

Tecnicas de Caracterización

Fraccionamiento por campo eléctrico (electroforésis)

$$\text{Movilidad electroforética} = \mu = \frac{V}{E} = \frac{Z}{f}$$

f = coeficiente de fricción = $A \eta$

Para una esfera $A = 6 \pi r$

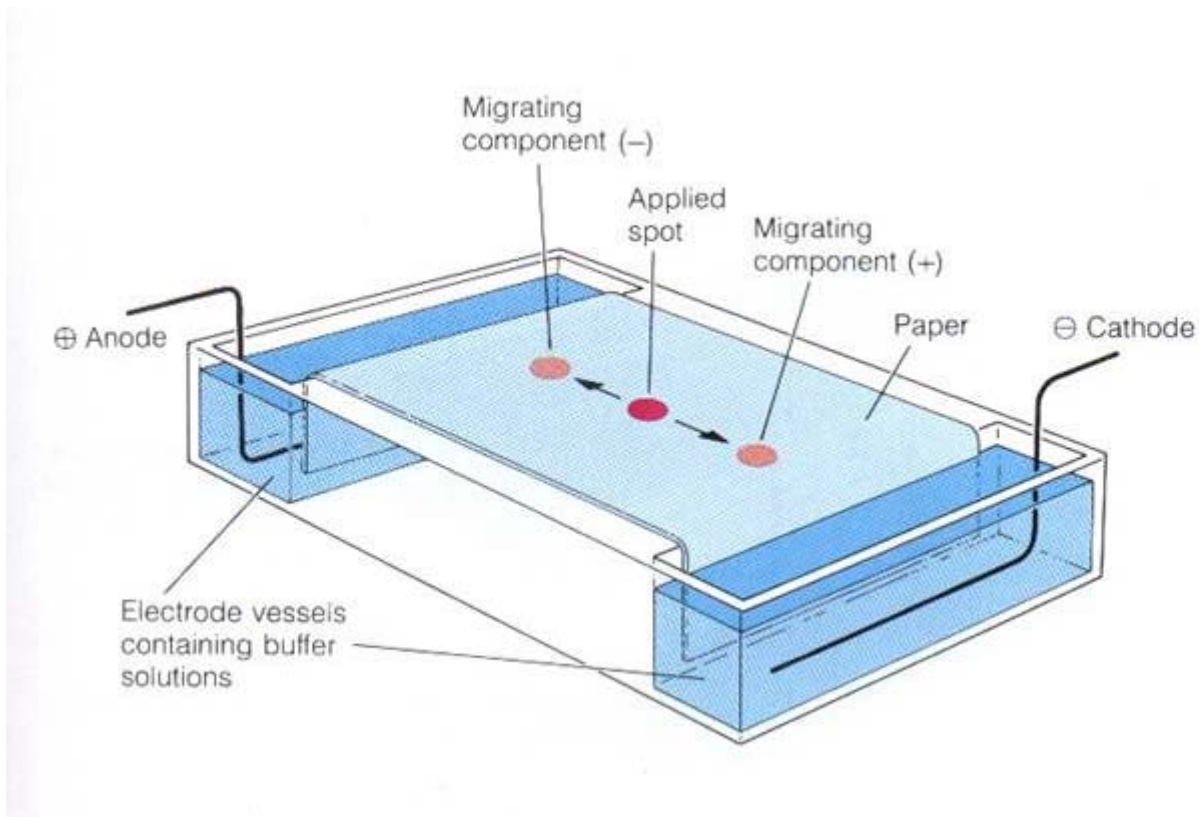
$$V = \frac{4}{3} \pi r^3$$

$$V_{\text{esp}} = V/PM$$

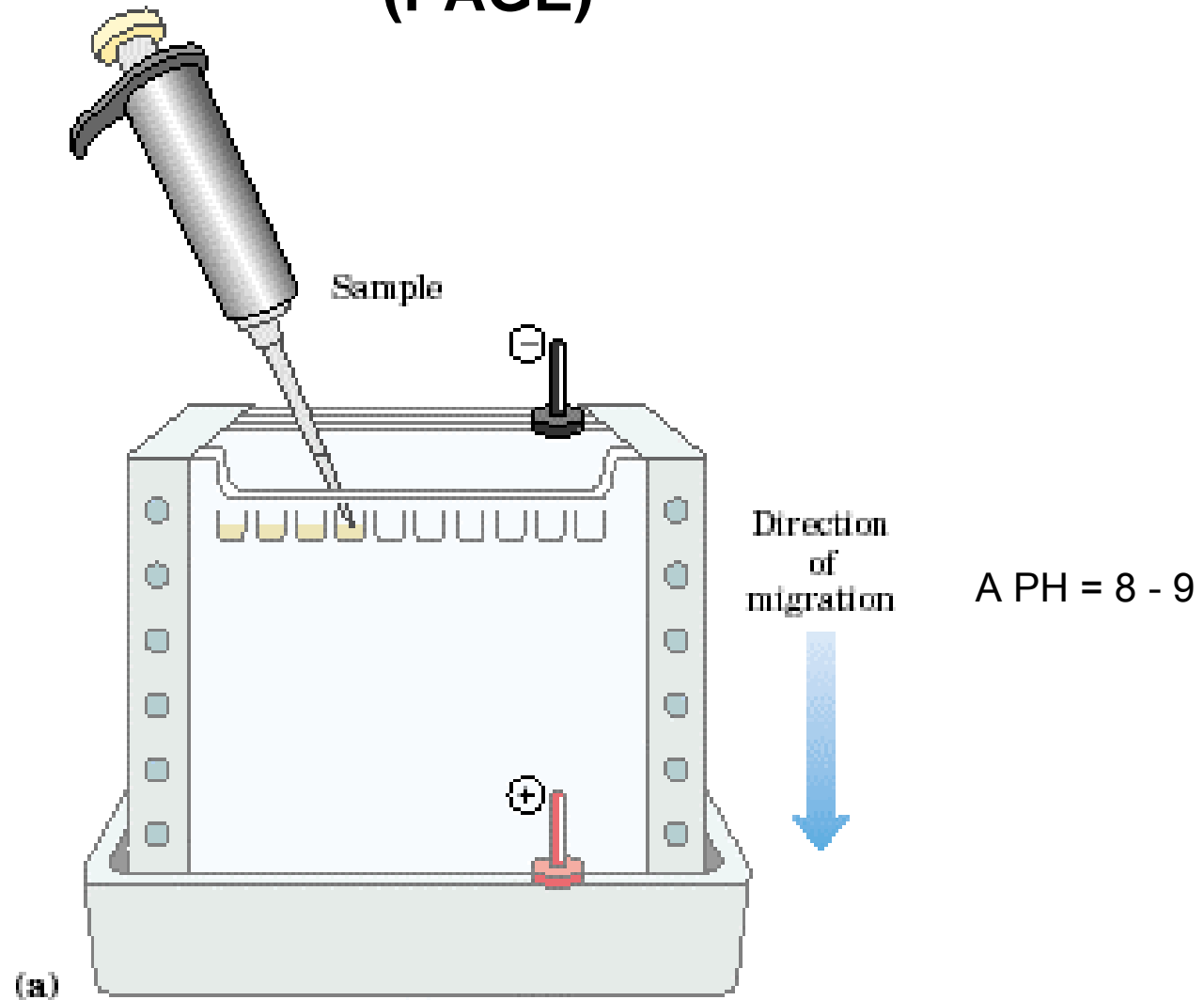
Electroforesis en papel

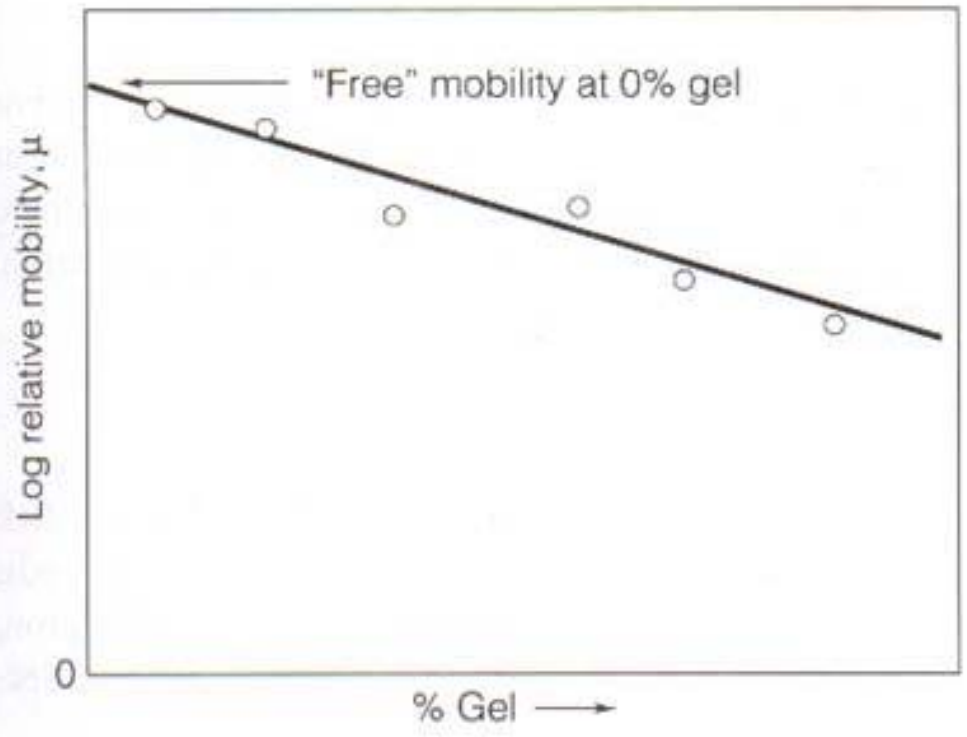
TOOLS OF BIOCH

Figure T2.1
Paper electrophoresis.

