TECNICAS DE FRACCIONAMIENTO

ULTRACENTRIFUGACION

Bibliografia:

-*"Centrifugation in Biology and Medical Science".*
P. Sheeler. (1981) John Wiley & Sons, Inc.

-*"Técnicas Instrumentales de Análisis en Bioquímica". Cap. 4.*
García-Segura et al., Editorial Síntesis, 1996.

INDICE

SEDIMENTACIÓN

Transporte de una partícula en solución sometida a una fuerza (gravitacional o centrífuga)

Para sedimentar una partícula sólo por acción de la gravedad, deben ser partículas grandes.

Para separar macromoléculas biológicas en suspensión por sedimentación debemos usar fuerza centrífuga (**CENTRIFUGACIÓN**)

Y aceleraciones altas!

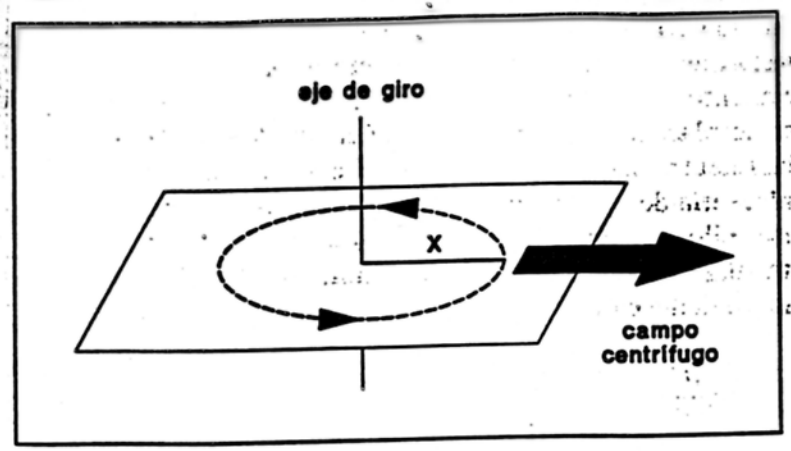


FIGURA 4.1. Representación del campo centrífugo, $G = \omega^2 X$, que actua sobre un punto situado a una distancia X del eje de giro, y sometido a una rotación cuya velocidad angular es ω.

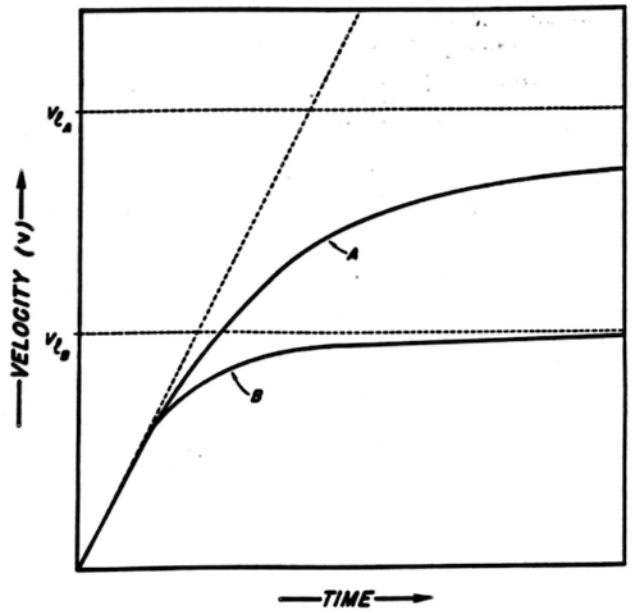


Fig. 2-1 Relationship between time and velocity for objects falling (or sedimenting) through a medium offering resistance. A heavy object (*A*) approaches a limiting velocity over a longer time interval than a light object (*B*).

Fuerza Centrífuga Relativa

Velocidad de operación de la centrífuga se expresa en "rpm" (revoluciones por minuto) que se pueden convertir en radianes:

$\omega = \pi \text{ rpm} / 30$

La magnitud de la fuerza centrífuga depende de la velocidad angular ω en radianes ($F = \omega^2 r$) pero es frecuentemente expresada relativa a la fuerza gravitacional g (fuerza centrífuga relativa, RCF):

$$RCF = \omega^2 r / 980 = \frac{(\pi \text{ rpm} / 30)^2 r}{980}$$

$$RCF = 1.119 \times 10^{-5} (\text{rpm})^2 r$$

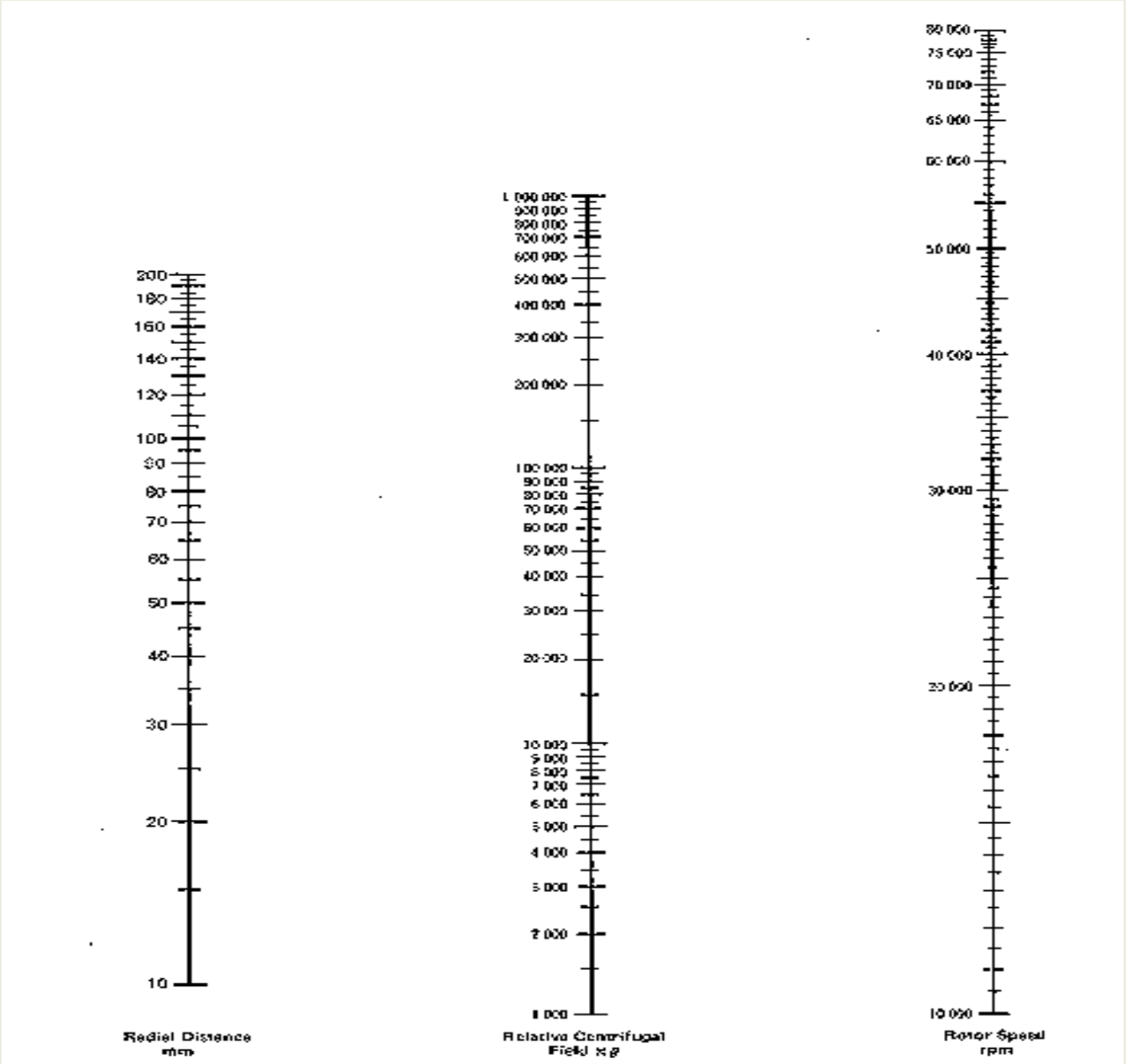
RCF = g, r = cm

Radio (r) mínimo (top)

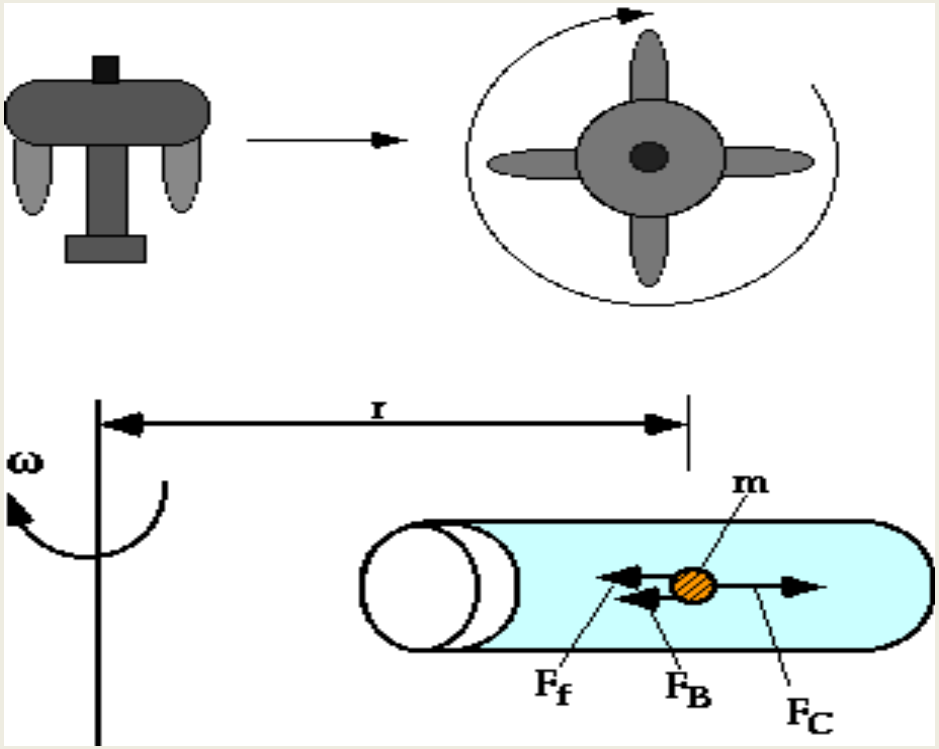
máximo (bottom)

promedio

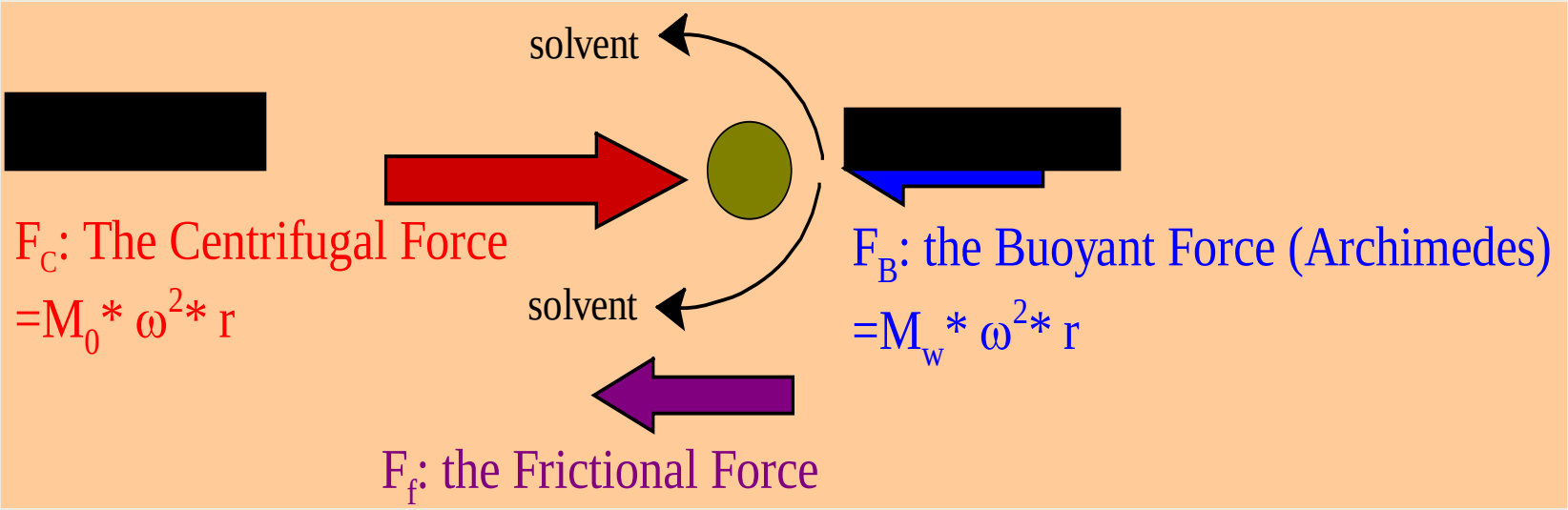
Equivalencia FCR (gess)- rpm en función de la distancia radial



Fuerzas que intervienen en Centrifugación



Sedimentation Theory



Centrifugal force = buoyant force + frictional force

1. The Centrifugal Force

$$Fc = m w^2 r$$

- m is the mass of particle
- ω (omega)= angular velocity (radians/sec)
- r is the radius of rotation

This equation says that the larger the molecule, or the faster the centrifugation, or the longer the axis of rotation, the greater the centrifugal force and the rate of sedimentation.

2.The Buoyant Force

$$FB = V \rho w^2 r$$

- The buoyant force opposes the centrifugal force.
- Where V: is the volume of the particle.
  - ρ: is the density of the displaced liquid.
  - w^2 r: angular acceleration

The net force= (Fc-FB) will determine whether a particle floats or sediments

- Particles with higher density will experience smaller buoyant force, and thus, sediment faster.

