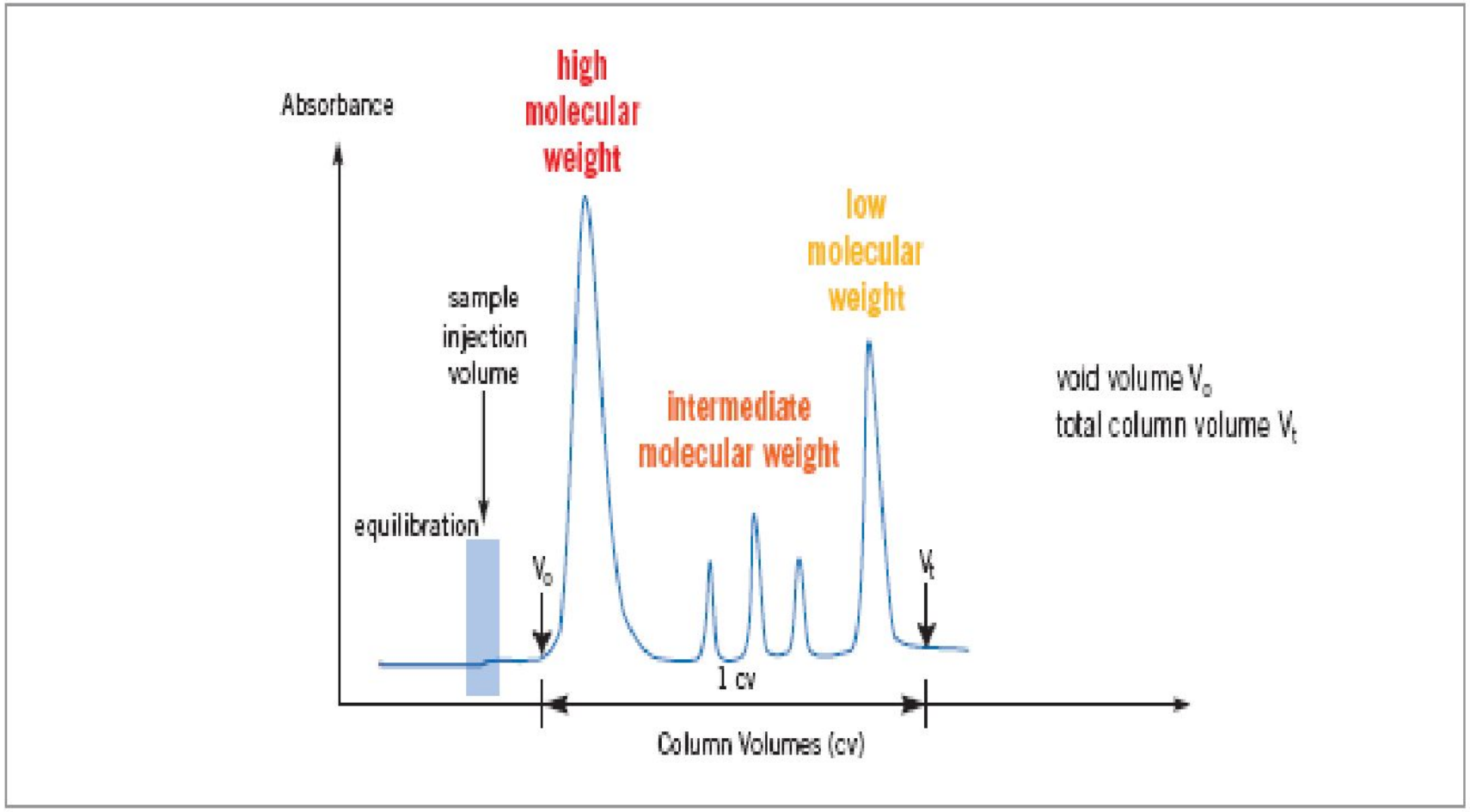
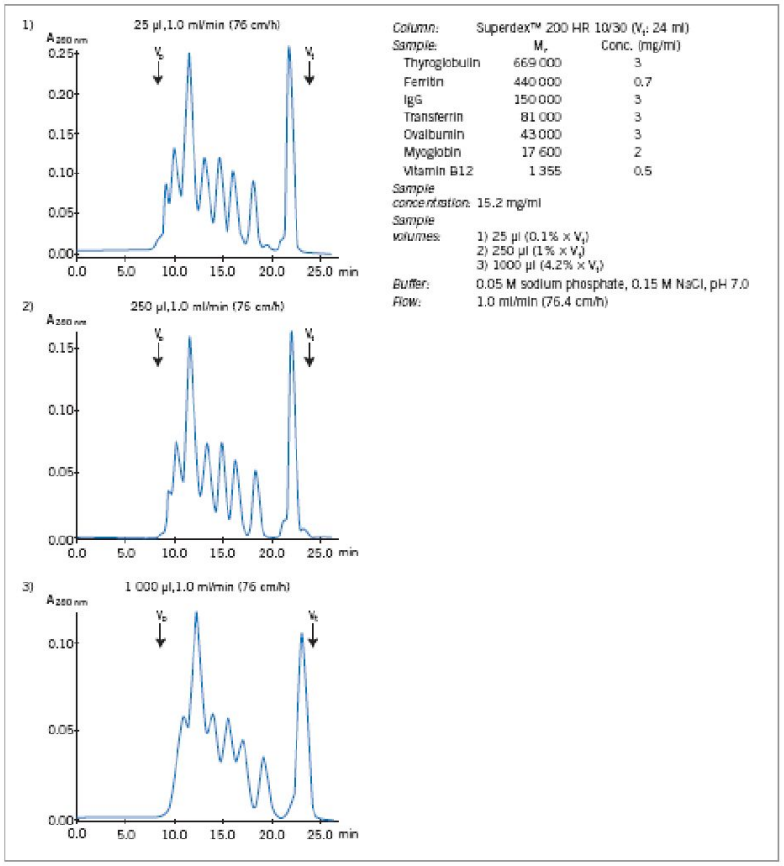


Figure 5.2

A schematic representation of size exclusion chromatography.


high
molecular
weight
Absorbance
low
molecular
weight
sample
injection
volume
intermediate
molecular weight
void volume V_o
total column volume V_t
equilibration
V_o
V_t
1 cv
Column Volumes (cv)

Влияние объема нанесения образца



Column:	Superdex™ 200 HR 10/30 (V_t: 24 ml)	
Sample:	M_r	Conc. (mg/ml)
Thyroglobulin	669 000	3
Ferritin	440 000	0.7
IgG	150 000	3
Transferrin	81 000	3
Ovalbumin	43 000	3
Myoglobin	17 600	2
Vitamin B12	1 355	0.5

Sample concentration: 15.2 mg/ml

Sample volumes:
1) 25 µl (0.1% × V_t)
2) 250 µl (1% × V_t)
3) 1000 µl (4.2% × V_t)

Buffer: 0.05 M sodium phosphate, 0.15 M NaCl, pH 7.0

Flow: 1.0 ml/min (76.4 cm/h)

Fig. 7. Influence of sample volume on resolution.