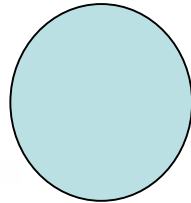


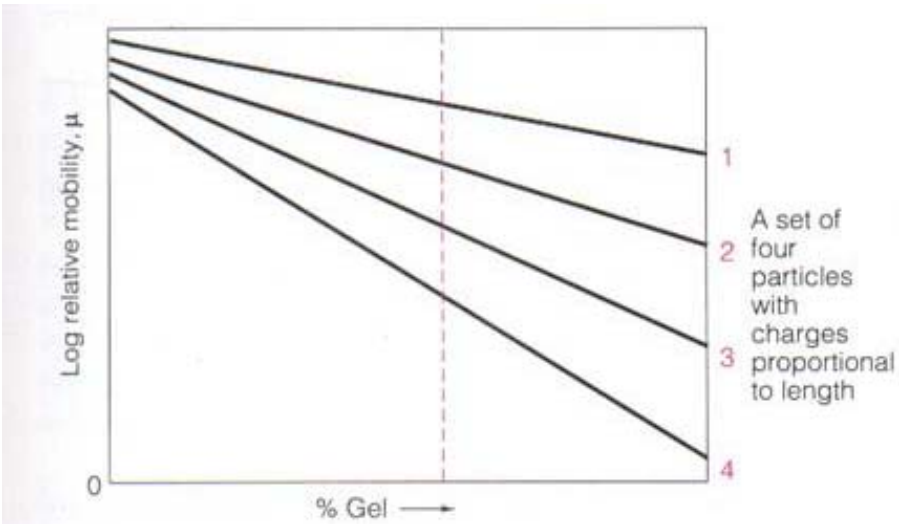
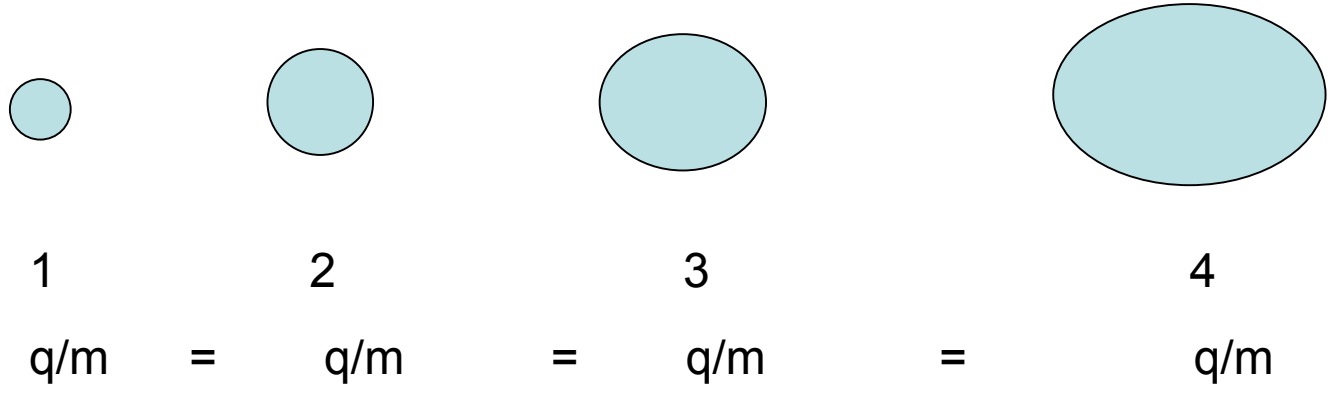
Electroforesis en gel de poliacrilamida (PAGE)



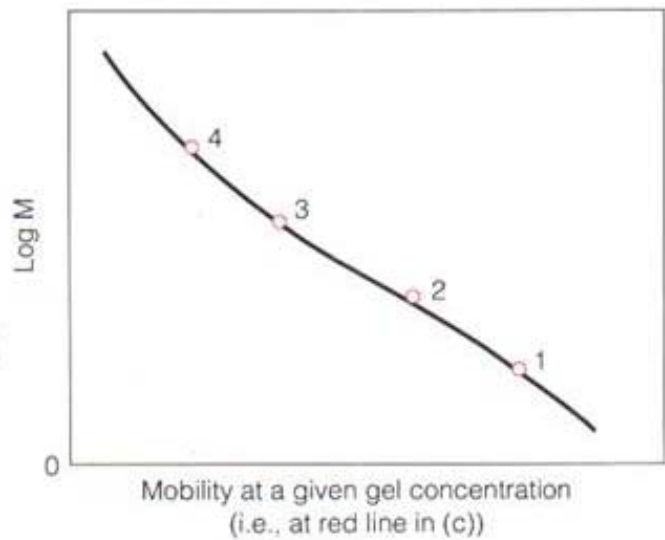


(a) Ferguson plot





(c) Ferguson plot observed when charge is proportional to length

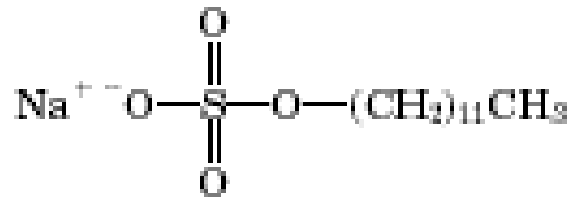


(d) Relationship between molecular weight (M) and mobility, at a given gel concentration for molecules like those shown in (c).

