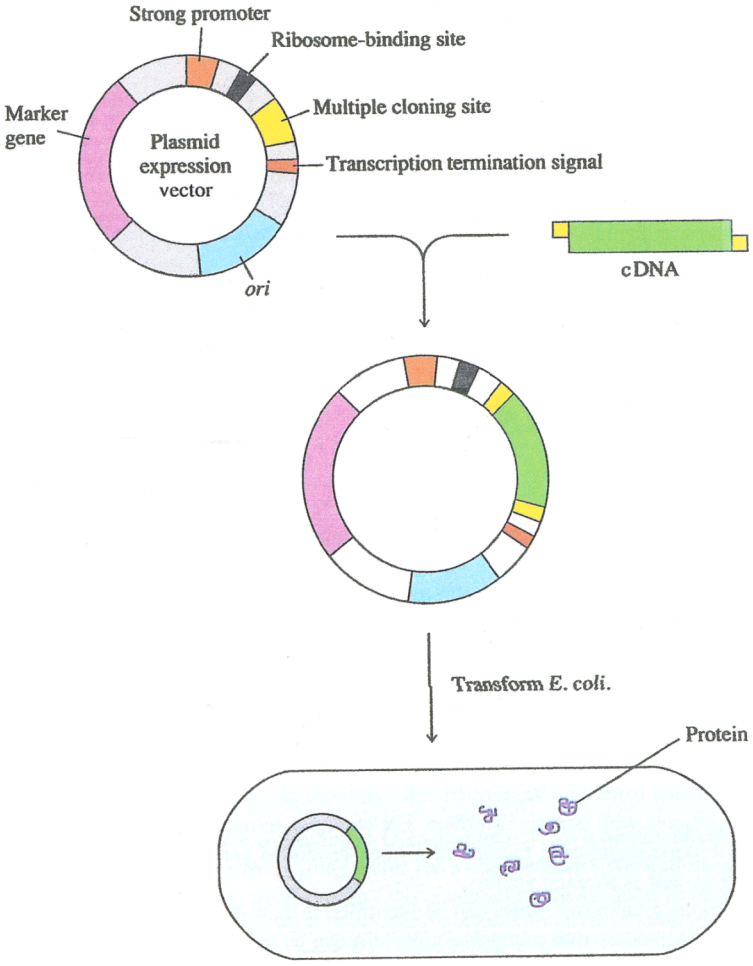
RECOMBINANT PROTEIN EXPRESSION



PROTEIN PURIFICATION BY COLUMN CHROMATOGRAPHY

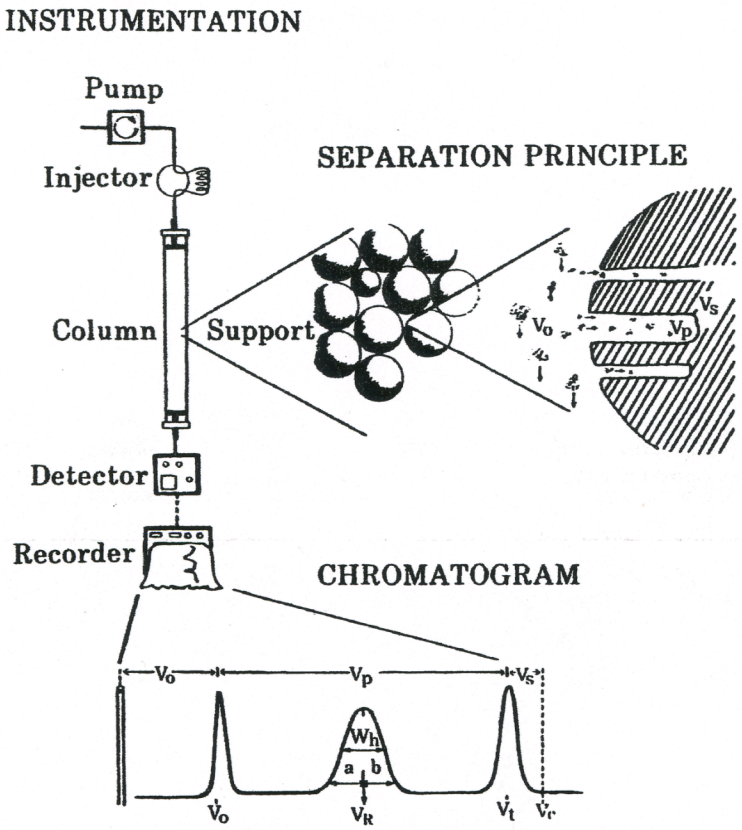


Figure 3-1. Fundamentals of gel filtration. Solutes injected into the column are separated according to decreasing size due to incompatibility between the solute dimension and the pore size of the support. V_0 = void volume between the support particles, V_p = pore volume and V_s = matrix volume of the 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.

COLUMN PACKING MATERIALS (STATIONARY PHASE, ABSORBENT)

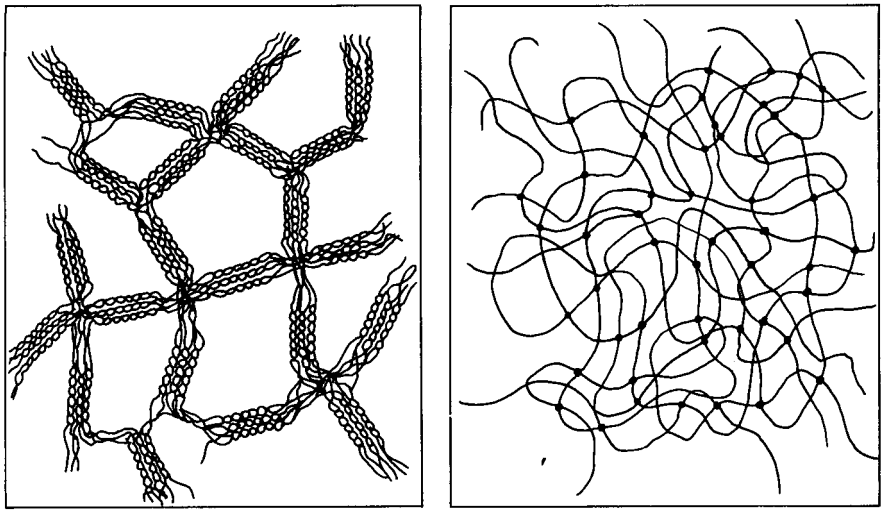
