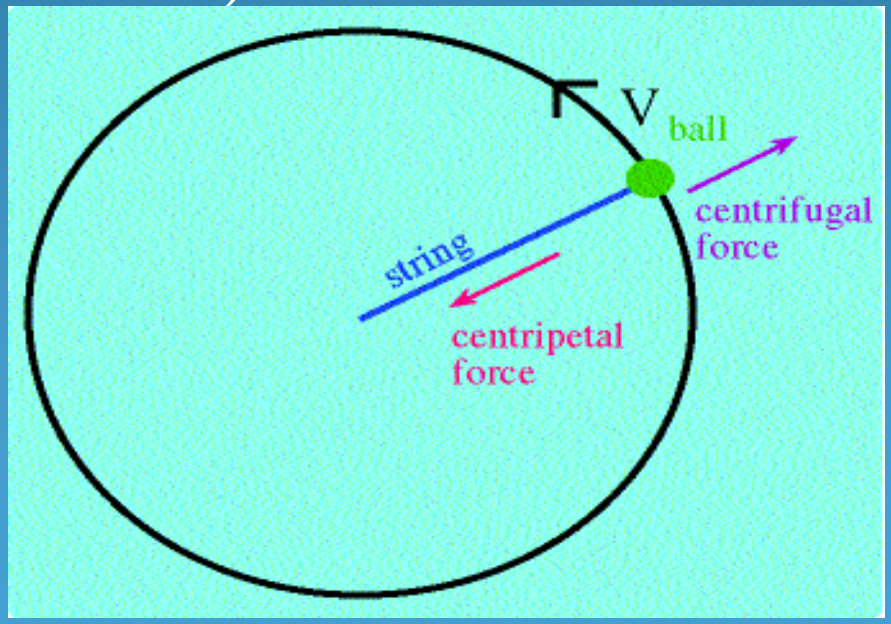
ULTRACENTRIFUGATION

Centrifugal force:

It is the apparent outward force that draws a rotating body away from the centre of rotation. It is caused by the inertia of the body as the body's path is continually redirected.

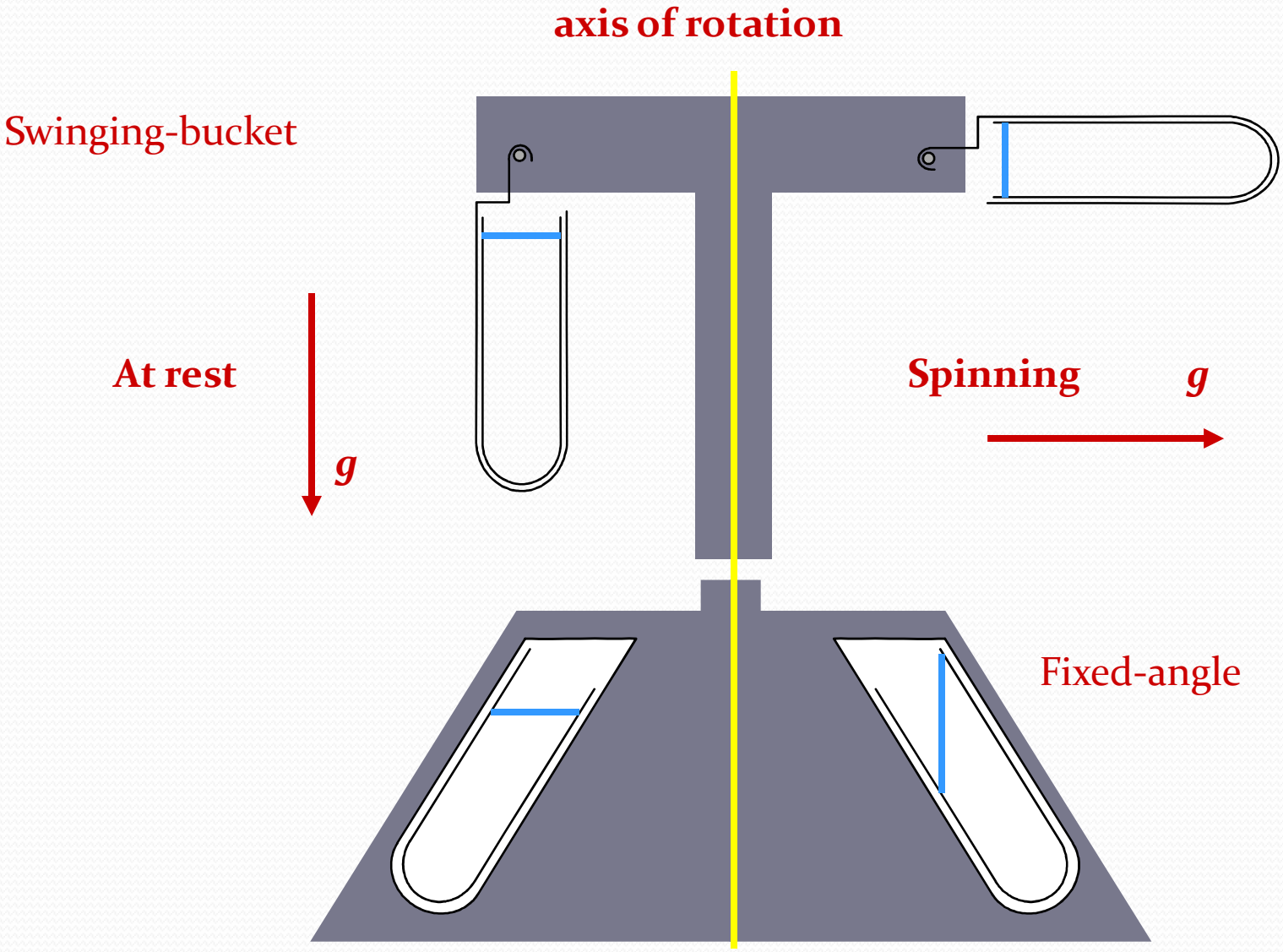
(Inertia - it is the resistance of any physical object to a change in its state of motion or rest, or the tendency of an object to resist any change in its motion.)