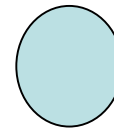
Figure T2.4

Efecto "colador"

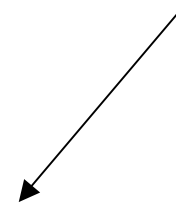
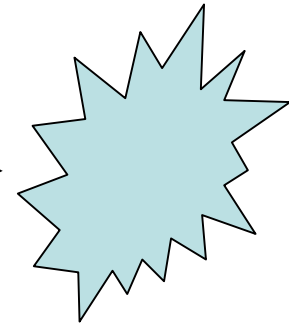
PAGE SDS



Sodium dodecyl sulfate
(SDS)



SDS →

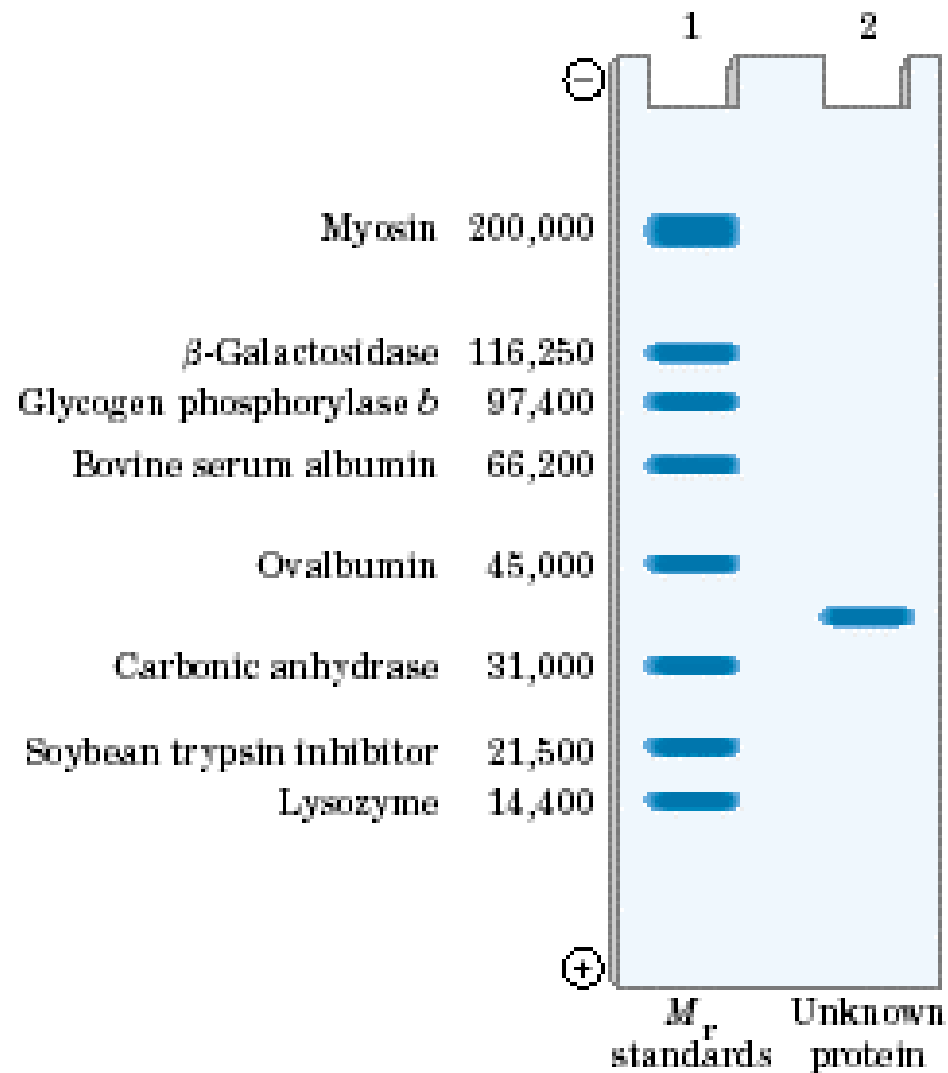


Movilidad = 1/ PM

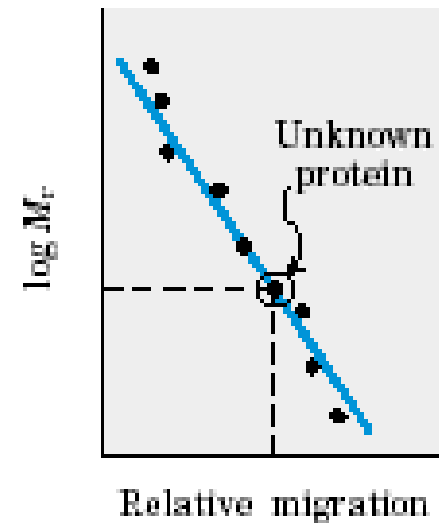
Tipos de Electroforesis

- PAGE Nativa
- PAGE SDS
- PAGE + condiciones reductoras
- Isoelectroenfoque
- Bidimensional