The Frictional force

$$F_f = f\,V$$

Frictional force (resistance of a molecule to movement)

- **v = velocity relative to the centrifuge tube**
- ***f* = frictional coefficient = 6 π η r**
- η: viscosity of medium
- r: radius of particle

- **The frictional coefficient** depends upon:

1. the size

2.shape of the molecule,

3.the viscosity **of the gradient material.**

- **The frictional coefficient *f* of a compact particle is smaller than that of an extended particle of the same mass.**

$Fc = m\ \omega^2\ r$

m = masa partícula

ω = velocidad angular rotor

r = radio rotor

$Fb = V\ \rho\ g$ (Arquímedes)

V = Volumen de la partícula

g= aceleración de la gravedad (ω^2 r)

ρ = densidad del solvente

$F_f = f\ (dx/dt)$ (Stokes)

$F_f = 6\ \pi\ \eta\ r\ (dx/dt)$

η = viscosidad del medio

f = coeficiente de fricción = 6 π η r (p. esférica)

dx/dt = velocidad de la partícula

La partícula alcanza una velocidad constante (v) cuando la fuerza neta es cero:

$Fc - (Fb + F_f) = 0$

DETERMINACIÓN DE LA VELOCIDAD DE SEDIMENTACIÓN

La partícula que sedimenta alcanza una velocidad constante (v) cuando la fuerza neta es cero: $Fc - (Fb + F_f) = 0$

Igualando las expresiones que describen las fuerzas anteriores:

$$(Fc)\ m\ \omega^2\ r = (Fb)\ V\ \rho\ g + (Ff)\ 6\ \pi\ \eta\ r\ (dx/dt)$$

y asumiendo que se trata de una partícula esférica de $V = 4/3\ \pi\ r^3$

y teniendo en cuenta que la densidad de la partícula $\rho_p = m/V$, se llegaría a la siguiente expresión:

$$dx/dt = 2\ \omega^2\ x\ r^2(\rho_p - \rho_m)/\ 9\eta$$

Siendo ρ_m= densidad del medio y η=viscosidad del mismo.

The Sedimentation Coefficient

- $v = dr/dt = \omega^2 \times S$, siendo $S = 2r^2(\rho_p - \rho_m)/ 9\eta$

ρ_p=density of spherical particle

r=radius of spherical particle(cm)

ρ_m=density of medium

η=viscosity of medium(cgs)

Cálculo de S:

Integrando: $\ln r/r_o = \omega^2 S (t - t_o)$

Factors increasing the Sedimentation Coefficient

- An increase in size of the particle.

(Fungi> Bacteria> Viruses)

increase of radius by factor 2 will increase S by 4.

- An increase in the difference between the density of the particle ρ_p & that of the medium ρ_m.
- A decrease in the viscosity of the medium.
- An increase in the force due to gravity.

Efecto de la viscosidad, densidad y concentración en S

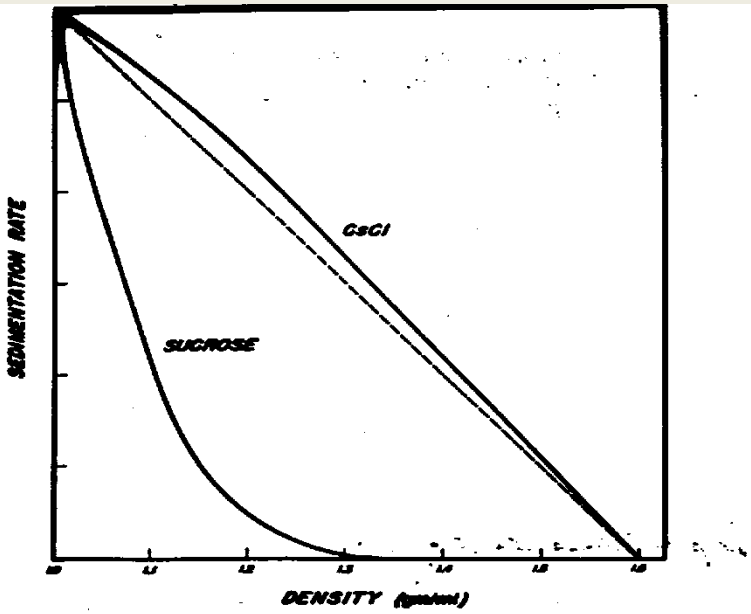


Fig. 2-8 Comparison of the relative sedimentation rates for a spherical particle that has a density of 1.6 in sucrose and CsCl solutions of different density. The two curves are different because the viscosities of sucrose and CsCl solutions vary differently in relation to solute concentration. The dashed curve shows the sedimentation rates that would occur if the viscosities of sucrose and CsCl solutions did not change.

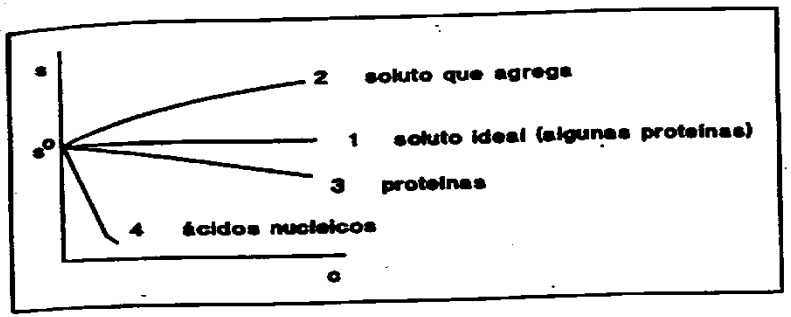
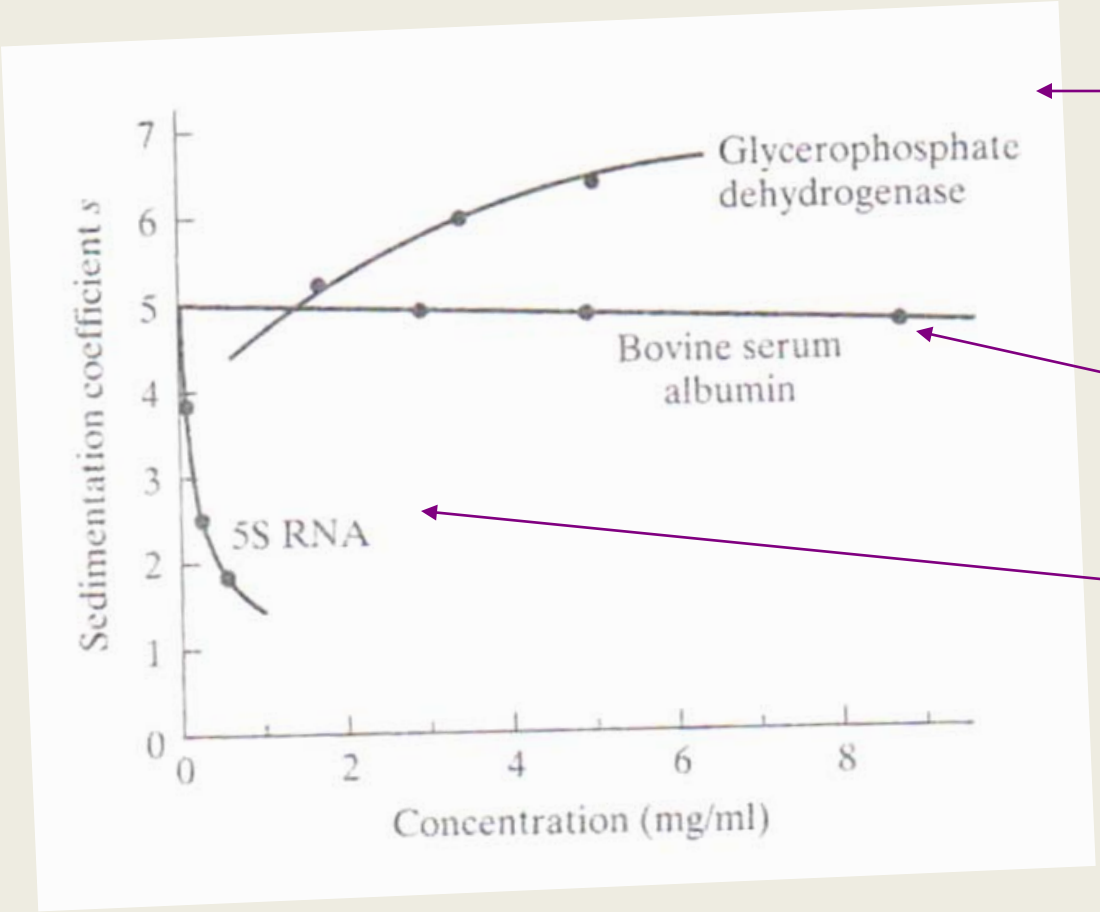


FIGURA 4.2. Dependencia del valor del coeficiente de sedimentación, s, con la concentración de soluto, C. s^0 es el valor del coeficiente de sedimentación extrapolado a concentración cero. Se ha considerado, de forma arbitraria, que el valor de este último parámetro es el mismo en todos los casos.

Efecto de la concentración en S



Se agrega a altas conc.

Proteína compacta

Molécula extendida

S varía con el coeficiente de fricción, que a su vez depende de la concentración de la macromolécula utilizada, más fuertemente para moléculas extendidas

Types of Centrifugation

There are basically two modes of centrifugation

1.-Analytical

- **determining hydrodynamic or thermodynamic properties of biomolecules in the absence of solid supports (vs. electrophoresis, chromatography)**
- **Relative MW**
- **Molecular shape**
- **Aggregation behavior**
- **Protein-protein interactions**

2.- Preparative :

- Cellular fractionation and/or separating coarse suspension
- removal of precipitates
- crude purification step
- Separation of complex mixtures
- Finer fractionation of cellular components
- Purification of proteins, nucleic acids, plasmids
- Characterization of molecular interactions

Rate Separations

1. Differential centrifugation.

- Separation is achieved primarily based on the size of the particles in differential centrifugation.
- It is commonly used in simple pelleting and in obtaining partially-pure preparation of subcellular organelles and macromolecules.
- For the study of subcellular organelles, tissue or cells are first disrupted to release their internal contents.
- This crude disrupted cell mixture is referred to as a homogenate.
- During centrifugation of a cell homogenate, larger particles sediment faster than smaller ones and this provides the basis for obtaining crude organelle fractions by differential centrifugation.
- A cell homogenate can be centrifuged at a series of progressively higher g-forces and times to generate pellets of partially-purified organelles.

- When a cell homogenate is centrifuged at 1000 x g for 10 minutes, unbroken cells and heavy nuclei pellet to the bottom of the tube.

- The supernatant can be further centrifuged at 10,000 x g for 20 minutes to pellet subcellular organelles of intermediate velocities such as mitochondria, lysosomes, and microbodies.

- Some of these sedimenting organelles can obtained in partial purity and are typically contaminated with other particles.

- Repeated washing of the pellets by resuspending in isotonic solvents and re-pelleting may result in removal of contaminants that are smaller in size.

- Obtaining partially-purified organelles by differential centrifugation serves as the preliminary step for further purification using other types of centrifugal separation (density gradient separation).

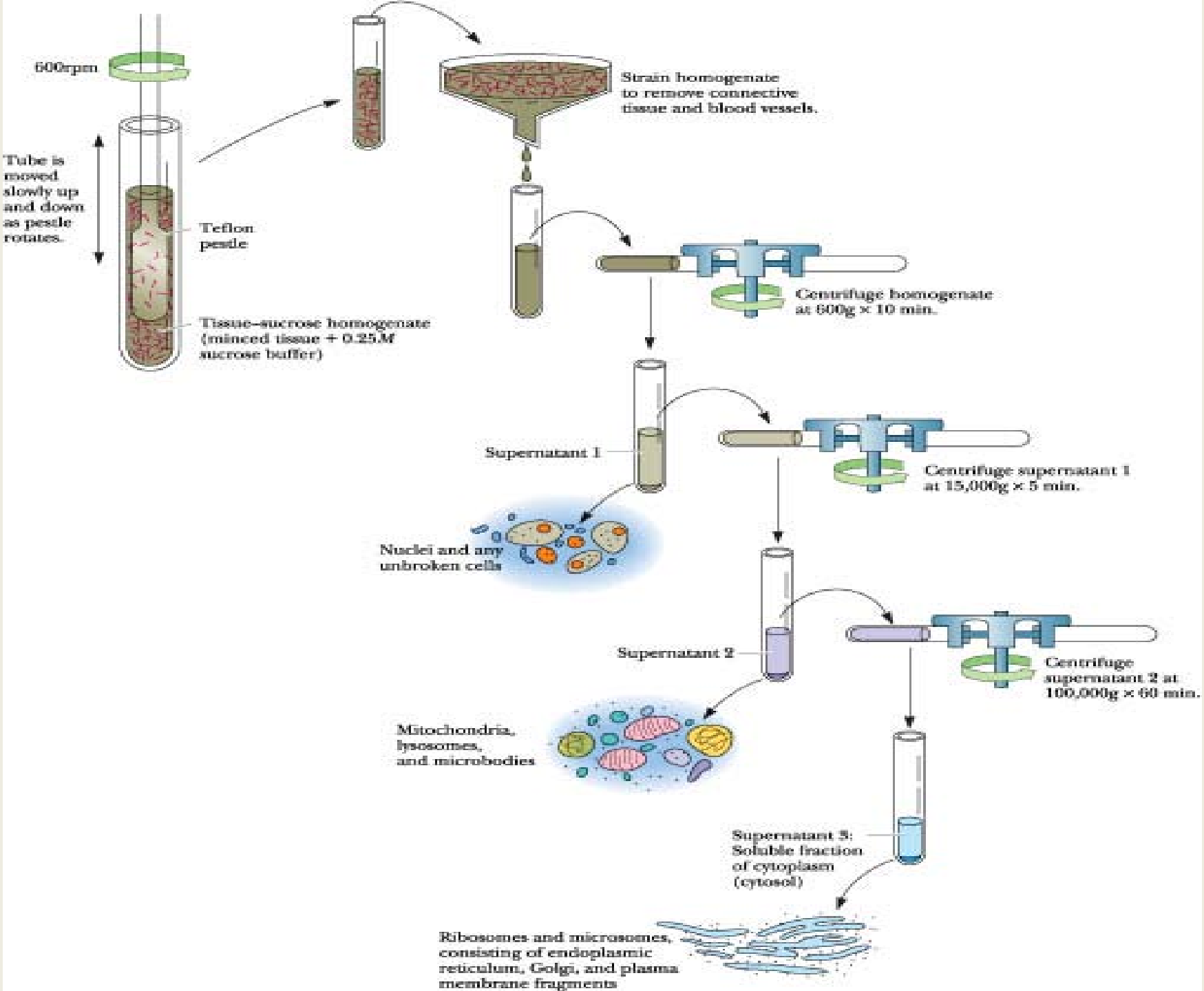
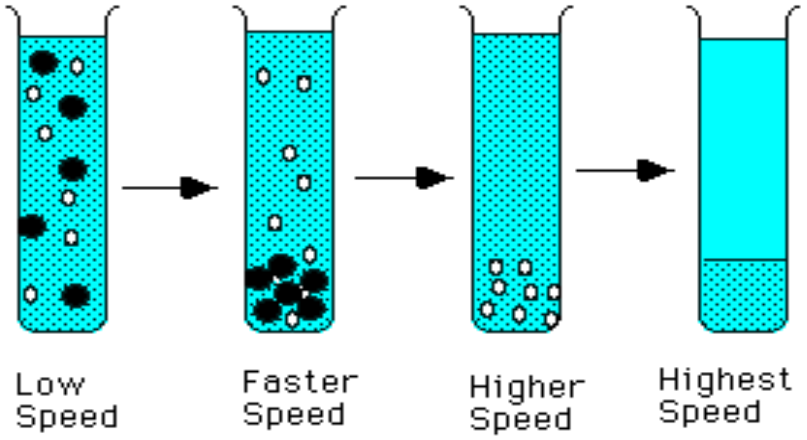

