

Гельфилтрация в очистке белков

Принципы биохромотографии

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)

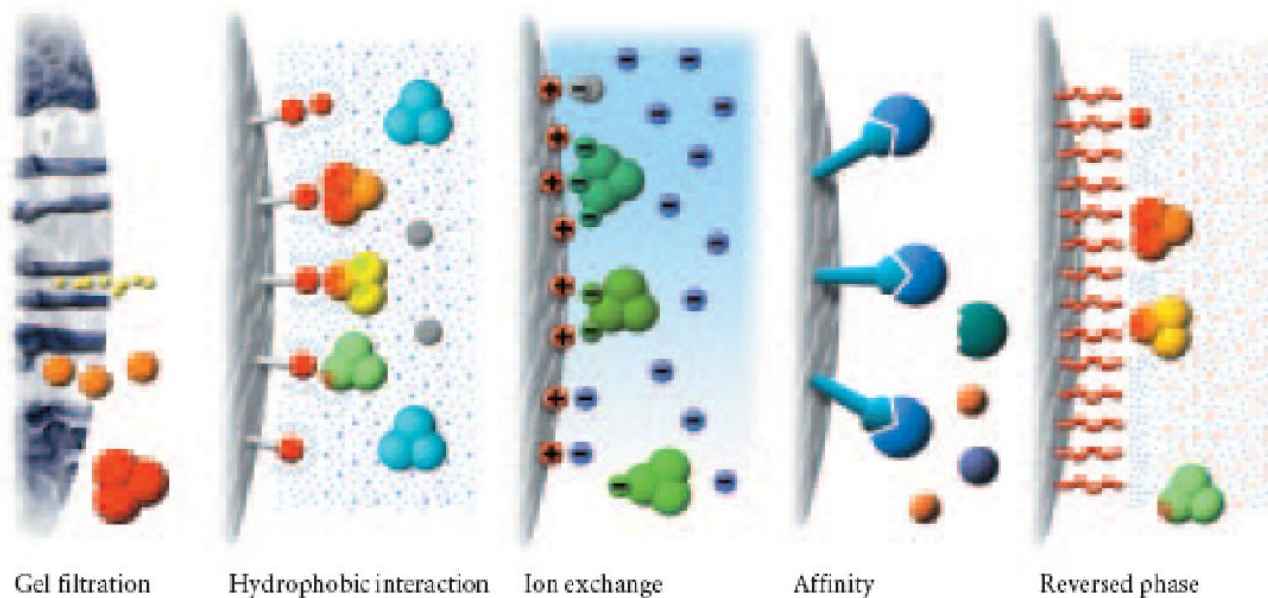


Fig. 1. Separation principles in chromatography purification.

Принципы гельфилтрации

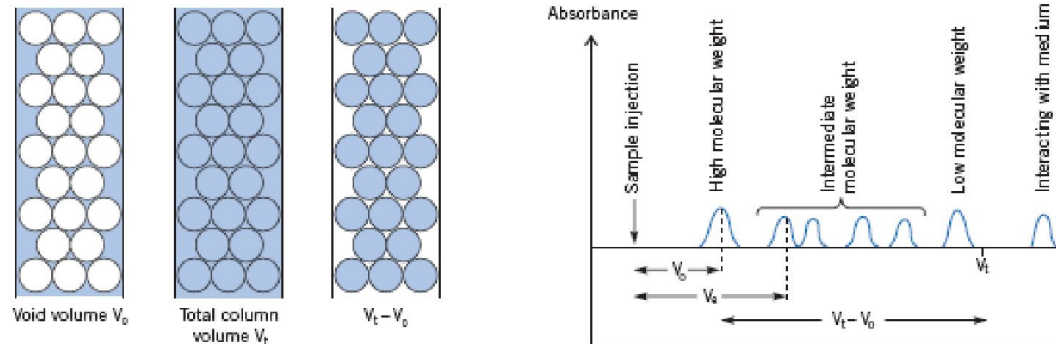
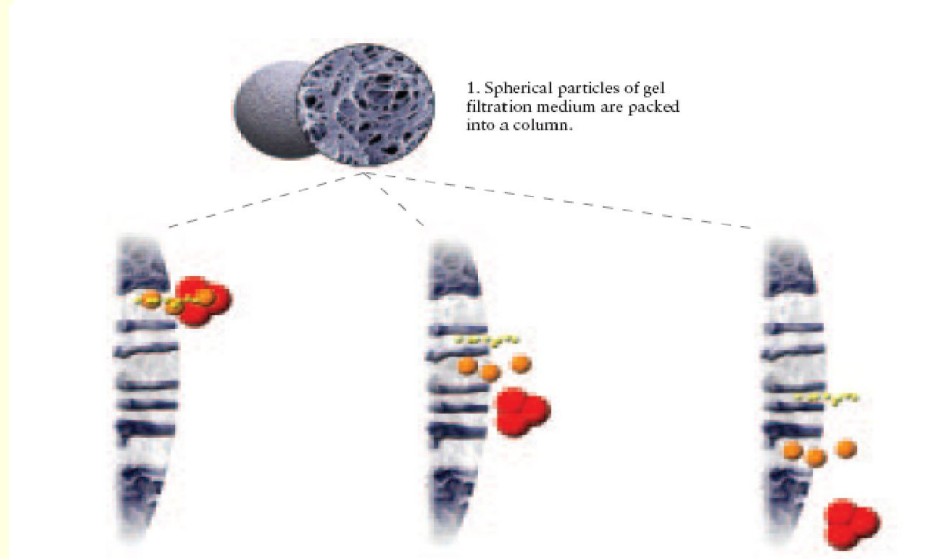


Fig. 2. Common terms in gel filtration.





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.

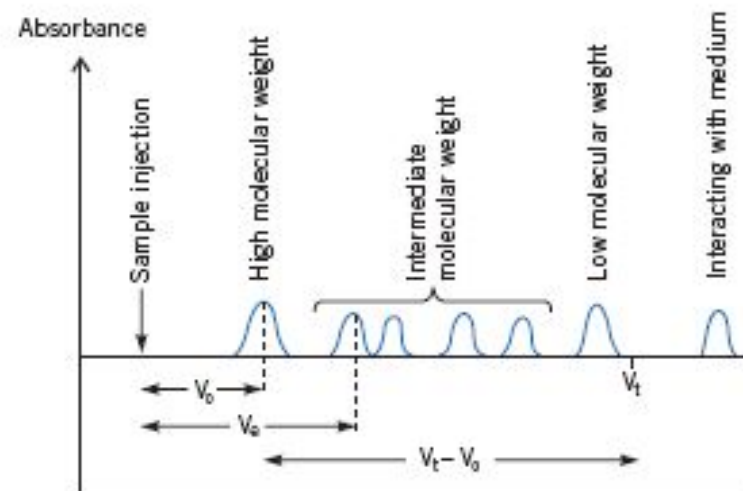
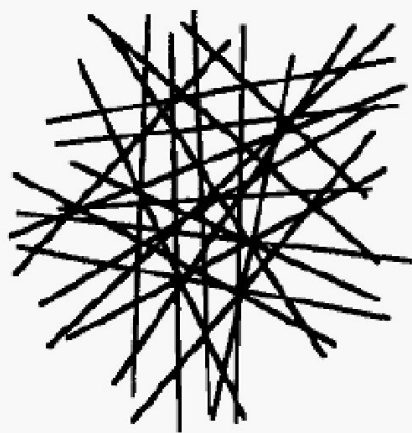
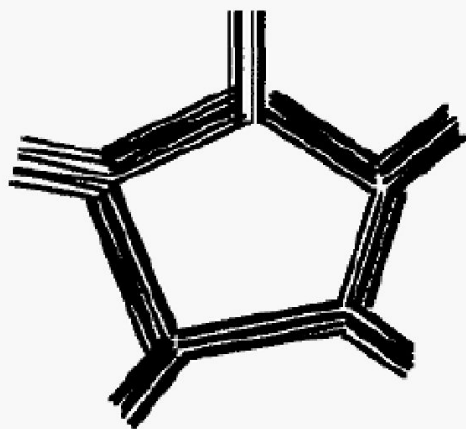


Fig. 3. Process of gel filtration.



A



B

Figure 64. Comparison of micro- and macroreticular gels.