Determinación de PM

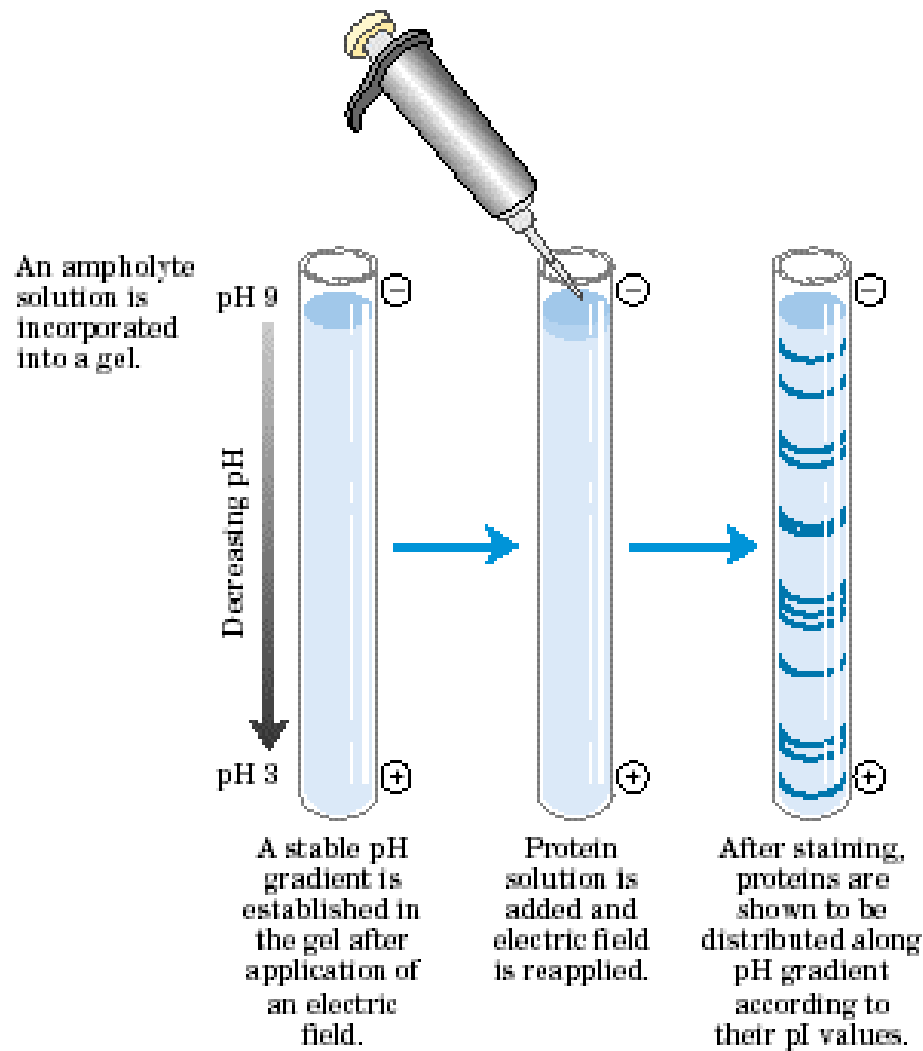


(a)



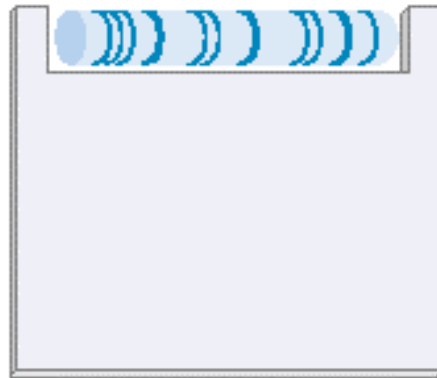
(b)

Isoelectrofocusing

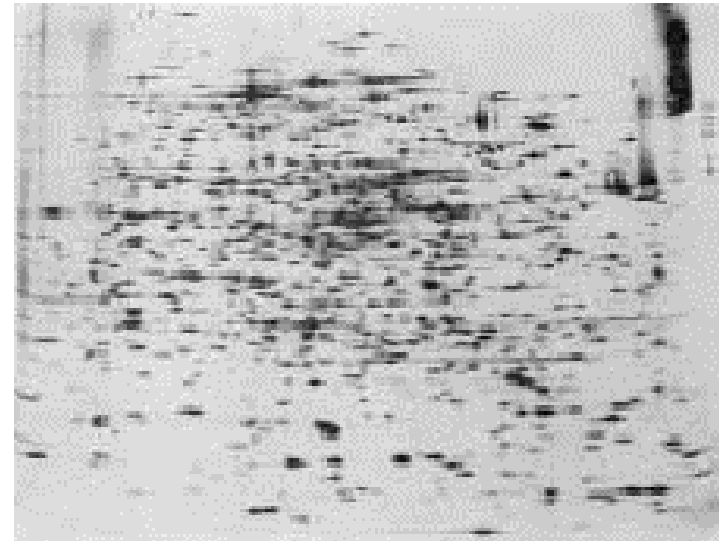
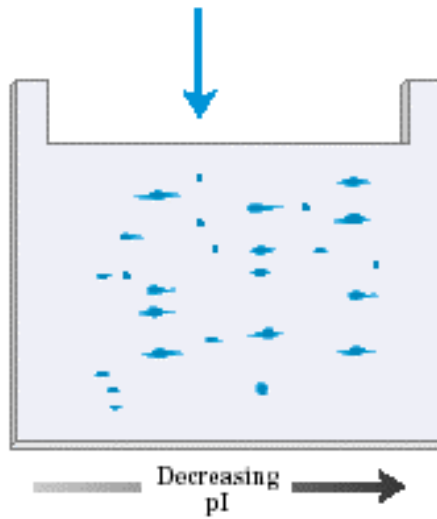


Bidimensional

Isoelectric focusing gel is placed on SDS polyacrylamide gel.



Second dimension
SDS polyacrylamide gel electrophoresis



(b)

Fraccionamiento basado en el coeficiente de reparto

El coeficiente de reparto caracteriza la distribución de una sustancia entre dos fases: la fase móvil y la fase estacionaria

$$K = C_s/C_m$$

Fases: liquido-liquido, liquido-solido

Fase estacionaria:

- Partículas de sólidos
- Partículas de sólidos con líquido

Equipamiento de una cromatografía

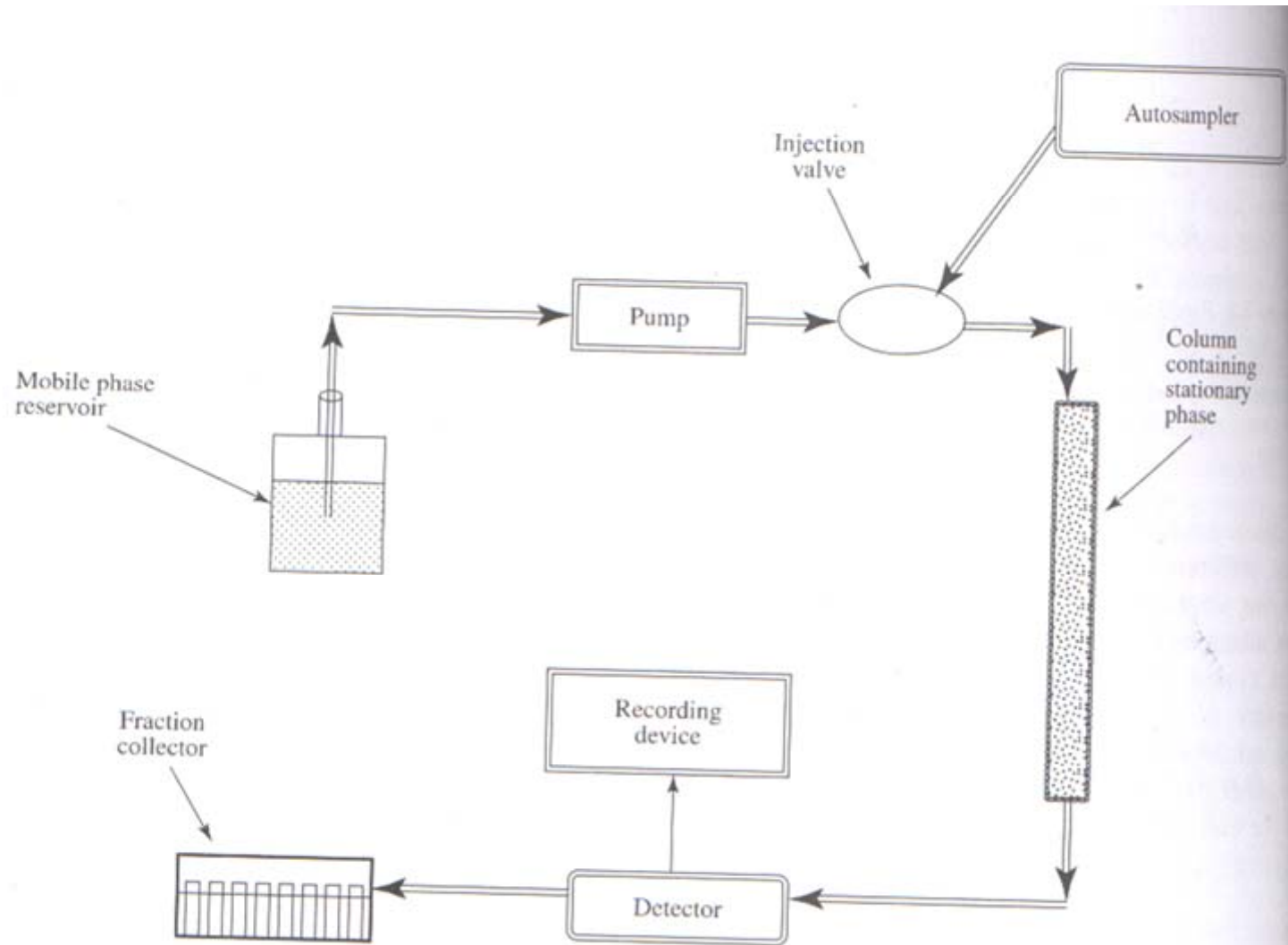
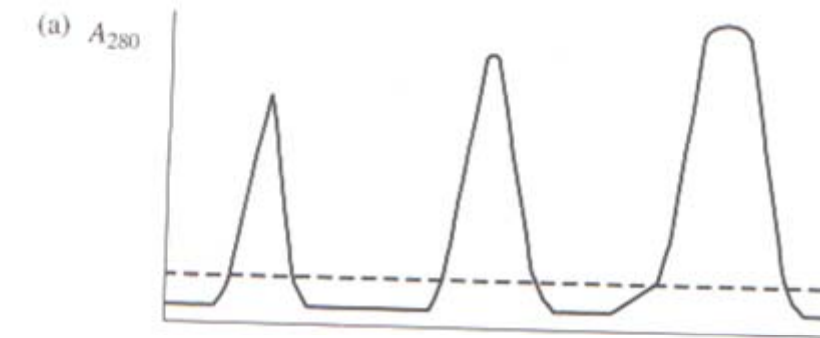
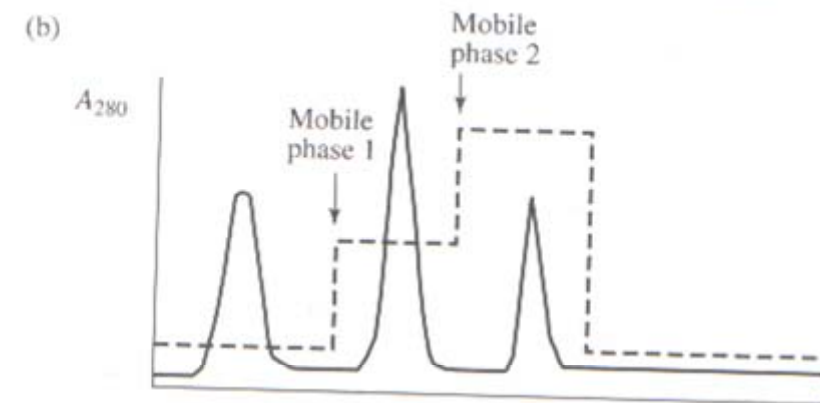


Figure 2.2. A typical liquid chromatography system. The direction of flow is shown by arrows. Sample is loaded via injection through a valve. If a large number of samples are required an autosampler may be used to reload the column repetitively after each chromatography.

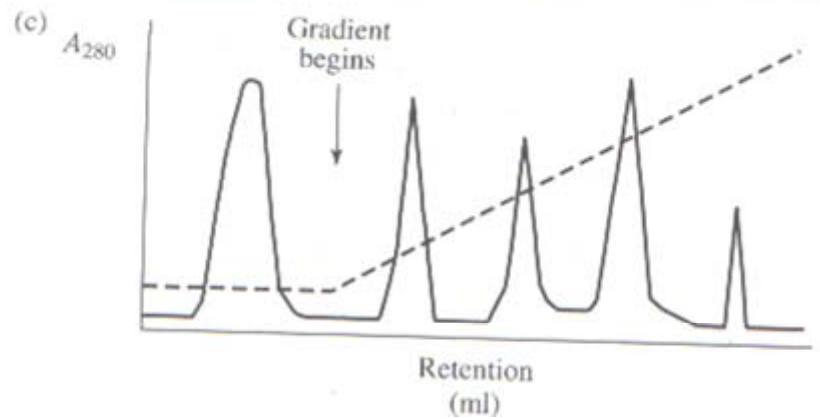
Elución



Contínua



Batch



Gradiente

Fases sólidas y estacionarias

Table 2.1. Selected stationary phase packings used in chromatography

Packing	Composition	Application
DEAE/CM-cellulose	Polysaccharide (cellulose)	Ion exchange chromatography, especially early in purification scheme
Glutathione-agarose	Polysaccharide (agarose)	Affinity chromatography and purification of GST fusion proteins
IDA-agarose	Polysaccharide (agarose)	IMAC (see Section 2.4.6)
Sephacryl S-300	Polysaccharide (dextran/bis acrylamide)	Gel filtration of proteins in Mass range 10–1500 kDa
Sephadex G-25	Polysaccharide (dextran)	Desalting of protein extracts by gel filtration
Superose 12	Polysaccharide (agarose)	Gel filtration FPLC in the Mass range 1–300 kDa
C-18 Silica	Silica	Reversed-phase HPLC of tryptic peptides
POROS	Poly(styrene-divinylbenzene)	Perfusion chromatography
DEAE/CM-MemSep	Polysaccharide (cellulose)	Membrane-based ion exchange chromatography of proteins