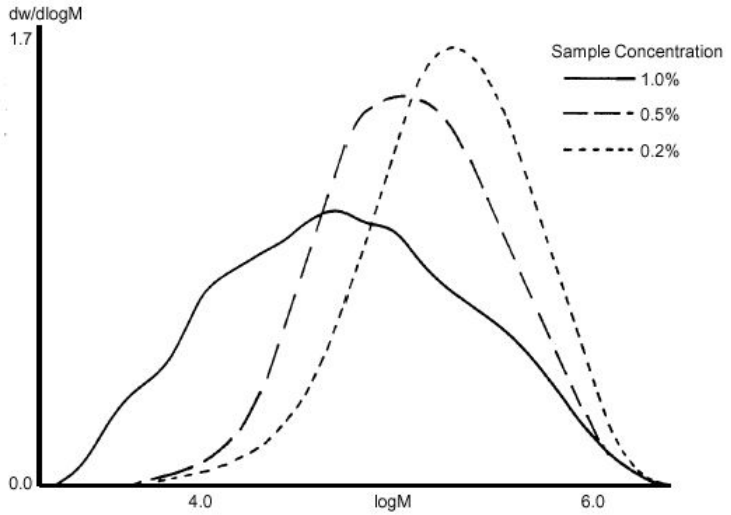
TB019: Overloading Effects

Keywords

GPC, Theory, Overloading Effects

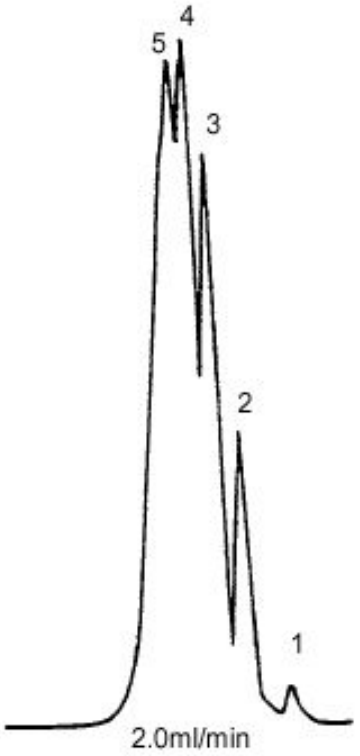
Solution viscosity is dependent upon molecular weight and concentration. At high sample concentrations increased solution viscosity can cause significant band broadening due to viscous streaming or viscous fingering on the trailing side of the solute band.

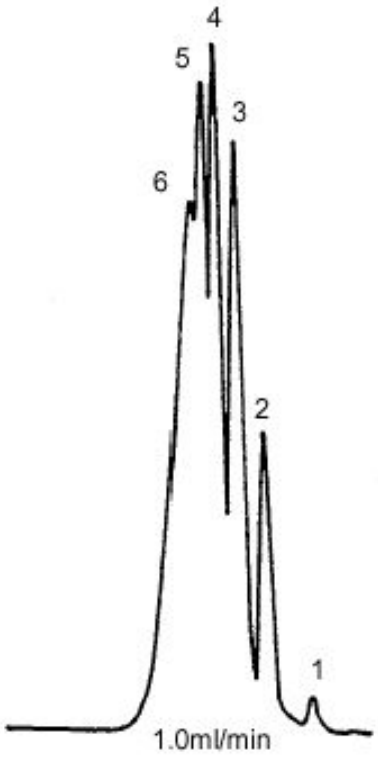
Columns:	2xPLgel 5µm MIXED-C, 300x7.5mm
Sample:	Broad Polystyrene
Eluent:	THF
Flow Rate:	1.0ml/min
Inj Vol:	200µl
Detector:	RI
Calibrants:	Polystyrene Standards

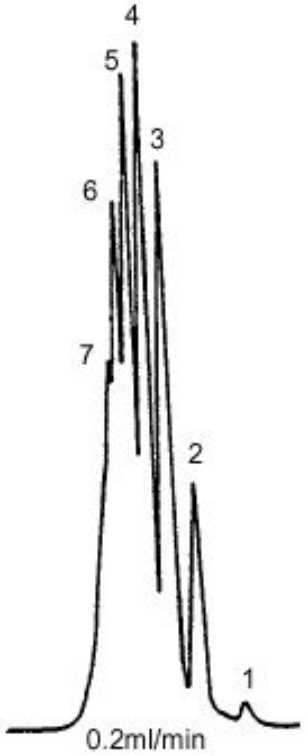


This technical bulletin demonstrates how the resolution of oligomers improves as flow rate is reduced. However, the run time is obviously increased as a result.

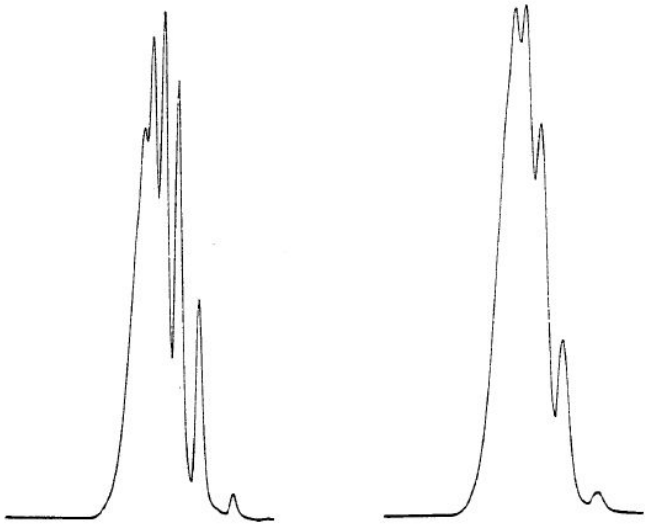
Sample:	Polystyrene standard 480
Column:	PLgel 5µm 100Å, 300x7.5mm
Eluent:	THF
Detector:	RI







Sample:	Polystyrene Standard 480
Column:	PLgel 5µm 100Å, 300x7.5mm
Eluent:	THF
Flow Rate:	1.0ml/min
Conc:	0.1%
Detector:	RI



Inj 20ul

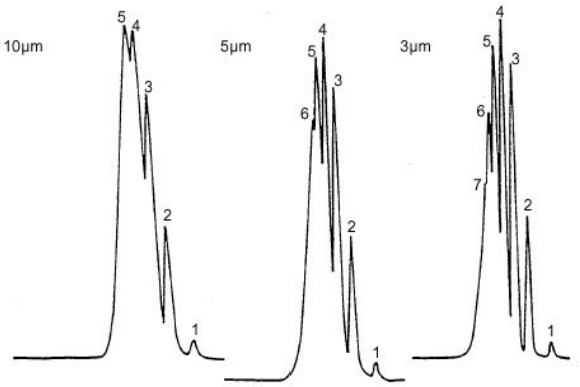
Inj 200ul

TB230: Effect of Particle Size on the Separation of Oligomers

Keywords
GPC, RI, PLgel, Oligomer Resolution, THF

As predicted by the Van Deemter equation, as the particle size of a column is decreased, so the resolution improves. The operating pressure will increase as the particle size of the packing is reduced.

Sample:	Polystyrene standard 480
Column:	PLgel 100Å, 300x7.5mm
Eluent:	THF
Flow Rate:	1.0ml/min
Detector:	RI