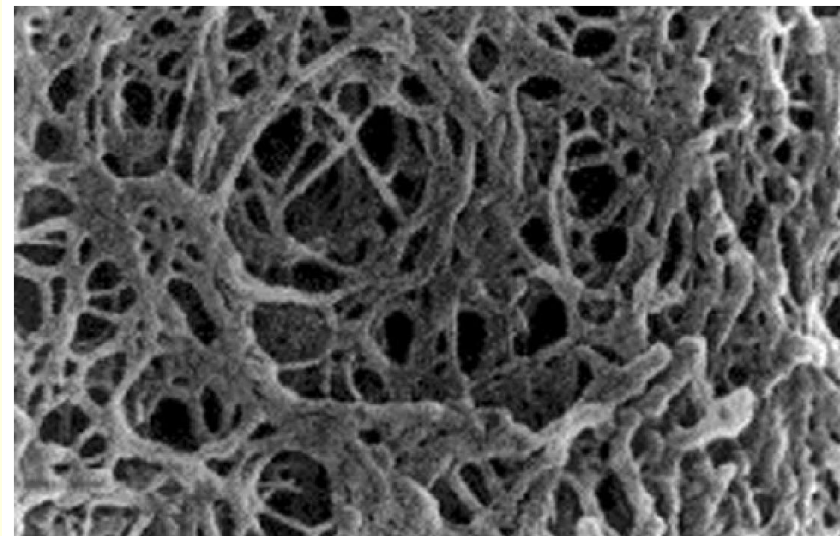


Figure 5.1

A scanning electron micrograph of a porous agarose gel (magnification: $\times 50\,000$).

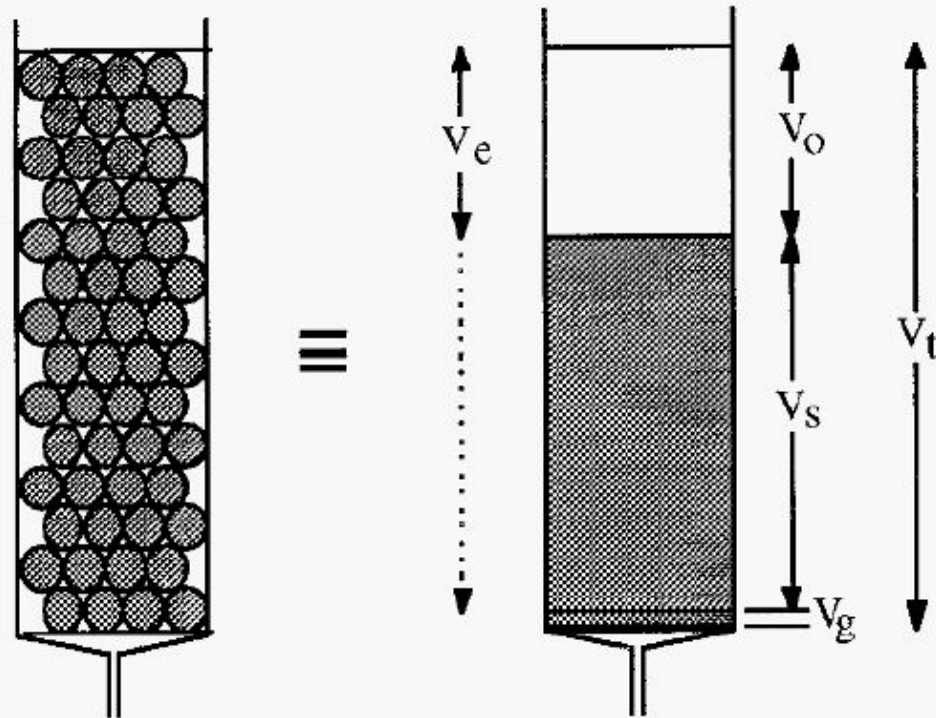
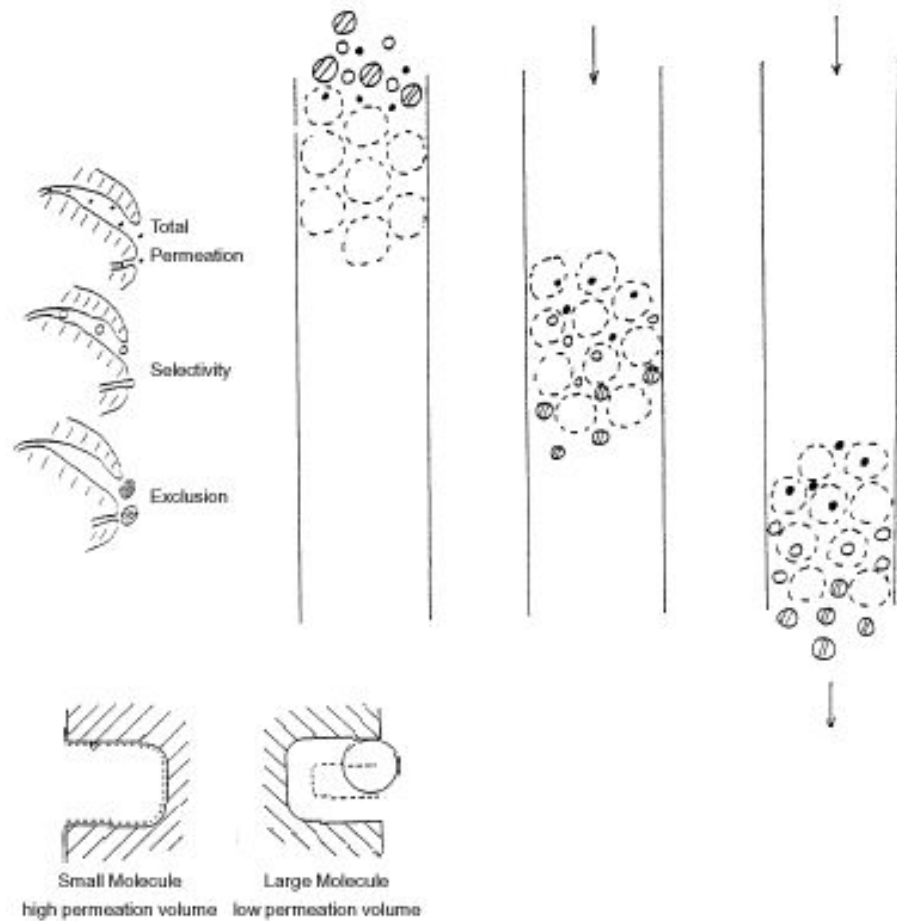


Figure 71. The volumes comprising the total volume of a molecular exclusion column. V_t is the total column volume, V_s is the volume of the water immobilised within the gel beads, V_o is the volume of the mobile water between the gel beads, V_g is the volume of the gel polymer strands and V_e is the 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



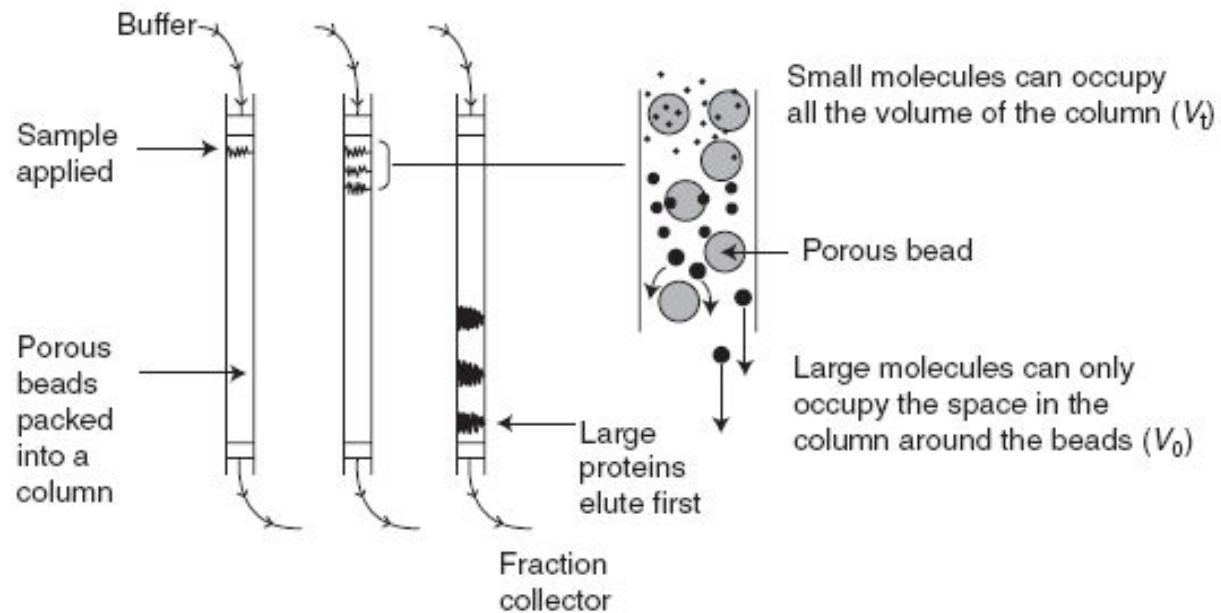
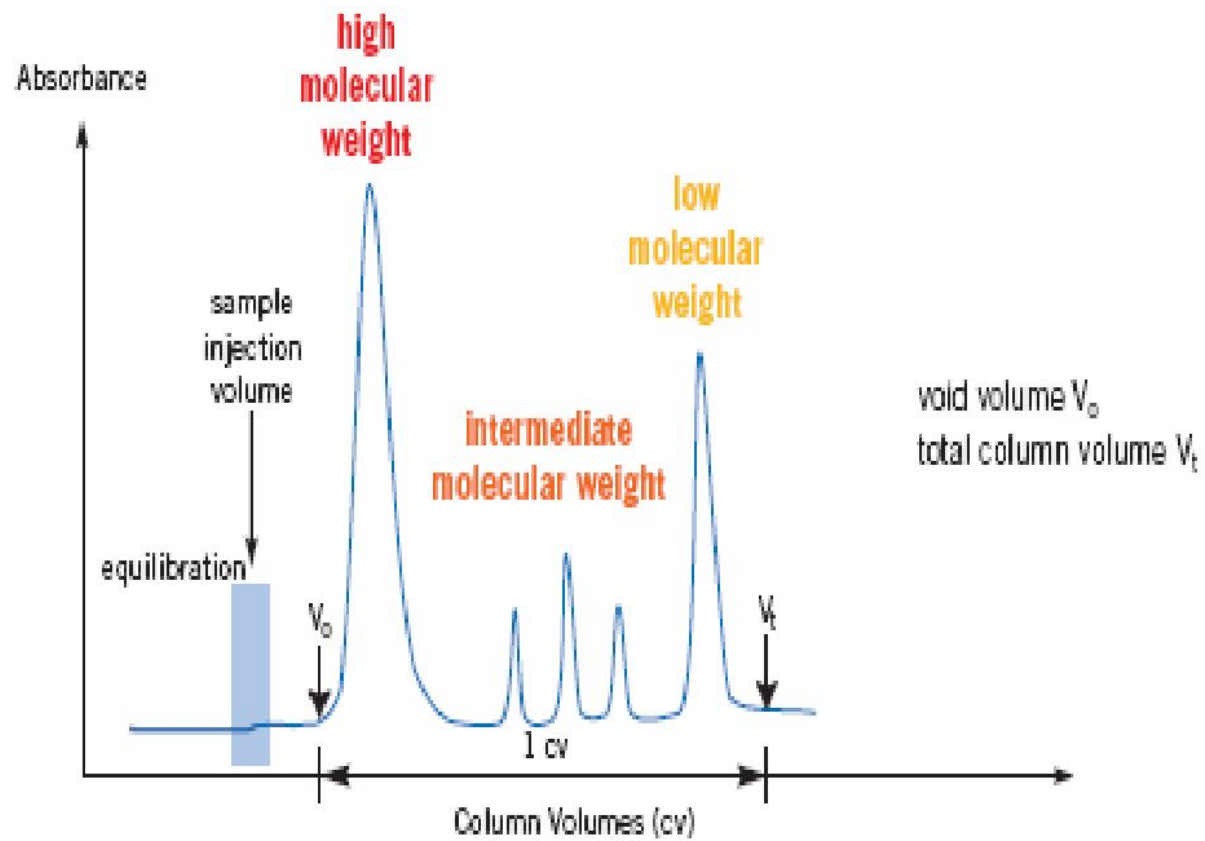