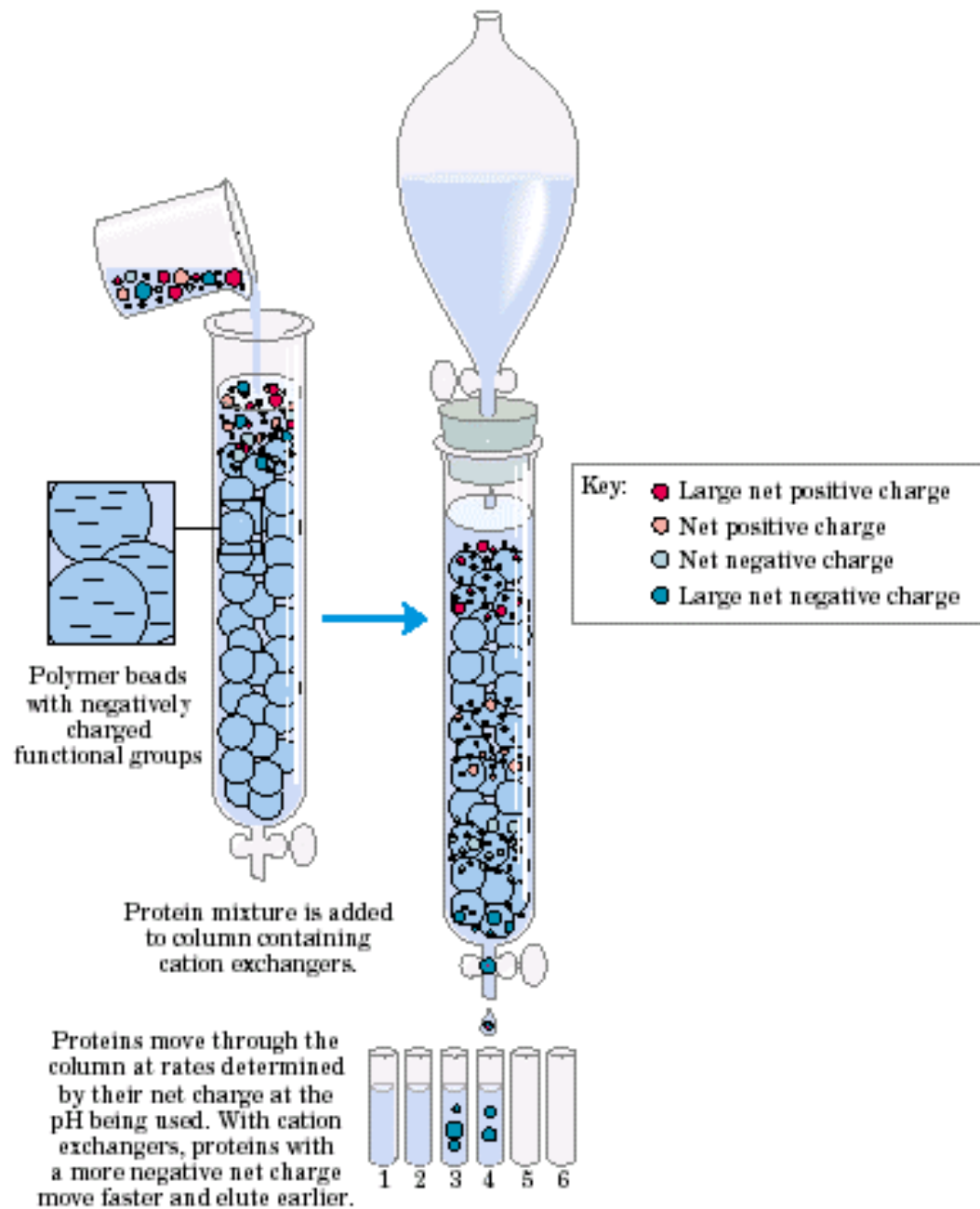
Tipos de cromatografías

Intercambio iónico

- Técnica independiente de la forma, tamaño y PM
- Soportes: Celulosa, agarosa, vinilbenceno
- Antes hacer un deslado (diálisis, filtración en gel)
- En general elución por gradiente (de PH o con agentes caotrópicos)

Table 2.2. Examples of ion exchange groups commonly used

Group	pH range	Chemical structure
<i>Cation exchangers</i>		
Sulphopropyl (SP)	2-12	$-(\text{CH}_2)_2-\text{CH}_2-\text{SO}_3^-$
Methyl sulphonate (S)	2-12	$-\text{CH}_2-\text{SO}_3^-$
Carboxymethyl (CM)	6-11	$-\text{O}-\text{CH}_2-\text{COO}^-$
<i>Anion exchangers</i>		
Quaternary ammonium (Q)	2-12	$-\text{CH}_2-\text{N}^+(\text{CH}_3)_3$
Diethylaminoethyl (DEAE)	2-9	$-\text{O}-\text{CH}_2-\text{CH}_2-\text{NH}^+(\text{C}_2\text{H}_5)_2$
Quaternary aminoethyl (QAE)	2-12	$-\text{O}-(\text{CH}_2)_2-\text{N}^+(\text{C}_2\text{H}_5)_2-\text{CH}_2-\text{CHOH}-\text{CH}_3$



(a)

Filtración molecular o Exclusión molecular

- Separa por distinto tamaño molecular (cutoff o limite de exclusion)
- Se puede usar para desalado
- Se puede usar para estimar PM
- Para purificación se usa generalmente hacia el final

TABLE 2.2. Examples of gel filtration resin

Resin	Fractionation range kDa (globular proteins)	Application
SEPHADEX- (dextran)		
G-10	<0.7	Desalting
G-25	1-5	Desalting
G-50	1.5-30	Peptide separation
G-75	3-80	Protein fractionation
G-100	4-150	Protein fractionation
G-150	5-300	Protein fractionation
G-200	5-600	Protein fractionation
SEPHAROSE- (agarose)		
6B	10-4000	Protein fractionation
4B	60-20 000	Protein fractionation/ligand immobilisation
2B	70-40 000	Fractionation of nucleic acids, particles and viruses
SEPHACRYL (dextran crosslinked with <i>N,N'</i> -methylene bisacrylamide)		
S-100 HR	1-100	Peptide/protein fractionation
S-200 HR	5-250	Protein fractionation
S-300 HR	10-1500	Protein fractionation
S-400 HR	20-8000	Protein fractionation of molecules with extended structures
S-500 HR	80-80 000	Large molecules and small particles
BIOGEL (polyacrylamide)		
P-2	0.1-1.8	Desalting
P-4	0.8-4	Desalting
P-6	1-6	Peptide separation
P-10	1.5-20	Fractionation of small proteins
P-30	2.5-40	Protein fractionation
P-60	3-60	Protein fractionation
P-100	5-100	Protein fractionation
P-150	15-150	Protein fractionation
P-200	30-200	Protein fractionation
P-300	60-400	Fractionation of extended structures
SUPERDEX- (dextran covalently bonded to highly crosslinked agarose)		
75	3-70	FPLC gel filtration
200	10-600	FPLC gel filtration
SUPEROSE- (cross-linked agarose)		
12	1-300	FPLC gel filtration
6	5-5000	FPLC gel filtration
FRACTOGEL- (polyvinyl chloride)		
TSK HW-40	0.1-10	HPLC gel filtration (peptides)
TSK HW-55	1-700	HPLC gel filtration (proteins)
TSK G2000SW	5-100	HPLC gel filtration (proteins)
TSK G3000SW	10-500	HPLC gel filtration (proteins)
TSK HW-65	50-5000	HPLC gel filtration (proteins)
TSK HW-75	500-50 000	HPLC gel filtration (complexes)

Cromatografía de fase reversa

- Se basa en “interacciones hidrofóbicas”
- La fase estacionaria son generalmente compuestos alifáticos de distinta longitud de cadena (C4, C8, C16 etc)
- Elución por gradiente (usando mezclas de agua y solventes orgánicos)
- Muy alta capacidad resolutive (mezclando distintas propiedades fisicoquímicas)

Cromatografía de Afinidad

- Reconocimiento de ligandos
- Alta resolución y especificidad (disminuye mucho el numero de pasos en la purificación)
- Alto tiempo de preparación