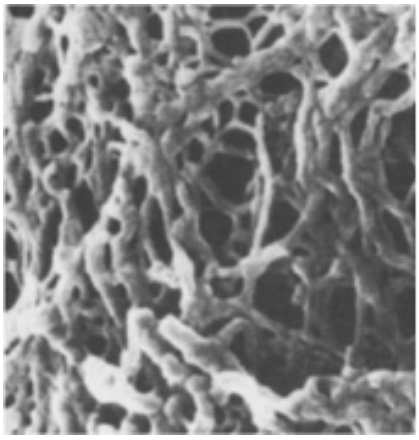


Figure 2.2. Schematic representation of agarose gel network (right), in comparison with a network such as Sephadex that is formed from random chains at similar polymer concentration. Note that the aggregates in agarose gels may actually contain 10 to 10^4 helices rather than the smaller numbers shown here. Reprinted with permission from Arnott, et al (1974), *J. Molec. Biol.* **90**, 269. Copyright by Academic Press Inc. (London) Ltd.



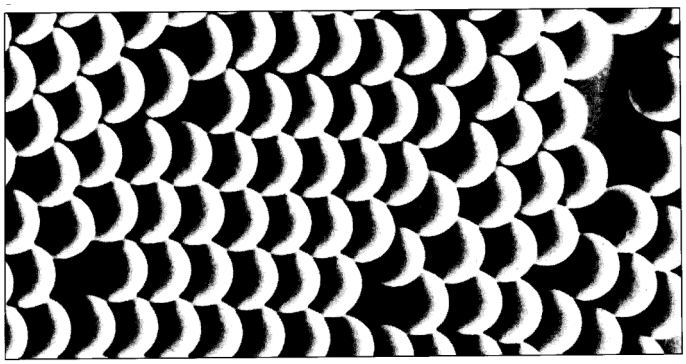


Fig. 8. An electron micrograph of MonoBeads showing their distinct monodispersity.