Figure 5.2

A schematic representation of size exclusion chromatography.



Влияние объема нанесения образца

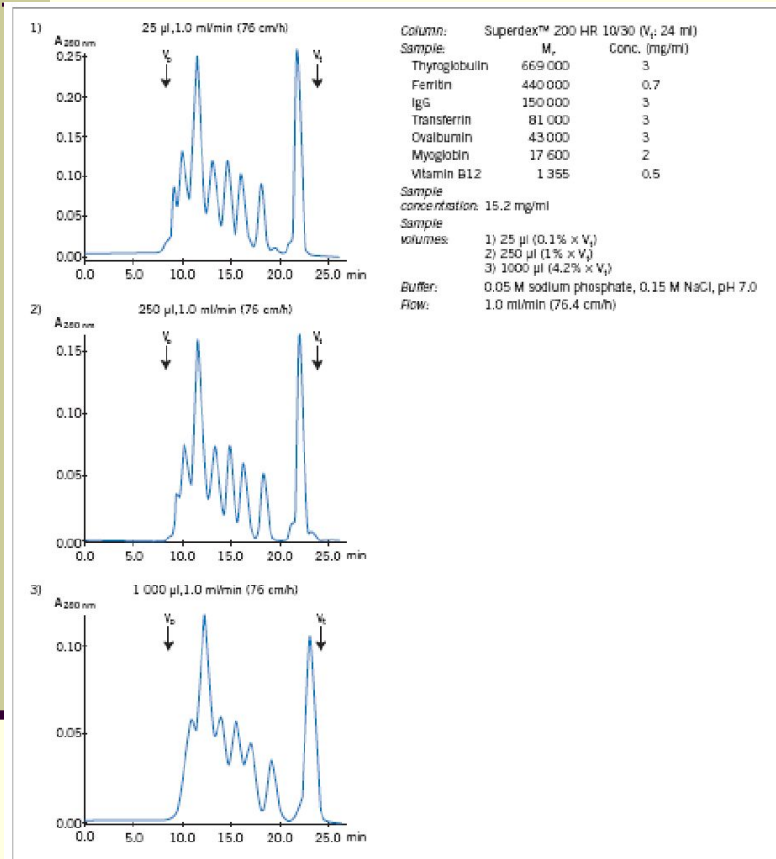


Fig. 7. Influence of sample volume on resolution.

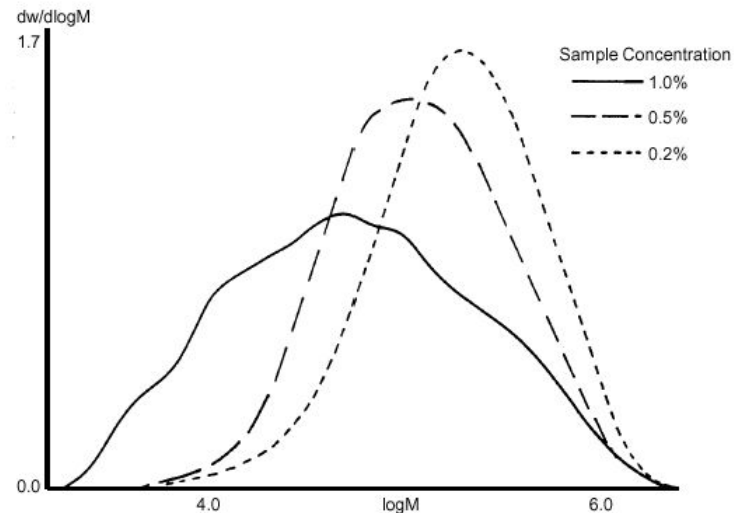
TB019: Overloading Effects

Keywords

GPC, Theory, Overloading Effects

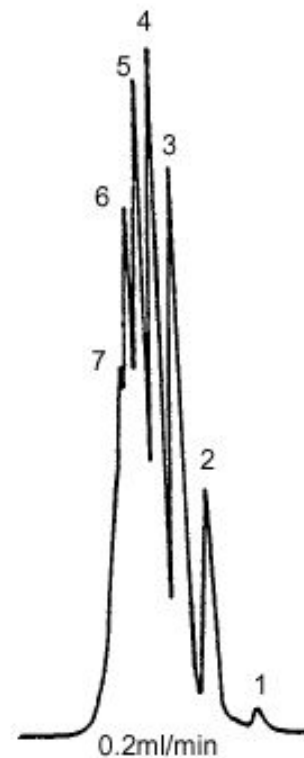
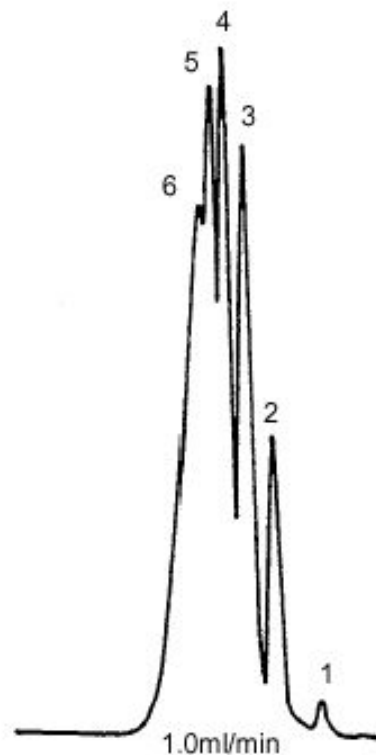
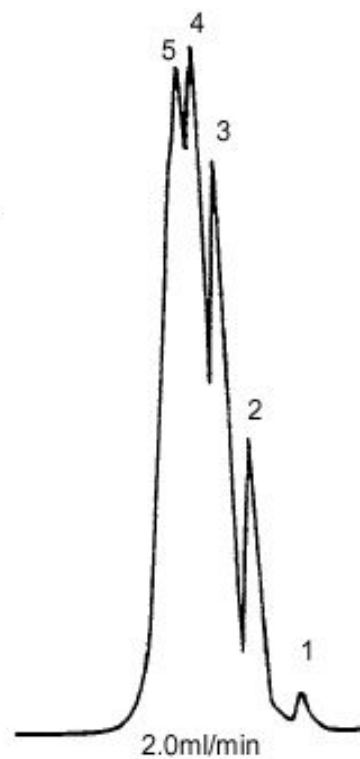
Solution viscosity is dependent upon molecular weight and concentration. At high sample concentrations increased solution viscosity can cause significant band broadening due to viscous streaming or viscous fingering on the trailing side of the solute band.