600rpm
Tube is moved slowly up and down as pestle rotates.
Teflon pestle
Tissue–sucrose homogenate (minced tissue + 0.25M sucrose buffer)
Strain homogenate to remove connective tissue and blood vessels.
Centrifuge homogenate at 600g × 10 min.
Supernatant 1
Nuclei and any unbroken cells
Centrifuge supernatant 1 at 15,000g × 5 min.
Supernatant 2
Mitochondria, lysosomes, and microbodies
Centrifuge supernatant 2 at 100,000g × 60 min.
Supernatant 3: Soluble fraction of cytoplasm (cytosol)
Ribosomes and microsomes, consisting of endoplasmic reticulum, Golgi, and plasma membrane fragments

Figure 2: Differential Centrifugation.

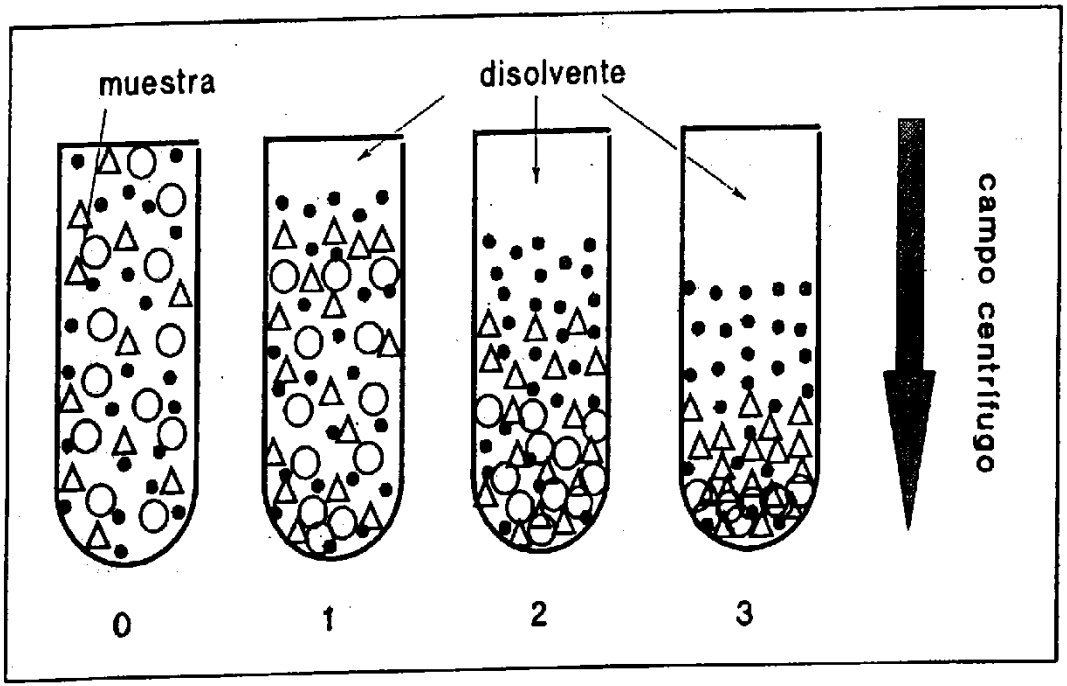


Enzymic subcellular markers

Fraction		*Marker*	*References*	*EC number*
Plasma membrane	baso-lateral	adenylate cyclase	1	4.6.1.1
		Na^+ K^+ ATPase	2	3.6.1.37
		receptors e.g. asialoglycoprotein	3	
	apical	5′-nucleotidase	2,4,5	3.1.3.5
		leucineaminopeptidase	6	3.4.11.1
		γ-glutamyltranspeptidase	7	2.3.2.12
Endoplasmic reticulum		glucose-6-phosphatase	8,22	3.1.3.9
		NADPH-cyt.c reductase	9	1.6.2.4
		epoxide hydrolase	10	3.3.2.3
Golgi apparatus	*trans* and middle region	galactosyltransferase	11,12	2.4.1.38
		sialyltransferase	11,12	2.4.99.1
		NADP-phosphatase	13	3.6.1.22
Mitochondria	inner membrane	succinate dehydrogenase	14	1.3.99.1
		cytochrome oxidase	15	1.9.3.1
		rotinone-insenstive NADH-cyt.c reductase	16	1.6.99.1
	outer membrane	monoamine oxidase	17	1.4.3.4
		kynurenine-3-hydroxylase	18	1.14.13.9
Lysosomes		acid phosphatase	19	3.1.3.2
		β-glucuronidase	19	3.2.1.31
		aryl sulphatase	20	3.1.6.1
Endosomes		monensin-activated Mg^{2+}-ATPase plus intact ligands	21	3.6.1.3
Peroxisomes		catalase	23	1.11.1.6
		carnitine palmitoyl transferase	24	2.3.1.21
Cytosol		lactate dehydrogenase	25	1.1.1.22

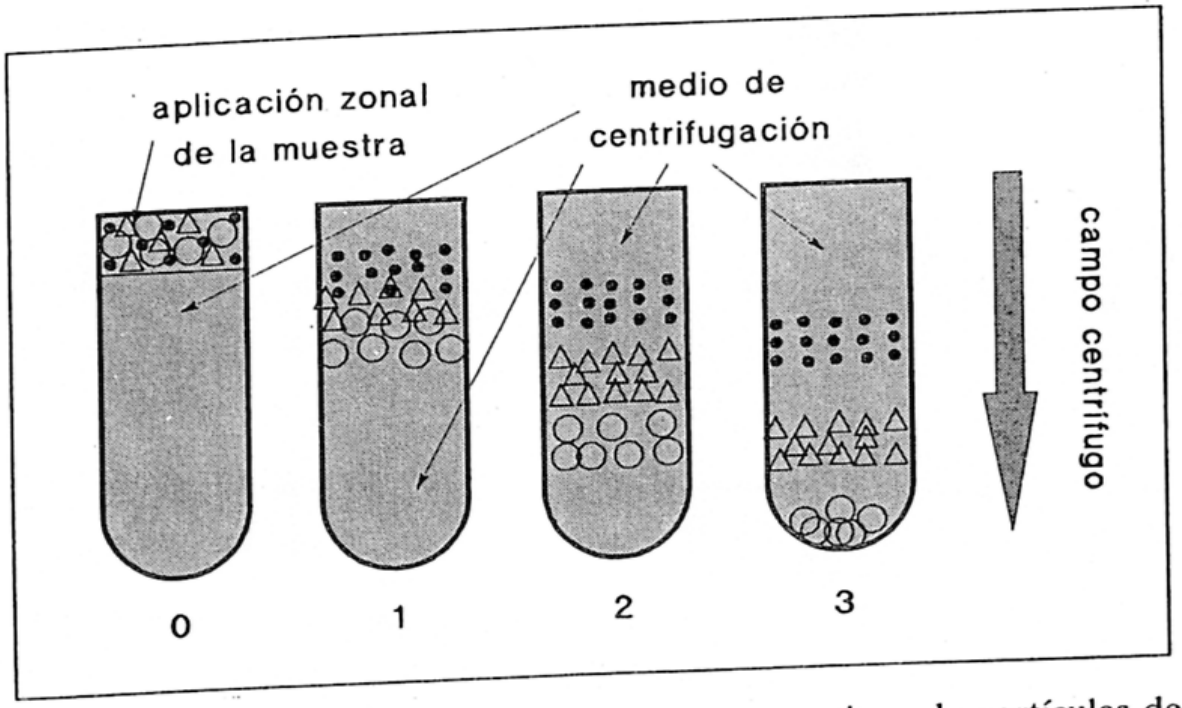
Detailed descriptions of the assay procedures are to be found in the references quoted, and many of the procedures are described together in reference 9. For a discussion of the distribution of baso-lateral and apical plasma membranes see reference 4 in Chapter 1. No specific enzyme markers exist for nuclear membranes, and the *cis* region of the Golgi apparatus. For further details of the reliability of these markers, see Chapter 1 and 2.

Problema de Cosedimentación



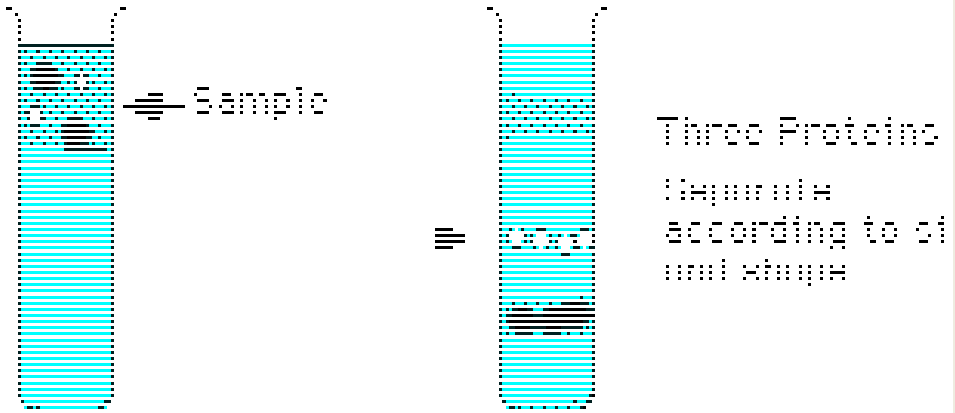
Centrifugación diferencial de una suspensión que contiene tres tipos de partículas de distinto coeficiente
ación, *s*. Los coeficientes de sedimentación decrecen en el orden (0) > (Δ) > (·). Se representan diferen-
os del proceso. La situación inicial es la correspondiente al gráfico (0), mientras que el gráfico (3) corres-
mpo al que han sedimentado todas aquellas partículas de mayor coeficiente de sedimentación. Se puede
: el material sedimentado contiene partículas de los tres tipos, aunque sean mayoritarias las de mayor *s*.

Centrifugación Zonal

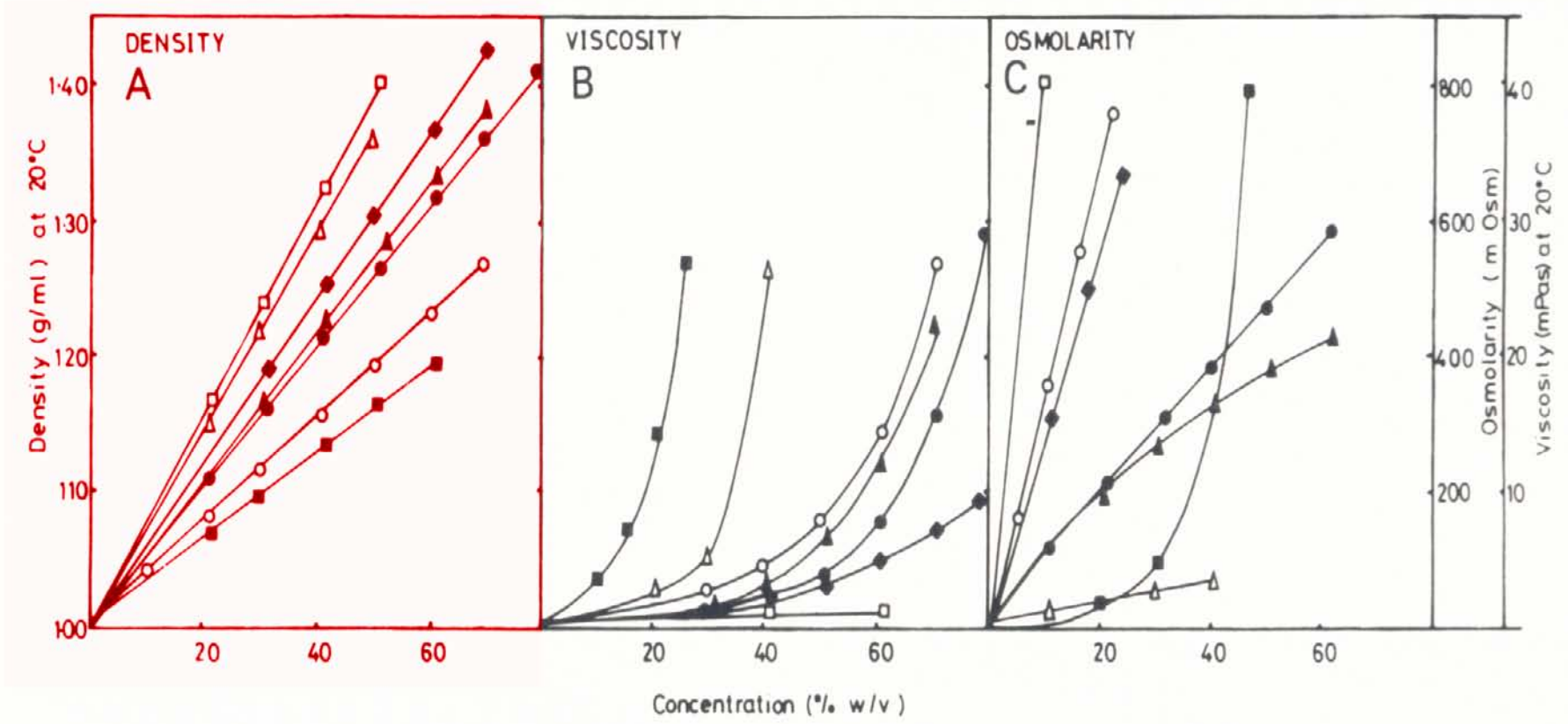


8. Centrifugación zonal de una suspensión que contiene tres tipos de partículas de diferente coeficiente
ntación. Estos decrecen en el orden (0) > (Δ) > (·). Se representan diferentes momentos del proceso. La
nicial es la correspondiente al gráfico (0), mientras que el gráfico (3) corresponde a un tiempo al que se
ado ya las bandas correspondientes a cada uno de los tres componentes considerados, ordenadas de
nayor s según el sentido de actuación del campo centrífugo. La muestra se ha aplicado de forma zonal
sobre el medio de centrifugación.