Centrifugation Theory and Practice

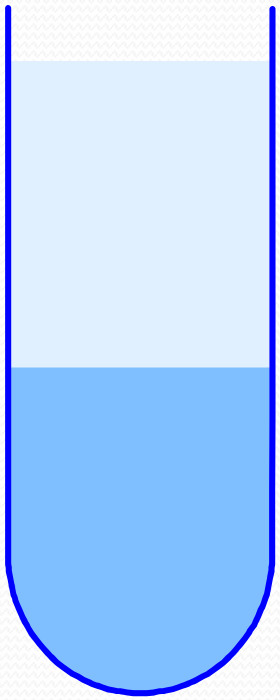
- Routine centrifuge rotors
- Density gradient theory

Centrifuge rotors

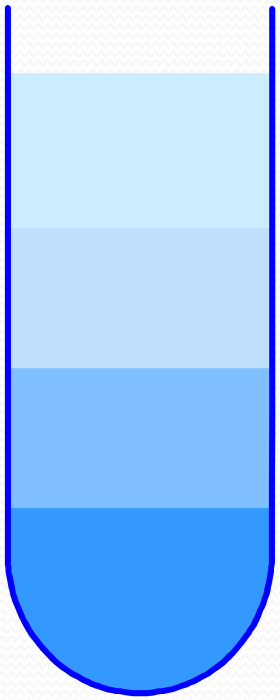


Density gradient centrifugation

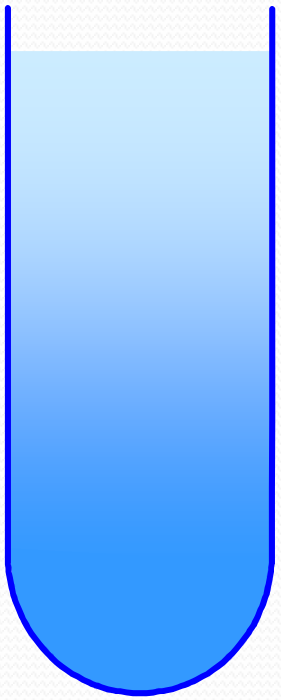
Density Barrier



