Chromatographic Protein Purification

Protein Property	Chromatography
Size	Gel filtration (GFC) Gel permeation (GPC) Size exclusion (SEC)
Charge	Ion exchange (IEC)
Hydrophobicity	Hydrophobic interaction (HIC) [Reverse phase (RPC)]
Specific binding	Affinity (AC)

Size-Exclusion Chromatography

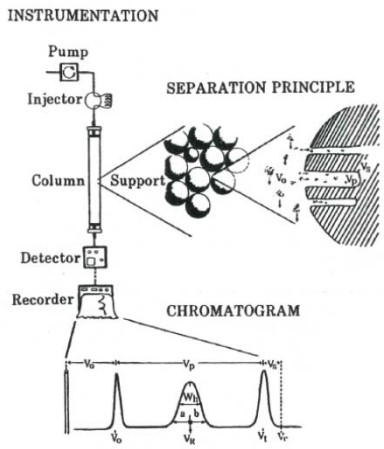
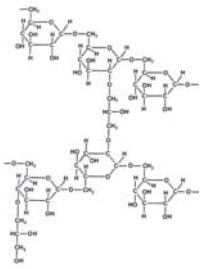
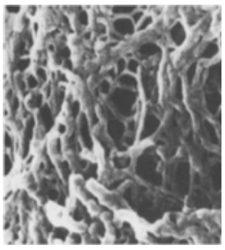


Figure 3-1. Fundamentals of gel filtration. Solutes injected into the column are separated according to decreasing size due to incompatibility between the solute dimension and the pore size of the support. V_0 = void volume between the support particles, V_p = pore volume and V_s = matrix volume of the support. V_R = elution volume of the solute, V_t = total liquid volume of the column and V_c = total geometric volume of the column. Column plate number $N = 5.54 \times (V_R/w_h)^2$ where w_h is the peak width at half peak height and b/a = peak symmetry at 10% peak height.

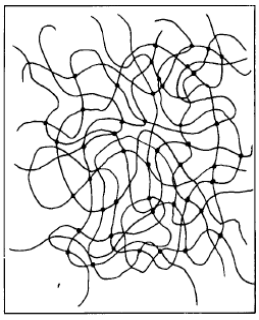
Size-Exclusion Chromatography

Sephadex



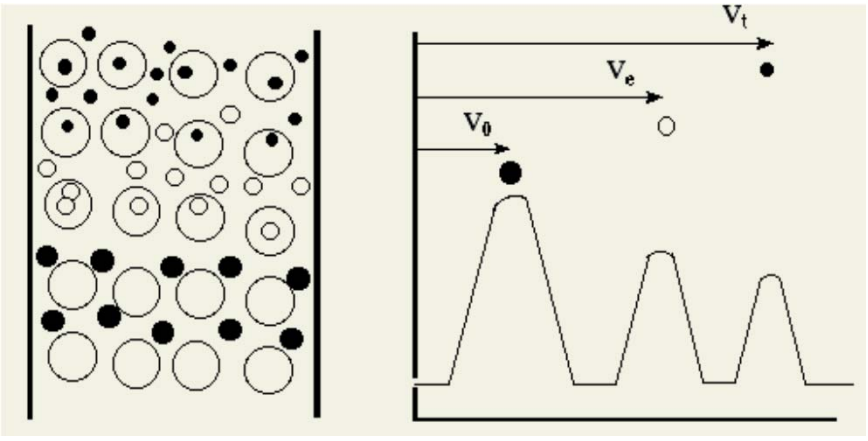


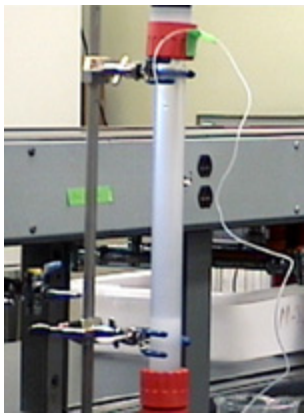
Scanning electro micrograph of 2% agarose gel



- Sephadex is a gel consisting of crosslinked dextran molecules. Dextran is a branched polymer of glucose molecules.
- Sephadex is formed into beads (20-300 um), and each bead thus comprises a 3D network of pores into which solute molecules, such as proteins, can diffuse.