Scanning electro micrograph of 2% agarose gel

CHROMATOGRAPHIC SEPARATION: ANALYSIS OF CHROMATOGRAM

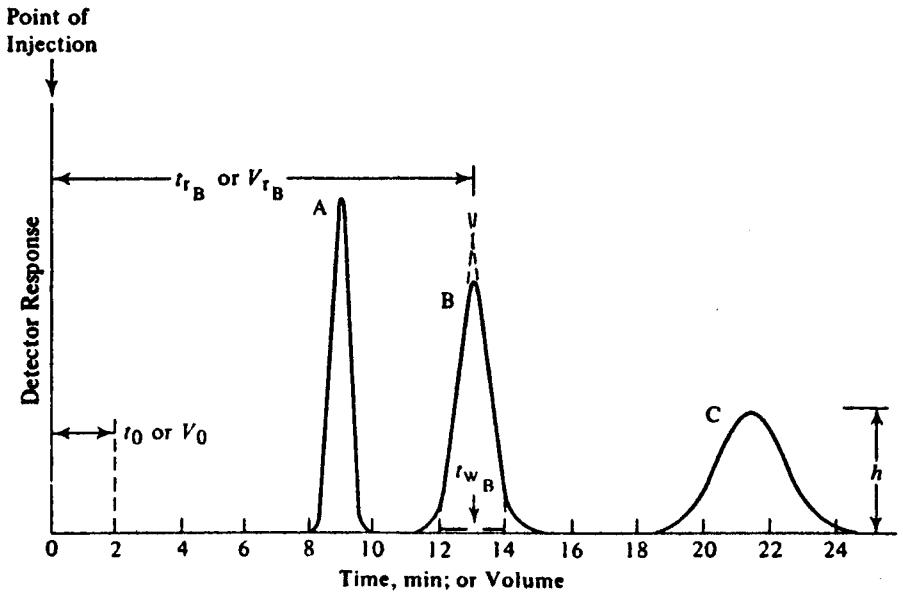
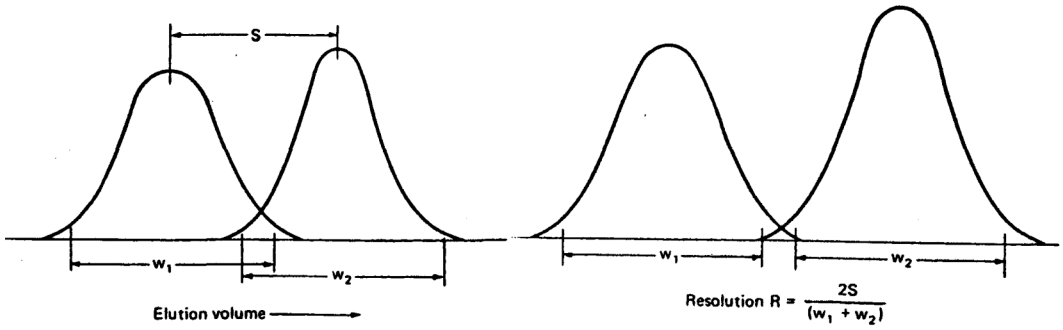


FIGURE 21.2. *Chromatogram of the three-component mixture of Figure 21.1.* t_0 = *time for solvent to traverse the column,* t_{r_B} = *retention time of substance B,* t_{w_B} = *peak basewidth of substance B,* h = *peak height. Units can also be given in terms of volume rather than time:* V_0, V_{r_B},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