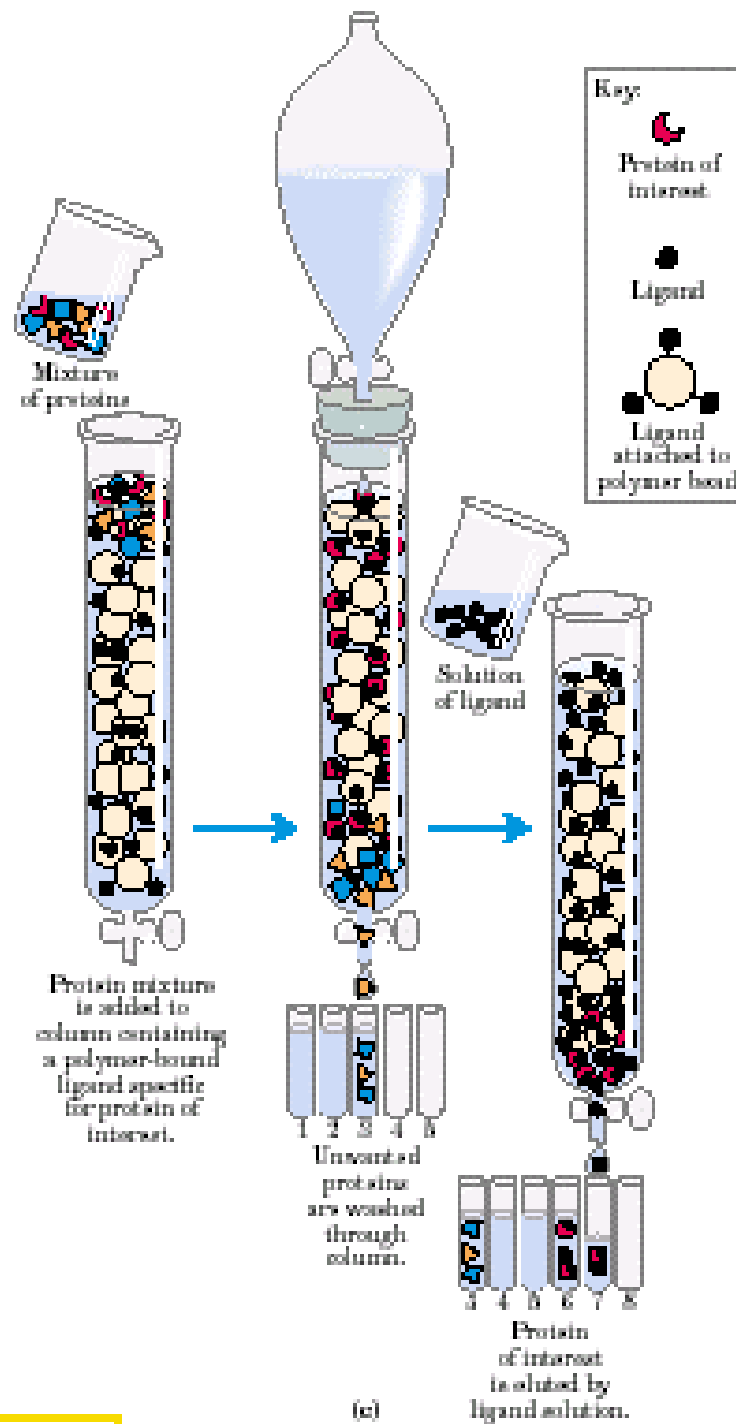
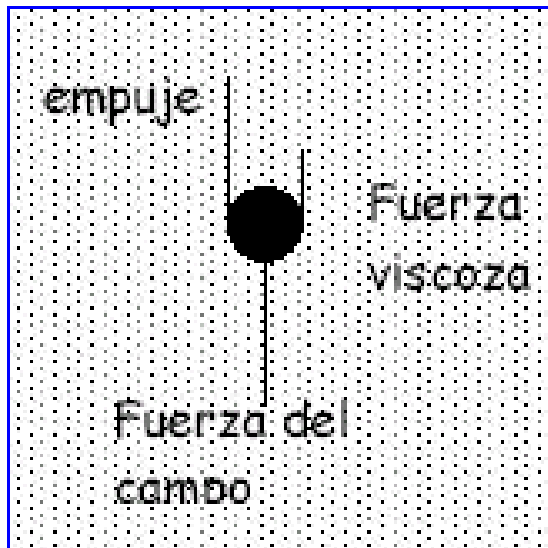


Table 2.4. Examples of group-specific ligands used in affinity chromatography

Ligand	Molecule purified
Substrates	Enzymes
Inhibitors	Enzymes
Co-factors	Enzymes
Avidin	Biotin-containing enzymes
Antibodies	Antigen
Antigen	Immunoglobulins
Proteins A and G	Immunoglobulins
Hormone	Receptor/binding protein
Glutathione	GST fusion proteins
Soyabean lectins	Glycoproteins
Oligo dT	PolyA mRNA
Oligo A	PolyU RNAs
Lysine	rRNA

Fraccionamiento basado en propiedades dinámicas



$$\text{Empuje} = m \bar{v}_2 g \rho$$

$$\text{fuerza viscosa} = f u$$

$$\text{fuerza del campo} = mg \text{ si es gravitatorio}$$

$$m \omega^2 x \text{ si es centrífugo}$$

Sedimentación

$$\frac{u}{\omega^2 r} = s = \frac{m(1 - \bar{v}_2 \rho)}{f}$$

$$s = \frac{M / N_0 (1 - \bar{v}_2 \rho)}{KT / D} = \frac{DM (1 - \bar{v}_2 \rho)}{RT}$$

$$s_{20,w} = s \frac{\eta(1 - \bar{v}_2 \rho)_{20,w}}{\eta_{20,w}(1 - \bar{v}_2 \rho)} = \text{coeficiente de sedimentación estándar}$$