Size-Exclusion Chromatography





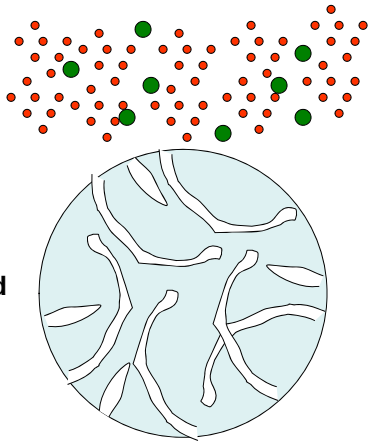
Fancy

Plain

4ºC (cold room)

70

Molecular sieve chromatography
(= gel filtration, Sephadex chromatography)



Sephadex bead

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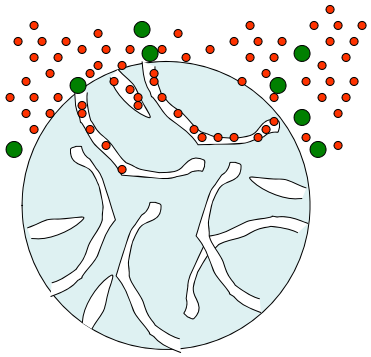
Molecular sieve chromatography

Sephadex bead



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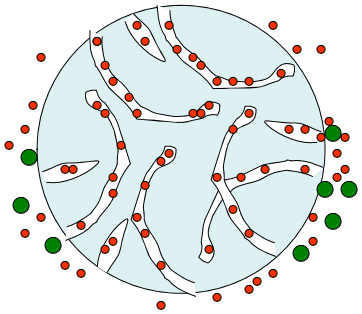
Molecular sieve chromatography

Sephadex bead



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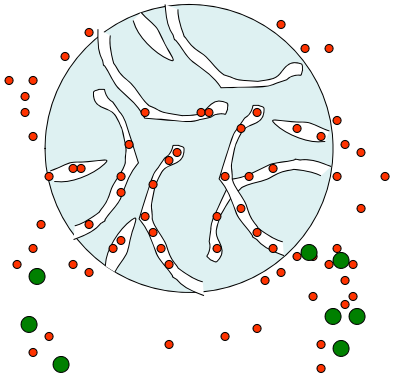
Molecular sieve chromatography

Sephadex bead



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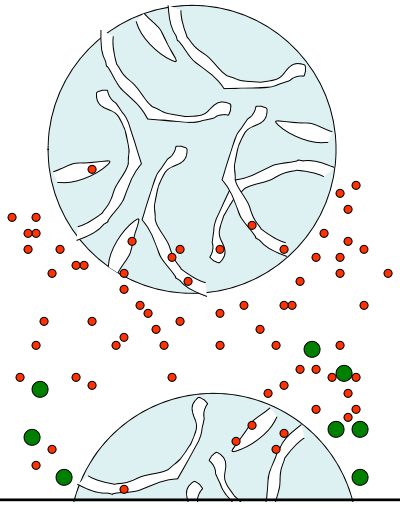
Molecular sieve chromatography

Sephadex bead



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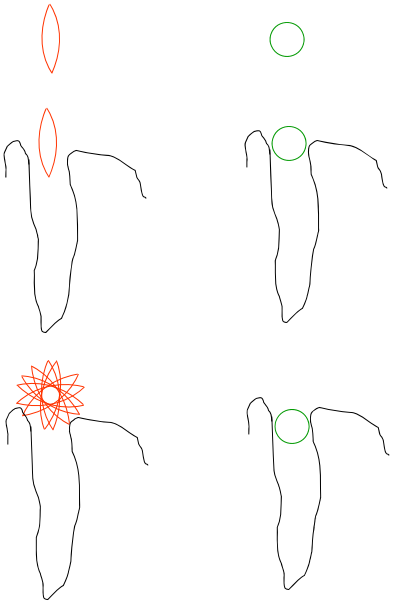
Larger molecules get to the bottom faster, and ….
Non-spherical molecules get to the bottom faster