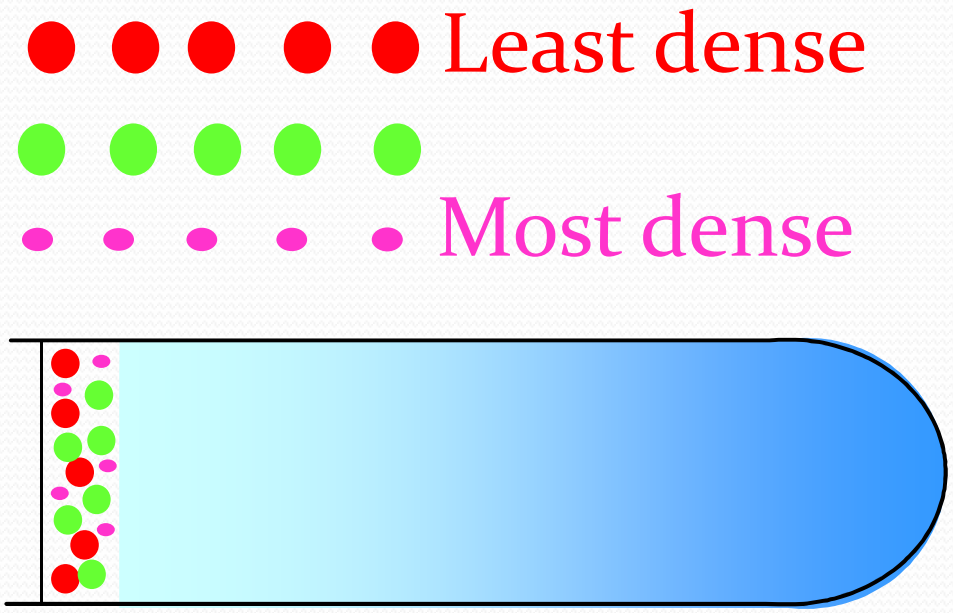
Discontinuous



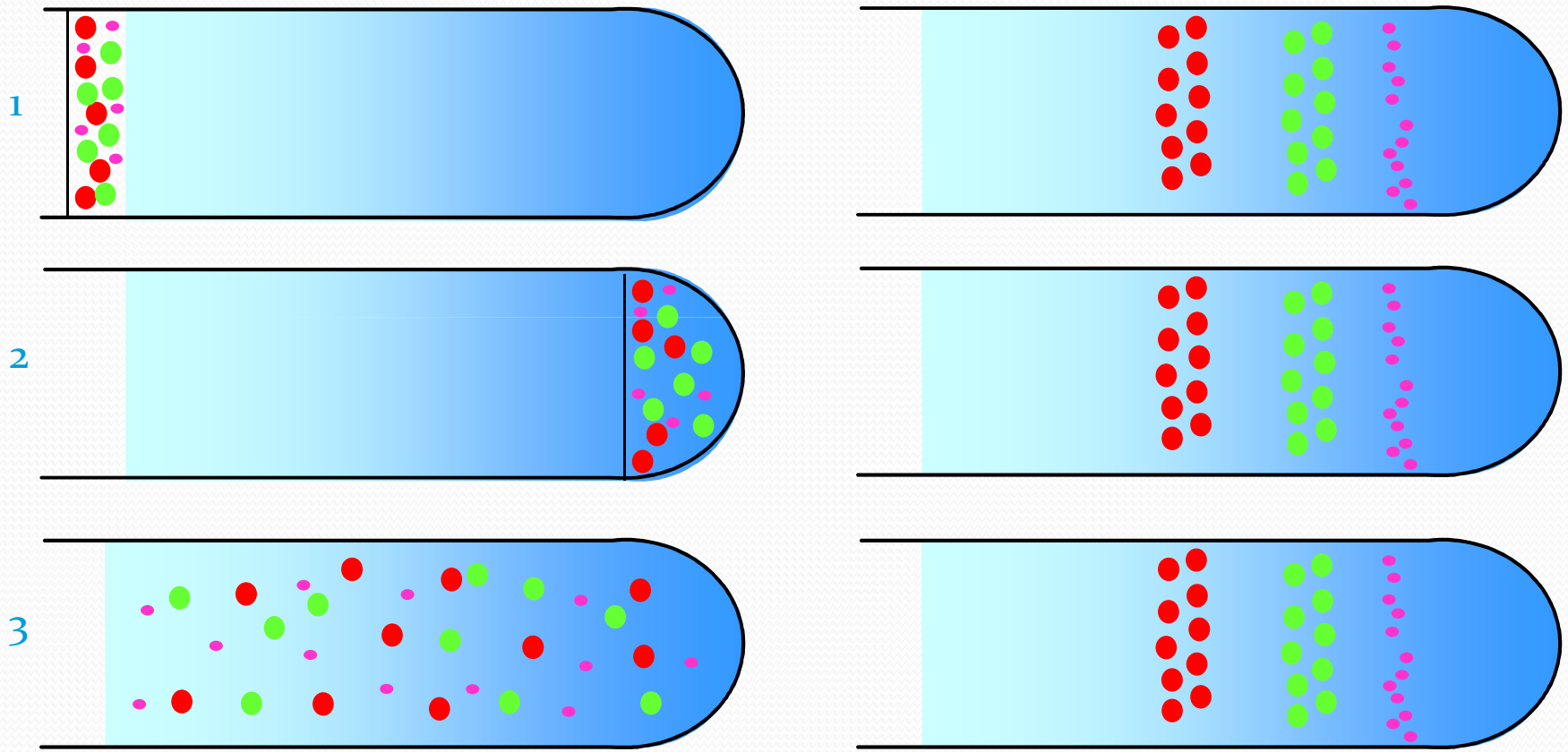
Continuous

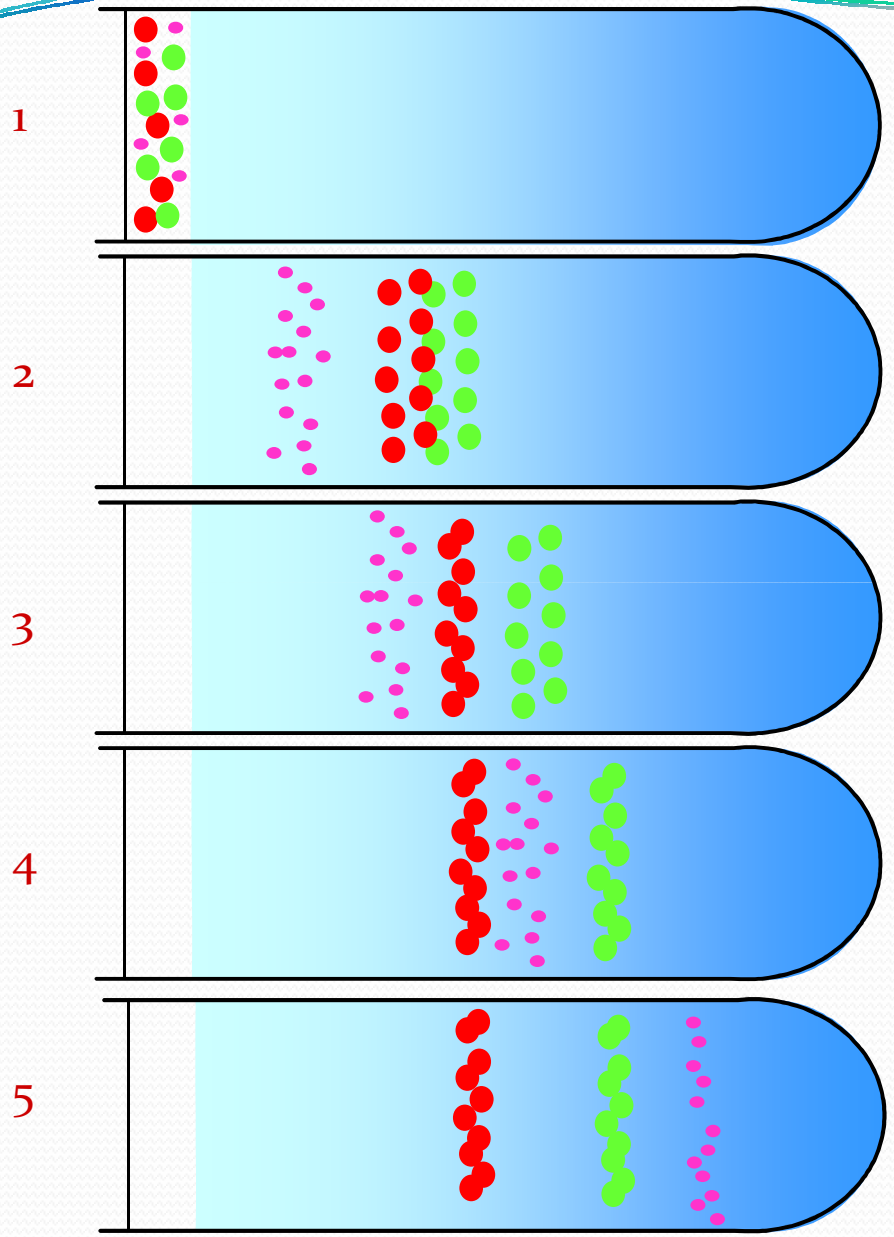


How does a gradient separate different particles?



3 Formats for separation of different density portions in a testtube.





Equilibrium density banding

ULTRACENTRIFUGATION