Влияние вязкости раствора

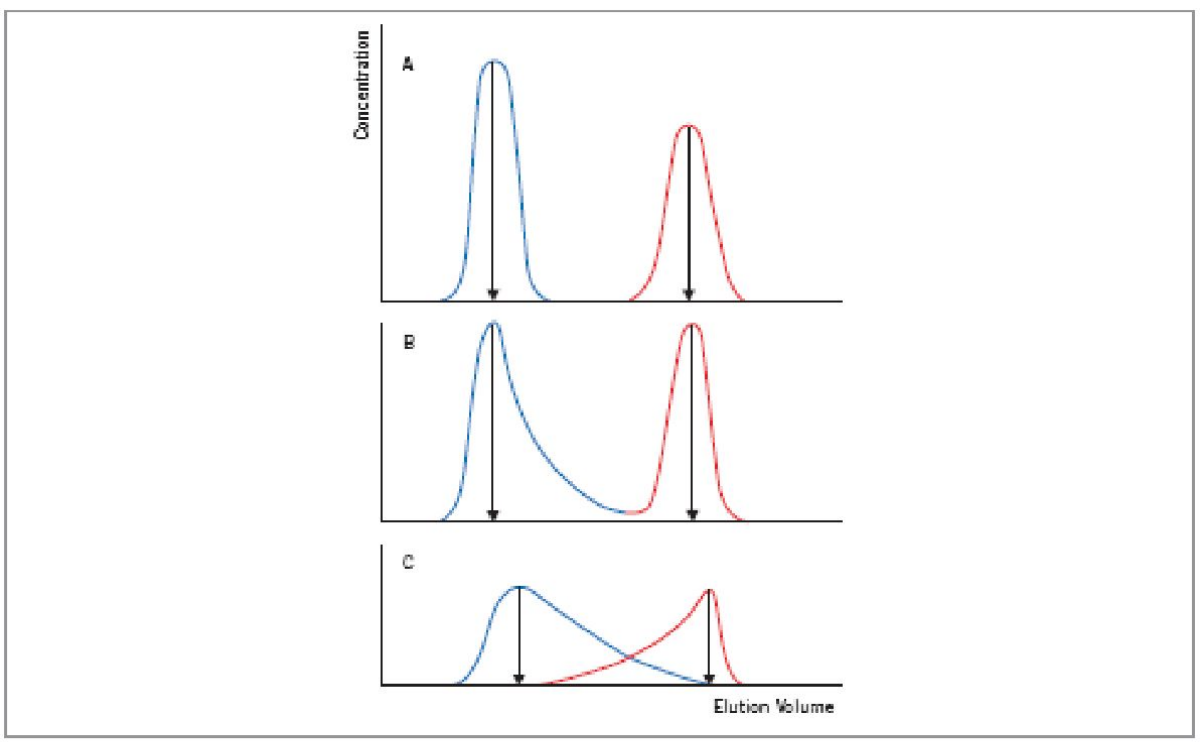
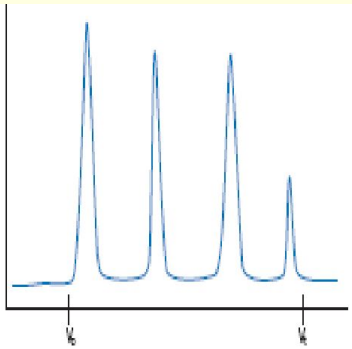


Fig. 13. Elution diagrams obtained when haemoglobin (blue) and NaCl (red) were separated. Experimental conditions were identical except that the viscosities were altered by the addition of increasing amounts of dextran. A deterioration of the separation becomes apparent. (A lower flow rate will not improve the separation.)

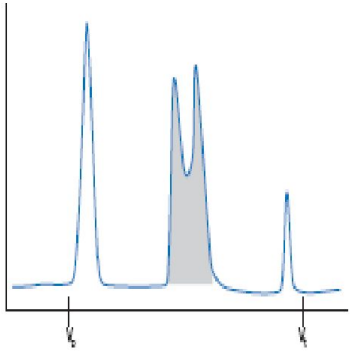

high efficiency
low efficiency

Кривые элюции



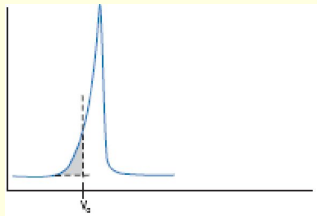
Satisfactory separation

Well resolved, symmetrical peaks.



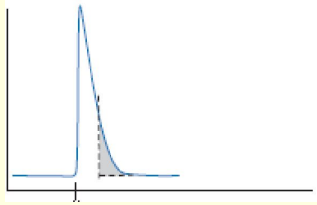
Poor resolution

Review factors affecting resolution (see page 13), including media selection, particle size, sample volume: column volume and flow rate.



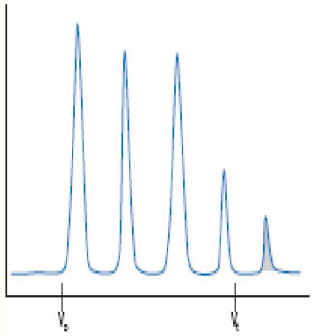
Leading peaks

Asymmetric peaks: sample elutes before void volume indicates channelling in column bed. Leading peaks can also be due to overpackin of column (packed at too high pressure or flo rate). Column may need to be repacked.



Tailing peaks

Asymmetric peaks: sample application uneve Check top of column if possible. Ensure mediu is evenly packed and that sample is applied wit out disturbing the packed bed. Tailing peaks can also be due to underpacking of column (packed at too low pressure or flow rate).



Late elution

Peaks seen after one column volume of buffer has passed through the column. Always include a wash step between runs to ensure removal of late eluting molecules.

Molecules may be binding non-specifically to gel filtration medium. If the interaction is ionic in nature, increasing the concentration of sodium chloride (up to 150 mM) may help. If the interaction is hydrophobic in nature, reducing salt concentration, increasing pH or adding a detergent or organic solvent may help.

Влияние скорости разделения

Column: Superdex 200 HR 10/30 (V_t 24 ml)

Sample:	M_r	Conc. (mg/ml)
Thyroglobulin	669 000	3
Ferritin	440 000	0.7
IgG	150 000	3
Transferrin	81 000	3
Ovalbumin	43 000	3
Myoglobin	17 600	2
Vitamin B12	1 355	0.5

Total sample concentration: 15.2 mg/ml

Buffer: 0.05 M sodium phosphate, 0.15 M NaCl, pH 7.0

Flow: 1) 0.25 ml/min (19.1 cm/h)
2) 1.0 ml/min (76.4 cm/h)