CHROMATOGRAPHIC SEPARATION: RESOLUTION



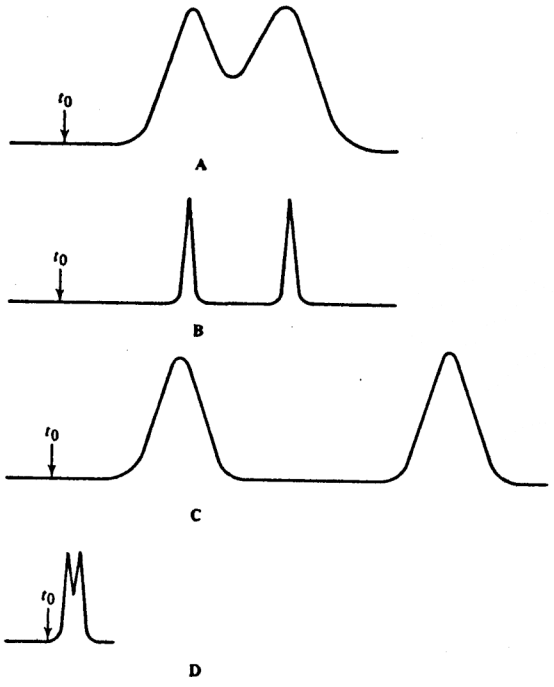
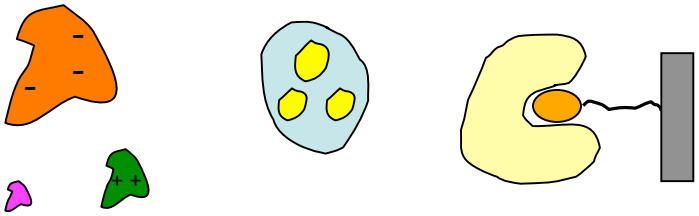


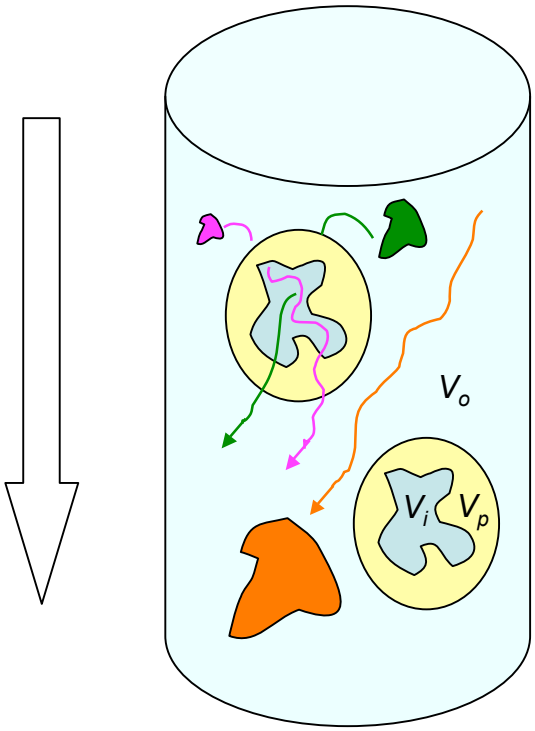
FIGURE 21.7. *Effect of selectivity, efficiency, and capacity factor on resolution. A: Poor resolution. B: Good resolution due to column efficiency. C: Good resolution due to column selectivity. D: Poor resolution due to low capacity factor despite adequate column efficiency and selectivity. Courtesy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

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)