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

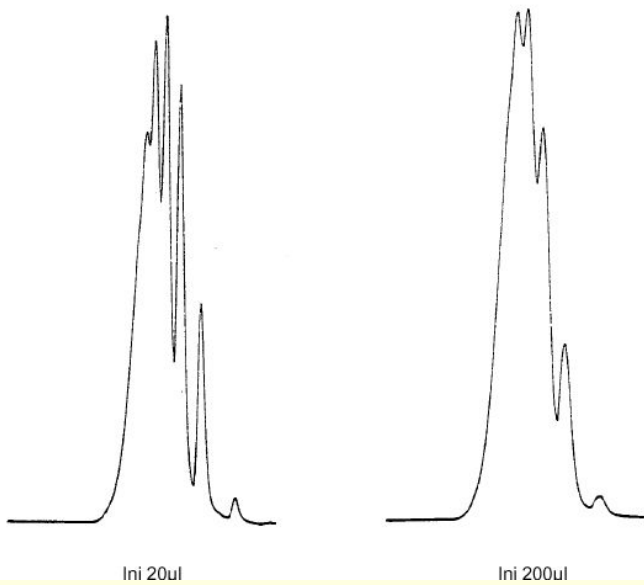


This technical bulletin demonstrates how the resolution of oligomers improves as flow rate is reduced. However, the run time is obviously increased as a result.

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



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



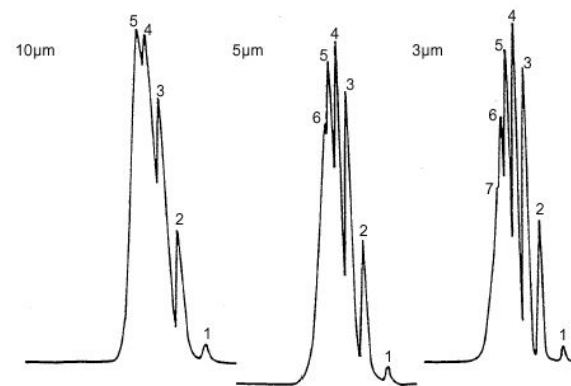
TB230: Effect of Particle Size on the Separation of Oligomers

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

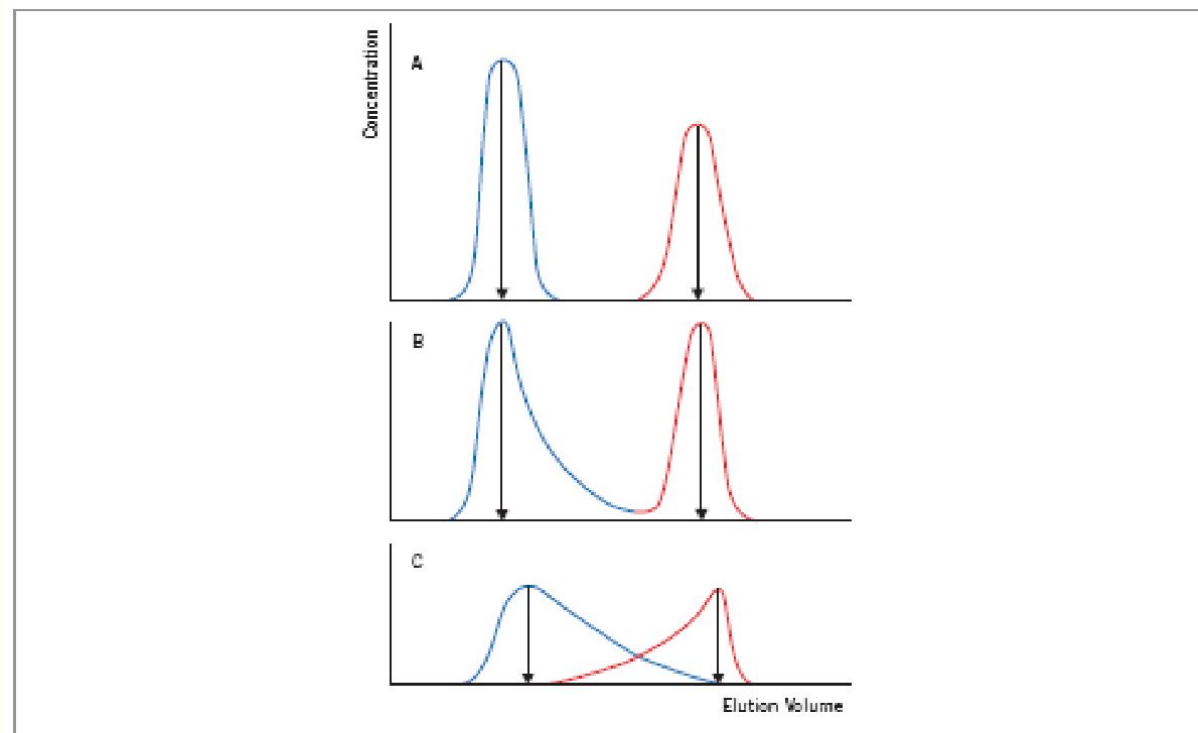
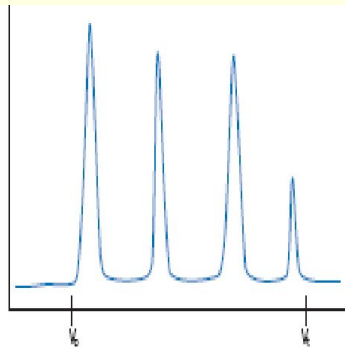


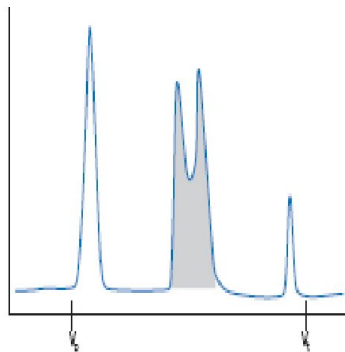
Fig. 13. Elution diagrams obtained when haemoglobin (blue) and NaCl (red) were separated. Experimental conditions were identical except that the viscosities were altered by the addition of increasing amounts of dextran. A deterioration of the separation becomes apparent. (A lower flow rate will not improve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



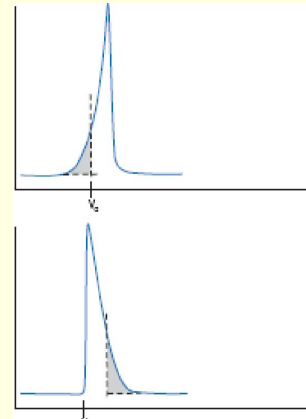
Кривые элюции



Satisfactory separation
Well resolved, symmetrical peaks.

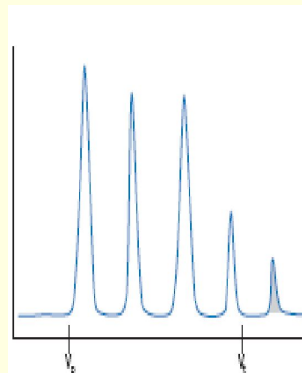


Poor resolution
Review factors affecting resolution (see page 13), including media selection, particle size, sample volume: column volume and flow rate.



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

Tailing peaks
Asymmetric peaks: sample application uneven. Check top of column if possible. Ensure medium is evenly packed and that sample is applied without 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