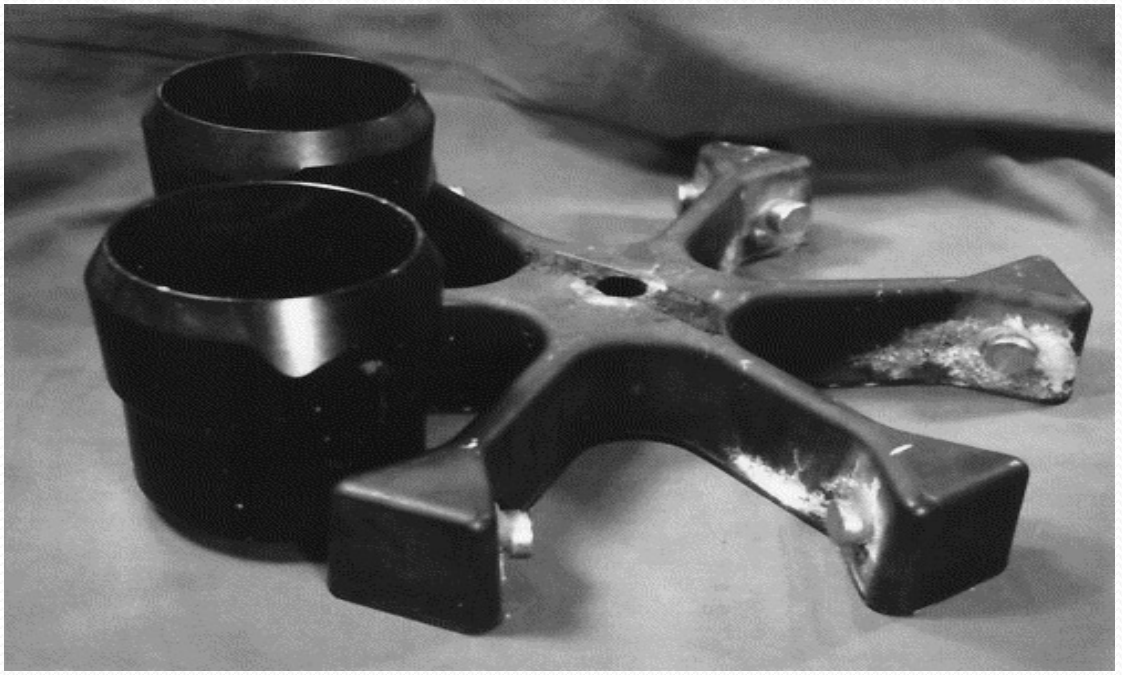
An important tool in biochemical research is the centrifuge, which through rapid spinning imposes high centrifugal forces on suspended particles, or even molecules in solution, and causes separations of such matter on the basis of differences in weight.