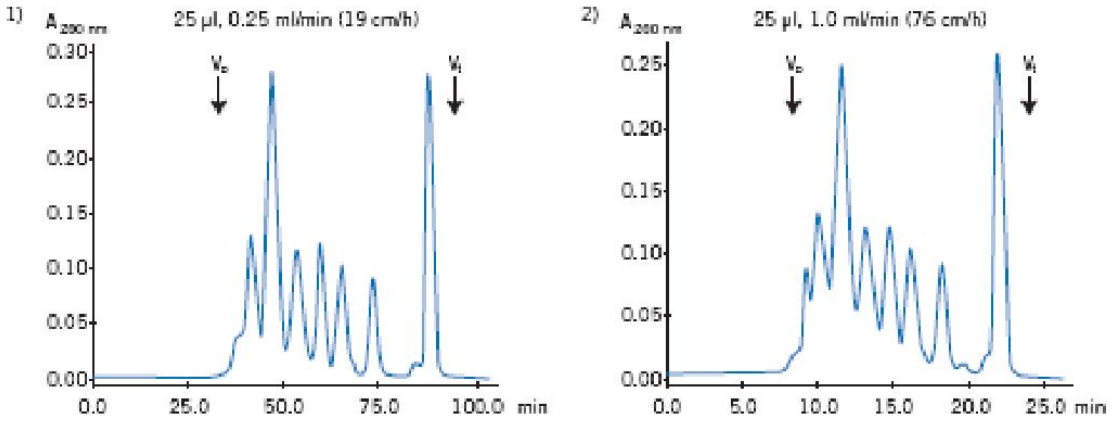
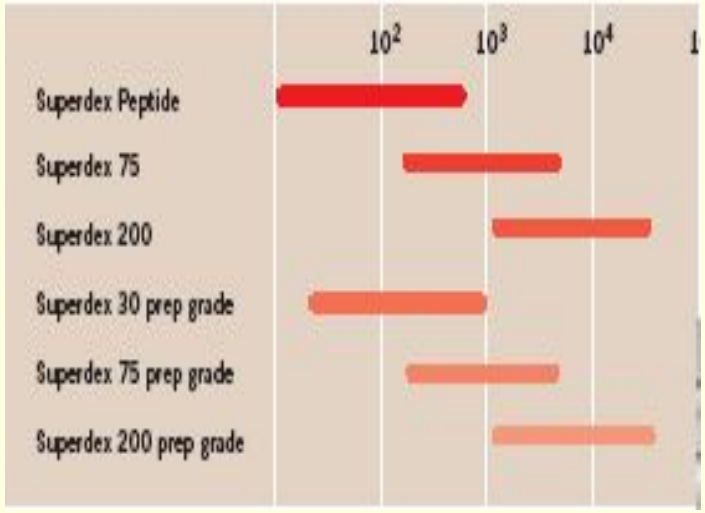
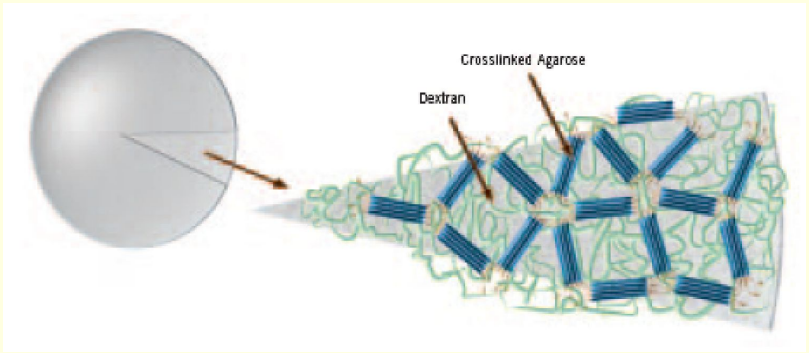
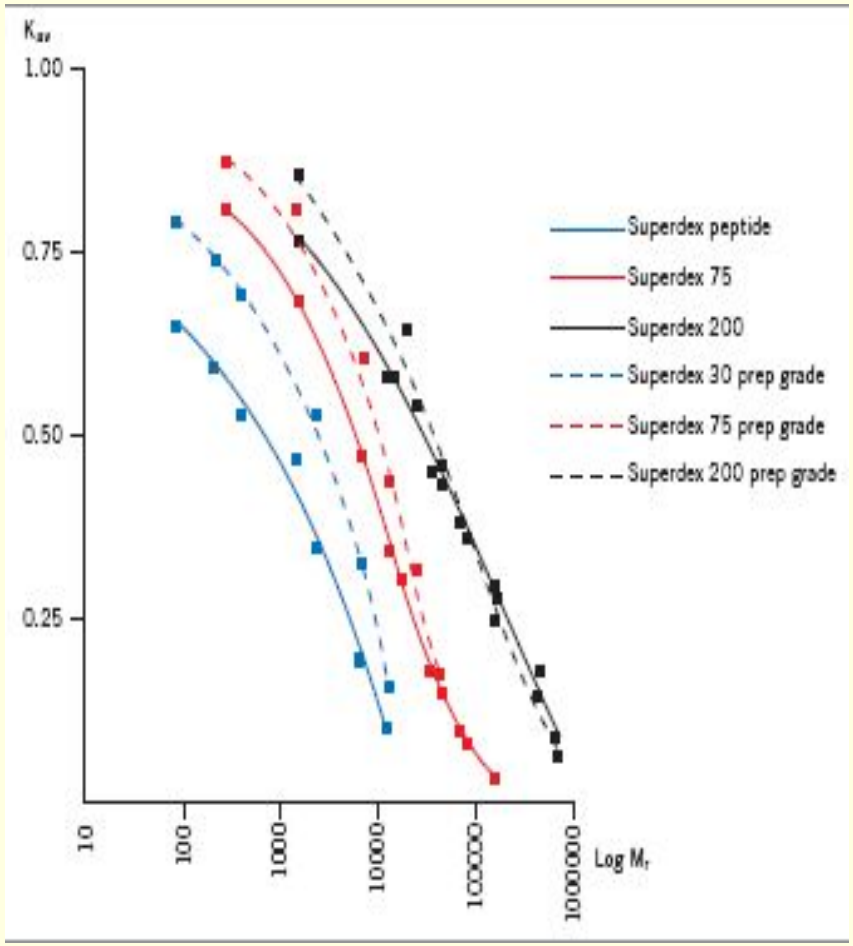


Fig. 14a. Influence of flow rate on resolution.