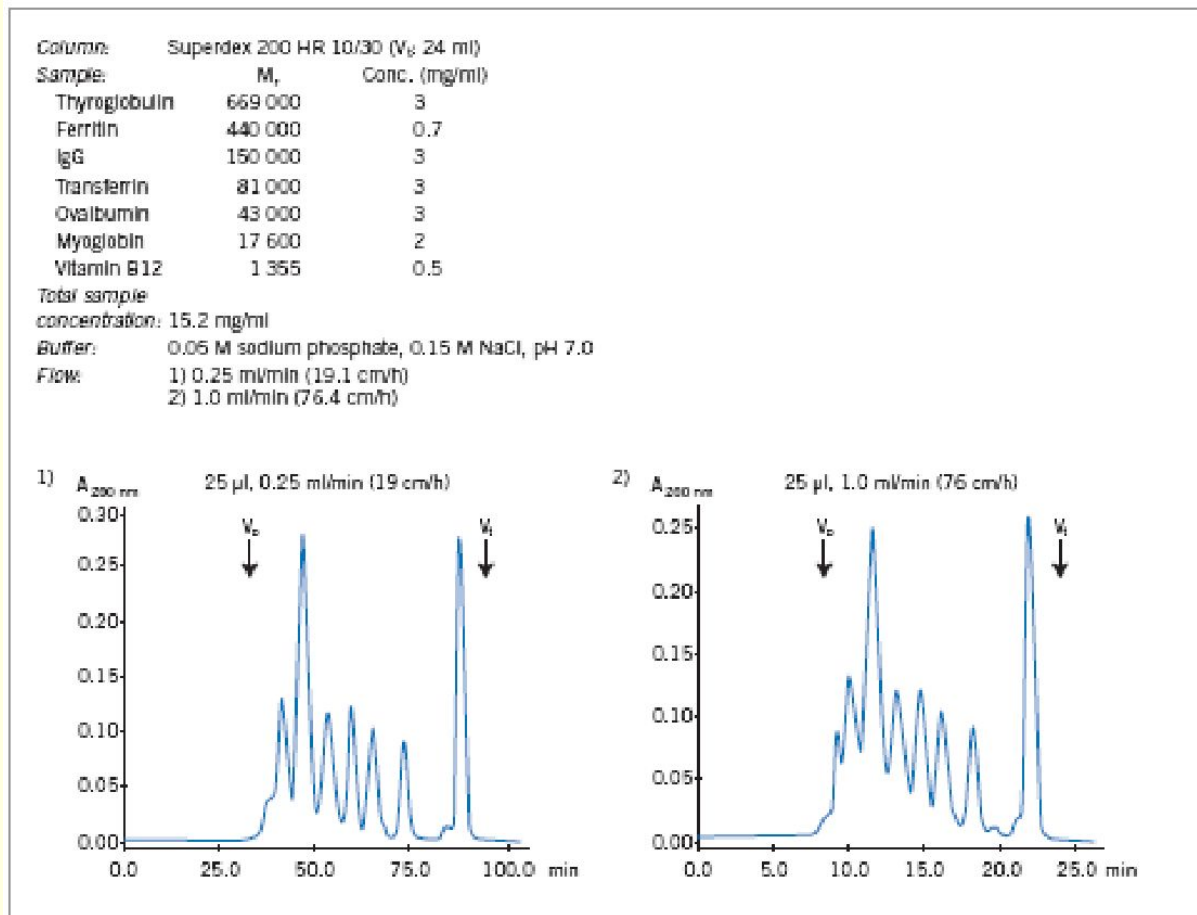
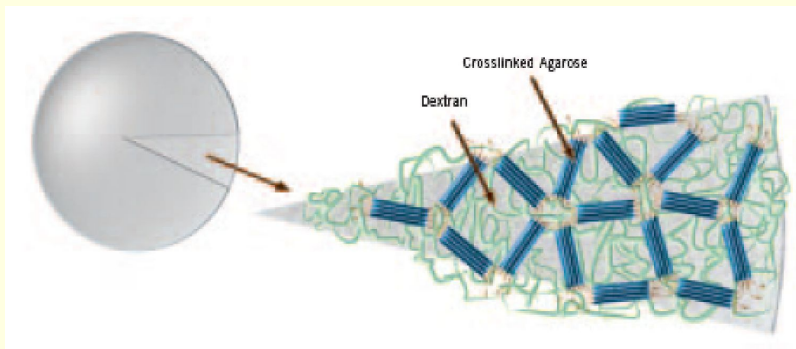
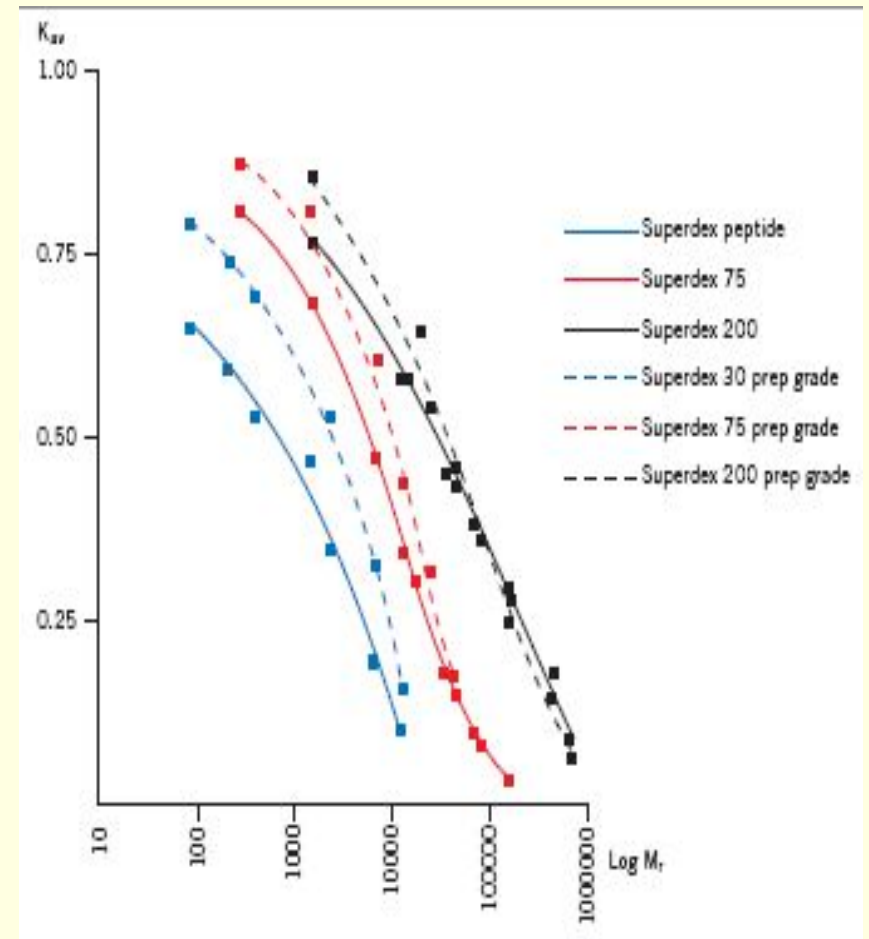
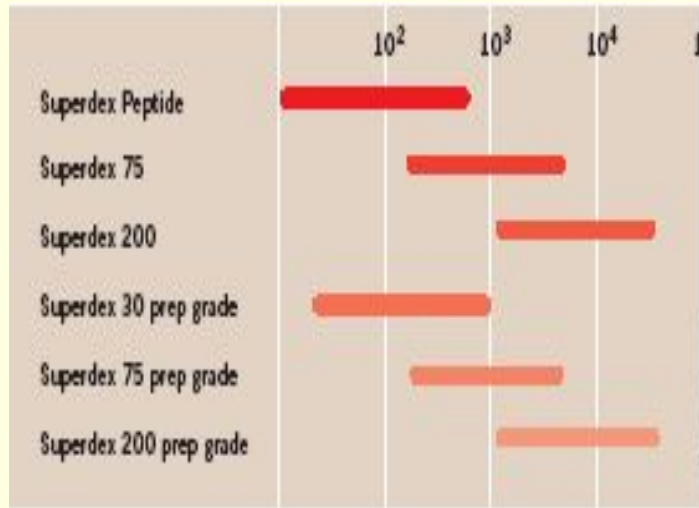


Fig. 14a. Influence of flow rate on resolution.