Figure 3b: Rate zonal centrifugation.



Dependencia de la Densidad, Viscosidad y Osmolaridad con la concentración de medio



The relationships between the concentration (% w/v) of gradient solutes and their density, viscosity and osmolarity are shown for Nycodenz (●——●), metrizamide (▲——▲), sodium metrizoate (♦——♦), CsCl (□——□), sucrose (○——○), Ficoll (■——■) and Percoll (△——△). Reproduced from (1).

Gradientes Discontinuos

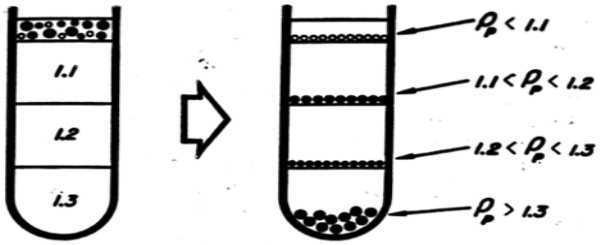


Fig. 5-5 Use of step gradients to selectively halt the sedimentation of families of particles falling within specific density ranges.

Dos formas de Centrifugación en gradientes de densidad

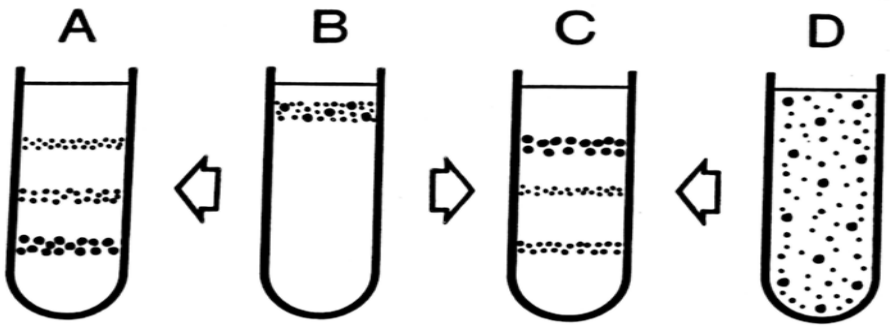


Fig. 5-2 *Rate* and *isopycnic* density gradient separations of particles. In a rate separation (i.e., from *B* to *A*), the particles become distributed in order of their sedimentation coefficients. In isopycnic separations (from *B* to *C* and from *D* to *C*) the particles become distributed in order of their densities. Note that the final distributions of particles (i.e., stages *A* and *C*) may not be the same. Isopycnic separations can sometimes be achieved by initially suspending the particles in a uniform solution of the gradient solute and allowing the gradient to form automatically during centrifugation (i.e., from *D* to *C*).

2. Density gradient centrifugation.

Density gradient centrifugation is the preferred method to purify subcellular organelles and macromolecules.

Discontinuous density gradients can be generated by placing layer after layer of gradient media such as sucrose in a tube with the heaviest layer at the bottom and the lightest at the top in either a discontinuous or continuous mode.

The cell fraction to be separated is placed on top of the layer and centrifuged.

Density gradient separation can be classified into two categories.

2a. Rate-zonal (size) separation.

2b. Isopycnic (density) separation.

PREPARACION DE GRADIENTES DE DENSIDAD

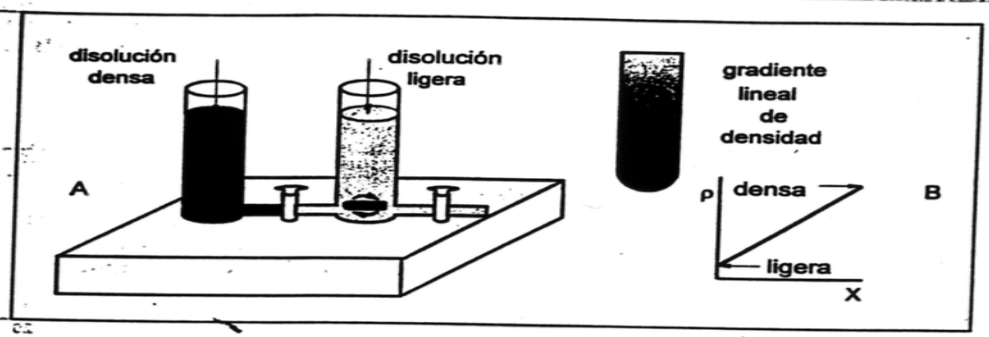


FIGURA 4.11. Formador de gradientes lineales (parte *A*). Al conectar el sistema de vasos comunicantes, de la cámara de mezcla (en la que está la barra magnética de agitación) va fluyendo un medio de densidad creciente, que se va depositando en el tubo de centrifugación, dando lugar al gradiente de densidad (ρ) (parte *B*), de menor a mayor hacia el fondo del tubo. En el fondo del tubo la densidad será la de la disolución densa, mientras que en la parte superior será la de la ligera.

Colocación de la muestra

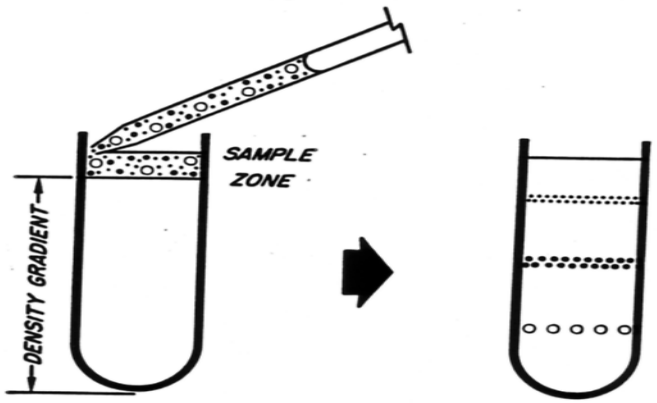


Fig. 4-1 Basic thesis of density gradient centrifugation. Sample containing a mixture of particles is layered onto the surface of the density gradient (left), and after centrifugation (right) the particles are distributed through the gradient as a series of zones.

Rate zonal (size) separation

- Rate-zonal separation takes advantage of particle size and mass instead of particle density for sedimentation.
- Examples of common applications include separation of cellular organelles such as endosomes or separation of proteins, such as antibodies.
- For instance, Antibody classes all have very similar densities, but different masses.
- Thus, separation based on mass will separate the different classes, whereas separation based on density will not be able to resolve these antibody classes.

Criteria for successful rate-zonal centrifugation:

- Density of the sample solution must be less than that of the lowest density portion of the gradient.
- Density of the sample particle must be greater than that of the highest density portion of the gradient.
- The pathlength of the gradient must be sufficient for the separation to occur.
- Time is important. If you perform too long runs, particles may all pellet at the bottom of the tube.

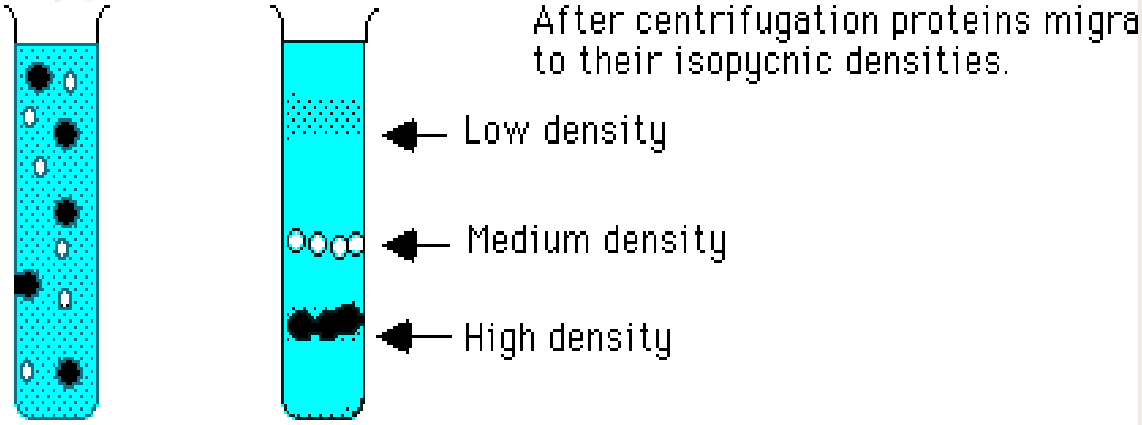
2b. Isopycnic separation

- In this type of separation, a particle of a particular density will sink during centrifugation until a position is reached where the density of the surrounding solution is exactly the same as the density of the particle.
- Once this quasi-equilibrium is reached, the length of centrifugation does not have any influence on the migration of the particle.
- A common example for this method is separation of nucleic acids in a CsCl gradient.

Criteria for successful isopycnic separation:

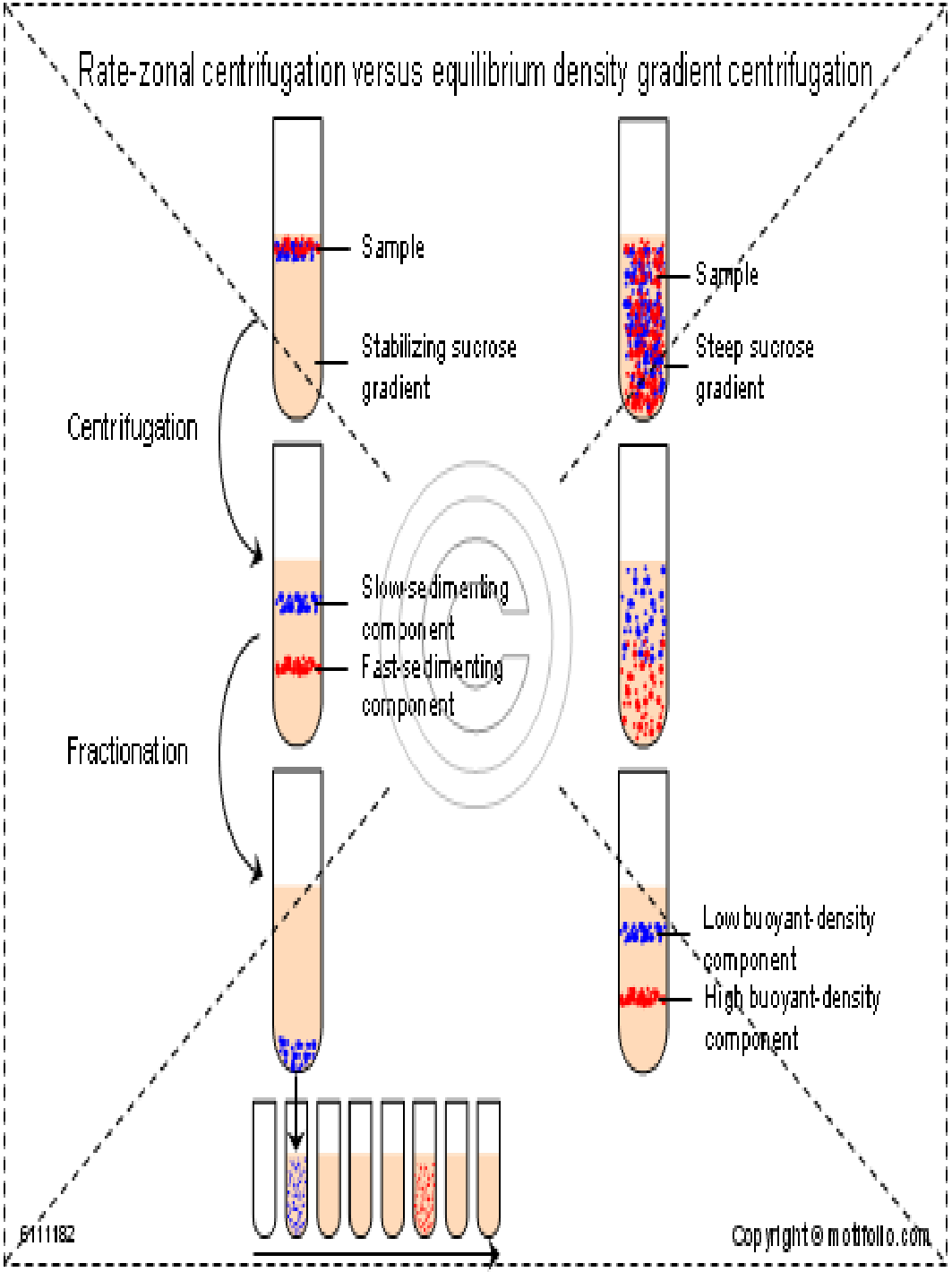
- Density of the sample particle must fall within the limits of the gradient densities.
- Any gradient length is acceptable.
- The run time must be sufficient for the particles to band at their isopycnic point. Excessive run times have no adverse effect.