La unidad Svedberg se refiere a 10^{-13} segundos

Tipos de protocolos en sedimentación

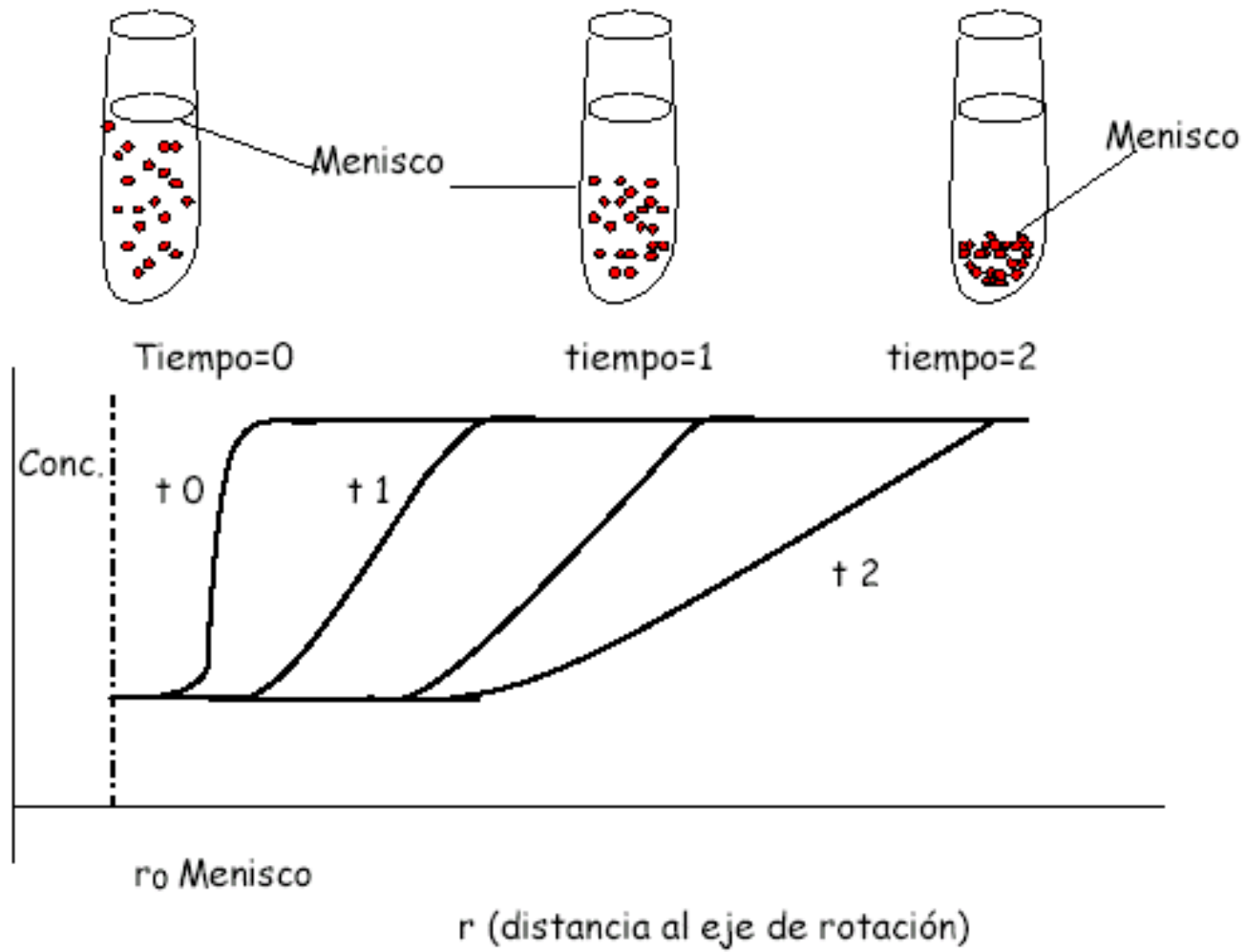
Centrifugación en un gradiente de densidad

zonal centrifugation: glicerol, sacarosa

Equilibrium centrifugation (isopycnic centrifugation) CsCl₂. DNA, lipoproteínas. También Ficoll, Percoll para células

Centrifugación por velocidad

Determinación de S

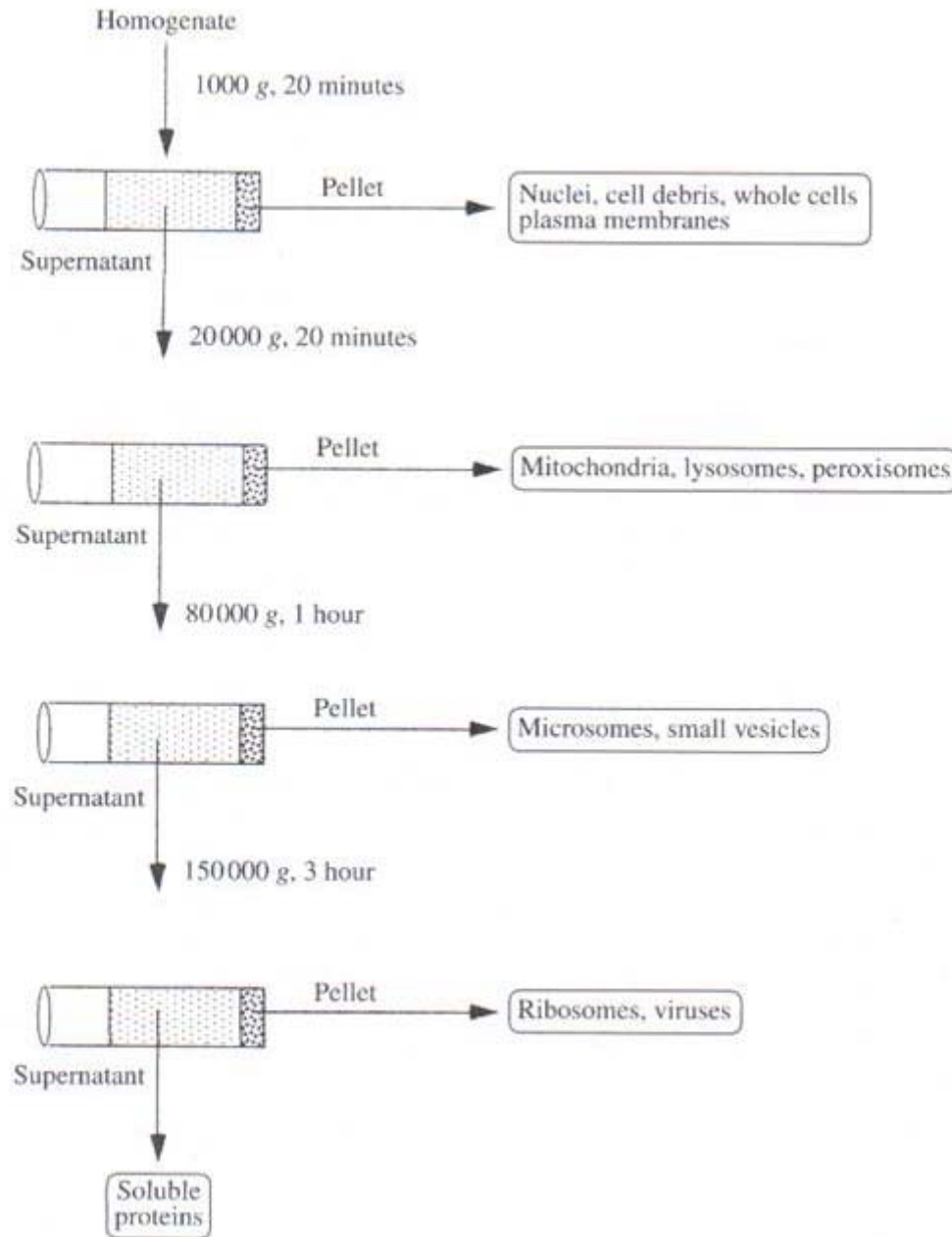


$$s = \frac{u_r}{\omega^2 r} = \frac{dr/dt}{\omega^2 r}$$

$$s = \frac{1}{\omega} \frac{d(\ln(r))}{dt}$$

aplicando logaritmo natural

$$\ln(r) = C + s\omega^2 t$$



7.9. Subcellular fractionation. A tissue homogenate may be fractionated into nuclear, mitochondrial, microsomal and soluble

Otras técnicas basadas en parámetros hidrodinámicos

Difusión

$$D = \frac{kT}{f}$$

Viscosidad

$$\eta_{sp} = \frac{\eta' - \eta_0}{\eta_0} = \text{viscosidad específica}$$

$$\lim_{c} \frac{\eta_{sp}}{c} = [\eta]$$

Técnicas de desalado

- Ultracentrifugación
- Diálisis
- Columnas de desalado