Носители для гельфильтрации Superdex



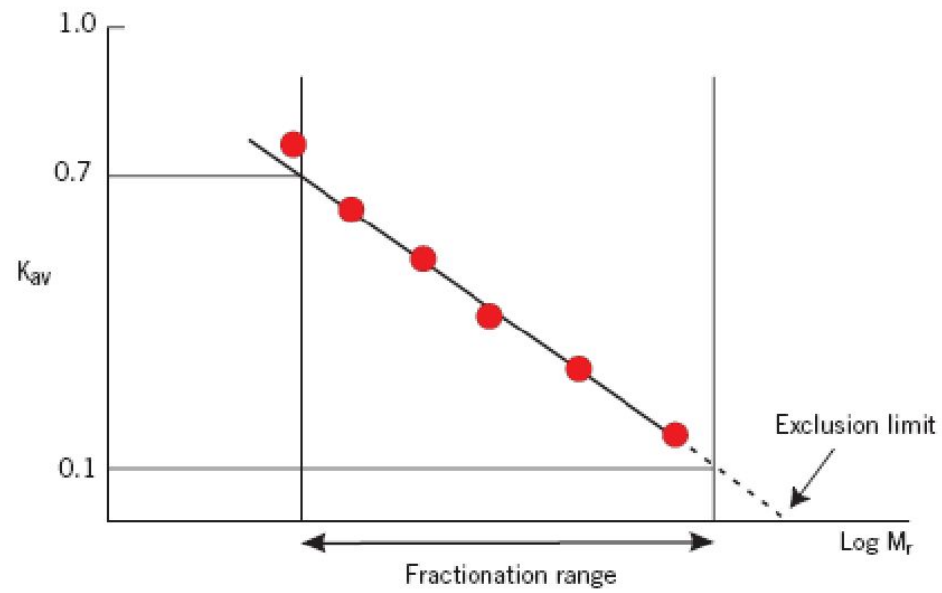
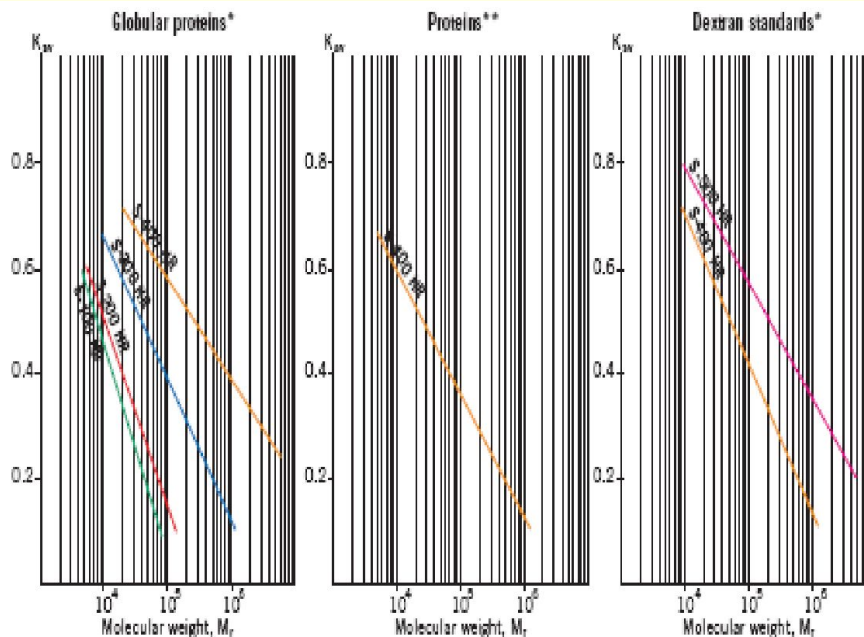
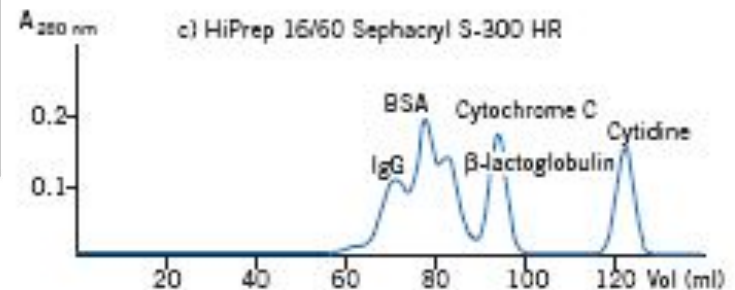
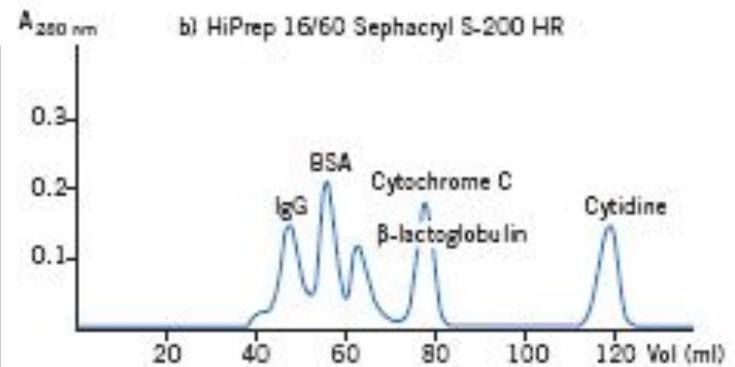
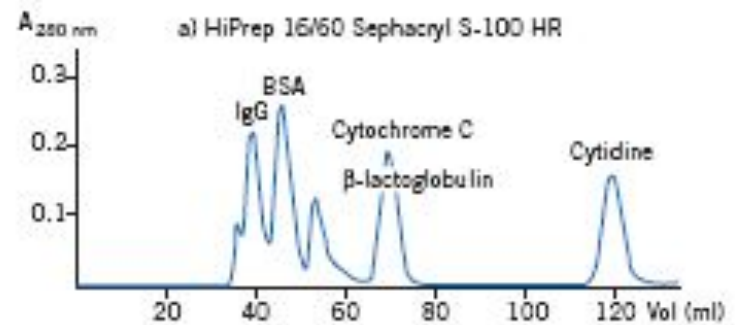
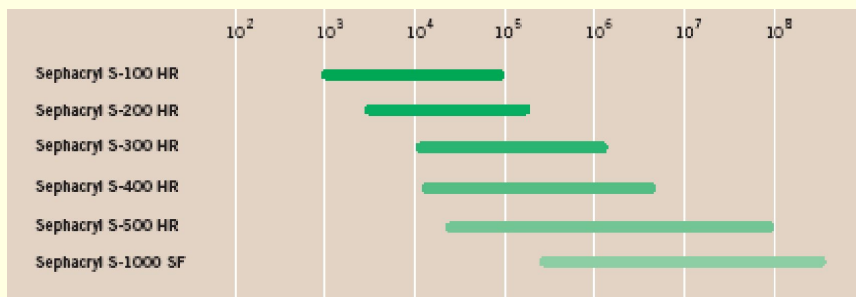


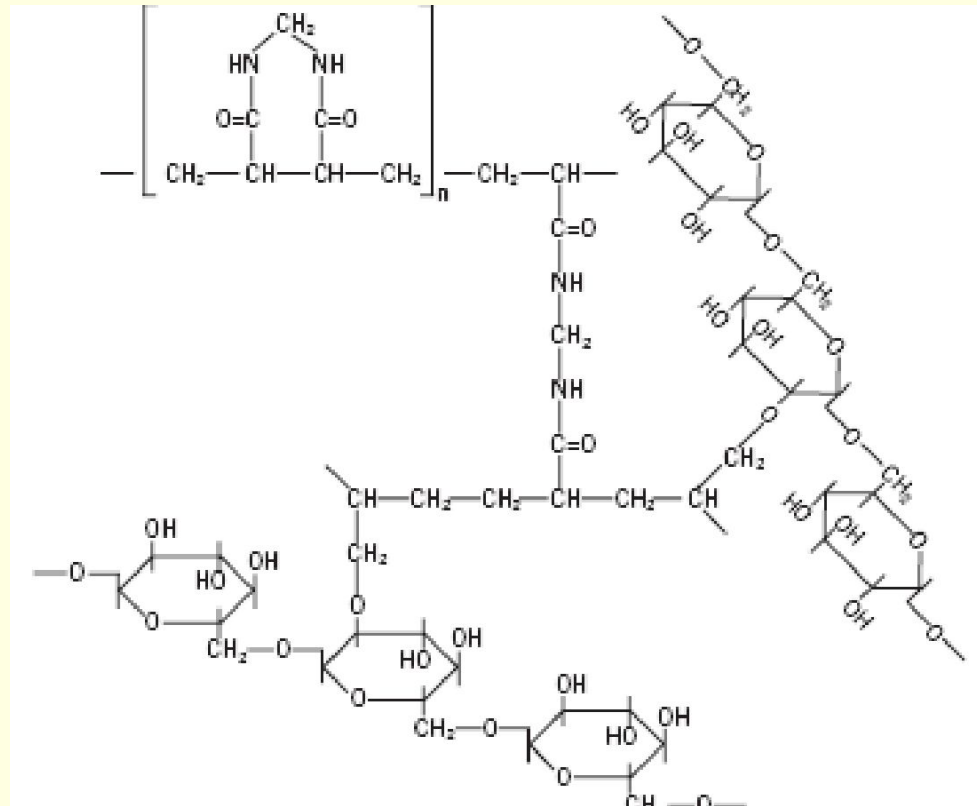
Fig. 10. Defining fractionation range and exclusion limit from a selectivity curve.