Носители для гельфильтрации Superdex







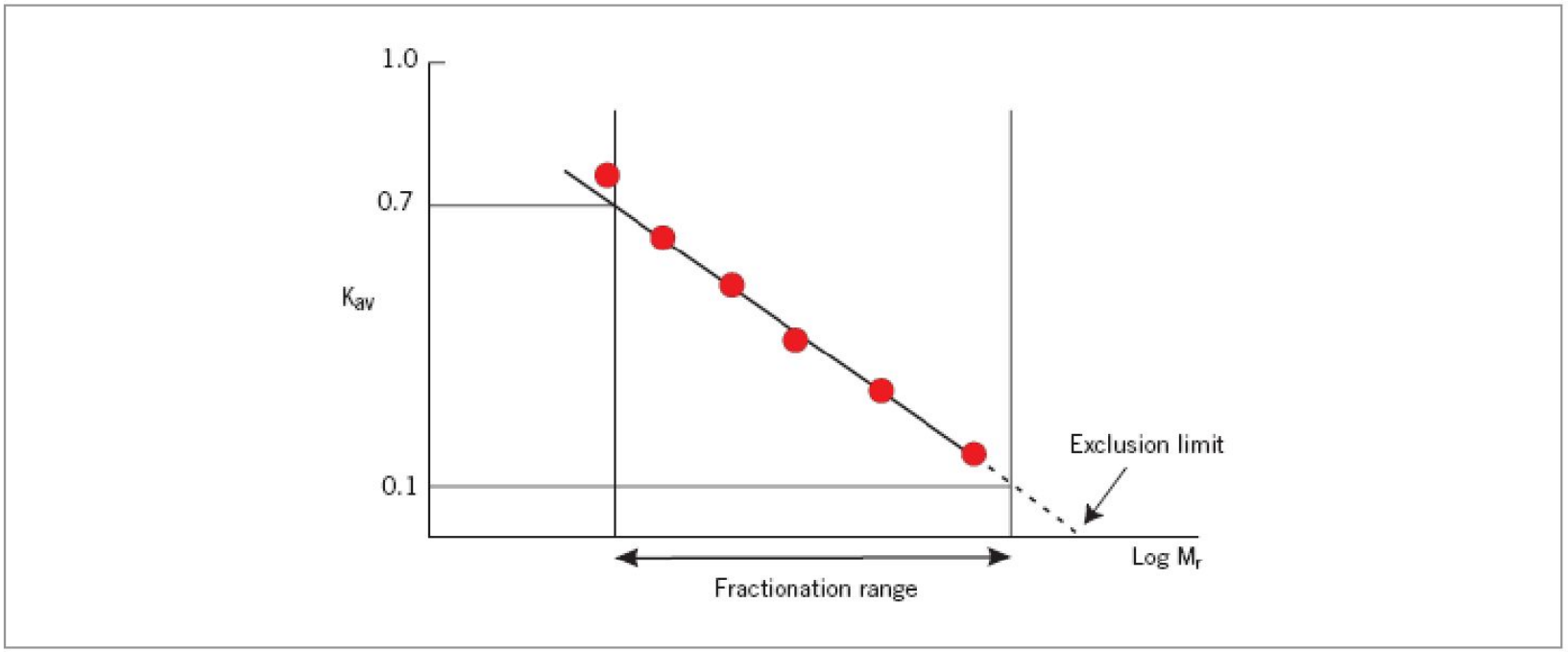
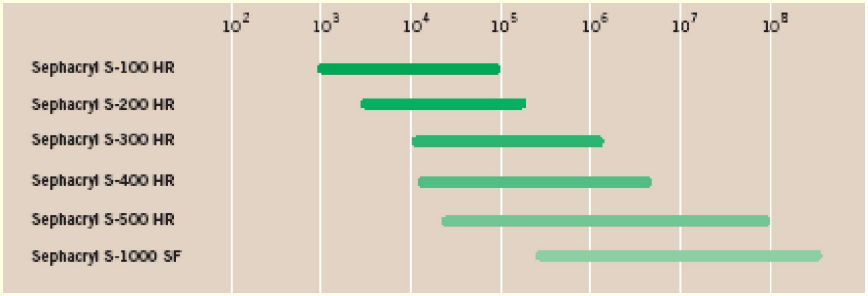
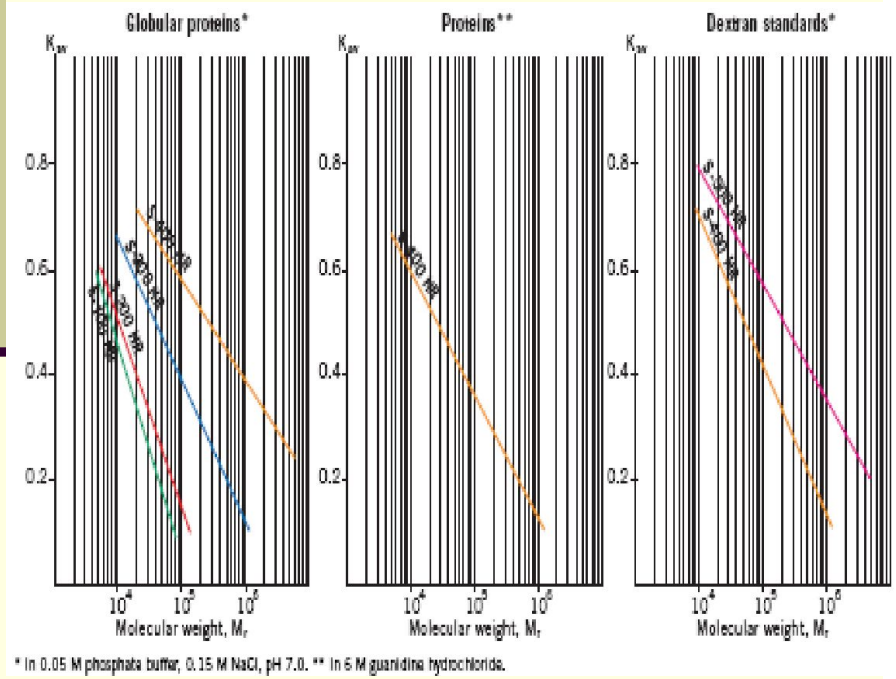
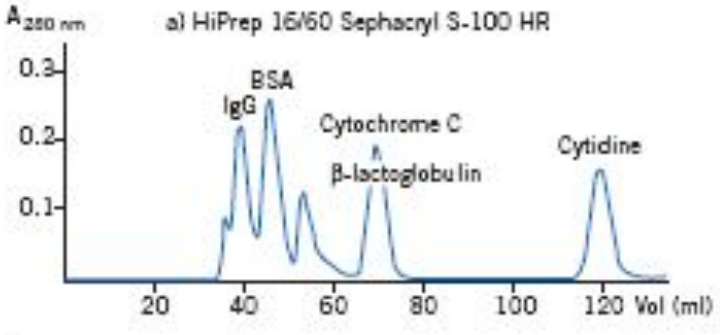


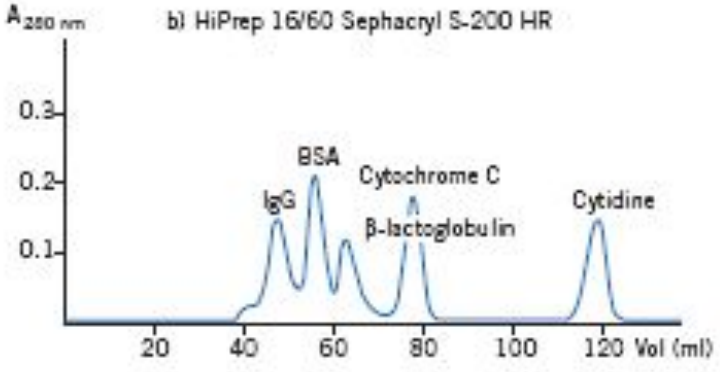
Fig. 10. Defining fractionation range and exclusion limit from a selectivity curve.