Example:

Red cells may be separated from plasma of blood, nuclei from mitochondria in cell homogenates, and one protein from another in complex mixtures

- . Proteins are separated by ultracentrifugation—very high speed spinning; with possibility of appropriate photography of the protein layers as they form in the centrifugal field, it is possible to determine the molecular weights of proteins.
- The **ultracentrifuge** is a centrifuge optimized for spinning a rotor at very high speeds, capable of generating acceleration as high as ***19600 km/s²***

  (around 50000 rpm).



Analytical Ultracentrifugation

- Analytical Ultracentrifugation (AUC) experiments give us a method for the direct measurement of basic thermodynamic properties of macromolecules in solution.
- Since sedimentation relies on the principal property of mass and centrifugal force, it is a valuable technique for a wide variety of solution conditions.

- **1) What can be done with AUC to characterize a sample?**
- a) Determine number of components and number of species; detection of impurities
- b) Molar mass of each species
- c) Kinds and stoichiometry of chemical reactions present in solution, including association with ligands, self-association.
- d)Shape and charge of the molecules, as inferred from their sedimentation frictional behavior.

- **2) Examples of molecules that can be analyzed:**
- a) Proteins
- b) Polysaccharides
- c) Nucleic acids
- d) Small molecules: drugs, ligands, gasses
- e) Large aggregates: viruses, organelles

- **3) Kinds of buffers and additional solutes:**
- a) BME, DTT,
- b) Triton X100. Tween-80
- b) Nucleotides
- c) Salts and neutral molecules that significantly affect density
- d) PEG, glycerol affect viscosity and desnity strongly
- e) 6 M Gdn and 8 M Urea

Analytical ultracentrifuge

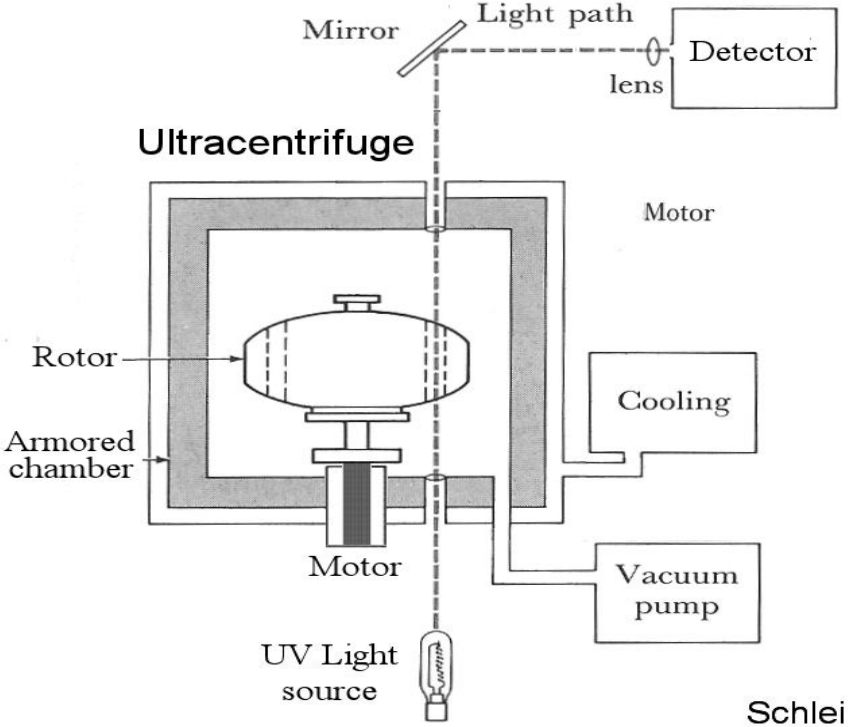
In an analytical ultracentrifuge, a sample being spun can be monitored in real time through an optical detection system, using ultraviolet light absorption and optical refractive index sensitive system.