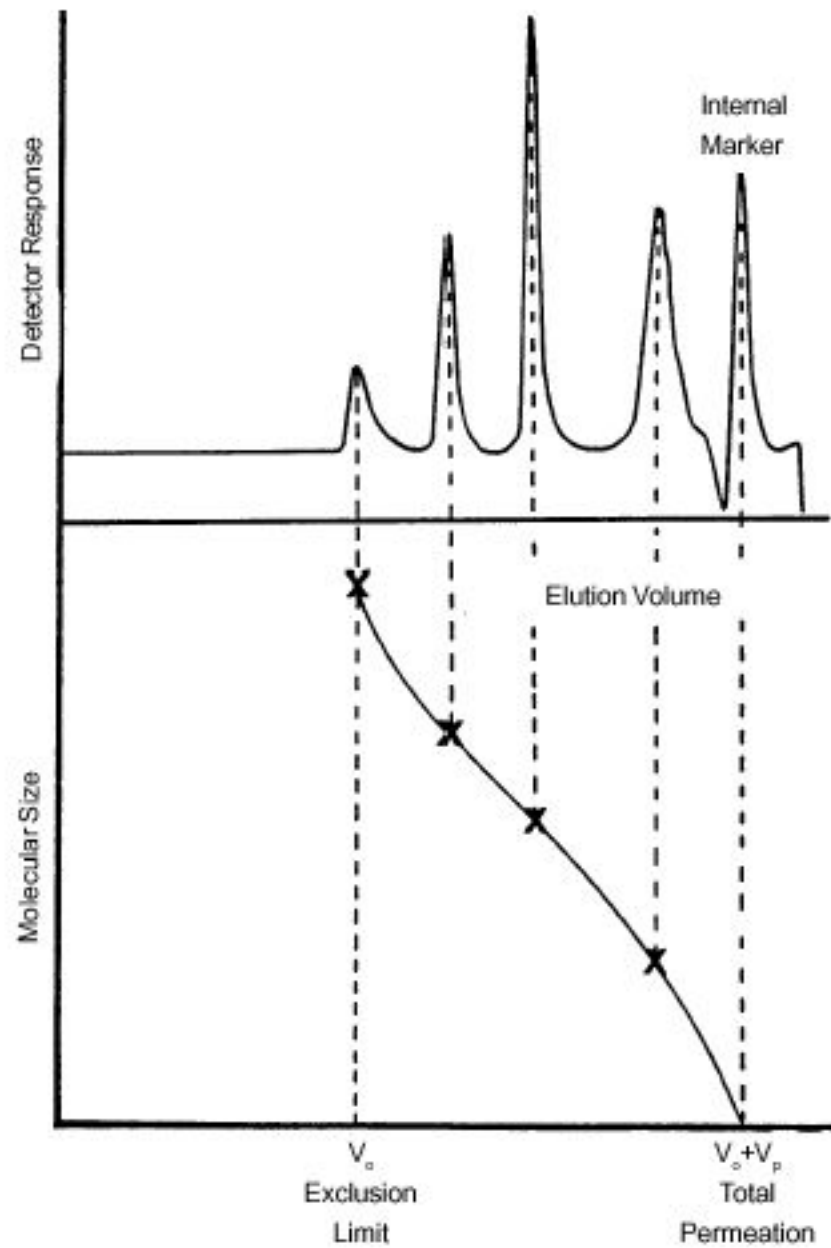
Носители для гельфильтрации Sephacryl



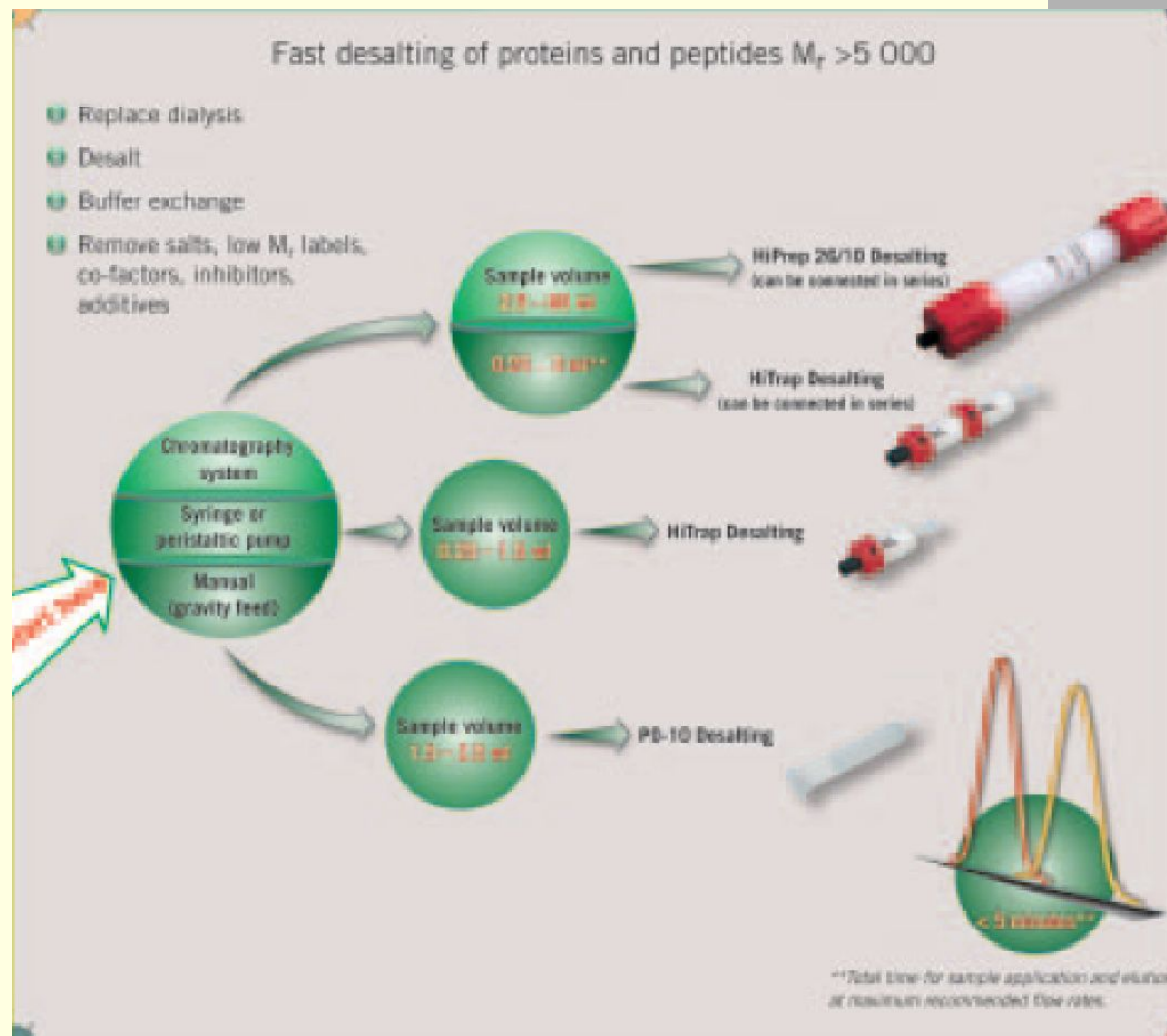
* In 0.05 M phosphate buffer, 0.15 M NaCl, pH 7.0. ** In 6 M guanidina hydrochloride.

Структура Sephacryl

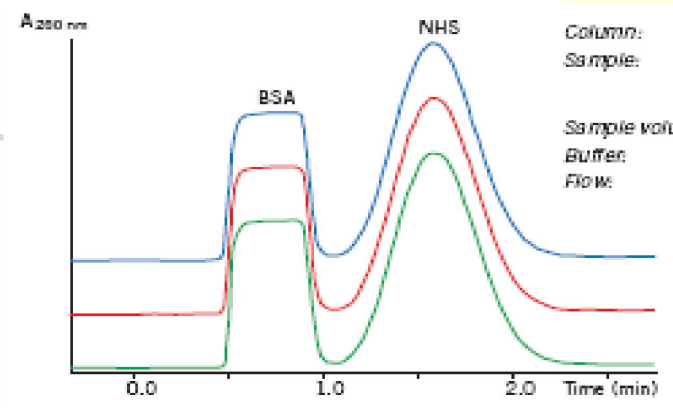
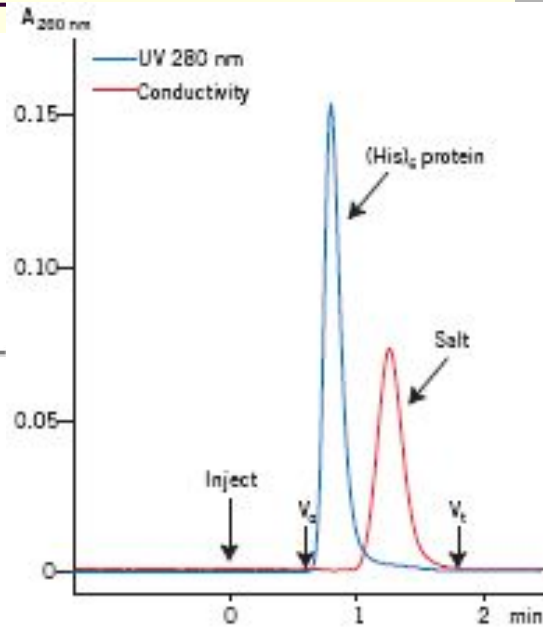
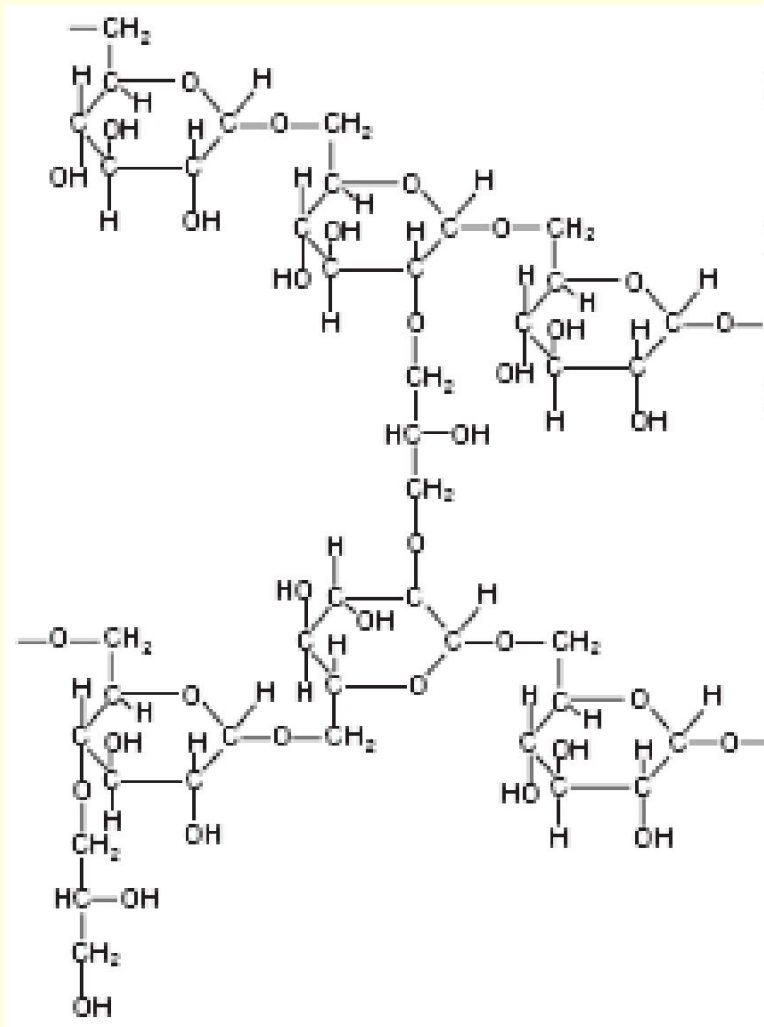