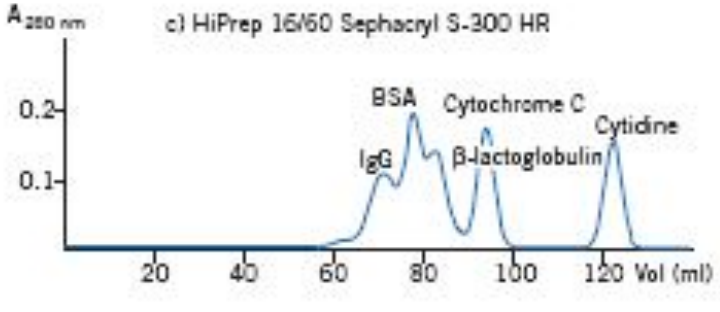
Носители для гельфильтрации Sephacryl



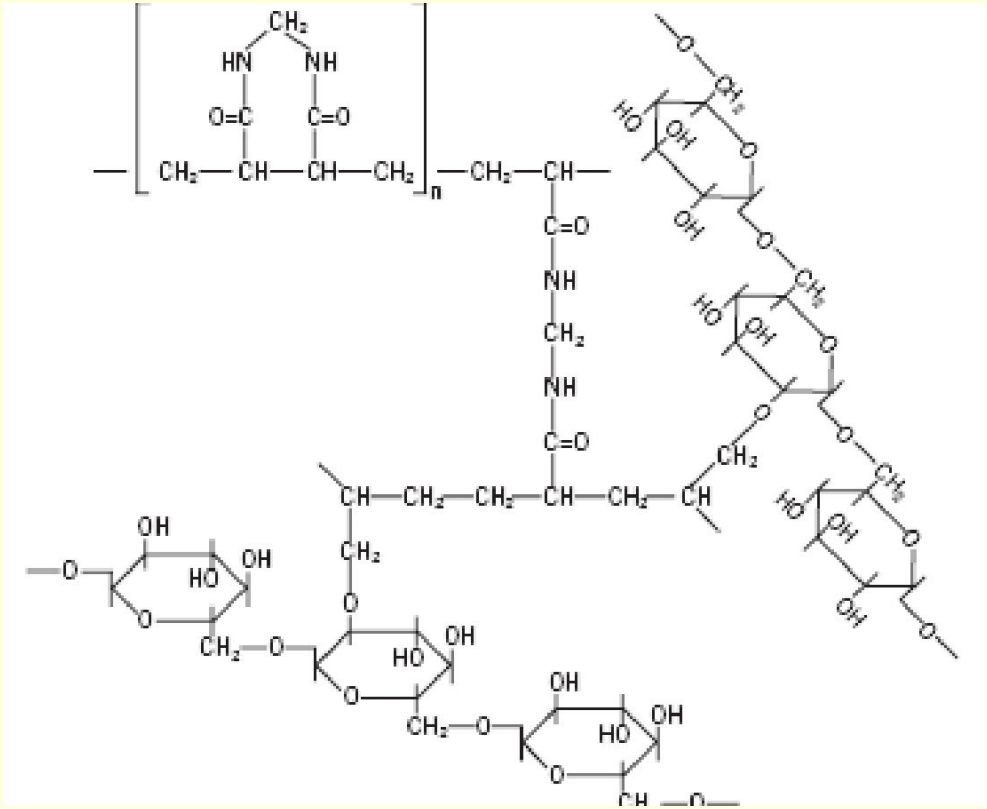


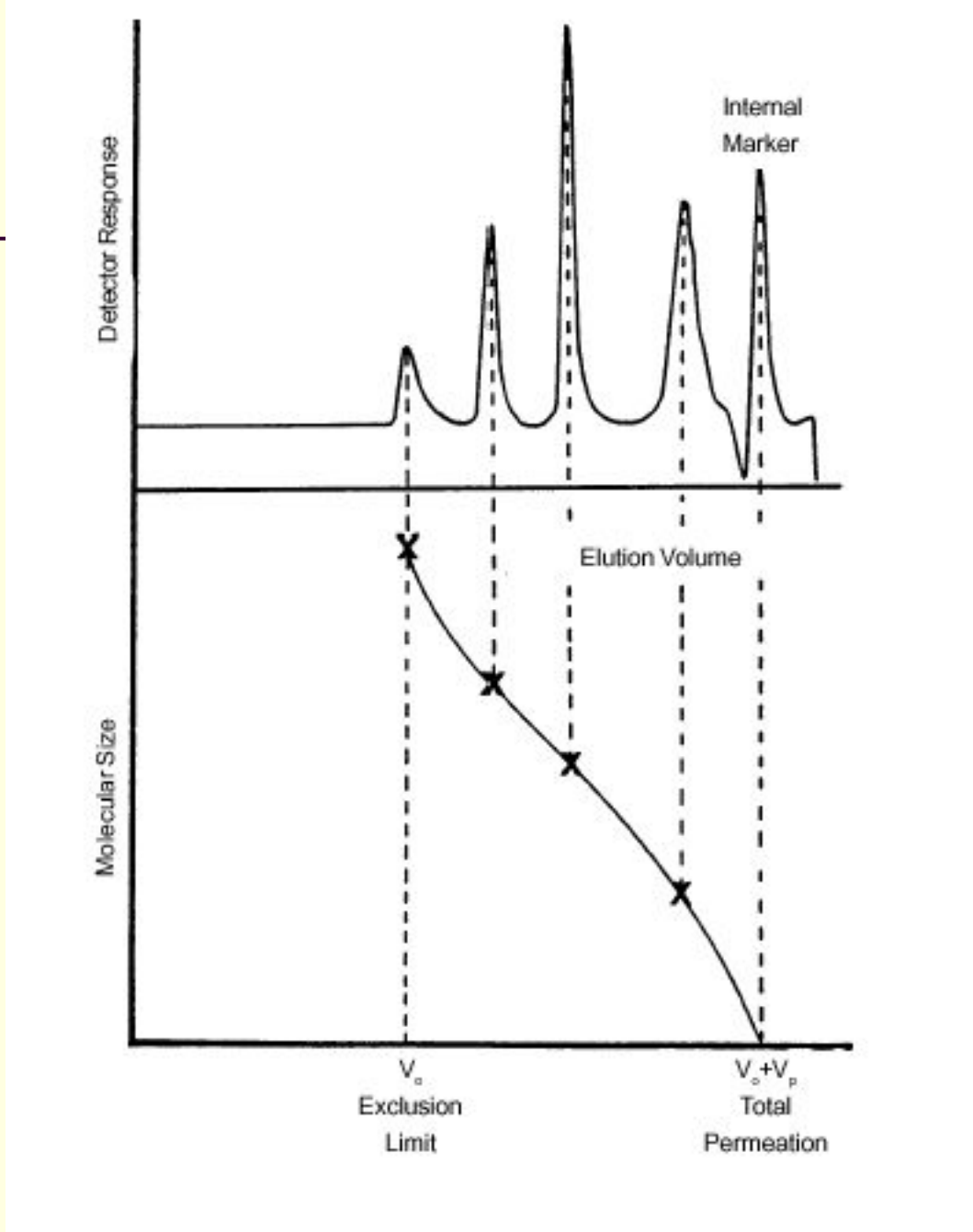




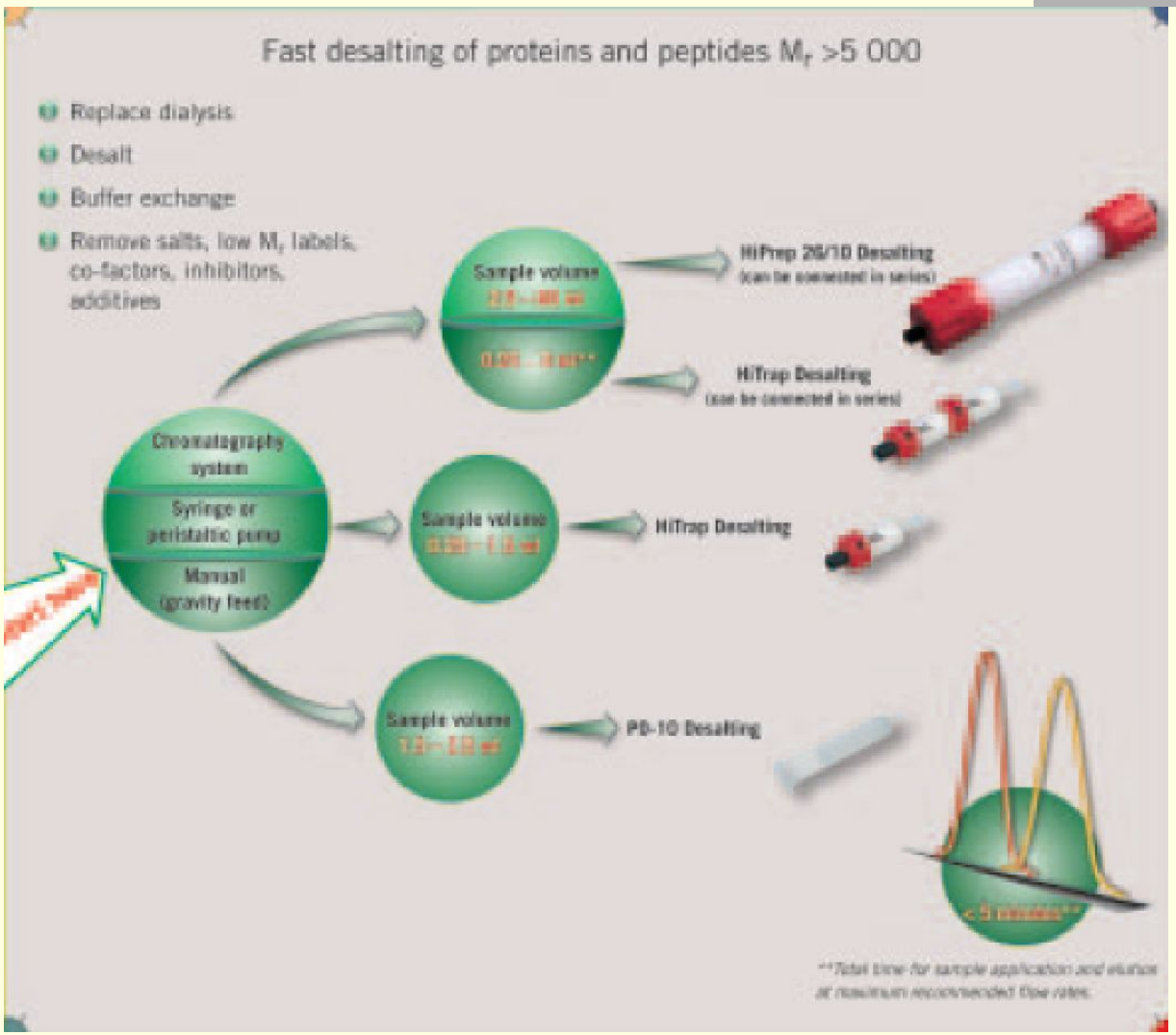


Структура Sephacryl

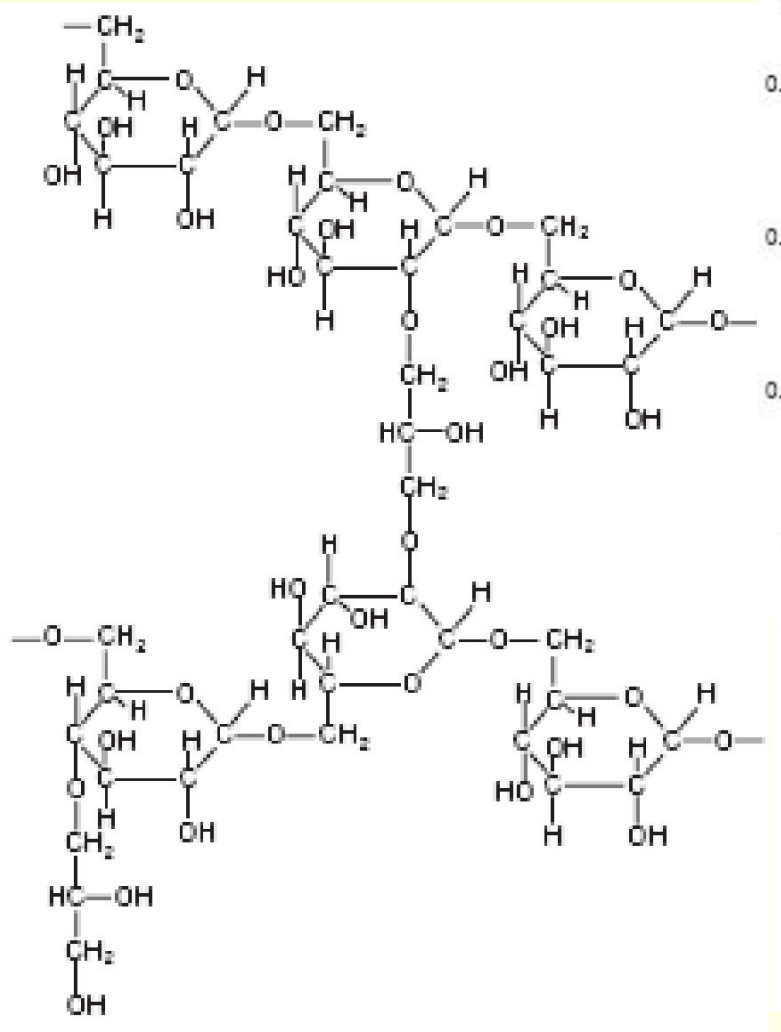


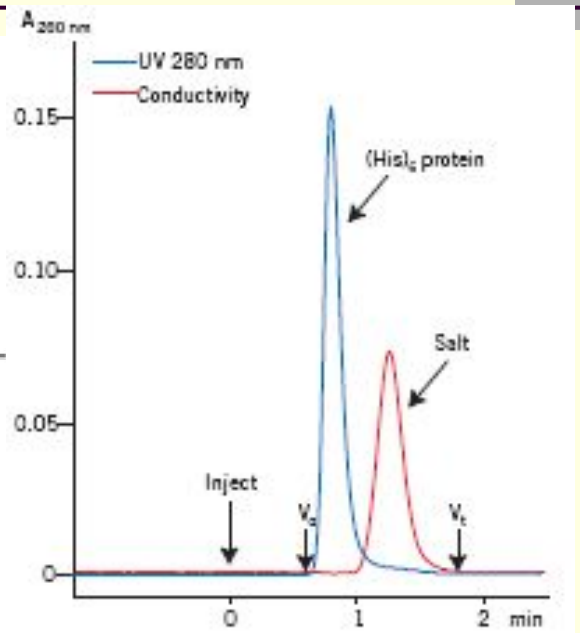

Detector Response
Internal
Marker
Elution Volume