GEL FILTRATION CHROMATOGRAPHY: PRINCIPLES

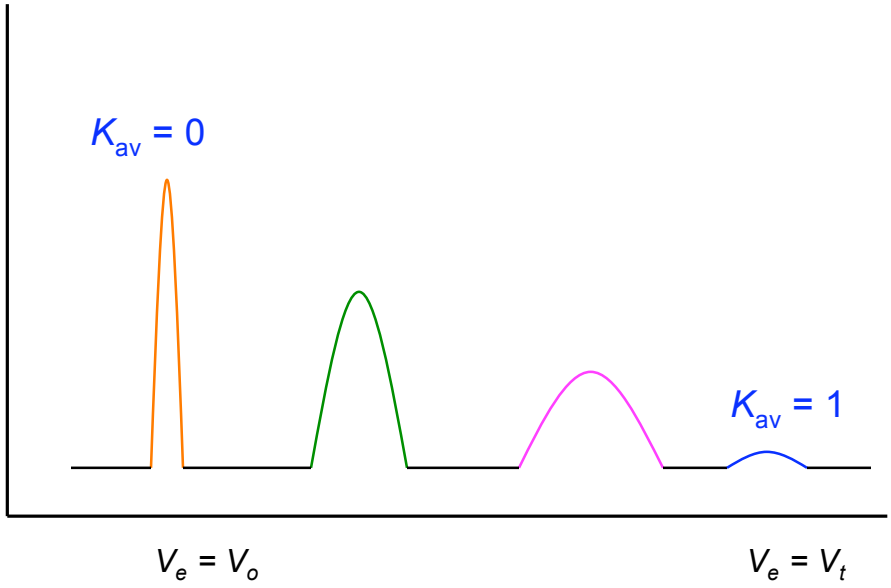


$$V_{total} = V_o\ (= 0.35 \times V_t) + V_p + V_i$$

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

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

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



GEL FILTRATION CHROMATOGRAPHY: SIZE SEPARATION

Table 5. Properties of Sephadex.

Gel type	Dry bead size µm	Fractionation range Globular proteins	Fractionation range Dextrans	Swelling factor ml/g
Sephadex G-10	40 – 120	– 700	– 700	2 – 3
Sephadex G-15	40 – 120	– 1 500	– 1 500	2.5 – 3.5
Sephadex G-25 Coarse	100 – 300	1 000 – 5 000	100 – 5 000	4 – 6
Sephadex G-25 Medium	50 – 150	1 000 – 5 000	100 – 5 000	4 – 6
Sephadex G-25 Fine	20 – 80	1 000 – 5 000	100 – 5 000	4 – 6
Sephadex G-25 Superfine	10 – 40	1 000 – 5 000	100 – 5 000	4 – 6
Sephadex G-50 Coarse	100 – 300	1 500 – 30 000	500 – 10 000	9 – 11
Sephadex G-50 Medium	50 – 150	1 500 – 30 000	500 – 10 000	9 – 11
Sephadex G-50 Fine	20 – 80	1 500 – 30 000	500 – 10 000	9 – 11
Sephadex G-50 Superfine	10 – 40	1 500 – 30 000	500 – 10 000	9 – 11
Sephadex G-75	40 – 120	3 000 – 80 000	1 000 – 50 000	12 – 15
Sephadex G-75 Superfine	10 – 40	3 000 – 70 000	1 000 – 50 000	12 – 15
Sephadex G-100	40 – 120	4 000 – 150 000	1 000 – 100 000	15 – 20
Sephadex G-100 Superfine	10 – 40	4 000 – 100 000	1 000 – 100 000	15 – 20
Sephadex G-150	40 – 120	5 000 – 300 000	1 000 – 150 000	20 – 30
Sephadex G-150 Superfine	10 – 40	5 000 – 150 000	1 000 – 150 000	18 – 22
Sephadex G-200	40 – 120	5 000 – 600 000	1 000 – 200 000	30 – 40
Sephadex G-200 Superfine	10 – 40	5 000 – 250 000	1 000 – 150 000	20 – 25

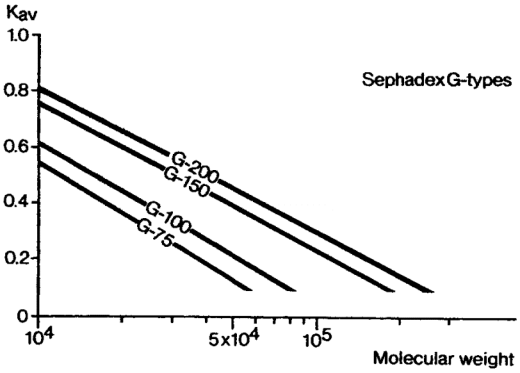
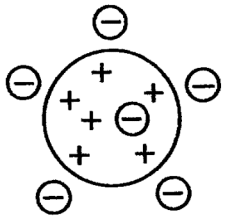
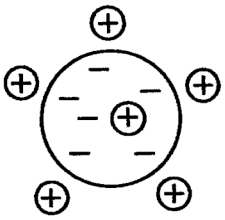


Fig. 24. Selectivity curves of Sephadex G-types, globular proteins.