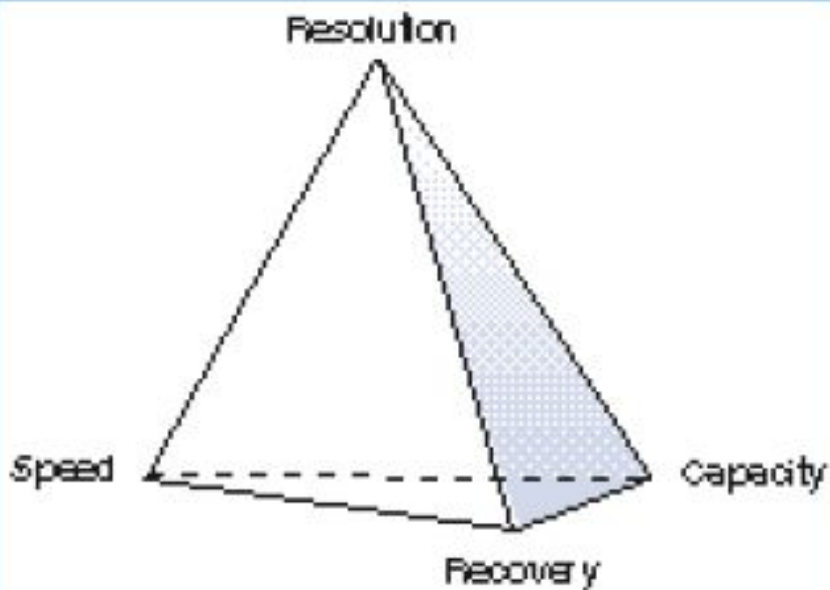


Смена буфера белков и пептидов с ММ больше 5000 Д



Отделение белка от продуктов реакции на колонках с Sephadex G25





Common Problems in Gel Permeation Chromatography, Possible Causes, and Their Remedies*

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 sample recovery	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
	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
Elution profile not reproducible	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
	Buffer composition may have changed since the previous experiment	Use fresh buffer
Low column flow rate	Some components of the sample may have changed or precipitated during storage	Try other storage conditions
	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
Leaky 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

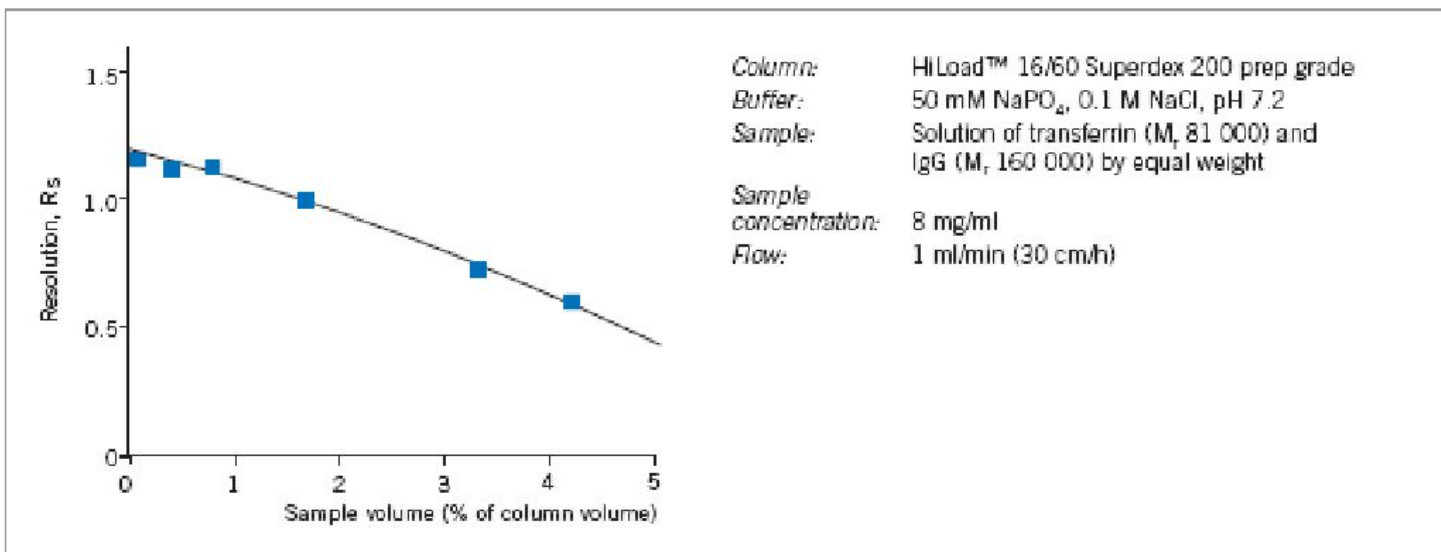


Fig. 8a. Influence of sample volume on the resolution of transferrin and IgG on prepacked HiLoad 16/60 Superdex 200 prep grade.

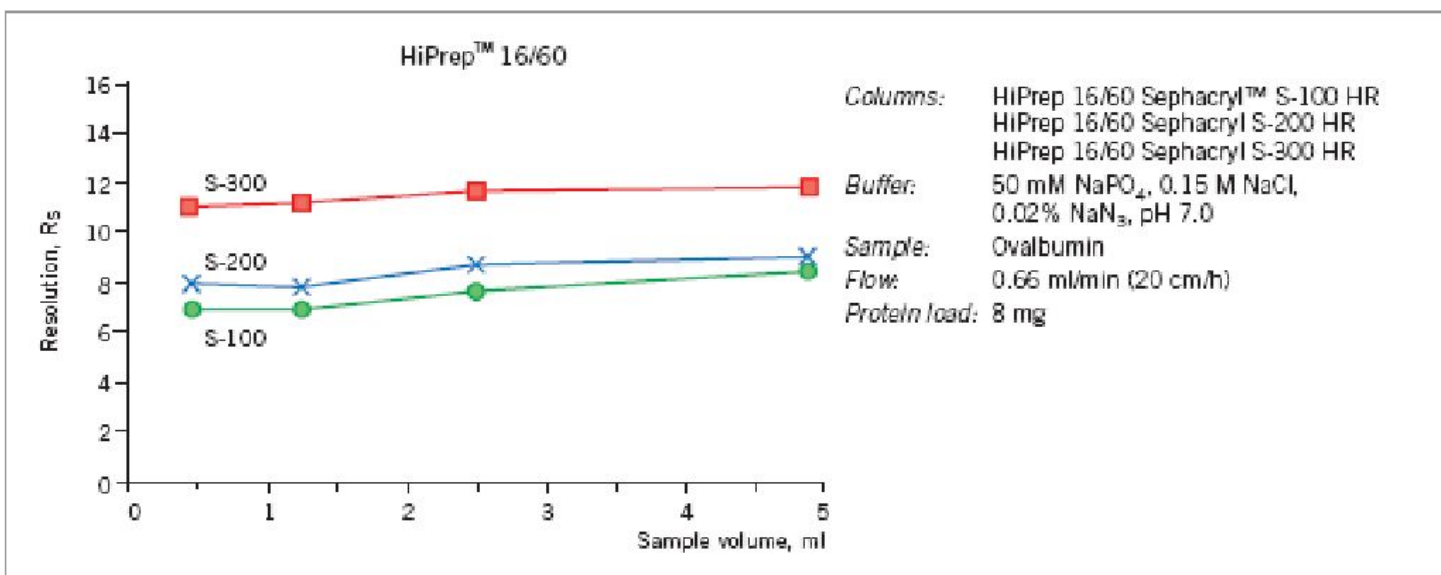


Fig. 8b. Influence of sample volume on the resolution of ovalbumin and IgG on different prepacked columns of HiPrep 16/60 Sephacryl High Resolution.

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