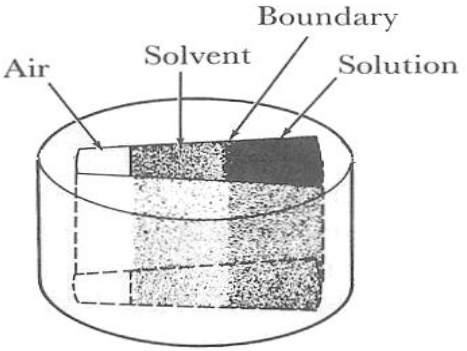
- ***Working***:-

#As the rotor turns, the images of the cell (proteins) are projected by an optical system on to film or a computer. The concentration of the solution at various points in the cell is determined by absorption of a light of the appropriate wavelength. This can be accomplished either by measuring the degree of blackening of a photographic film or by the deflection of the recorder of the scanning system and fed into a computer.

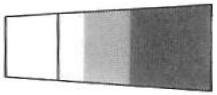
This allows the operator to observe the separation of the sample concentration versus the axis of rotation (lateral axis of the tube)
as a result of the applied centrifugal field. Two kinds of experiments are commonly performed on these instruments: sedimentation velocity experiments and sedimentation equilibrium experiments.


BECKMAN
Optima XL-A
IBM

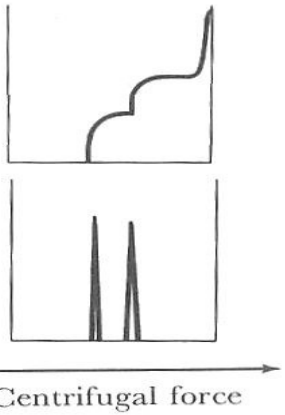




Sample Cell



Schleirin Optics (schematic)



Rotor

Sedimentation velocity experiments aim to interpret the **entire** time-course of sedimentation, and report on the shape and molar mass of the dissolved macromolecules, as well as their size-distribution.

Sedimentation velocity experiments can also be used to study reversible chemical equilibria between macromolecular species, by monitoring the number and molar mass of macromolecular complexes.

#Sedimentation equilibrium experiments are concerned only with the **final** steady-state of the experiment, where sedimentation is balanced by diffusion opposing the concentration gradients, resulting in a time-independent concentration profile.

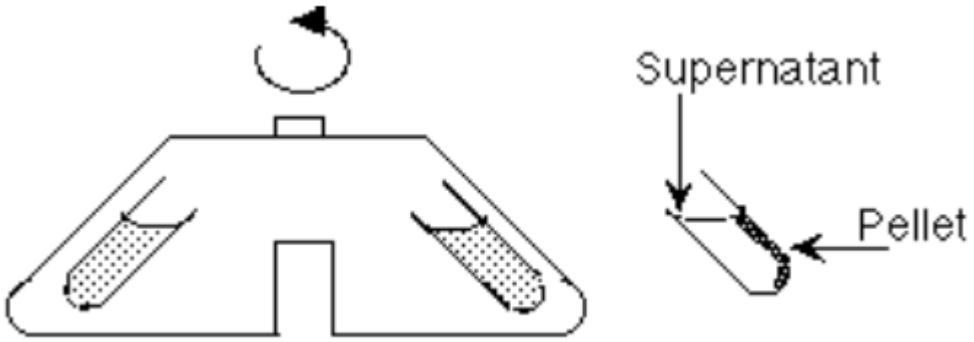
- Analytical centrifugation involves measuring the physical properties of the sedimenting particles such as sedimentation coefficient or molecular weight.

•Preparative Centrifuge:

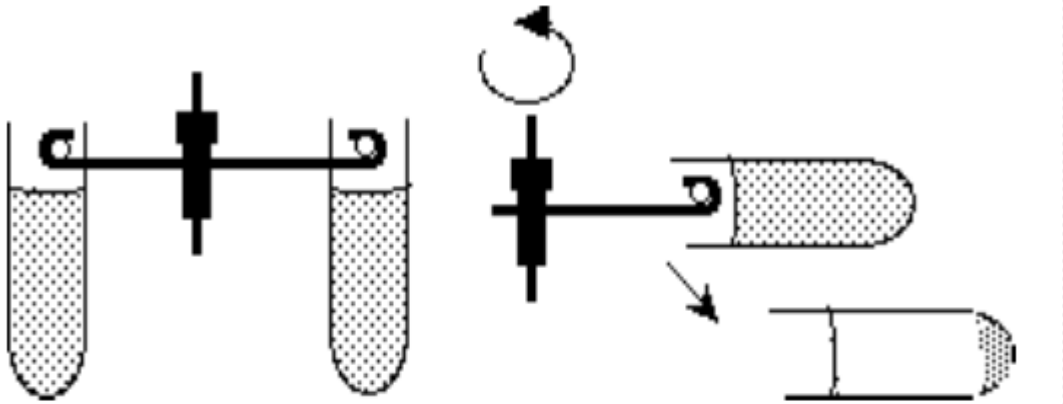
▪Preparative ultracentrifuges are available with a wide variety of rotors suitable for a great range of experiments.