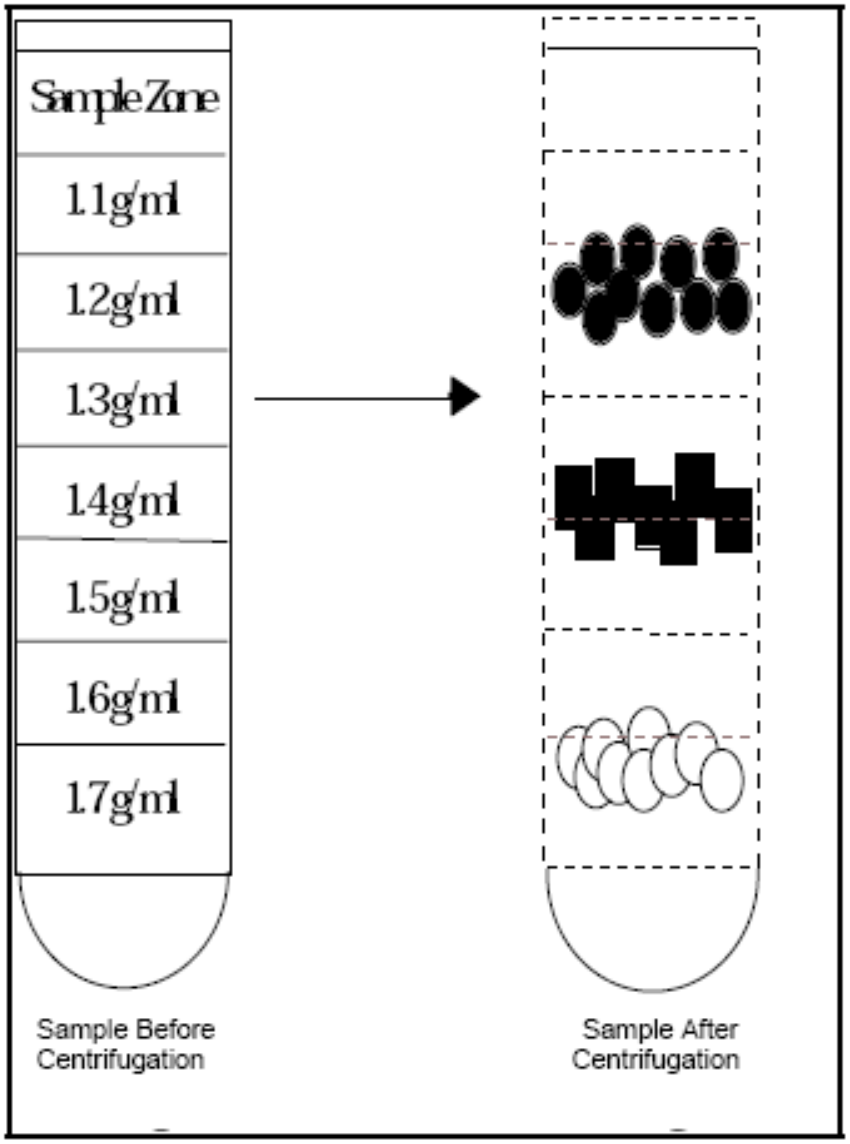
Figure 4: Isopycnic separation with a self-generating gradient



The sample is evenly distributed through-out the centrifuge tube
centrifugation.

Figure 2. RATE-ZONAL (SIZE) SEPARATION





Gradientes Discontinuos

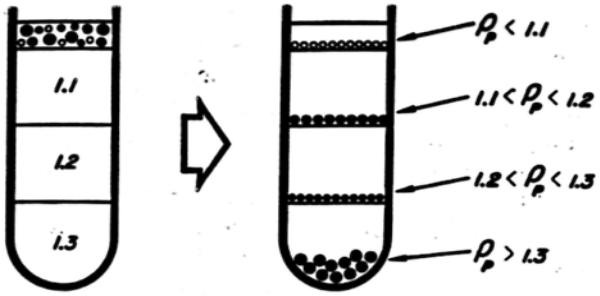


Fig. 5-5 Use of step gradients to selectively halt the sedimentation of families of particles falling within specific density ranges.

Dos formas de Centrifugación en gradientes de densidad

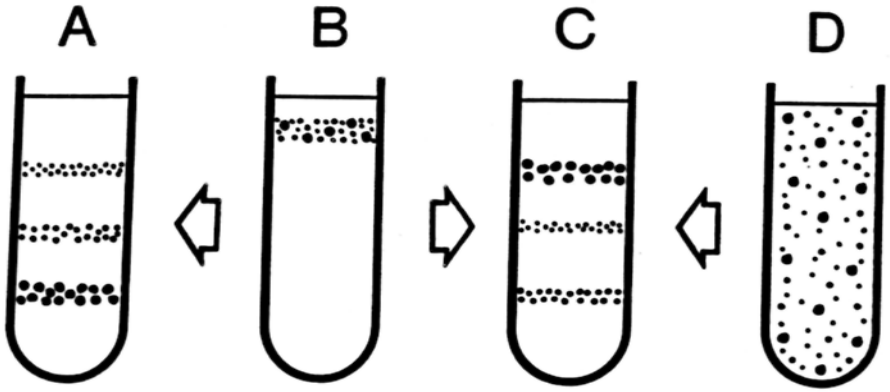
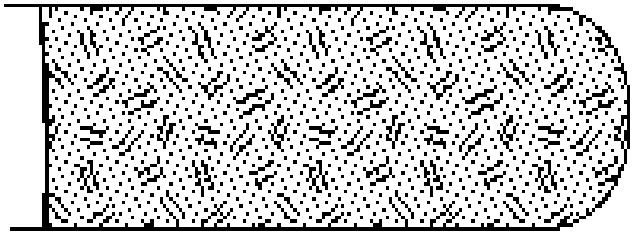


Fig. 5-2 *Rate* and *isopycnic* density gradient separations of particles. In a rate separation (i.e., from *B* to *A*), the particles become distributed in order of their sedimentation coefficients. In isopycnic separations (from *B* to *C* and from *D* to *C*) the particles become distributed in order of their densities. Note that the final distributions of particles (i.e., stages *A* and *C*) may not be the same. Isopycnic separations can sometimes be achieved by initially suspending the particles in a uniform solution of the gradient solute and allowing the gradient to form automatically during centrifugation (i.e., from *D* to *C*).

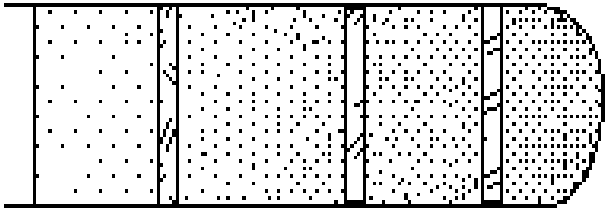
GRADIENTES DE DENSIDAD AUTOGENERADOS

(a) Before centrifugation. CsCl and sample uniformly distributed



CsCl DNA

(b) After centrifugation. CsCl redistributes giving density gradient. Nucleic acid species form bands at "equal density" levels.



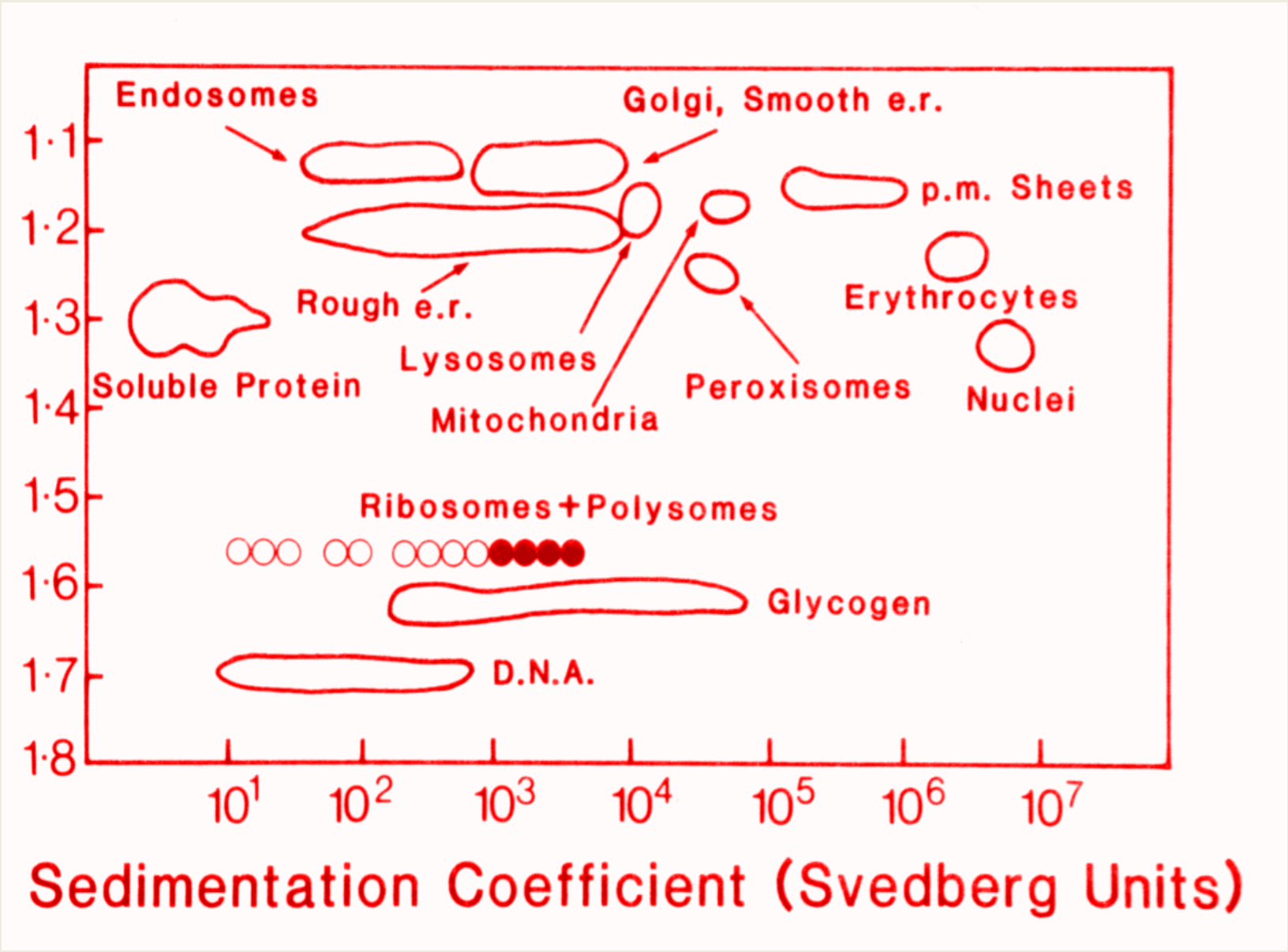

Density
Endosomes
Golgi, Smooth e.r.
p.m. Sheets
Rough e.r.
Erythrocytes
Lysosomes
Soluble Protein
Peroxisomes
Nuclei
Mitochondria
Ribosomes + Polysomes
Glycogen
D.N.A.
1·1
1·2
1·3
1·4
1·5
1·6
1·7
1·8
10^1 10^2 10^3 10^4 10^5 10^6 10^7
Sedimentation Coefficient (Svedberg Units)

Table 2. Applications of density gradient media for isopycnic separations.

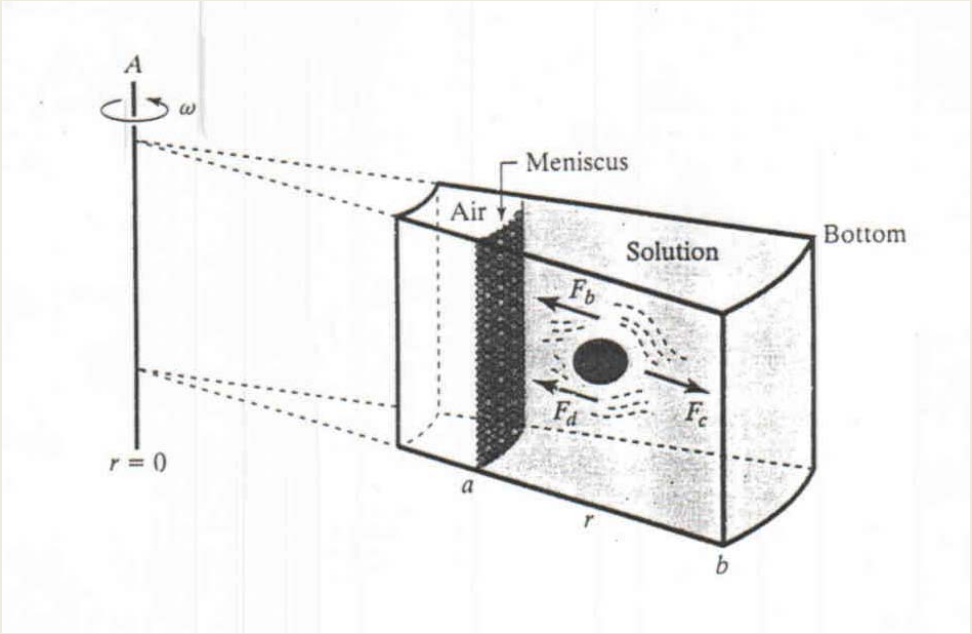
Gradient media	Cells	Viruses	Organelles	Nucleoproteins	Macro-molecules
Sugars (e.g sucrose)	+	+ + +	+ + +	+	-
Polysaccharides (e.g Ficoll)	+ +	+ +	+ +	-	-
Colloidal silica (e.g Percoll)	+ + +	+	+ + +	-	-
Iodinated media (e.g Nycodenz)	+ + + +	+ +	+ + + +	+ + +	+
Alkali metal salts (e.g. CsCl)	-	+ +	-	+ +	+ + + +

+ + + + excellent, + + + good, + + good for some applications, + limited use, - unsatisfactory

Source: D. Rickwood, T.C. Ford, J. Steensgard (1994) *Centrifugation essential data,* John Wiley & Sons Ltd. U.K.