ION EXCHANGE CHROMATOGRAPHY: RESINS



ANION exchanger with exchangeable counter-ions

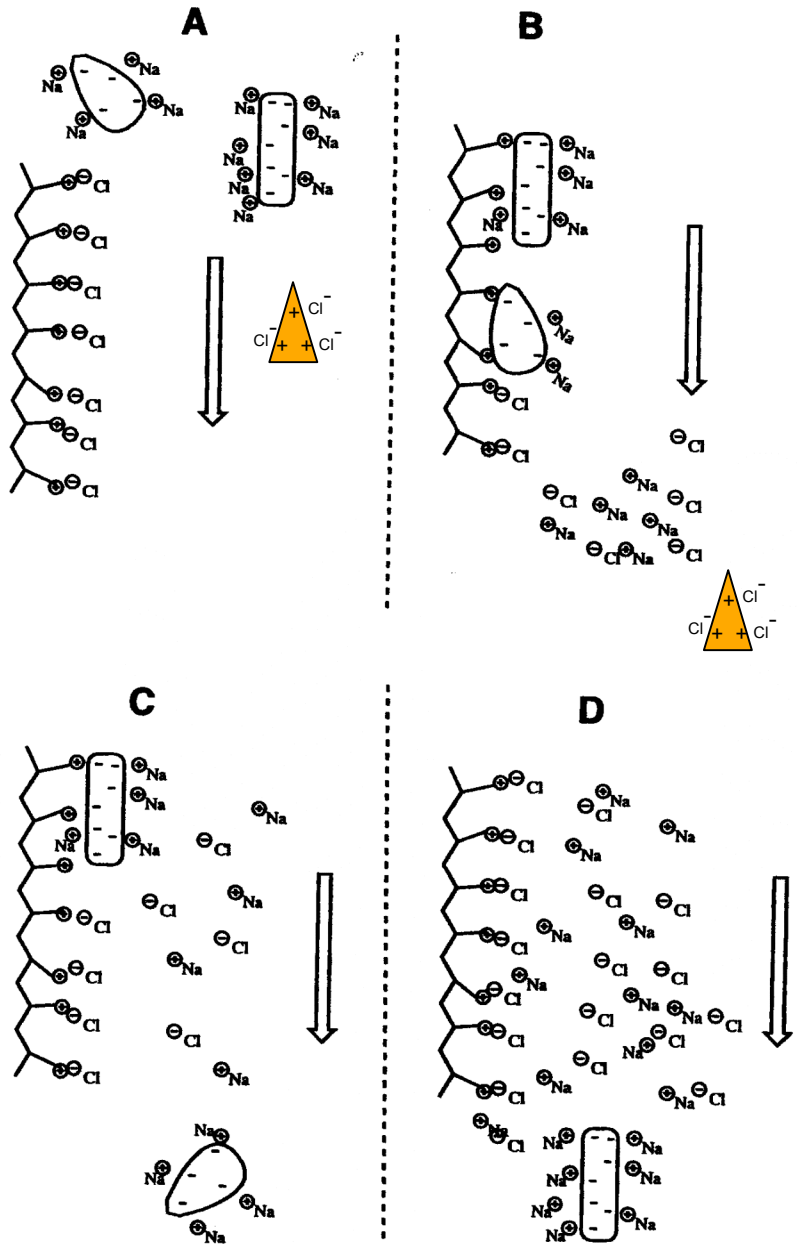


CATION exchanger with exchangeable counter-ions

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

ION EXCHANGE CHROMATOGRAPHY: PRINCIPLES



PROTEIN PURITY DETERMINATION BY SDS-PAGE

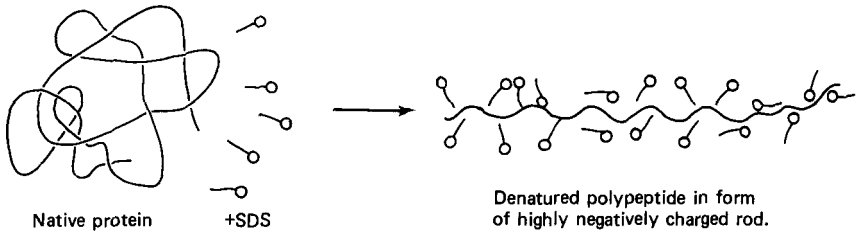


Figure 10.3. The action of dodecyl sulfate in denaturing proteins.