▪ Most rotors are designed to hold tubes that contain the samples.

▪ *Swinging bucket rotors* allow the tubes to hang on hinges so the tubes reorient to the horizontal as the rotor initially accelerates.



- *Fixed angle rotors* are made of a single block of metal and hold the tubes in cavities bored at a predetermined angle.



- *Zonal rotors* are designed to contain a large volume of sample in a single central cavity rather than in tubes. Some zonal rotors are capable of dynamic loading and unloading of samples while the rotor is spinning at high s

- They can also be used for gradient separations, in which the tubes are filled from top to bottom with an increasing concentration of a dense substance in solution.
- Sucrose gradients are typically used for separation of cellular organelles.
- Gradients of caesium salts are used for separation of nucleic acids.
- After the sample has spun at high speed for sufficient time to produce the separation, the rotor is allowed to come to a smooth stop and the gradient is gently pumped out of each tube to isolate the separated components.

- The other form of centrifugation is called **preparative ultracentrifugation** and the objective is to isolate specific particles which can be reused. Two types.

1)Differential ultracentrifugation: **Differential centrifugation** is a common procedure in microbiology and cytology used to separate certain organelles from whole cells for further analysis of specific parts of cells. In the process, a tissue sample is first homogenised to break the cell membranes and mix up the cell contents. The homogenate is then subjected to repeated centrifugations, each time removing the pellet and increasing the centrifugal force. Finally, purification may be done through equilibrium sedimentation, and the desired layer is extracted for further analysis.

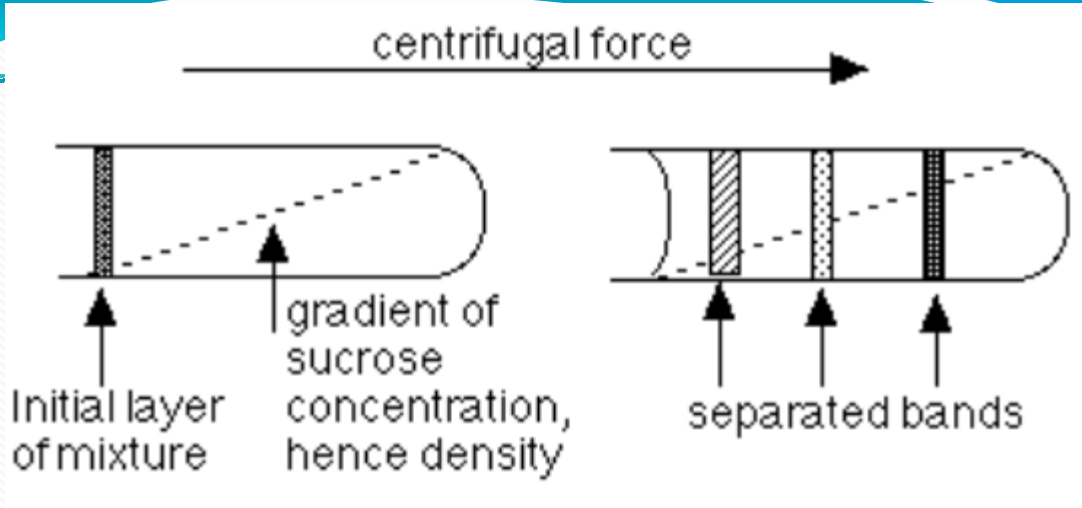
2) Density gradient. Based on denstiy difference

- There are two types of density gradient centrifugations under preparative centrifugation such as:
- **ZONAL(or)RATE**
- **ISOPYCNIC**

•ZONAL(or)RATE centrifugation:

- Mixture to be separated is layered on top of a **SUCROSE, or FICOLL, GRADIENT**(increasing concentration down the tube)
- Provides gravitational stability as different species move down tube at different rates.
- Forming separate bands.