Partícula	Densidad (ρ)	Gradiente
	g/ml	
RNA	2	Cs_2SO_4
DNA	1.7	CsCl
Ribosomas	1.55	CsCl
Proteínas	1.3	Glicerol, sacarosa, Ficoll
Organelos	1 – 1.3	Sacarosa, Ficoll, Percol
Lipoproteínas	1	KBr

Experimento de velocidad de sedimentación. Solución de una proteína a determinada concentración, c, la sometemos a centrifugación. Se empieza a mover hacia el fondo del tubo y se genera una zona clara, sólo solvente cerca del menisco y un **frente móvil** que limita solvente de solución



Si seguimos la velocidad con que se mueve ese **frente móvil** (en una ultracentrífuga analítica), podemos **determinar S**, **coeficiente de sedimentación** para esa proteína

Corrientes de convección en distintos Rotores

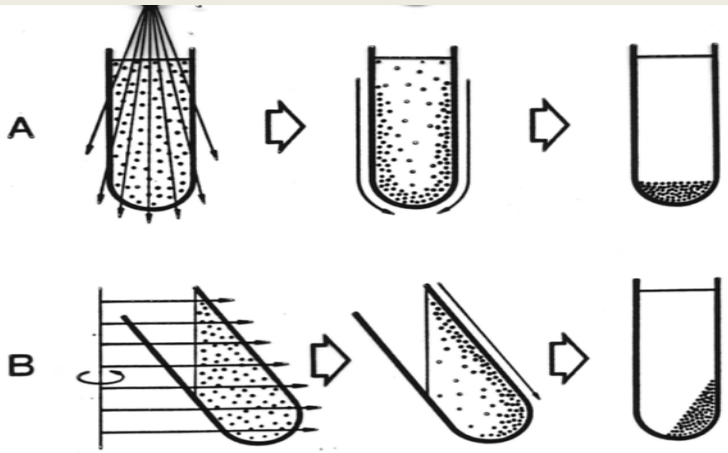


Fig. 3-5 Convection in swinging-bucket (*A*) and fixed-angle (*B*) rotors leads to bulk movement of particles along the walls of the centrifuge tube. See text for details.

Curso de sedimentación en Centrifugación Analítica

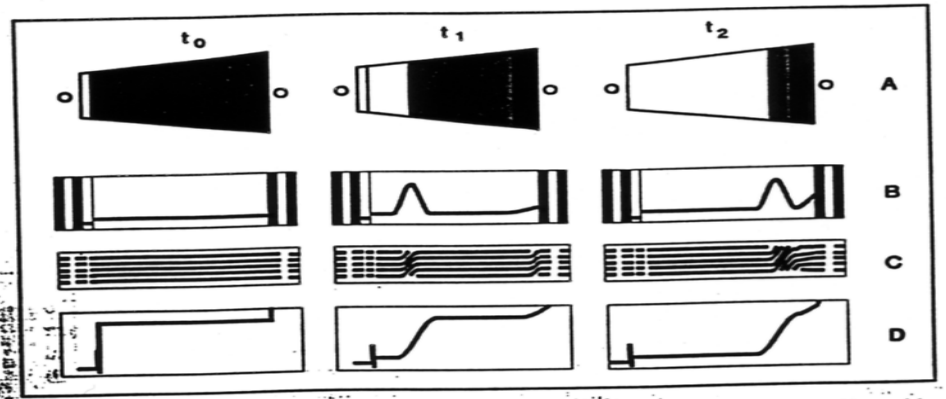


FIGURA 4.23 Representación gráfica de una célula de sector a diferentes tiempos de centrifugación (parte *A*). Inicialmente, antes de comenzar el proceso (tiempo t_0), hay una disolución homogénea (zona sombreada). Transcurrido un tiempo t_1 el soluto ha ido sedimentando, por lo que en la zona de la célula próxima al menisco sólo hay disolvente; el soluto se ha desplazado hacia el fondo (zona sombreada más oscura). A mayor tiempo de centrifugación ($t_2 > t_1$) el soluto se ha desplazado más aún hacia el fondo, y algunas moléculas ya han sedimentado. También se muestra el resultado de los análisis con los tres sistemas ópticos de observación, schlieren (*B*), refractométrico (*C*) y absorbancia (*D*), a los diferentes tiempos considerados. La zona de discontinuidad disolvente/disolución aparece como un pico (schlieren), como un cambio en las propiedades birrefringentes del sistema (refractométrico), y como un incremen-to de absorción debido a la presencia de soluto (absorbancia).

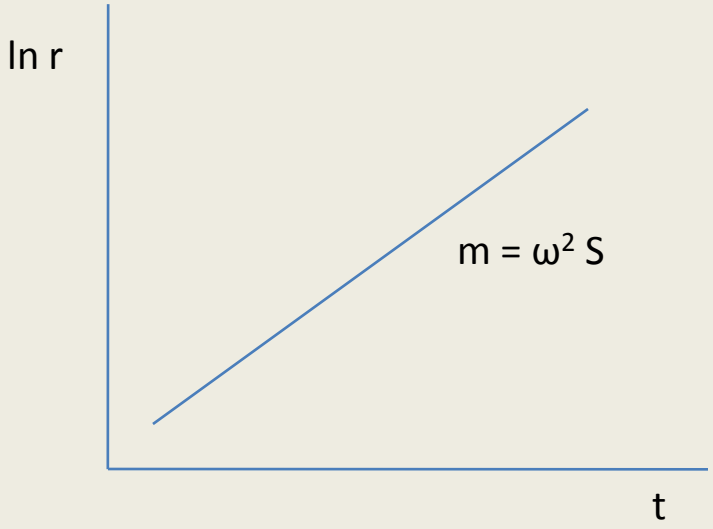
Determinación de S

$v = dr/dt = \omega^2 r S$

Reordenando: $dr/r = \omega^2 S \, dt$

Integrando: $\ln r = = \omega^2 S t$

Representando lnr frente a t se obtiene recta de pendiente $\omega^2 S$



ω es conocido

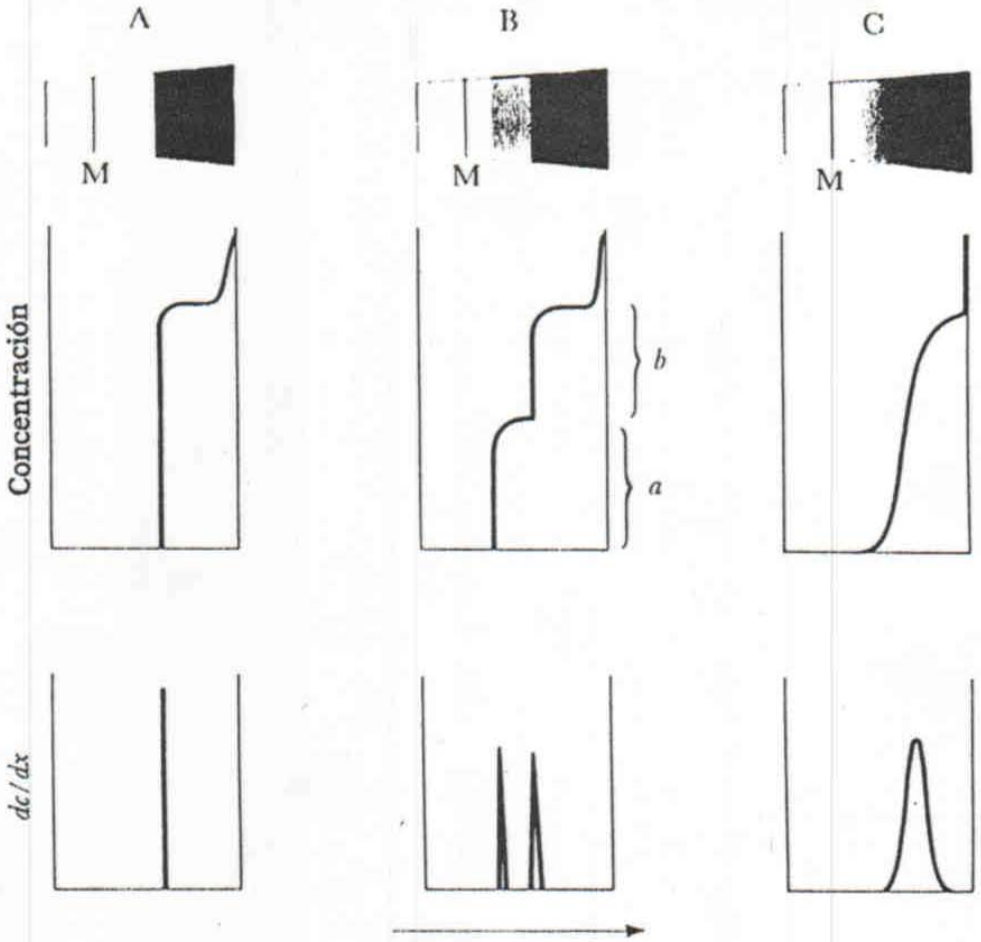
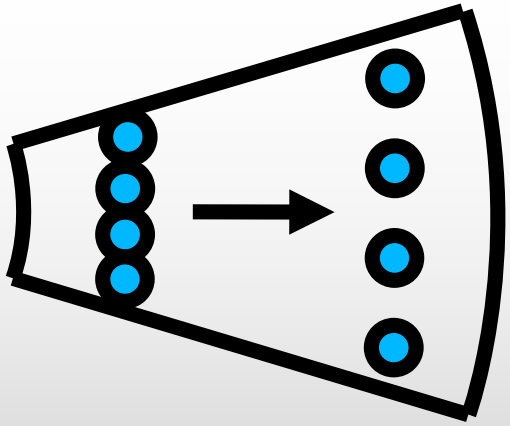
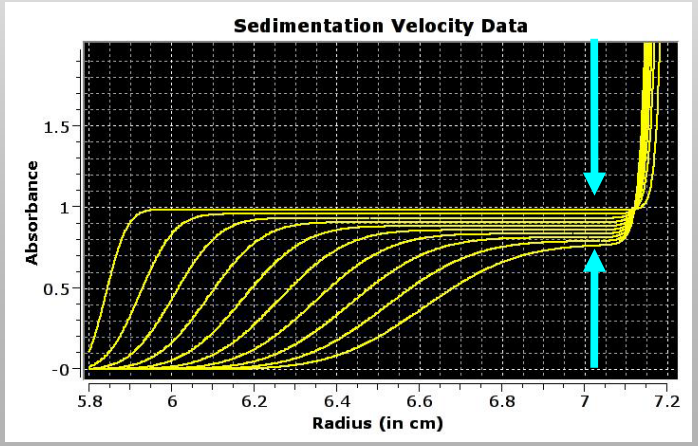


FIGURA 11-7
Distribución de las concentraciones después de un determinado período de tiempo para celdas llenas con distintas disoluciones: (A) un solo tipo de molécula de soluto con un solo coeficiente de sedimentación; (B) dos componentes, cada uno de ellos con un coeficiente de sedimentación distinto; y (C) una mezcla heterogénea con toda una serie de valores de *s*. Nótese el material que se acumula en el fondo de la celda. La serie inferior de gráficos muestra el gradiente de concentración; esto equivale a una fotografía de schlieren. La letra M indica el menisco.

Radial Dilution:





Radial Dilution occurs because of the cell's sector shape. Molecules sedimenting towards the outside of the cell will dilute as they sediment.

All like molecules - no matter at what position they are - will dilute at the same rate, causing a reduction in the observed optical density. At any given time, this dilution is the same at each point in the cell.

Radial Dilution can be observed through a reduction in the plateau absorbance in successive scans

History of Ultracentrifuge

1. ***Theodor Svedberg*** invented the analytical ultracentrifuge in 1923, and won the Nobel Prize in Chemistry.

2. ***Edward Greydon Pickels*** invented the vacuum ultracentrifuge

- Vacuum allowed a reduction in friction generated at high speeds.

- Vacuum systems also enabled the maintenance of constant temperature.

2. Type of Centrifuge

2-1.Low-speed centrifuges

- Also called: microfuge, Clinical, Table top or bench top centrifuges
- Max speed ~ 20,000 rpm
- Operate at room temperature
- Fixed angle or swinging bucket can be used
- Commonly used for rapid separation of coarse particles
  - **E.g. RBC from blood, DNA from proteins, etc.**
  - **The sample is centrifuged until the particles are tightly packed into pellet at the bottom of the tube. Liquid portion, supernatant, is decanted.**

2-2. High-speed Centrifuges

Preparative centrifuges.

- Max speed ~ 80,000 rpm
- Often refrigerated, and requires vacuum to operate
- Fixed angle or swinging bucket can be used
- Generally used to separate macromolecules (proteins or nucleic acids) during purification or preparative work.
- Can be used to estimate sedimentation coefficient and MW,
  - No optical read-out

Ultracentrifuge

- The **ultracentrifuge** is a centrifuge optimized for spinning a rotor at very high speeds, capable of generating acceleration as high as 1,000,000 g (9,800 km/s²).

- There are two kinds of ultracentrifuges, the preparative and the analytical ultracentrifuge.

- Both classes of instruments are used in molecular biology, biochemistry and polymer science.