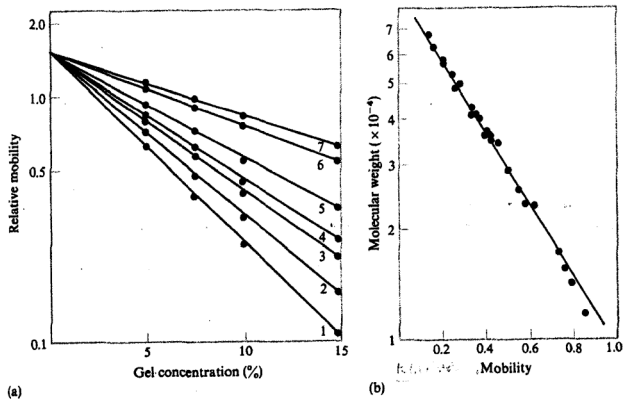
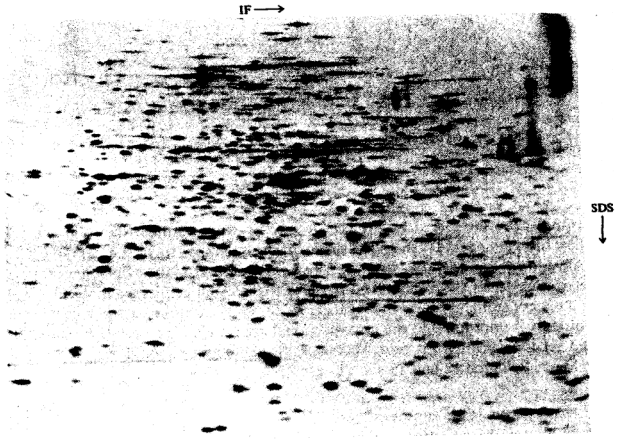
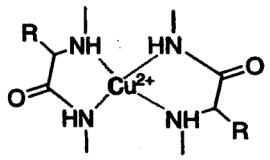


Figure 12-18

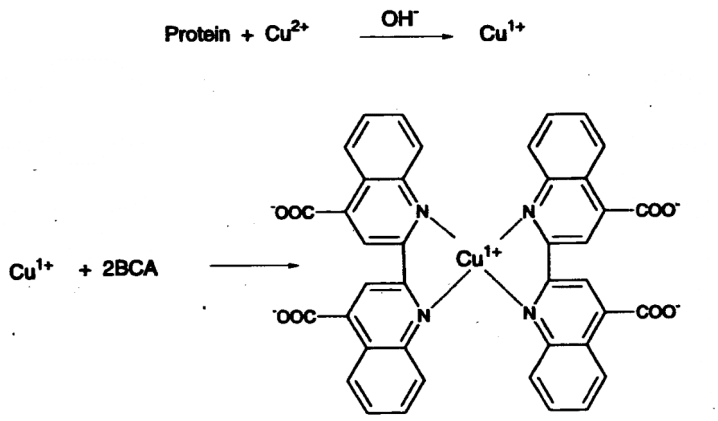
SDS polyacrylamide-gel electrophoresis of proteins. **(a)** Relative mobility of seven proteins as a function of the concentration of the acrylamide gel. The molecular weights of the proteins range from 14,000 d for the coat protein of phage R17 (curve 7) to 60,000 d for catalase (curve 1). **(b)** Relative mobility of various proteins in 10% acrylamide gels as a function of molecular weight. [After K. Weber and M. Osborn, in *The Proteins*, 3d ed., vol. 1 (New York: Academic Press, 1975), p. 179.]



PROTEIN CONCENTRATION DETERMINATION BY BCA ANALYSIS



Putative cupric complex with peptide bond.