~infrequent orientation

Non-spherical molecules get to the bottom faster

76

Analysis of Chromatogram

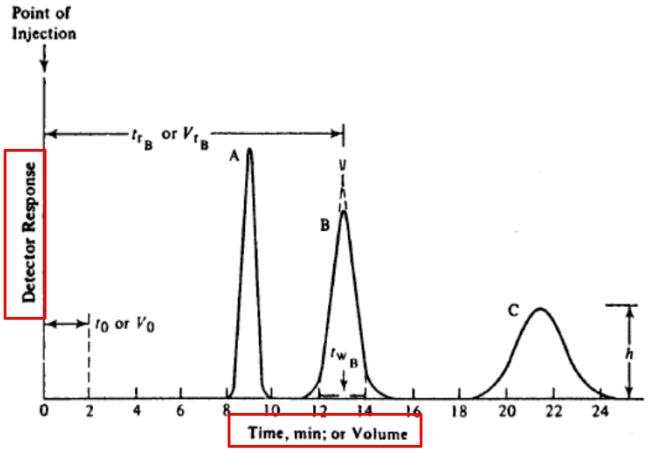
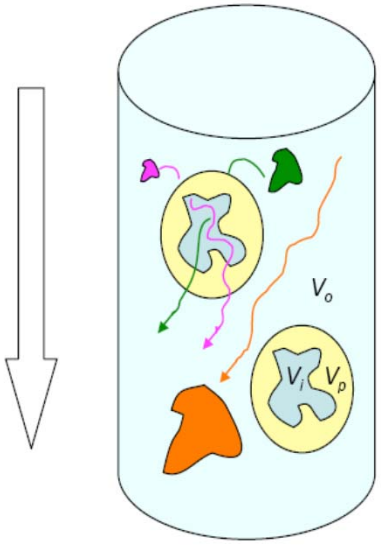


FIGURE 21.2. *Chromatogram of the three-component mixture of Figure 21.1. t_0 = time for solvent to traverse the column, t_{r_B} = retention time of substance B, t_{w_B} = peak basewidth of substance B, h = peak height. Units can also be given in terms of volume rather than time: V_0, V_{r_B}, V_{w_B}, and so forth.*

Analysis of Chromatogram



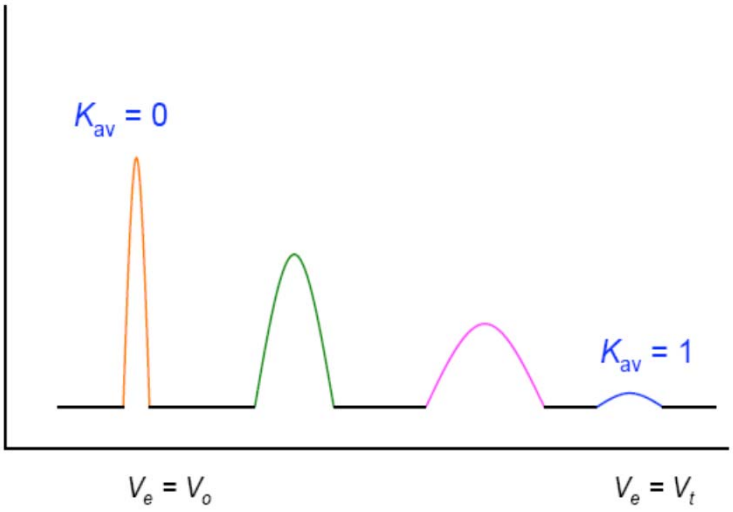
$$V_{total} = V_o\ (= 0.35 \times V_t) + V_p + V_i$$

$$K_{av} = \frac{V_e - V_0}{V_i + V_p} = \frac{V_e - V_0}{V_t - V_0}$$

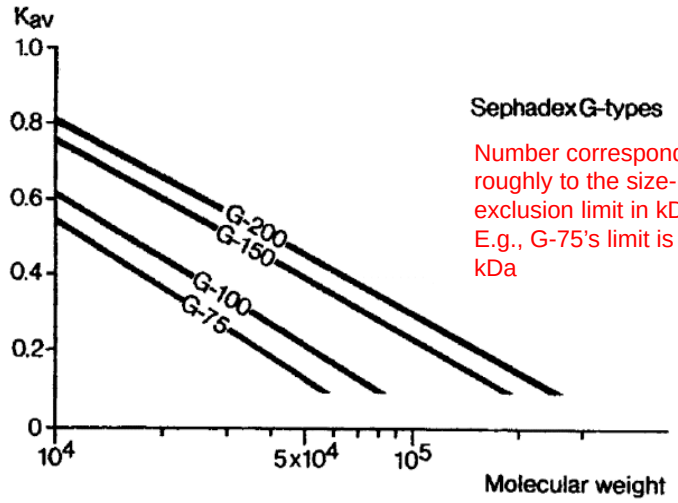
$$0 \leq K_{av} \leq 1$$

$$K_{av} = -a \times \log MW + b$$

Analysis of Chromatogram



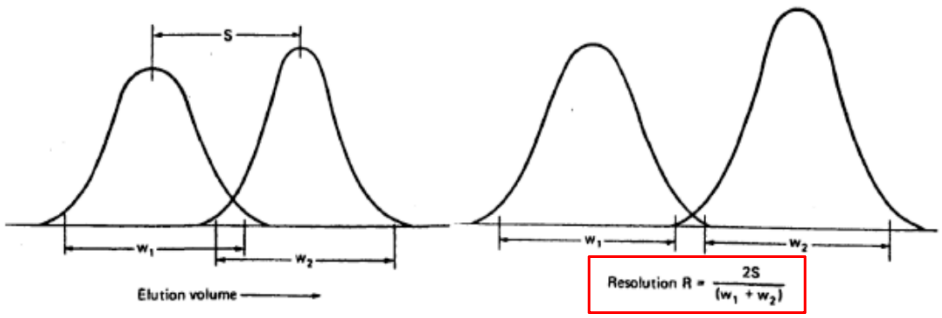
Kav vs. log MW



Number correspond roughly to the size-exclusion limit in kDa. E.g., G-75's limit is ~75 kDa