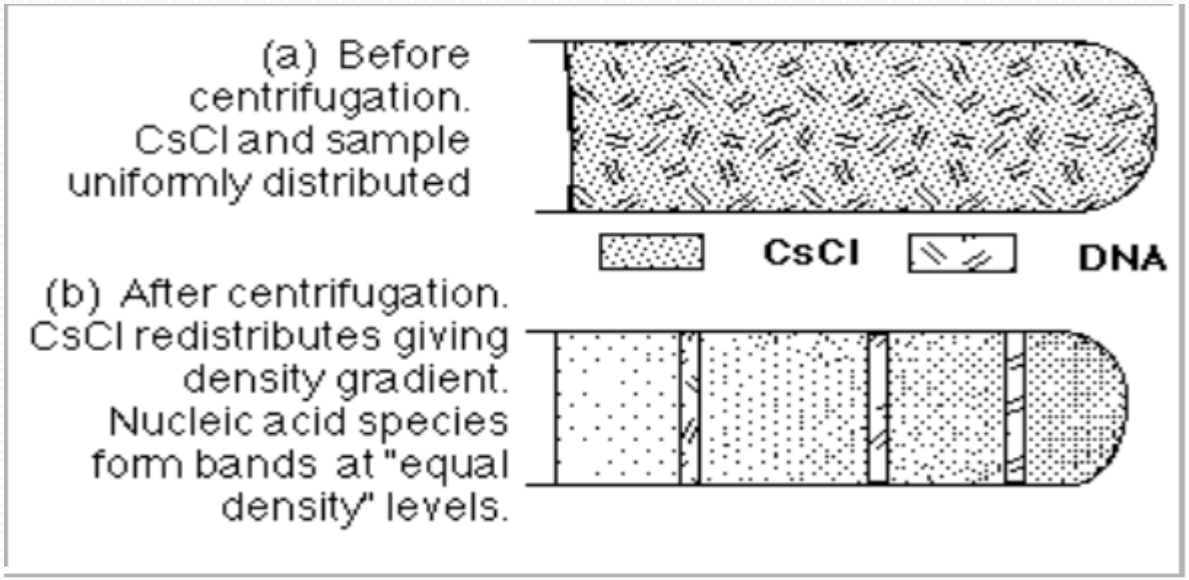
Sedimenting force on particle
= Mass x centrifugal field
= $\mathbf{m\omega^2 r}$



- Species are separated by differences in
- **SEDIMENTATION COEFFICIENT (S)=Rate of movement down tube/ Centrifugal force**
- S is increased for particle of LARGER MASS
- S is also increased for MORE COMPACT STRUCTURES of equal particle mass.
- Mild, non-denaturing procedure, useful for protein purification, and for intact cells and organelles.

ISOPYCNIC centrifugation:

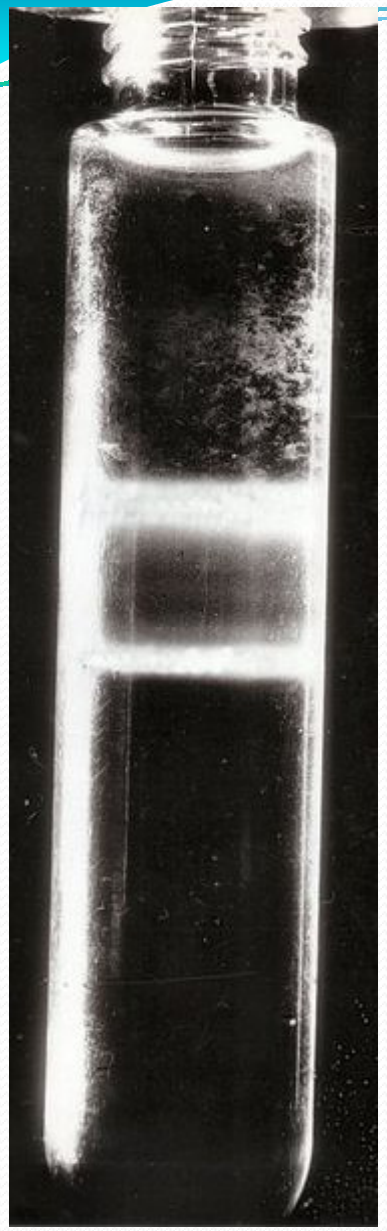
- **Isopycnic** means "of the same density."
- Molecules separated on EQUILIBRIUM POSITION, NOT by RATES of sedimentation.
- Each molecule floats or sinks to position where density equals density of **CsCl** solution.
- Then no net sedimenting force on molecules.
- **Isopycnic = Equal density** and separation is on basis of DIFFERENT DENSITIES of the particles.



•The term "isopycnic" is also encountered in biophysical chemistry and usually in reference to a process of separating particles, sub cellular organelles, or other substances on the basis of their density.

• Isopycnic centrifugation refers to a method wherein a density gradient is either pre-formed or forms during high speed centrifugation, after this gradient is formed particles move within the gradient to the position having a density matching their own . This technique is extremely powerful.

•Following example shows the isopycnic centrifugation:



•Viruses purified by isopycnic centrifugation.

• A density gradient was formed during high speed centrifugation of a solution of caesium chloride and the two virus types come to rest at points corresponding to their density.

• The tube is about 10cm long.

BENCHTOP CENTRIFUGE

Thank You