BCA reaction.

bicinchoninic acid

ENZYME ACTIVITY ASSAY

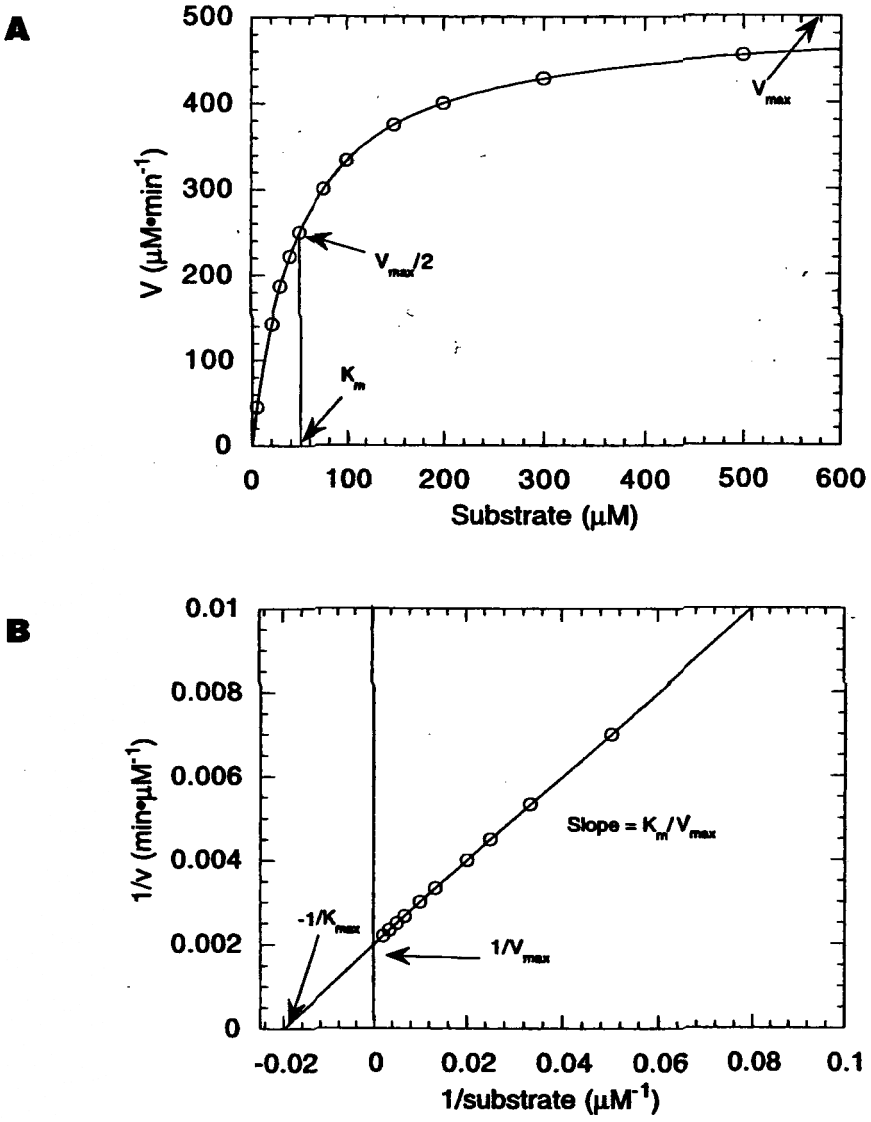


FIGURE 8-1 *(A)* Michaelis-Menten plot and *(B)* Lineweaver-Burk plot. K_m = 50 μM, V_{max} = 500 μM/min.

PROTEIN PURIFICATION TABLE

Step	Protein (mg)	Total activity (milliunits)	Specific activity (milliunits/mg)	Yield (%)	Purification (fold)
Crude extract	1070	890	0.8	-	-
$(NH_4)_2SO_4$ precipitation	400	580	1.5	65	1.9
Gel Filtration Chromatography (Sephadex G50)	38	278	7.3	31	9.0
Ion Exchange Chromatography (Q-Sepharose)	2	96	58.0	11	73.0

One unit (U) of enzyme activity is the amount of enzyme that hydrolyzes 1 μmole of substrate per minute at 37°C