Molecular Size
V_o
Exclusion
Limit
V_o+V_p
Total
Permeation

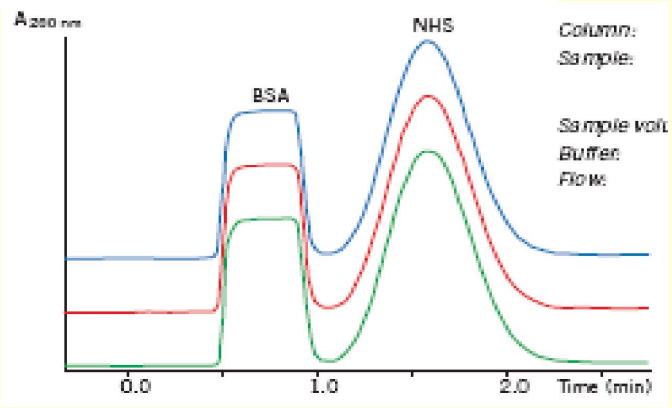
Смена буфера белков и пептидов с ММ больше 5000 Д

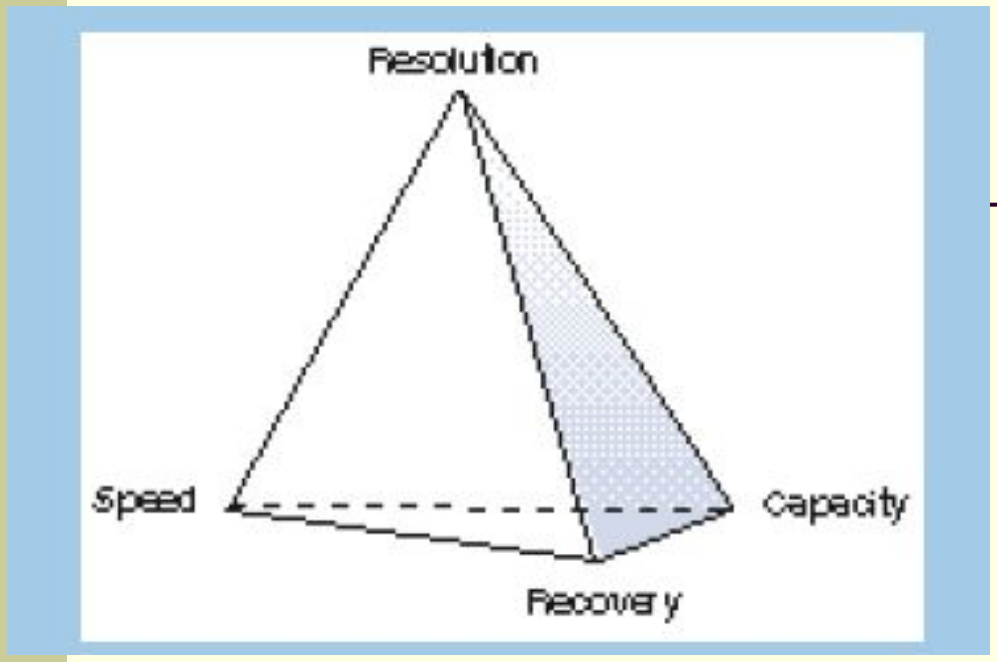


Отделение белка от продуктов реакции на колонках с Sephadex G25









Common Problems in Gel Permeation Chromatography, Possible Causes, and Their Remedies[a]