Fig. 24. Selectivity curves of Sephadex G-types, globular proteins.

Chromatographic Resolution



Chromatographic Resolution

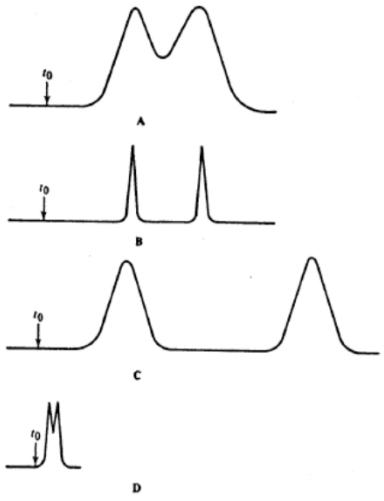
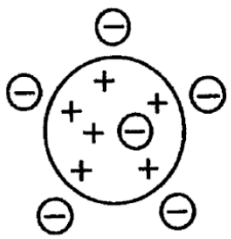
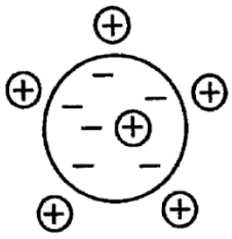


FIGURE 21.7. *Effect of selectivity, efficiency, and capacity factor on resolution. A: Poor resolution. B: Good resolution due to column efficiency. C: Good resolution due to column selectivity. D: Poor resolution due to low capacity factor despite adequate column efficiency and selectivity. Courtesy of Varian Associates.*

Ion Exchange Resins w/ exchangeable counter ions



ANION exchanger with exchangeable counter-ions

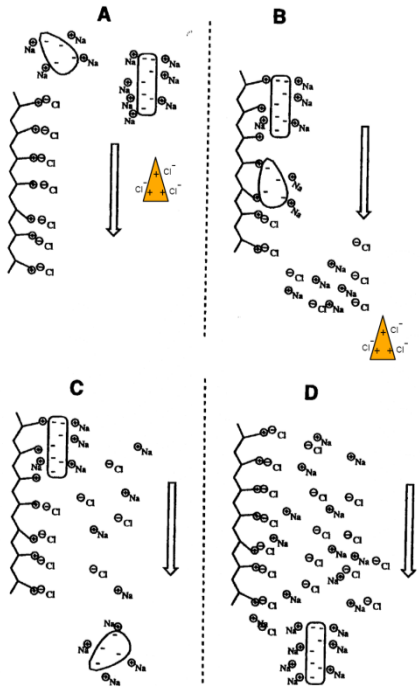


CATION exchanger with exchangeable counter-ions

Functional groups used in Ion-exchange resin

TABLE 4-2
Ion Exchange Groups Used in the Purification of Proteins

FORMULA	NAME	ABBREVIATION
Strong anion		
$-CH_2N^+(CH_3)_3$	trimethylaminoethyl	TAM
$-C_2H_4N^+(C_2H_5)_3$	triethylaminoethyl	TEAE
$-C_2H_4N^+(C_2H_5)_2CH_2-CH(OH)CH_3$	diethyl-2-hydroxypropylamino-ethyl	QAE
Weak anion		
$-C_2H_4N^+H_3$	aminoethyl	AE
$-C_2H_4N^+H(C_2H_5)_2$	diethylaminoethyl	DEAE
Strong cation		
$-SO_3^-$	sulpho	S
$-CH_2SO_3^-$	sulphomethyl	SM
$-C_3H_6SO_3^-$	sulphopropyl	SP
Weak cation		
$-COO^-$	carboxy	C
$-CH_2COO^-$	carboxymethyl	CM



pH < pI
: Protein is ___ charged
→ _____ exchange column (e.g. ____)

pH > pI
: Protein is ___ charged
→ _____ exchange column (e.g. ____)

*Ion exchange chromatography usually uses increasing salt gradient (e.g. NaCl from 0-1 M) to elute proteins.

Cf. What about size-excl. chrom.?