2-3. Ultracentrifuge

The most advanced form of centrifuges: (specialized and expensive)

- Used to precisely determine sedimentation coefficient and MW of molecules, Molecular shape, Protein-protein interactions
- Uses very high speed and/or RCF
- Uses small sample size (< 1 ml)
- Uses relatively pure sample
- Built in optical system to analyze movements of molecules during centrifugation

Analytical Ultracentrifuge

	Centrifuge Classes		
	Lowspeed	**High-speed**	**Ultra/micro-ultra**
Maximum Speed (rpm $x10^3$)	10	28	100/150
Maximum RCF ($x10^3$)	7	100	800/900
Pelleting applications			
Bacteria	Yes	Yes	(Yes)
Animal and plant cells	Yes	Yes	(Yes)
Nuclei	Yes	Yes	(Yes)
Precipitates	Some	Most	(Yes)
Membrane fractions	Some	Some	Yes
Ribosomes/Polysomes	-	-	Yes
Macromolecules	-	-	Yes
Viruses	-	Most	Yes

( ) = can be done but not usually used for this purpose.

Before

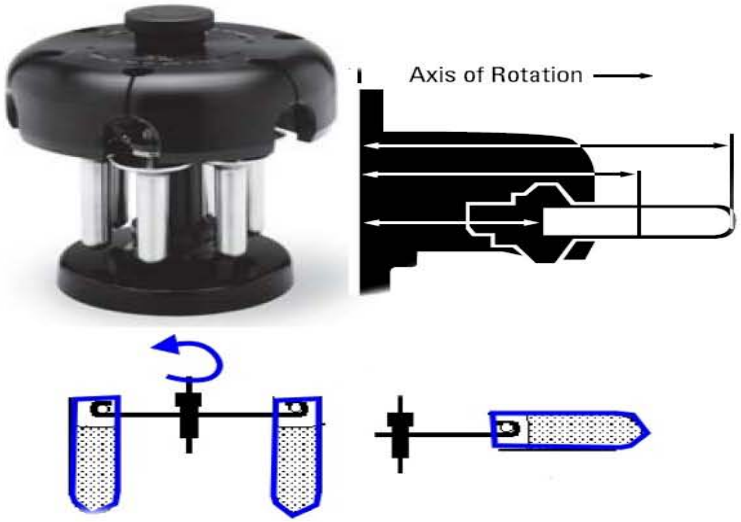
After

1. Types of Rotors

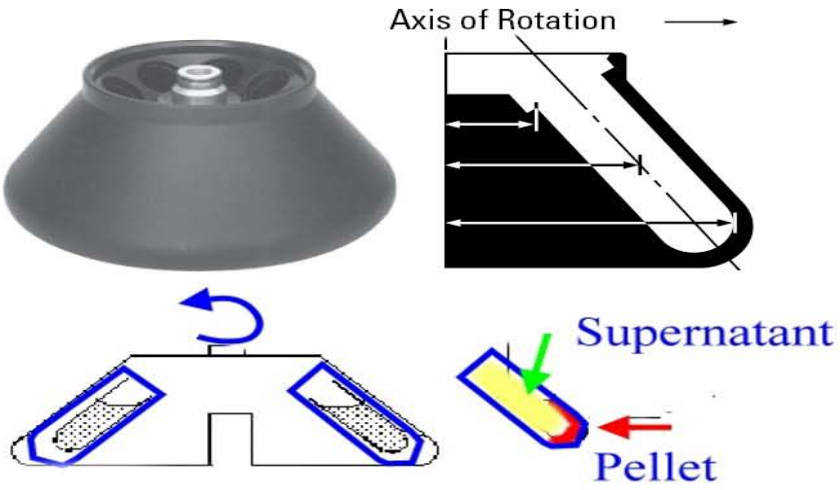
swinging bucket rotors:

fixed-angle rotors:

Other types include vertical rotors and continuous-flow rotors



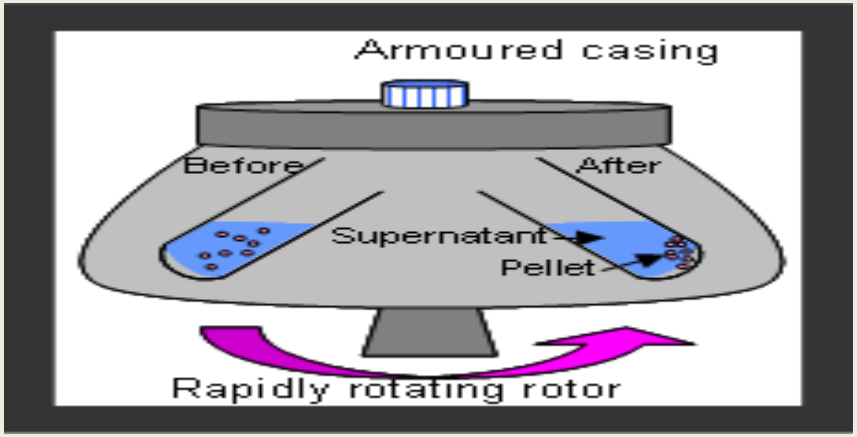
Swinging Bucket



Fixed-angle

Fixed-angle rotors

- Sample tubes are held fixed at the angle of 20 – 45^{o} in the rotor cavity.
- When the rotor begins to rotate, the solution in the tubes reorients.
- This rotor type is most commonly used for pelleting applications.
- Examples include pelleting bacteria, yeast, and other mammalian cells.
- It is also useful for isopycnic separations of macromolecules such as nucleic acids.



Swinging bucket rotors

- Sample tubes are loaded into individual buckets that hang vertically while the rotor is at rest.
- When the rotor begins to rotate the buckets swing out to a horizontal position.
- Useful when samples are to be resolved in density gradients.
- The longer pathlength permits better separation of individual particle types from a mixture.
- However, this rotor is relatively inefficient for pelleting.

Vertical rotors

- Sample tubes are held in vertical position during rotation.
- This type of rotor is not suitable for pelleting applications but is most efficient for isopycnic (density) separations due to the short pathlength.
- Applications include plasmid DNA, RNA, and lipoprotein isolations.

Table 3. Types of rotors and their applications.

Type of rotor	Pelleting	Rate-zonal Sedimentation	Isopycnic
Fixed-angle	Excellent	Limited	Variable*
Swinging-Bucket	Inefficient	Good	Good**
Vertical	NS	Good	Excellent
Zonal	NS	Excellent	Good

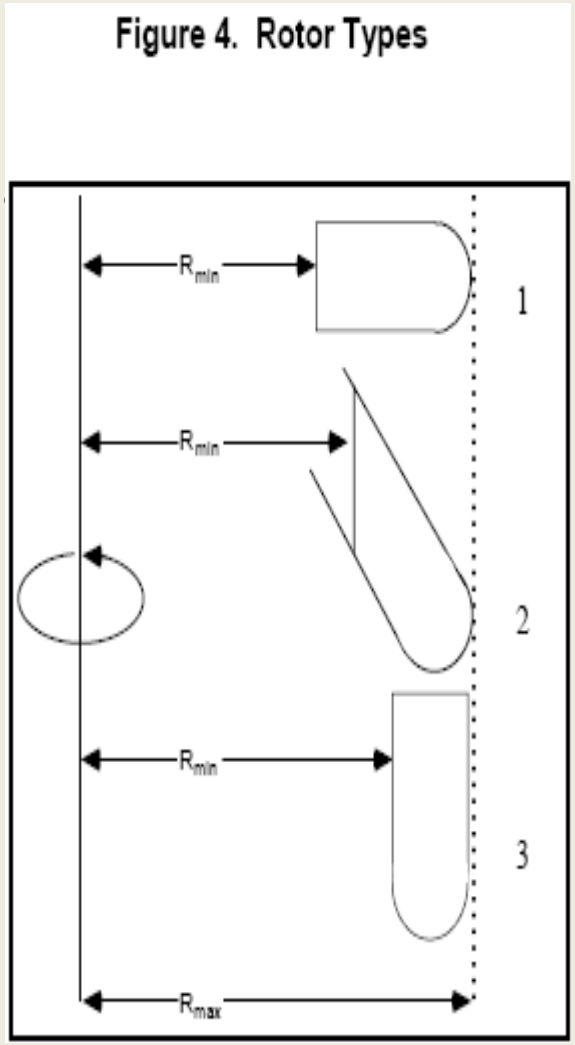
NS = not suitable

*Good for macromolecules, poor for cells, and organelles

**Good for cells and organelles, caution needed if used with CsCl

Types of rotor

1. Horizontal swing – out rotors
2. Fixed – angle rotors.
3. Vertical rotors.
4. Continuous - flow rotors.



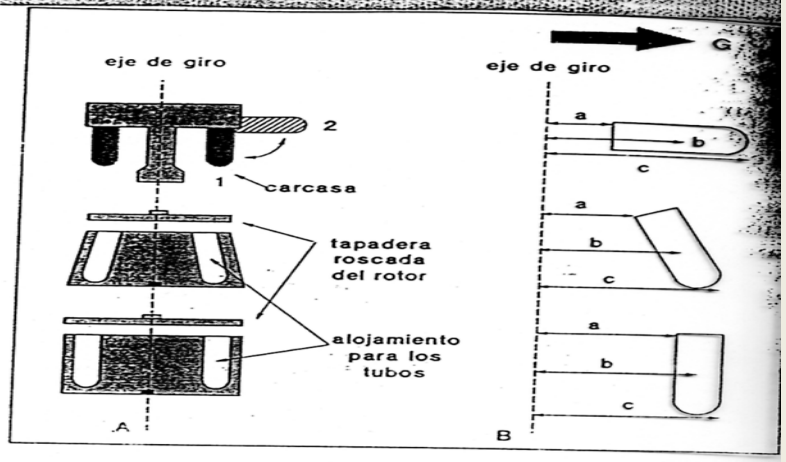


FIGURA 4.4. (Parte *A*): esquema de un rotor flotante (I), uno angular (II), y uno vertical (III). En el caso del flotante, la posición (1) corresponde a la situación de reposo de las carcasas, y la posición (2) a la que adquieren bajo la acción del campo centrífugo G; la flecha de doble punta hace referencia al movimiento de flotación que experimentan las carcasas. (Parte *B*): distancias mínima "a", máxima "c" y promedio "b" al eje de giro, para un tubo sometido a la acción de un campo centrífugo en cada uno de los tres tipos de rotores.

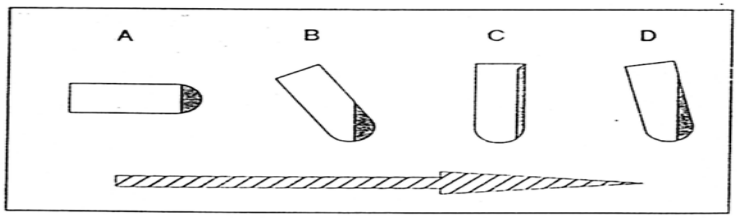


FIGURA 4.5. Disposición de los depósitos de material sedimentado (zonas sombreadas) en los tubos de centrifugación, en los casos de un rotor flotante (*A*), uno angular (*B*), uno vertical (*C*), y uno "casi vertical" (*D*). La flecha representa la dirección de actuación del campo centrífugo aplicado.

Applied Centrifugation

- **Parameters you need to know:**

1. Type of rotor:
   - fixed angle, swinging bucket, vertical

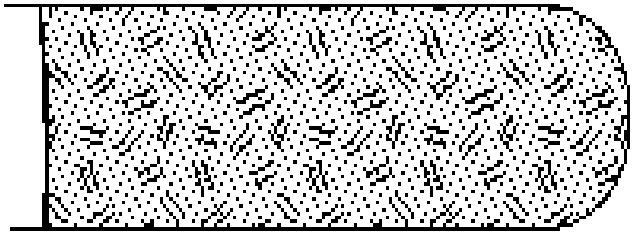
2. Type of centrifuge:
   - Low speed , high Speed, ultracentrifuge

3. Type of centrifugation
   - Differential, preparative, or analytical

– Also, the **Speed and duration** of centrifugation

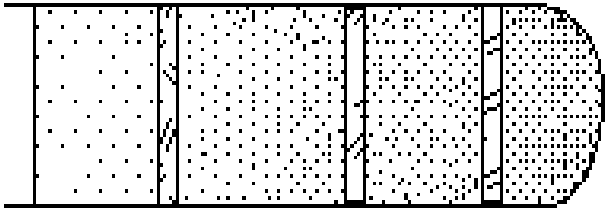
GRADIENTES DE DENSIDAD AUTOGENERADOS

(a) Before centrifugation. CsCl and sample uniformly distributed



CsCl DNA

(b) After centrifugation. CsCl redistributes giving density gradient. Nucleic acid species form bands at "equal density" levels.