Problem	Cause	Remedy
Poor peak resolution	Short column	Use long column
	High flow rate	Use low flow rate
	Large sample volume	Keep sample volume below 5% of the column bed volume
	Large "dead space"	Reduce the length of the tube between end of the column and fraction collector
	Poor column packing	Check column packing with blue dextran
	Incorrect fractionation range and grade of matrix	Use a matrix with correct fractionation range and grade
Poor sample recovery	Sample may be precipitated due to very low or excessive salt concentration in the buffer	Check the solubility of the protein
	Protein adsorbed to the matrix	Include detergent or carbohydrate (in case of a lectin) in the buffer. Reduce ionic strength of the buffer
	Proteolysis	Include protease inhibitors in elution buffer
	Harsh elution conditions, which can remove essential cofactors or can dissociate protein subunits, resulting in a partial loss of activity	Try mild conditions
Elution profile not reproducible	Buffer composition may have changed since the previous experiment	Use fresh buffer
	Some components of the sample may have changed or precipitated during storage	Try other storage conditions
Low column flow rate	Column outlet may be partially closed	Check the column outlet and reopen
	Sample precipitate on top of the gel-bed	Scrape the surface of the gel-bed and remove the precipitate. To obtain even gel-bed surface, aspirate the top 1 – 2 cm of matrix and gently apply onto the column. Allow to settle the gel before continuing with the elution.
	Salt deposit inside tubing, obstructing flow rate	Change the tubing
	Gel matrix compressed due to excessive pressure	Repack column
	Air bubbles in the tubing resisting flow	Degas buffer and matrix before packing
	Leaky tubing	Check tubing and replace, if necessary

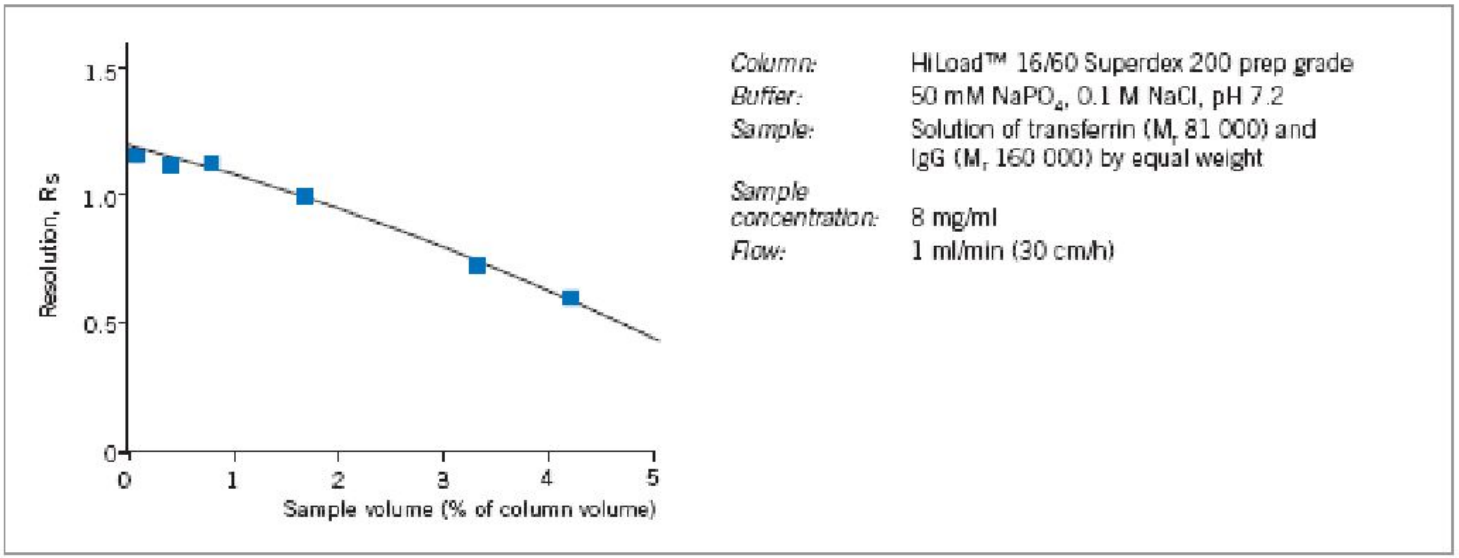


Fig. 8a. Influence of sample volume on the resolution of transferrin and IgG on prepacked HiLoad 16/60 Superdex 200 prep grade.

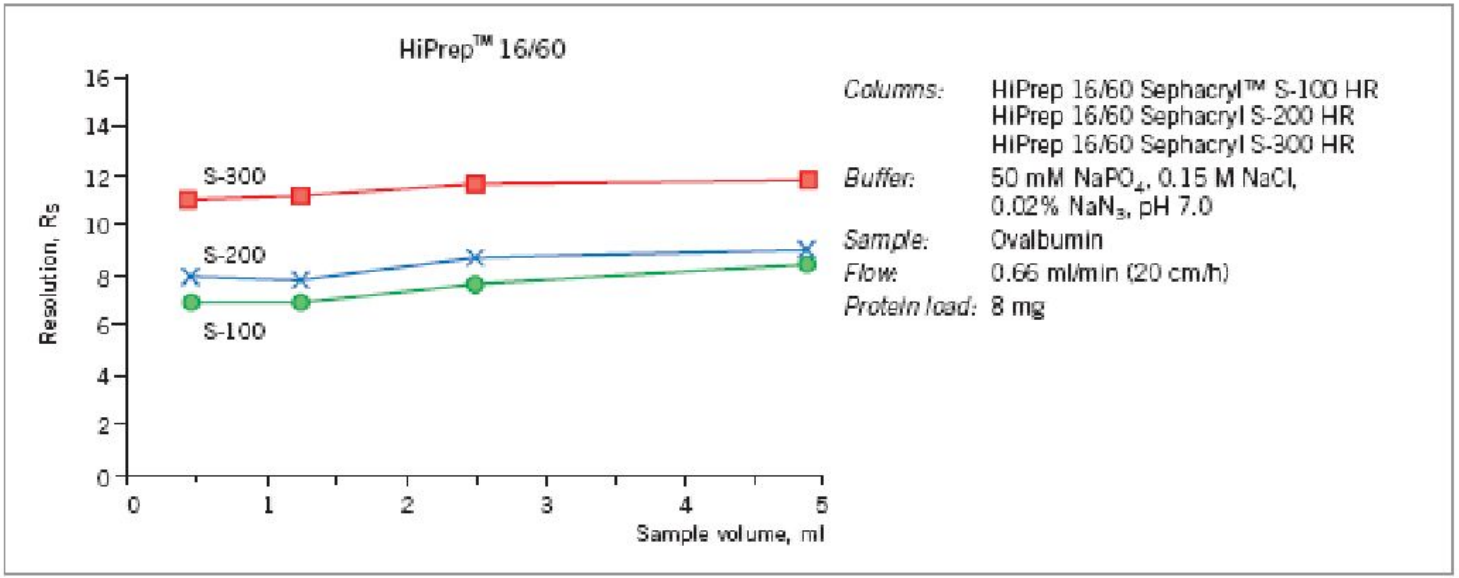


Fig. 8b. Influence of sample volume on the resolution of ovalbumin and IgG on different prepacked columns of HiPrep 16/60 Sephacryl High Resolution.

lectivity, as shown for Superdex in Figure 9. The curve has been obtained by plotting a artition coefficient K_{av} against the log of the molecular weight for a set of standard protein ee Chapter 3 Gel filtration in theory for calculation of K_{av}).