SEPARACION DE DNAs en GRADIENTES DE CsCl

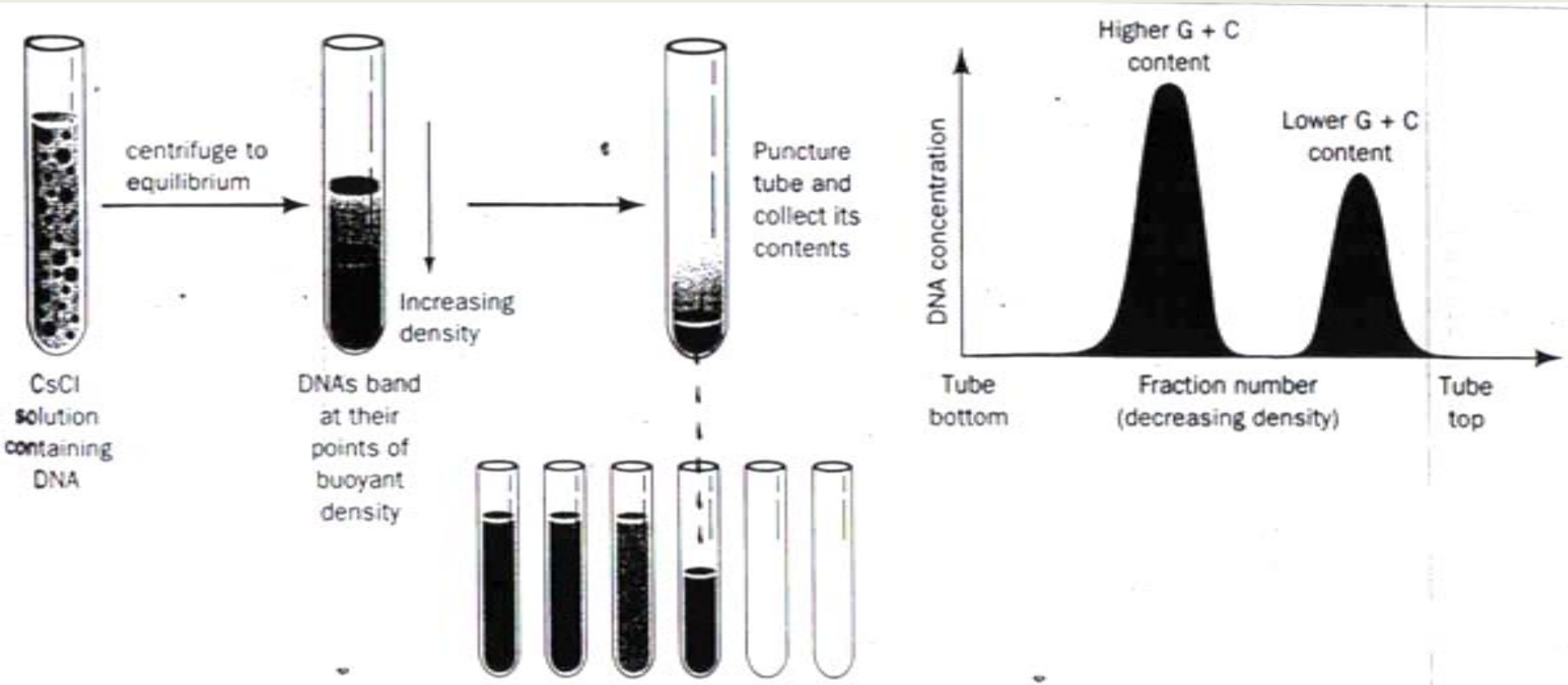
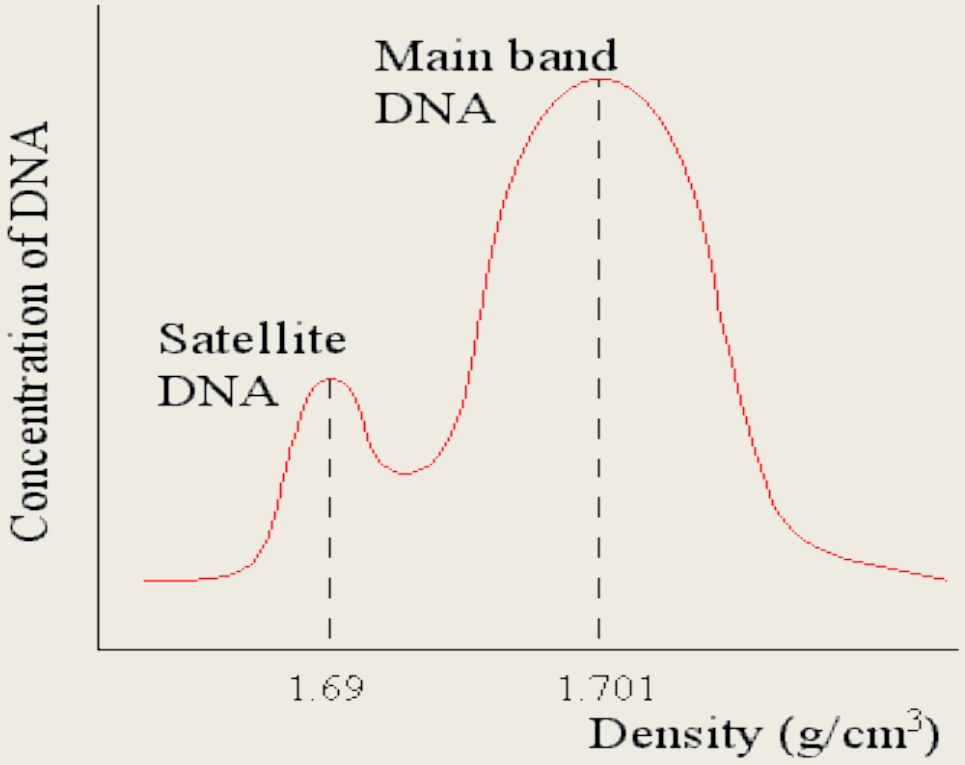


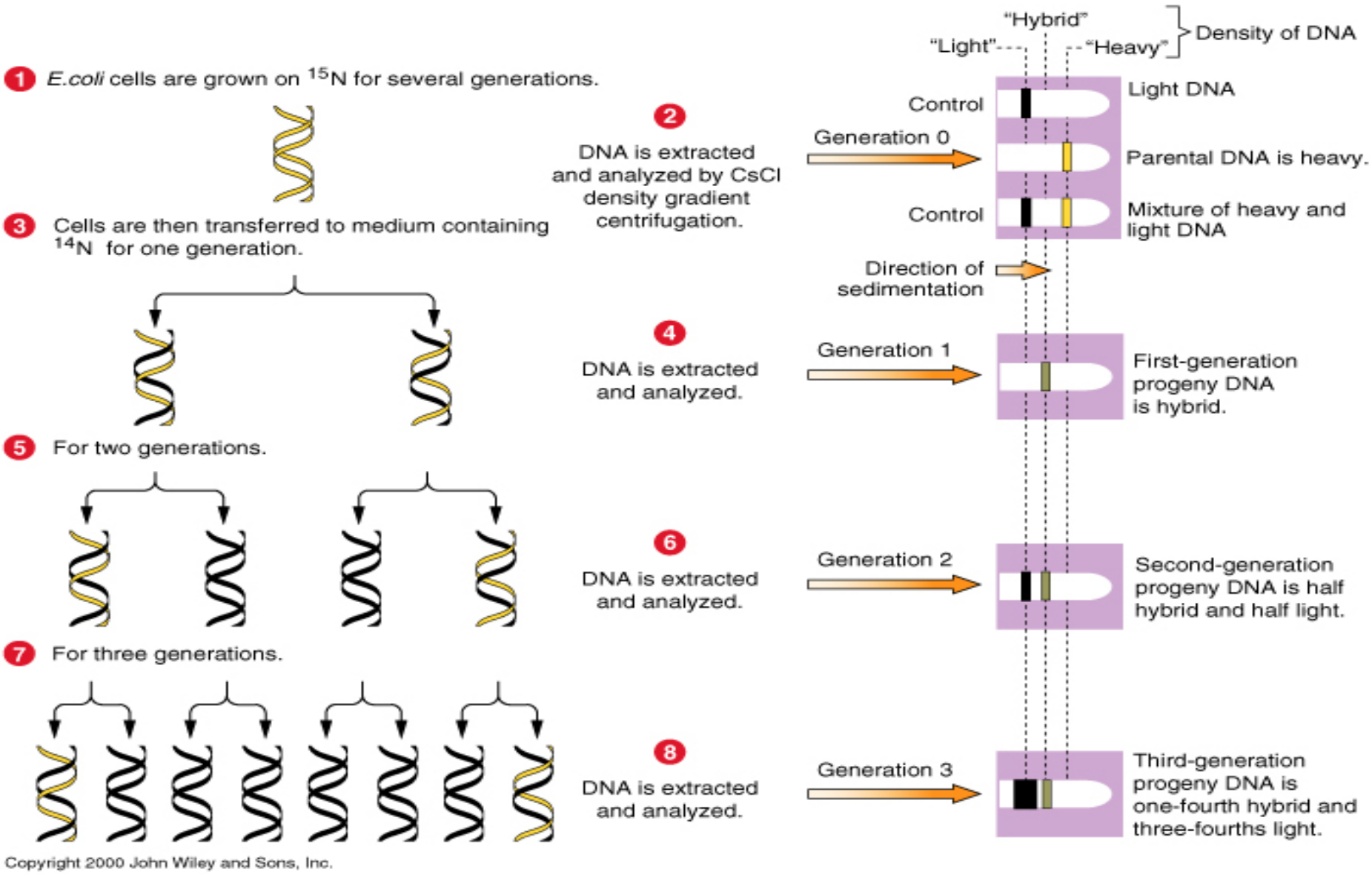
FIGURE 28-31. The separation of DNAs according to base composition by equilibrium density gradient ultracentrifugation in CsCl solution. An initially 8*M* CsCl solution forms a density gradient that varies linearly from ~1.80 g·cm^{-3} at the bottom of the centrifuge tube to ~1.55 g·cm^{-3} at the top. The amount of DNA in each fraction is estimated from its UV absorbance, usually at 260 nm.

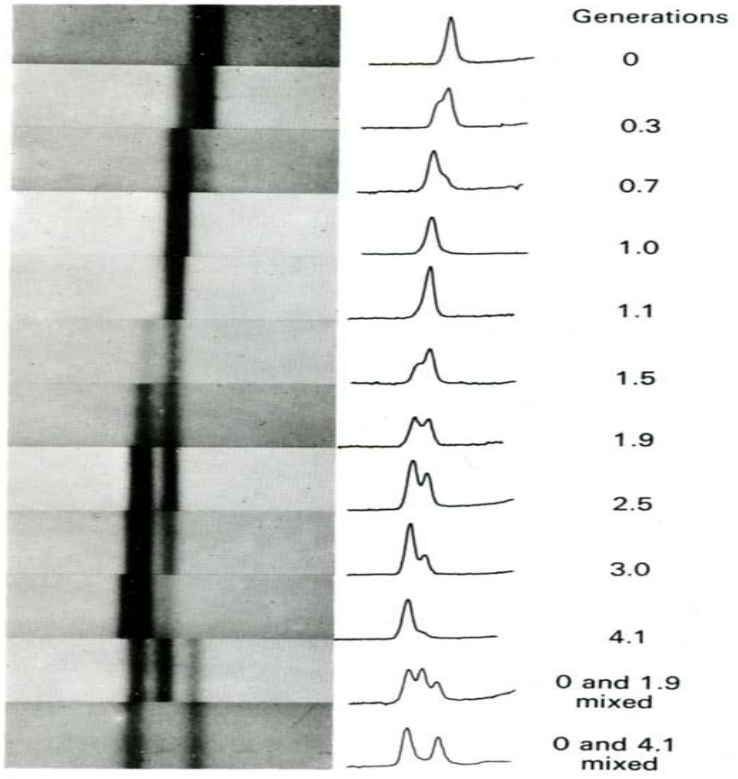
SEPARACION DE DNAs en GRADIENTES DE CsCl

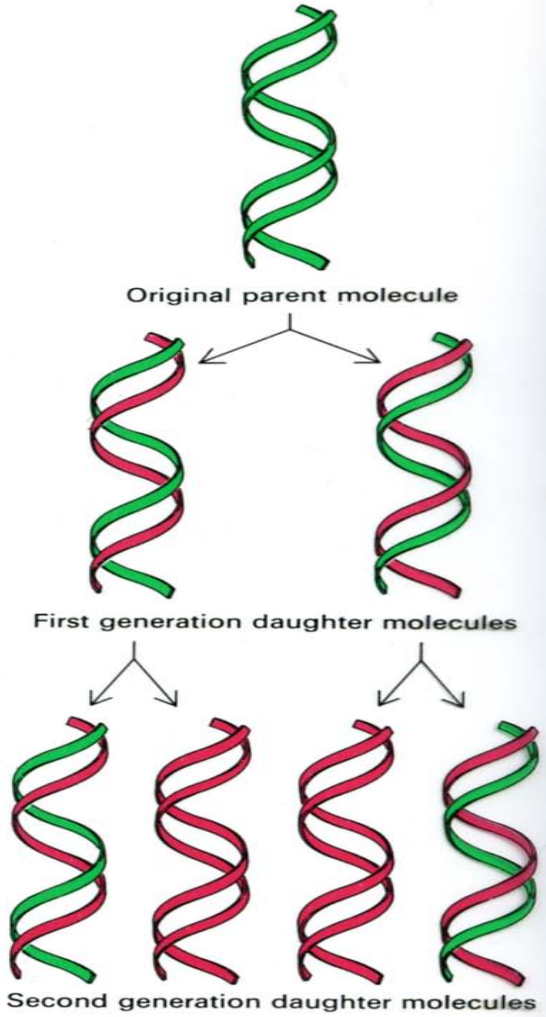


Research work Related to Ultracentrifugation

2- Meselson and Stahl's experiment







Figures 4-14 and 4-15, page 85

Stryer: *Biochemistry,* Fourth Edition

GRADIENTES ISOCINETICOS

-Gradientes continuos de densidad en los que la v de sedimentación es CONSTANTE a lo largo del gradiente y proporcional a S.

-La v de sedimentación es independiente de la longitud del tubo.

- Un ejemplo muy utilizado: Gradiente de sacarosa 5-20%.

- La distancia recorrida por un soluto es proporcional a S (la distancia recorrida es proporcional al volumen recogido hasta llegar a la posición del soluto).

- Podemos generar una recta patrón con solutos de S conocidos y representarla en función de la distancia recorrida (volumen recogido). Interpolar el volumen de la muestra cuyo S se quiere determinar.

- En el caso de proteínas, a través de la expresión $M^{2/3} / M^{2/3} = S_1 / S_2$ se puede determinar la Masa Molecular de la proteína 1 por comparación con la proteína 2 que debe tener forma y densidad similar a 1.

-No requiere purificación previa (ventaja respecto de Ultracentrifugación analítica), pero es más impreciso.

Factor K

Each rotor is given a value for k (the clearing constant, or clearing factor), which is an estimate of the time (in hours) required to pellet a particle of known sedimentation coefficient at the maximum speed of the rotor. The lower the value of k, the shorter the time required to sediment a given particle. The relationship is given by $t = k / S$.

S = Sedimentation Coefficient in water at 20 ° expressed in Svedbergs

It is possible to compute k, but it is easiest to use the manufacturers tabulated values

Using tabulated k values, however, the comparison of rotors is even easier, as the equation above becomes:

$$t_1 = k_1 t_2 / k_2$$

where the t and k values are the time and k factor for each rotor.